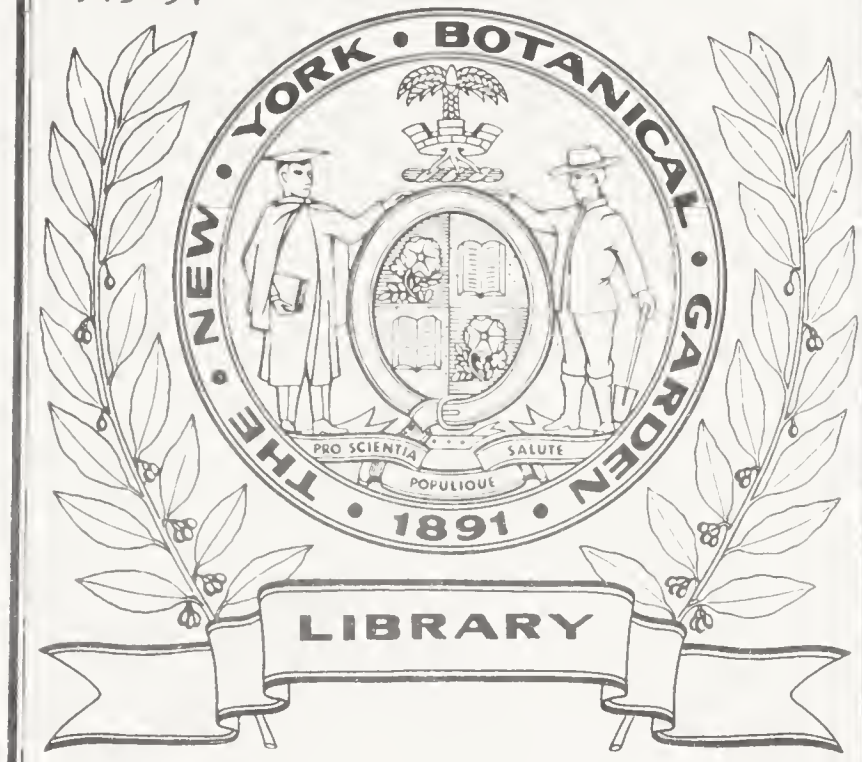

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The Taxonomy, Host Range and Geographic Distribution of the Genus Pythium

By

JOHN T. MIDDLETON

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THE TAXONOMY, HOST RANGE AND GEOGRAPHIC DISTRIBUTION OF THE GENUS PYTHIUM

JOHN T. MIDDLETON

INTRODUCTION

This investigation was prompted by the need for a study of the genus *Pythium* which would contribute toward a better understanding of the species, their taxonomic status, and their phylogeny.

The genus has been treated systematically at least five times since its establishment in 1858. Several distinctly different dispositions have been made. New species from various hosts from widely scattered areas throughout the world have been described. A review of the literature concerning the species indicates some of them to be synonymous, others of doubtful validity and still others imperfectly delineated. Since 1931–1932, when the last account of the genus was published, a comparatively large number of species have been described but not given any taxonomic treatment. In the following discussion an attempt is made to assemble information from many scattered papers and indicate the salient parts. New data resulting from observation, research and analysis are presented and already existing data are amplified and evaluated.

An effort was made to secure from various hosts and localities as many cultures as were available of the described species as it was assumed that a study of a group of isolates would be more representative than the study of a single isolate and would enable the investigator to determine the extent and nature of variation within a natural system. The majority of the cultures employed in this study were supplied by various individuals interested in the genus. Wherever possible the host and locality from which the fungus was obtained are given. Cultures listed as being from Baarn are from the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Keys for the identification of the species were often found to be inadequate, emphasis frequently being placed upon the host infected or the growth habit of the fungus when grown on certain culture media. The need for a classification of the species became more apparent as the work progressed. A natural system of classification based upon the morphology of the species seemed most desirable; a key utilizing the principles of this system is therefore supplied.

To further aid students of the genus and plant pathologists encountering members of the genus as plant pathogens, information relative to host and geographic distribution is included. In addition to a summary and condensation of the available literature on this phase of the work, the extent and variety of hosts have been materially amplified. This information is appended to the discussion of the individual species and cross-indexed for the reader's convenience.

MORPHOLOGICAL OBSERVATIONS

Mycelium

The mycelium of *Pythium* is made up of rather fine filaments, measuring from 1 to 8 μ, usually 2 to 5 μ, in diameter. The filaments are cylindrical, usually irregularly branched, hyaline and coenocytic when young. As the thallus matures, septa are laid down in a random fashion, serving to separate the empty portions of the hyphae, formed by the apically directed protoplasmic movement, from the full ones. The hyphae may be either intramatrical or extramatrical, usually both when grown on solid substrates. When a multicellular host is invaded the hyphae develop both intercellularly and intracellularly.

The method by which the hyphae penetrate the cell wall is not clear. Appressoria are usually formed when the hyphae make a surface contact. Appressoria may be simple or complex, straight, curved or globular in shape, becoming more or less flattened when applied to a firm surface. They may be discerned readily because of their dense protoplasmic contents. Though at times confused with certain filamentous sporangial types, they may be distinguished from these by their failure to develop zoospores. Although appressoria are found in both filamentous and spherical sporangial types, they seem to be most abundant and most readily produced in the former.

Sporangia

Sporangial germination in *Pythium*, as previously indicated, is characterized by the passage of the protoplasm in an undifferentiated state through a short or long emission tube into an evanescent apical vesicle in which the zoospores are formed. This process takes place in ten to fifty, and usually in fifteen to twenty, minutes. Sometimes the term sporangium is used in place of the term vesicle as used here; the structure from which the vesicle arises in turn is called pre- or prosporangium. The writer concurs with the opinion given by Fitzpatrick (1930) that this terminology only leads to confusion, particularly in *Phytophthora* where no vesicle is produced and the structure gives rise to zoospores directly.

There are two principal types of sporangia in the genus *Pythium*, filamentous and spheroidal. The occurrence of these two distinctly different types of sporangia provides a convenient basis for separation of the species.

The filamentous sporangia may be indistinguishable from the vegetative thallus or somewhat inflated. When strictly filamentous, they may be simple or branched, acrogenous or intercalary, but in any case cut off from the supporting hypha by a septum. They usually may be recognized by their rather dense protoplasmic content. The sporangia of *Pythium gracile*, *P. dictyosporum*, and *P. monospermum* are good examples of the strictly filamentous type.

Pythium aphanidermatum, *P. arrhenomanes*, *P. graminicolum*, and *P. torulosum* exemplify the inflated filamentous type of sporangia, usually more

or less complex in structure, consisting of several communicating dactyloid or lobulate elements which extend outward in all directions. The individual lobes vary from 8 to 20 μ in diameter and are densely filled with protoplasm. The sporangia of *P. periilum* and *P. myriotylum*, though typically inflated filamentous, are more or less intermediate in form between *P. monospermum* and *P. arrhenomanes*. The sporangia of *P. periilum* are composed of sparingly distributed swollen digitate elements which may or may not be arranged in complex units; they also have entirely undifferentiated vegetative elements. *P. myriotylum* is much like *P. periilum* with regard to its sporangia except that the inflated lobulate elements are more numerous.

The spheroidal sporangia vary considerably in size, shape, and position on the supporting hyphae. The range in size in a single species may be as much as 30 μ. This is well illustrated in *Pythium debaryanum* in which the measurement of 200 sporangia gave a range of from 8.1 to 39.4 μ in diameter. (Measurements taken of 200 sporangia of each species studied are omitted here as including them would not contribute to the usefulness of this study.) The sporangia are usually spherical to elliptical in shape in most species, as exemplified in *P. debaryanum*, *P. spinosum* and *P. ultimum*. Frequently, however, they are spherical to subspherical, ovate to obovate, pyriform and truncate. All these sporangial shapes may be found in a single species, as exemplified by *P. irregulare*.

A sporangium unique in the genus is exhibited in *Pythium anandrum*, *P. helicoides*, *P. oedochilum*, *P. palingenes*, and *P. polytylum*. These species possess sporangia which are usually spherical to obovate and possess a sessile apical papilla.

Several species, including *Pythium megalacanthum* and *P. proliferum*, possess proliferous sporangia. Proliferation manifests itself in two ways: the secondary sporangium is formed within the previously emptied sporangium; the supporting hypha of the secondary sporangium grows up through the primary sporangium to form the secondary structure outside and above the primary one.

The sporangia of the spheroidal type may be acrogenous, intercalary, or laterally sessile. When they are acrogenous, the sporangiophore may vary in length from 5 to 400 μ, no definite length being associated with a particular species. Intercalary sporangia are usually single but may be grouped. Grouped sporangia fall into two classes: in one the sporangia are of individually spherical parts contiguous to similar structures by means of an undifferentiated filament; in the other they are spherical and separated from the supporting hyphae by septa but connected serially to form a catenulate structure. *Pythium acanthicum* and *P. oligandrum* are examples of the former and *P. intermedium* typifies the latter.

When properly treated, the sporangia germinate in a characteristic and peculiar manner. The sporangium, whether filamentous or spheroidal, gives rise to a delicate emission tube which is usually from 2 to 5 μ in diameter and

which varies from 5 to 500 μ in length. The protoplasm of the sporangium flows into an evanescent vesicle; here cleavage of the protoplasm takes place to produce the zoospores. In rare instances the protoplasm may be discharged from the vesicle in an undifferentiated state, collecting at the periphery of the vesicle and delineating the zoospores exogenously. In no instance are zoospores formed within the sporangium.

The sporangia of all filamentous strains invariably germinate by the production of zoospores. Though the sporangia of spheroidal strains usually produce zoospores they frequently germinate by the production of one to six germ tubes. These spheroidal structures which germinate by germ tubes have been occasionally referred to as conidia. It is true that asexual reproductive bodies which do not give rise to spores cannot correctly be termed sporangia. Conidia are, however, commonly considered to be deciduous and are borne on stalks which are morphologically distinguishable from the mycelium from which they arise, except perhaps the micronemal conidia of Saccardo. No spheroidal asexual reproductive bodies in this genus are considered truly deciduous nor are they borne on supporting hyphae that are distinguishable morphologically from the mycelium. The use of the word deciduous is qualified here, for in species producing catenulate reproductive bodies those at the apex are frequently dislodged, not akin to falling off at maturity, the basal series remaining in position; it is contended that this is not truly a deciduous condition, especially as no new structure is produced in its place subsequent to its removal. Further, these reproductive organs may germinate either by the production of zoospores or of germ tubes depending on environmental factors. The one exception is *Pythium ultimum* in which species zoospore formation is unknown. In view of these facts it seems desirable to designate the asexual reproductive structures in this group of species as sporangia rather than as pseudosporangia, pseudoconidia or conidia, inasmuch as morphologically and phylogenetically only one structure is under consideration.

Zoospores

The zoospores are reniform, possessing two lateral cilia which arise from a hilum located on the concave side. The number of zoospores produced in a vesicle varies from two to 125, possibly more. Information pertinent to the cytology of zoospore formation in *Pythium* is lacking; a detailed cytological study of a similar process occurring in *Plasmopara halstedii* (Farlow) Berl. and de Toni is given by Nishimura (1926). After an active stage in which the zoospores move rapidly about in water, they encyst and assume a spherical form varying from 4 to 18 μ, mostly from 8 to 12 μ, in diameter. Zoospores are monoplanetic and ordinarily germinate by the formation of a germ tube. Repeated emergence has been observed rarely in species possessing large zoospores, the spores sending out an evacuation tube from 2 to 3 μ wide and from 5 to 30 μ long, capped by a vesicle in which usually a single, though rarely from two to six zoospores are formed. A single zoospore of some species

may undergo as many as six repeated emergences. These escape from the vesicle, encyst, and then germinate by a germ tube. This process has been erroneously referred to as diplanetism. Since only one type of swarmspore is produced a true diplanetic condition does not exist; rather, typical spore germination merely recurs; this phenomenon is termed repetitional emergence or secondary spore formation.

Cornu (1872) is apparently the first to observe repetitional emergence in his study of *Pythium proliferum* in which he observes the production of secondary zoospores. Butler (1907) portrays this behavior in his consideration of *P. diacarpum*, referring to it as diplanetism. Atkinson (1909) corrects Butler's interpretation, calling it secondary spore formation rather than diplanetism. Drechsler (1930b) also mistakenly refers to the repeated emergence of zoospores of *P. butleri* and *Phytophthora* spp. as diplanetism.

Oogonia

Oogonia exhibit less variation in shape than sporangia; they are usually spherical to subspherical when acrogenous and elliptical to limoniform when intercalary, and always delimited from the supporting hyphae by a septum. Oogonia vary from 6 to 75 μ in diameter. The measurement of 200 oogonia of each species studied showed that though each species had a certain range and mean size, the ranges and means were so very similar, overlapping and often identical, that no taxonomic value could be assigned to them; these data are therefore omitted.

Structures resembling oogonia, if not identical with them, have been observed to produce germ tubes under suitable conditions, which again emphasizes the fact that the oogonium is a vegetative structure and that it may also serve as an asexual reproductive body. This has been demonstrated in *Pythium ultimum* by the writer (Tompkins *et al.* 1939).

The wall of the oogonium is thin, about 1 μ thick, and may have either a smooth or an echinulate surface. The echinulations are merely extensions of the oogonial wall and may or may not be cut off from the oogonium by septa. The spines may be short or long, conical or curved, with sharp or blunt apices, or may be digitate, mammiform, or papillate.

The spines of *Pythium irregulare*, *P. mamillatum*, and *P. megalacanthum* are often proliferous, becoming much elongated. A single spine in *P. mamillatum* formed an oogonium at its tip which was fecundated by a monoclinous antheridium to produce an apparently normal oospore. Instances of this sort are exceptional.

The smooth or echinulate character of the oogonial wall and the type of echinulation are specific characters. Utilization of these characteristics is made in the classification of the species.

At maturity the protoplasm of the oogonium differentiates into two not very clearly defined regions, the central region becoming the oosphere and the peripheral region the periplasm. It is the oosphere that becomes the

oospore upon fertilization, the periplasm either contributing to the oospore wall or degenerating and not becoming a part of the oospore.

Antheridia

Soon after the oogonial initial is formed the antheridia generally become discernible. They may vary in size, shape, number, and place of origin. An antheridium may be allantoid, clavate, globose, suborbicular, or trumpet-shaped, borne terminally or intercalarly. The antheridial stalk is as often short as long and is frequently absent. The number of antheridia per oogonium varies from none to twenty-five and possibly more, though usually numbering between one and four.

There are three types of antheridia, based on their point of origin; they are hypogynal, monoclinous, and diclinous. Any of these may make only apical contact with the oogonium or may apply its entire length, in any case producing a short, narrow fertilization tube that penetrates the oogonial wall and permits the passage of the antheridial gonoplasm into the oogonium.

A hypogynous antheridium is one surmounted by an oogonium; the antheridium is formed either within the oogonial stalk or at the apex of the oogonial stalk adjacent to the oogonium, never laterally disposed. A monoclinous antheridium originates at any site along the oogonial stalk, while a diclinous antheridium arises from any hypha of the same thallus other than the oogonial branch.

The term "androgynous" is often employed in the discussion of the point of origin of antheridia in this and related genera. "Androgynous" does not accurately describe the antheridial habit because it means only that the antheridia and oogonia occur together and does not explain the way in which the antheridia and oogonia are really associated. The use of the words "androgynous" and "diclinous" as antinyms does not seem justified and it appears advisable to supplant "androgynous" with "monoclinous."

The origin and morphology of the antheridium is a specific characteristic and is considered a valuable criterion for specific identification.

Oospores

A single oospore is regularly formed in an oogonium, though occasionally two and even three have been observed. The oospores vary from 4 to 48 μ in diameter and are spherical with a smooth or reticulate wall which may be either thin (0.6 to 1.8 μ thick) or inspissate (1.8 to 3.8 μ thick). Oospores which fill the oogonial cavity are termed plerotic, while those that do not fill it are called aplerotic.

The oospore is filled with rather opaque, granular protoplasm usually containing a single reserve globule and a single refringent body. However, four species, *Pythium helicoides*, *P. oedochilum*, *P. polytylum*, and *P. palingenes*, constitute an exception, having several (from 5 to 30) reserve globules and several (from 2 to 8) refringent bodies.

Oospore germination is not commonly observed and is unknown in some species. Germination may be accomplished either by the production of one or several germ tubes or by the formation of zoospores. Oospore germination is most commonly seen in *Pythium acanthicum* and *P. vexans*.

The plerotic or aplerotic condition of the oospore and the thickness and type of the oospore wall are characters which are sufficiently stable to permit their use in the morphological segregation of the species of this genus.

Homothallism

The genus *Pythium* seems to be consistently homothallic. Sparrow (1931b) indicates that *Pythium adhaerens*, though possessing diclinous antheridia, is homothallic. Vanterpool (1939) reports that this is the case in *P. arrhenomanes*, *P. butleri* (= *P. aphanidermatum*), *P. myriotylum*, *P. torulosum*, and *P. complectens* (= *P. vexans*). More recently Wen-chun Ho (Ho *et al.* 1941) demonstrated that *P. graminicolum* is homothallic. The writer substantiates these observations and presents additional instances of homothallism.

In order to determine whether or not certain species were homothallic, isolates were cultured from individual hyphal tips and single zoospores. The fungus was first grown in Petri dishes on corn meal agar, a small block of agar containing the fungus was then removed and plated out on plain water agar. From two to four days later a piece of agar was removed containing an individual hyphal tip; this in turn was plated out on a substrate favorable for sexual reproduction. In single zoospore cultures, the fungus on water agar plates was allowed to develop until sporangia were produced. Subsequently either a fairly large agar block was removed and placed under irrigation in a glass vessel or the entire plate was irrigated (a glass vessel was tilted so that slowly running tap water completely covered the agar block and flowed freely over the surface); either technique was equally successful. Immediately following zoospore production the excess liquid was withdrawn and a few milliliters of liquid containing actively swimming zoospores were drawn into a pipette and removed to another Petri dish of plain water agar. Here the zoospores were singled out and allowed to germinate; subsequent to germination they were transferred to a substrate appropriate for sexual reproduction. This technique was repeated a number of times for several different isolates of a single species.

Whenever an isolate capable of reproducing sexually was used, cultures arising both from single hyphal tips and individual zoospores produced the sexual stage. Furthermore, the sexual stage exhibited through these two techniques was identical with that of the original isolate in all respects, emphasizing the stability and utility of the type and position of the oogonium and type, disposition and origin of the antheridium, if any, in specific identification.

Species which have been demonstrated by the author to be homothallic by both techniques are: *Pythium acanthicum*, *P. anandrum*, *P. aphaniderma-*

tum, *P. aristosporum*, *P. arrhenomanes*, *P. debaryanum*, *P. dissotocum*, *P. graminicolum*, *P. helicoides*, *P. indigoferae*, *P. irregulare*, *P. mamillatum*, *P. monospermum*, *P. myriotylum*, *P. oedochilum*, *P. oligandrum*, *P. paroecandrum*, *P. periilum*, *P. periplocum*, *P. perniciosum*, *P. polymastum*, *P. rostratum*, *P. salpingophorum*, *P. spinosum*, *P. tardicrescens*, *P. torulosum*, *P. vexans*, and *P. volutum*.

Employing the hyphal tip procedure outlined, *Pythium ultimum* was found to be homothallic. Inasmuch as zoospores are not produced in this species it was impossible to determine whether similar results would have been obtained had this method been used.

Two isolates tentatively assigned to *Pythium arrhenomanes* which have failed to produce the sexual phase on all media so far tried were used in making single zoospore cultures. The sexual apparatus failed to appear in the numerous zoospore cultures made.

Zoospores produced as the result of oospore germination in *Pythium acanthicum* were also used in making single zoospore cultures. The results obtained were identical with those secured from employing zoospores from sporangia.

Most species of *Pythium* exhibit antheridia which would usually be considered as typical of a homothallic fungus. The few species displaying antheridia generally thought unique to a heterothallic fungus are in reality those of a homothallic one. *P. arrhenomanes* has been thought heterothallic by some because of the frequent failure of the species to produce the sexual stage, this failure being associated with the absence of compatible sexual strains. The results cited above demonstrate that the failure to produce the sexual stage is due to some factor other than a heterothallic condition.

GROWTH AND REPRODUCTION

The fungi were grown on several kinds of culture media so that information on mycelial growth and production of reproductive organs might be obtained. The media used were potato-dextrose agar (100 g. potato tuber, 20 g. dextrose, 15 g. agar, 1 l. water), oatmeal agar (60 g. ground oatmeal, 10 g. agar, 1 l. water), lima bean agar (60 g. ground lima bean, 15 g. agar, 1 l. water), corn meal agar (50 g. yellow corn meal, 15 g. agar, 1 l. water), plain water agar (15 g. agar, 1 l. water), and pea broth (crushed canned peas steeped in water for twelve hours).

Stock cultures of the fungi were maintained on potato-dextrose agar slants, stored at 25° C. All cultures grew satisfactorily at this temperature.

All species grew on the media used. The maximum aerial mycelial development occurred on potato-dextrose agar, the growth habit of the fungi being best exhibited on this substrate. Limited aerial growth was produced on the oatmeal, lima bean, and corn meal agars, while little if any aerial development occurred on water agar and pea broth.

Several growth habits may be recognized when the fungi are cultivated on

potato-dextrose agar. These may be described as arachnoid, cumulous, pulvinate, radiate, and rosette. Some species have both pulvinate and arachnoid and intermediate habits of growth, which makes distinction between these types sometimes difficult if not impossible. The species may be arranged in groups according to growth habit. No species may be identified accurately solely on its macroscopic growth characteristics, for the various strains of a single species vary and species morphologically dissimilar may have identical growth habits. The amount of aerial mycelium varies with the strains used. The arachnoid and pulvinate groups contain the largest number of species, the rosette, radiate, and cumulous groups containing fewer.

The arachnoid habit of growth is common to *Pythium afertile*, *P. anandrum*, *P. aphanidermatum*, *P. arrhenomanes*, *P. artotrogus*, *P. debaryanum*, *P. graminicolum*, *P. helicoides*, *P. myriotylum*, *P. nagaii*, *P. oedochilum*, *P. oligandrum*, *P. paroecandrum*, *P. perniciosum*, *P. salpingophorum*, *P. spinosum*, and *P. ultimum*.

The pulvinate habit of growth is exhibited by *Pythium acanthophoron*, *P. aristosporum*, *P. megalacanthum*, *P. monospermum*, *P. periilum*, *P. plerosporon*, *P. polymastum*, *P. polymorphon*, *P. pulchrum*, *P. scleroteichum*, *P. splendens*, *P. tardicrescens*, and *P. volutum*.

The rosette habit of growth is well displayed by *Pythium deliense*, *P. diameson*, *P. indigoferae*, *P. mamillatum*, *P. rostratum*, *P. torulosum*, and *P. vexans*.

Only three species exhibit the radiate habit of growth; they are: *Pythium hypogynum*, *P. torulosum*, and *P. vexans*.

Cumulous growth is common to *Pythium acanthicum* and *P. periplocum*.

Asexual and sexual reproductive organs are usually produced within from three to eighteen days when the fungi are grown on potato-dextrose, oatmeal, lima bean, corn meal, and water agars; only rarely were sexual organs produced in pea broth. Corn meal and water agar proved to be the most satisfactory media for studying sexual reproduction. Very little difficulty was encountered in inducing production of sexual organs in the majority of species. Oogonia, antheridia, and oospores are formed more frequently intramatrically than extramatrically. The oospores of *Pythium arrhenomanes*, *P. graminicolum*, and *P. scleroteichum* frequently were aborted if the pH of the media was above 6.0. Oospores of these species were more perfectly formed on corn meal agar than on any other substrate. In the event oospores fail to form on corn meal agar the addition of humus extract (Rands and Dopp 1933) may assist in their production. Johann (1928) devised a grated carrot agar which facilitates the formation of oospores in species renitent to produce them in standard media. If neither of the above remedies alleviate the condition media preparations including whole or macerated portions of the host plant from which the fungus was obtained may be employed. Frequently the addition of a small amount of soil rectifies the situation.

The production of asexual reproductive bodies was most abundant when the fungi were grown in pea broth for five days, the mycelial weft then being washed and placed in a continually irrigated container. Similar, though generally not as satisfactory, results were obtained when corn meal, lima bean, and oatmeal agar blocks containing the mycelium of the fungus were subjected to irrigation. Continual irrigation seems to be necessary for zoospore formation in most species, though a few produce them in limited numbers without continuous watering. This is probably true because of the necessity of removing staling products produced by the fungi and for the maintenance of an adequate oxygen supply, both of which functions this process accomplishes. Species in which it is difficult to induce production or germination of asexual reproductive bodies often yield when lima bean agar blocks containing the fungus are suitably irrigated; the process may be hastened by the addition of a small amount of soil or decaying organic matter.

No differences that could be used as an aid to specific segregation were found in the numbers and types of reproductive organs produced on the various agars.

TEMPERATURE-GROWTH RELATIONS

The effect of temperature upon the mycelial development of various *Pythium* spp. in the writer's collection was determined.[1]

Isolations of the species were grown on corn meal agar prepared from a concentrate produced by the Digestive Ferments Company, Detroit, Michigan. Glass culture tubes, 2.1 by 20.0 cm., were used. They were provided with a dam at the open end, made by heating the glass and indenting one side. These culture tubes were originally described and used by Tompkins and Gardner (1935). About 13 ml. of corn meal agar, pH 6.0, was added to each tube and allowed to cool at a uniform depth with the tube in a horizontal position, the dam preventing the escape of the melted agar.

The tubes containing the medium were inoculated near the dam at the open end with small squares of potato-dextrose agar containing the mycelium of the fungus cut from two-day-old Petri dish cultures. Inoculated tubes were left at room temperature for twenty-four hours, the extent of growth at the close of that period being indicated by a wax pencil mark on the tube; subsequent measurements were made from this point. Two tubes of each culture were placed in a horizontal position in controlled temperature chambers ranging from 1° to 46° C. at 3° intervals.

The cultures were incubated for a 72-hour period. The average growth of the fungi, in millimeters, over 24-hour periods at the various temperatures is presented in table 1.

[1] Grateful appreciation is expressed to Professor M. W. Gardner, Division of Plant Pathology, University of California, for the facilities afforded for this phase of the work.

TABLE 1. Mycelial growth of *Pythium* spp. on Difco corn meal agar at various temperatures.*

Organism	Average growth in millimeters over 24-hour period at															
	1° C.	4° C.	7° C.	10° C.	13° C.	16° C.	19° C.	22° C.	25° C.	28° C.	31° C.	34° C.	37° C.	40° C.	43° C.	46° C.
P. acanthicum	0	0	0	3	5	7	10	12	15	18	18	14	7	4	0	0
P. acanthophoron	0	0	0	0	1	2	5	7	9	7	3	2	t	0	0	0
P. afertile	1	3	7	8	10	13	17	19	21	22	23	20	11	t	0	0
P. allantocladon	0	0	3	5	7	10	13	15	16	17	18	6	0	0	0	0
P. anandrum	1	3	8	10	13	15	18	20	21	22	10	t	0	0	0	0
P. aphanidermatum	0	0	0	6	11	16	24	29	34	41	49	51	47	44	5	t
P. aphanidermatum var. *hawaiiensis*	0	0	0	3	6	9	14	17	20	22	29	31	30	26	4	1
P. araiosporon	1	3	5	7	10	12	16	20	22	24	26	22	6	0	0	0
P. aristosporum	0	t	3	6	9	12	15	17	19	20	19	7	t	0	0	0
P. arrhenomanes	0	t	2	6	9	12	18	20	22	25	22	19	7	3	0	0
P. arrhenomanes var. *canadensis*	0	t	3	5	7	10	15	19	20	23	21	15	2	0	0	0
P. arrhenomanes var. *hawaiiensis*	0	0	3	6	9	12	16	19	22	25	28	22	3	0	0	0
P. arrhenomanes var. *philippinensis*	0	t	2	4	7	10	13	15	18	22	23	22	2	0	0	0
P. artotrogus	t	4	8	11	14	19	25	31	34	36	26	5	t	0	0	0
P. artotrogus var. *macracanthum*	t	5	7	11	15	19	24	27	30	34	30	6	0	0	0	0
P. ascophallon	0	0	1	2	3	5	10	13	15	18	18	10	0	0	0	0
P. butleri	0	0	0	3	6	10	20	26	31	36	38	41	38	37	6	1
P. complectens	0	0	t	2	3	5	10	13	16	17	16	13	0	0	0	0
P. complens	0	1	2	4	5	6	8	10	11	12	13	11	2	0	0	0
P. debaryanum	1	4	7	11	15	18	24	28	30	32	28	14	1	0	0	0
P. debaryanum var. *pelargonii*	t	3	7	11	15	18	23	27	30	33	34	23	1	0	0	0
P. deliense	0	0	0	2	5	10	18	23	26	31	34	35	28	27	t	0
P. diameson	0	0	4	7	9	11	13	15	16	15	7	t	0	0	0	0
P. dissotocum	1	3	5	7	9	10	12	15	16	17	16	8	0	0	0	0
P. echinulatum (cf. *P. acanthicum*)	0	0	2	4	6	8	12	15	17	19	20	18	11	3	0	0
P. epiphanosporon	0	t	1	3	6	8	13	16	18	15	10	8	1	0	0	0
P. euthyhyphon	0	t	2	4	6	8	10	12	14	16	17	9	1	0	0	0
P. fabae	1	2	6	9	13	16	20	23	25	23	17	4	t	0	0	0
P. graminicolum	0	t	3	7	10	12	17	20	23	27	22	16	2	0	0	0
P. helicoides	0	0	0	t	2	6	18	23	28	33	36	38	35	15	2	0
P. hyphalosticton	0	t	2	4	7	10	15	19	21	24	24	19	6	0	0	0
P. hypogynum	1	2	3	4	5	6	7	9	11	13	15	16	1	t	0	0
P. indigoferae	0	0	0	t	1	3	6	9	10	11	12	9	t	0	0	0
P. intermedium	1	4	7	9	12	16	19	21	23	24	19	2	0	0	0	0

t = trace.

* Additional confirmatory data can be supplied upon request.

TABLE 1—(*Continued*)

Organism	Average growth in millimeters over 24-hour period at															
	1° C.	4° C.	7° C.	10° C.	13° C.	16° C.	19° C.	22° C.	25° C.	28° C.	31° C.	34° C.	37° C.	40° C.	43° C.	46° C.
P. irregulare	0	t	4	8	13	18	22	27	32	36	30	14	2	0	0	0
P. leiohyphon	0	0	t	4	6	8	12	15	18	15	12	6	t	0	0	0
P. leucosticton	0	0	1	3	5	7	10	12	14	16	16	12	t	0	0	0
P. mamillatum	2	3	7	11	14	17	21	24	25	26	23	13	0	0	0	0
P. mastophorum	0	0	t	3	5	7	10	11	12	7	1	0	0	0	0	0
P. megalacanthum	0	t	1	2	3	5	7	9	7	3	t	0	0	0	0	0
P. monospermum	0	2	3	5	8	10	12	14	16	18	20	17	1	0	0	0
P. myriotylum	0	0	0	t	5	9	18	21	25	30	37	42	43	40	1	0
P. nagaii	0	t	1	3	5	7	10	11	12	14	15	8	1	0	0	0
P. oedochilum	0	0	0	3	6	9	15	18	21	23	20	16	1	t	0	0
P. oligandrum	0	0	0	5	9	13	17	23	25	28	30	23	7	t	0	0
P. oryzae	1	3	5	7	9	11	14	16	18	20	16	10	0	0	0	0
P. paroecandrum	0	0	2	10	14	18	21	24	29	28	24	5	1	0	0	0
P. periilum	0	0	3	7	11	14	20	24	27	30	33	27	2	t	0	0
P. periplocum	0	0	0	t	6	14	23	27	31	34	35	32	22	1	0	0
P. perniciosum	0	3	5	7	9	10	12	14	16	18	19	15	4	0	0	0
P. piperinum	0	0	t	1	3	5	8	12	14	16	17	14	t	0	0	0
P. plerosporon	1	3	5	7	9	11	14	17	18	21	23	11	3	t	0	0
P. polyandron	0	0	2	5	8	10	14	18	20	23	25	19	4	0	0	0
P. polycladon	0	0	t	2	5	8	12	15	17	18	12	4	0	0	0	0
P. polycladon var. *chamaihyphon*	0	0	t	2	5	7	10	14	18	20	20	6	0	0	0	0
P. polymastum	0	3	6	8	10	12	14	16	18	20	19	18	7	0	0	0
P. polymorphon	t	4	7	10	12	14	18	20	21	22	17	12	0	0	0	0
P. pulchrum	0	1	4	7	9	11	14	16	18	13	10	1	0	0	0	0
P. rhizophthoron	0	0	2	5	8	11	17	21	24	27	28	12	1	0	0	0
P. rostratum	0	1	2	3	4	5	6	7	8	6	4	t	0	0	0	0
P. salpingophorum	0	1	6	10	15	19	24	28	31	33	33	11	t	0	0	0
P. scleroteichum	0	0	0	2	5	8	16	21	24	21	8	0	0	0	0	0
P. spaniogamon	0	t	2	4	9	12	17	21	26	27	23	12	5	0	0	0
P. spinosum	0	2	6	11	17	20	28	32	33	33	26	9	3	0	0	0
P. splendens	0	1	3	5	7	10	13	15	17	21	21	13	0	0	0	0
P. splendens var. *hawaiianum*	0	0	t	6	8	13	23	28	34	36	35	14	0	0	0	0
P. tardicrescens	0	t	1	3	4	5	7	8	9	4	t	0	0	0	0	0
P. teratosporon	0	t	1	3	5	6	7	8	9	9	7	5	1	0	0	0
P. thysanohyphalon	0	t	2	4	6	8	10	12	14	16	17	11	t	0	0	0
P. torulosum	0	t	2	3	5	7	9	12	13	14	12	11	0	0	0	0
P. ultimum	1	5	7	11	15	17	24	28	30	34	33	17	1	0	0	0
P. vexans	0	t	2	3	5	7	9	12	13	15	14	9	0	0	0	0
P. volutum	t	1	3	4	5	6	8	9	10	8	1	0	0	0	0	0

Very little variation is noticeable in the conspecific isolates at different temperatures; this is in distinct contrast to the rather wide variation noticeable in congeneric isolates at different temperatures. Tucker (1931) reports similar observations in his study of the relation between temperature and growth of *Phytophthora*, a genus closely related to *Pythium*.

It is readily seen that the behavior of conspecific isolates is most uniform at their upper temperature limit; the minimum and optimum values are more variable. The species may be segregated into groups, separation being based upon maximum temperature tolerance.

Growth occurred at 43° C. in all cultures of *Pythium aphanidermatum*, *P. deliense*, *P. helicoides*, *P. myriotylum*, *P. butleri*, and *P. aphanidermatum* var. *hawaiiensis*. *P. butleri* and *P. aphanidermatum* var. *hawaiiensis* are considered synonymous with *P. aphanidermatum* for morphological reasons. The temperature response is presented here as evidence of their physiological similarity.

Some isolates of *Pythium aphanidermatum* are capable of growing at 46° C.; no other species of *Pythium* grows at this temperature.

Another group of isolates grew at 40° C. but not at 43° C.; this included *Pythium acanthicum*, *P. afertile*, *P. arrhenomanes*, *P. echinulatum*, *P. graminicolum*, *P. hypogynum*, *P. oedochilum*, *P. oligandrum*, *P. periilum*, *P. periplocum*, and *P. plerosporon*. Only six of the fifteen isolates of *P. arrhenomanes* grew at 40° C.; the remainder failed to grow at 40° C., but grew at 37° C. Likewise, only three of nine cultures of *P. graminicolum* grew at 40° C., the balance having their maxima at 37°. Two cultures of *P. oligandrum* did not grow at 40° even though a single isolate did.

For morphological reasons a culture received by the writer as *Pythium echinulatum* is considered synonymous with *P. acanthicum;* the temperature values for the two isolates are identical.

The majority of *Pythium* spp. fall in the group having a maximum temperature of 37° C. These species are: *P. acanthophoron*, *P. araiosporon*, *P. aristosporum*, *P. arrhenomanes*, *P. arrhenomanes* var. *canadensis*, *P. arrhenomanes* var. *hawaiiensis*, *P. arrhenomanes* var. *philippinensis*, *P. artotrogus*, *P. complens*, *P. debaryanum*, *P. debaryanum* var. *pelargonii*, *P. epiphanosporon*, *P. euthyhyphon*, *P. fabae*, *P. graminicolum*, *P. hyphalosticton*, *P. indigoferae*, *P. irregulare*, *P. leiohyphon*, *P. paroecandrum*, *P. perniciosum*, *P. piperinum*, *P. polyandron*, *P. polymastum*, *P. rhizophthoron*, *P. salpingophorum*, *P. spaniogamon*, *P. spinosum*, *P. teratosporon*, *P. thysanohyphallon*, and *P. ultimum*.

Pythium arrhenomanes and *P. graminicolum*, though previously listed as developing slightly at 40° C., are more properly grouped with species having a maximum temperature of 37° C.

The three varieties of *Pythium arrhenomanes* and *P. epiphanosporon*, *P. hyphalosticton*, *P. leiohyphon*, *P. leucosticton*, *P. polyandron*, *P. rhizophthoron*, *P. spaniogamon*, and *P. thysanohyphallon* are all morphologically

identical and considered synonymous with *P. arrhenomanes*. The temperature-growth relations of these species are likewise identical; their temperature values are cited as evidence of their physiologic similarity.

Pythium complens and *P. monospermum* have similar temperature values attesting their physiologic synonymity; these two species are also morphologically indistinguishable.

Pythium teratosporon compares favorably with *Phytophthora drechsleri* in its temperature-growth requirements, with which it is now considered synonymous.

Pythium araiosporon, *P. debaryanum* var. *pelargonii*, and *P. fabae* exhibit temperature values comparable with those of *P. debaryanum*, which they resemble in their general growth habit. *P. araiosporon*, *P. debaryanum* var. *pelargonii*, and *P. fabae* are similar in their morphology and are treated as synonymous with *P. debaryanum*.

A smaller group of species have 34° C. as their limiting temperature. These are: *Pythium allantocladon*, *P. anandrum*, *P. ascophallon*, *P. complectens*, *P. diameson*, *P. dissotocum*, *P. intermedium*, *P. mamillatum*, *P. oryzae*, *P. polycladon*, *P. polycladon* var. *chamaihyphon*, *P. polymorphon*, *P. pulchrum*, *P. rostratum*, *P. splendens*, *P. torulosum*, and *P. vexans*.

Morphologically, *Pythium allantocladon*, *P. ascophallon*, *P. complectens*, and *P. vexans* are identical and must be considered synonymous species. *P. vexans*, having priority in description, is maintained as the valid binomial. The temperature values are presented as evidence of the physiologic similarity of these organisms.

For morphological reasons, *Pythium dissotocum* and *P. oryzae* are considered identical, *P. dissotocum* being designated as the valid nomen. The temperature-growth responses of these two organisms are identical and are cited here as additional evidence of synonymy.

A relatively few species comprise the group having 31° C. as their maximum temperature. These are *P. mastophorum*, *P. megalacanthum*, *P. scleroteichum*, *P. tardicrescens*, and *P. volutum*.

Though the optimum temperature varies within a group of isolates of a single species, in general it may be said that the optimum temperature is usually about 10° below the maximum.

No generalizations may be made with regard to the minimum temperatures owing to the extreme elasticity in the range of temperature toleration of the various species.

There are several species which are able to grow at 1° C. These are: *Pythium afertile*, *P. anandrum*, *P. debaryanum*, *P. dissotocum*, *P. hypogynum*, *P. intermedium*, *P. mamillatum*, *P. plerosporon*, and *P. ultimum*.

Pythium acanthophoron is unique in being unable to grow at temperatures below 13° C.

It is interesting to note that isolates of a species from different climes have identical temperature values. This is well exemplified in *Pythium aphanider-*

matum, *P. arrhenomanes*, *P. graminicolum*, *P. irregulare*, *P. myriotylum*, *P. periplocum*, *P. ultimum*, and *P. vexans* (including synonyms). Apparently the climatic source of an isolation does not determine its ability to develop at a given temperature. As a specific example, cultures of *P. arrhenomanes* from Mauritius, Hawaii, and various parts of the United States are all capable of growing at 7° C. Similarly, isolations of *P. splendens* from Hawaii, Malaya, and the United States are capable of mycelial development at 7° C.

Literature relevant to the temperature requisites of *Pythium* spp. is rather limited so far as number of species is concerned. Gottlieb and Butler (1939) report that *P. aphanidermatum* does not grow at 5° C., that maximum mycelial development takes place at 37°, with only slight development at 45°. Tasugi and Takatuzi (1935) give the cardinal points as 10°, 30°, and 40° C. Wager (1931) states the optimum temperature to be 35° and the maximum 45°; no growth occurred at 5° C.

According to Vanterpool (1938), *Pythium aristosporum* grows most rapidly at 30° C., exhibiting only a trace of growth at 35°; no minimum temperature is presented. According to the writer's observations, the optimum temperature ranges between 25° and 31°, with somewhat better growth at 28° C. The maximum is about 2° higher than that recorded by Vanterpool.

Rands and Dopp (1934) report *Pythium arrhenomanes* capable of growing over a range of temperatures from 6° to 38° C., with the optimum about 30°. Vanterpool indicates that optimum temperature for growth of *P. arrhenomanes* is 30°, the maximum 35°; no minimum is given. Several isolates of the writer's collection grew at 4°; the optimum and maximum values are similar to those of Rands and Dopp.

Braun (1925) reports the cardinal temperatures of *Pythium debaryanum* as 6°, 27°, and 35.5° C. *P. debaryanum* var. *pelargonii*, described by Braun, has a minimum of 3°, an optimum of 30° and a maximum of 35.5°. Braun distinguishes this new variety from *P. debaryanum*, in part, by its lower minimum temperature. As previously indicated, no degree of significance can be attached to the minimum and optimum temperature values because they vary somewhat in conspecific isolates. Braun's use of the minimum temperature as an accessory factor in segregating his variety from the species is questionable; the writer does not consider it a valid criterion for separation. Though Braun points out some morphologic differences between the species and the variety, they are not believed sufficiently great to retain *P. debaryanum* var. *pelargonii* as a valid nomen. Saksena (1935) reports *P. debaryanum* to have an optimum temperature of 30° C. and a maximum of 35°.

According to Saksena, *Pythium deliense*, though able to grow at 15° C., cannot grow at 10°. The optimum temperature for growth is 35°; the maximum is stated to be intermediate between 40° and 45°.

Vanterpool (1938) reports the optimum temperature of *Pythium graminicolum* to be 25° C.; the maximum is coincidental with the optimum. No

instances of this sort have been observed among the isolates of *P. graminicolum* used in this study.

An incomplete study of the relation of temperature to growth of *Pythium indigoferae* by Saksena reveals the optimum of the fungus to be about 30° C., the maximum about 35°; no minimum value is recorded.

Younkin and Melhus (1939) report the optimum temperature of *Pythium irregulare* as 30° C., stating further that no growth occurred at 0° and 40°.

Saksena records *Pythium mamillatum* as having an optimum temperature for growth of 30° C. and a maximum of 35°; no minimum value is given.

The only published work dealing with the temperature values of the type species of the genus, *Pythium monospermum*, is that of Ito and Tokunaga (1933). They state that the optimum temperature of this fungus lies between 28° C. and 32°. No minimum or maximum temperatures are presented.

The temperature-growth values published by Harter and Whitney (1927b) for *Pythium scleroteichum* are not in harmony with those recorded by the writer. Harter and Whitney list the minimum as 8°, optimum 29°, and maximum 38.5° C.; the writer's values are: minimum 10°, optimum 25°, and maximum 31°. A comparison of the type culture with the Harter and Whitney fungus was not possible.

Sawada and Chen (1926) report the optimum temperature for the growth of *Pythium spinosum* as 30° C.; no further values are given.

Braun states the minimum, optimum, and maximum temperatures for the growth of *Pythium splendens* are 11°, 30°, and 37.5° C., respectively.

Vanterpool reports that *Pythium tardicrescens* is able to grow over a temperature range of 15° to 30°, with the optimum near 25° C. The writer was able to grow the fungus at temperatures ranging from 4° to 31°, with the optimum about 25° C. In the same paper, Vanterpool gives data indicating that *P. volutum* grows at 15° C. and 30°, but not at 35°; the optimum is given as 25°. A culture of *P. volutum* received from Vanterpool grew at 1° C. and 31°; the optimum growth occurred at 25°.

The temperature values of *Pythium ultimum* listed above in table 1 are in accord with reports of other investigators. Jones (1935) reports a minimum temperature value of approximately 4°, an optimum of 28°, and a maximum of 40° C. Likewise Wager (1931) has found the minimum temperature of *P. ultimum* to be rather low, about 4°. The optimum was 30° and the maximum about 40°. According to Pirone *et al.* (1933), the minimum value is somewhat lower than 9°, the optimum approximately 30°, and the maximum 38°. Alexander, Young, and Kiger (1931) report the optimum temperature of *P. ultimum* to be from 25° to 30°, the minimum about 10°; no maximum value is given.

In summation it may be said that temperature-growth relations are a specific feature and may be used as a criterion for the identification of species. The utilization of the ability of a species to grow at certain temperatures is particularly helpful as an adjunct to morphological characteristics in specific

identification. The temperature-growth relations cannot, however, be used to distinguish between species possessing filamentous or spherical sporangia, since there is no correlation between cardinal temperatures and morphologic characters.

TAXONOMY OF THE GENUS

Pringsheim (1858) created the genus *Pythium* in 1858, placing it in the family Saprolegniaceae. His description of the genus is as follows:

"*Pythium* n.g. Schwarmsporen aussen vor der Oeffnung der Sporangien aus deren Inhalt gebildet, sich nicht hautend. Schlauche die enterleerten Sporangien weder durchwachsend noch seitliche Sporangien treibend. Oosporen einzeln in jedem oogonium."

Pythium monospermum and *P. entophytum* were the first described species. With the subsequent transfer of *P. entophytum* to *Lagenidium entophytum* by Zopf (1890), *P. monospermum* became the type species.

Cornu (1872) includes *Pythium* in his treatise on the Saprolegniaceae published in 1872. In 1881 de Bary (1881b) published a series of articles dealing with the genera *Artotrogus*, *Peronospora*, *Phytophthora*, and *Pythium*. He points out the similarity of the genera, indicates their probable interrelationships and concludes that they should be placed in the family Peronosporaceae.

In 1888 Berlese and de Toni in Saccardo's *Sylloge Fungorum* (1888) return to Pringsheim's placement of the genus *Pythium* in the Saprolegniaceae. Four years later a monograph on the genus was presented by Fischer (1892) in which he refers *Pythium* to the Peronosporaceae. This work is noteworthy for the creation of the subgenera *Aphragmium*, *Nematosporangium*, and *Sphaerosporangium* and the sections *Orthosporangium* and *Metasporangium*. The subgenera *Aphragmium* and *Nematosporangium* represent groups of species possessing filamentous sporangia. *Aphragmium* is distinguished from *Nematosporangium* as devoid of a septum delimiting the sporangium from the mycelium. The subgenus *Sphaerosporangium* embraces species producing spheroidal sporangia cut off from the sporangiophore by a septum. The author describes the sporangial habits found in the subgenus *Sphaerosporangium: Orthosporangium* includes species in which the sporangium remains attached to the sporangiophore during zoospore formation; in *Metasporangium* the sporangia may be either fixed or deciduous with zoospore production occurring in either case, or the sporangia may fail to form zoospores and may germinate by means of germ tubes.

Schröter (1897) in 1897 placed the genus *Pythium* in a newly created family, the Pythiaceae, assigned to the order Saprolegniales. The subgenus *Nematosporangium* is elevated to generic rank and included in the Pythiaceae. Schröter designates *Nematosporangium* as having filamentous sporangia and *Pythium* as having spherical or limoniform sporangia. Two subgenera,

Aphragmium Fischer and *Eunematosporangium* (*Nematosporangium* Fischer), are included in the newly elevated genus *Nematosporangium*. The subgenus *Aphragmium* is retained in Fischer's sense while *Eunematosporangium* is considered synonymous with the subgenus *Nematosporangium* Fischer. *Pythium*, according to Schröter's treatment, is synonymous with Fischer's subgenus *Sphaerosporangium*. Schröter also presents two new subgenera for his genus *Pythium: Eupythium*, including species with smooth-walled oogonia, and *Artotrogus*, including species with echinulate-walled oogonia.

The writer favors abandonment of the subgenera *Aphragmium* and *Nematosporangium* and the two sections *Orthosporangium* and *Metasporangium* and suggests that the use of subgenera and sections in the genus *Pythium*, *sensu* Pringsheim, be discontinued. The presence or absence of a septum delimiting a sporangium cannot be considered a valid taxonomic criterion; the character is too variable. The subgenus *Sphaerosporangium* is sufficiently descriptive but does not aid materially in clarification. The mode of sporangial germination is largely controlled by conditions existing in the immediate environment. The sporangium of most *Pythium* spp. may germinate either by the formation of zoospores or by the production of germ tubes. When sporangia are introduced into fresh running water they nearly invariably germinate by the production of zoospores. If similar sporangia are placed in agar blocks without any surface water other than the water film naturally present, the sporangia invariably germinate by the formation of germ tubes. These observations are true not only for the species included in Fischer's *Sphaerosporangium*, but in his *Aphragmium* and *Nematosporangium* as well. Utilization of the mode of sporangial germination, which may be regulated by environmental conditions, should be avoided as a taxonomic feature.

Schröter's treatment of the genus is questionable in many respects. His inclusion of *Pythium monospermum* in *Nematosporangium* is irreconcilable with Pringsheim's delineation of *Pythium*. *P. monospermum* is the type species of the genus *Pythium* created by Pringsheim. Schröter's delineation of *Pythium* is identical with Fischer's subgenus *Sphaerosporangium* created to include species possessing spherical or limoniform sporangia. The adoption of the genera *Nematosporangium* and *Pythium*, *sensu* Schröter, seems unjustifiable because they are in direct opposition to the genus as originally described by Pringsheim. However, if the genus *Pythium* is to be divided into two groups following Schröter's plan, *Nematosporangium* would be embraced in the genus *Pythium* since *Pythium* was described first, making abandonment of *Nematosporangium* mandatory; a new genus would have to be proposed to replace *Pythium*, *sensu* Schröter.

Butler in his monograph on *Pythium* published in 1907 follows Fischer's treatment in retaining the various species within this single genus. Butler considers the subgenus *Nematosporangium* of doubtful validity, grouping all species possessing filamentous sporangia within the subgenus *Aphragmium*.

In 1930 Sideris (1930) proposed to revive the taxonomic plan proposed by Schröter. Sparrow (1931c) published a criticism of Sideris' paper, suggesting the replacement of the genus *Nematosporangium* by *Pythium* and the transference of *Pythium* spp. possessing inflated filamentous sporangia to the genus *Rheosporangium*, erected by Edson (1915b). Sparrow further proposed either that *Pythium* spp. possessing spherical or limoniform sporangia and all *Phytophthora* spp. be placed in *Sphaerosporangium*, elevating it to generic rank, or that *Pythium* spp. be placed in *Phytophthora*. These suggestions contribute little to the solution of the problem. The genus *Phytophthora*, though related to *Pythium* in the broader sense, comprises a group of species easily distinguishable from *Pythium*. Further, *Pythium* spp. with filamentous or inflated filamentous sporangia exhibit too many common characteristics to permit valid generic segregation.

The mode of zoospore production has been the principal distinguishing character for the separation of the genera *Pythium* and *Phytophthora*; this feature may be retained as a criterion of generic segregation despite recurring criticism if its value and limitations are thoroughly understood and appreciated. Under suitable environmental conditions the sporangia of the genus *Pythium* give rise to an emission tube capped by a vesicle; the undifferentiated contents of the sporangium pass into the vesicle and there through a series of cleavages is differentiated into zoospores. The vesicle then ruptures and the zoospores are set free. In *Phytophthora* zoospores are produced within the sporangium in the absence of any vesicle; liberation is accomplished through disruption of the sporangium wall, or through dissolution of a differentiated region, the papilla. Rare instances are on record wherein the sporangia of *Phytophthora* have given rise to vesicles. It must be borne in mind that in these aberrant cases the protoplasm does not flow into the vesicle in an undifferentiated state, but rather already differentiated into zoospores. Inasmuch as the production of a vesicle is an asexual form of reproduction and as such should not be considered an unfailing process, the use of the vesicle for generic segregation should not be over-emphasized; this does not preclude its use as a character indicative of generic distinction or for convenience in separating the genera. There are several other characters which serve to distinguish further *Pythium* from *Phytophthora*. The amphigynous type of antheridium predominant in the genus *Phytophthora* is unique and not encountered in the genus *Pythium;* that the antheridium is a component part of the sexual phase can be considered a valid criterion for separation. The two species of *Phytophthora* which possess paragynous antheridia are readily separated from *Pythium* species possessing similar antheridia. Sporangiophore branching is unusual in *Pythium* and not uncommon in *Phytophthora*. The habits of growth of *Pythium* and *Phytophthora* are different enough for general macroscopic distinction. Though there is no one infallible feature for generic segregation of these two genera, there are a

number of characteristics which make separation feasible, and though difficult to outline in abbreviated form, in practice separation is easily done.

In this taxonomic study the generic tenets of Butler (1907) and Matthews (1931) are followed and the genus is retained in the older and broader sense; the taxonomic study presented by Sideris (1930), patterned after Schröter's, is not considered herein.

KEY TO THE SPECIES

For species not included in the key refer to "Doubtful Species."

Sporangium filamentous.
- Sporangium undifferentiated from vegetative hypha; not inflated.
  - Sexual reproduction present.
    - Oogonium smooth.
      - Antheridium delimited by septum.
        - Oospore smooth.
          - Oospore plerotic.
            - Antheridium monoclinous or diclinous, one to two per oogonium ..... 1. *P. monospermum*
            - Antheridium diclinous, one per oogonium ..... 2. *P. marinum*
          - Oospore aplerotic.
            - Oospore wall inspissate.
              - Catenulate spherical asexual reproductive bodies present ..... 3. *P. perniciosum*
              - Catenulate spherical asexual reproductive bodies absent.
                - Antheridium monoclinous, occasionally diclinous.
                  - Antheridial cells one to five, mostly two to three per oogonium, of autonomous origin ..... 4. *P. dissotocum*
                - Antheridium diclinous, never monoclinous.
                  - Antheridial cells one, rarely two per oogonium, of autonomous origin ..... 5. *P. gracile*
                  - Antheridial cells one to four per oogonium, as lateral prolongations of a single antheridial branch ..... 6. *P. adhaerens*
            - Oospore wall not inspissate.
              - Antheridium monoclinous or diclinous, one to five per oogonium ..... 7. *P. angustatum*
              - Antheridium diclinous, never monoclinous ..... 8. *P. apleroticum*
          - Oospore wall reticulate ..... 9. *P. dictyosporum*
      - Antheridium not delimited by septum ..... 10. *P. tenue*
    - Oogonium papillate ..... 11. *P. papillatum*
  - Sexual reproduction absent ..... 12. *P. afertile*

Sporangium somewhat differentiated from vegetative hypha; inflated.
- Sexual reproduction present.
  - Oogonium smooth.
    - Oospore plerotic.
      - Catenulate spherical asexual reproductive bodies present ..... 13. *P. catenulatum*
      - Catenulate spherical asexual reproductive bodies absent.
        - Antheridium monoclinous; origin proximal to oogonium.
          - Antheridial cells one to two per oogonium; not greatly inflated, allantoid-clavate; oogonium small, 12.0 to 19.0 μ in diameter ..... 14. *P. torulosum*
          - Antheridial cells two to six per oogonium; inflated, crook-necked-clavate; oogonium large, 16.5 to 28.6 μ (Subr.), often larger, 24 to 36 μ in diameter ..... 15. *P. graminicolum*
        - Antheridium diclinous; origin not proximal to oogonium.
          - Antheridial cells arising from separate antheridial branches.

Antheridial branches simple; antheridial cells few, one to two per oogonium....16. *P. inflatum*
Antheridial branches frequently divided; antheridial cells many, up to 25 per oogonium....17. *P. arrhenomanes*
Antheridial cells arising from a single antheridial branch...18. *P. periilum*
Oospore aplerotic.
Antheridium monoclinous, occasionally diclinous.
Antheridial branch usually straight; oogonial branch curved toward the antheridium.
Oogonium and antheridium frequently formed in connection with lateral sporangial complexes....19. *P. indigoferae*
Oogonium and antheridium never formed in connection with lateral sporangial complexes....20. *P. deliense*
Antheridial branch rarely straight; oogonial branch not curved toward the antheridium.
Antheridial cell typically intercalary, sometimes terminal, inflated; one to two per oogonium....21. *P. aphanidermatum*
Antheridial cell typically terminal, never intercalary, not greatly inflated; one to six, mostly two to three, per oogonium....22. *P. tardicrescens*
Antheridium diclinous, occasionally monoclinous.
Antheridial branch frequently helicoid about the oogonial branch....23. *P. volutum*
Antheridial branch not helicoid about the oogonial branch.
Origin of antheridial branch proximal to oogonium; vegetative prolongations rare; antheridial cells crook-necked, with apical contact with oogonium....24. *P. aristosporum*
Origin of antheridial branch not proximal to oogonium; vegetative prolongations common; antheridial cells crook-necked, with basal and apical contact with the oogonium 25. *P. myriotylum*
Oogonium echinulate....26. *P. periplocum*
Sporangium spheroidal.
Sexual reproduction present.
Oogonium smooth.
Oospore smooth.
Oospore plerotic.
Sporangium proliferous....27. *P. salpingophorum*
Sporangium not proliferous.
Antheridium absent....28. *P. conidiophorum*
Antheridium present.
Antheridium monoclinous or hypogynous.
Antheridial cell hypogynous, fertilization tube long and tenuous....29. *P. hypogynum*
Antheridial cell monoclinous, originating in close proximity to the oogonium, may be reduced to a hypogynous cell or short lateral process, fertilization tube short and stout. 30. *P. rostratum*
Antheridium monoclinous or diclinous, not hypogynous....31. *P. iwayamai*
Oospore aplerotic.
Sporangium proliferous.
Oospore wall inspissate.
Oospore containing a single reserve globule.
Antheridium typically monoclinous, rarely diclinous....32. *P. proliferum*
Antheridium typically diclinous, rarely monoclinous...33. *P. marsipium.*
Oospore containing several reserve globules.
Sporangium spherical....34. *P. polytylum*
Sporangium ovoid to obovoid.

Antheridial cell elongate, cylindrical, curved, regular in contour....35. *P. helicoides*
Antheridial cell elongate, cylindrical, curved, irregular and wavy in contour.
Oogonia typically acrogenous....36. *P. oedochilum*
Oogonia typically laterally and tangentially intercalary....37. *P. palingenes*
Oospore wall not inspissate....38. *P. nagaii*
Sporangium not proliferous.
Antheridium absent....39. *P anguillulae-aceti*
Antheridium present.
Antheridium typically hypogynous, rarely monoclinous...40. *P. pulchrum*
Antheridium typically monoclinous or diclinous, never hypogynous.
Antheridial branch falcate, sigmoid....41. *P. polymorphon*
Antheridial branch not falcate, not sigmoid.
Antheridium typically sessile, originating immediately adjacent to the oogonium.
Oospore wall inspissate....42. *P. ultimum*
Oospore wall not inspissate....43. *P. paroecandrum*
Antheridium typically stalked, not originating immediately adjacent to the oogonium.
Sporangium terminal....44. *P. splendens*
Sporangium terminal or intercalary.
Antheridium crook-necked, clavate, the apex bell-shaped, closely appressed to the oogonium and frequently fused with it....45. *P. vexans*
Antheridium crook-necked, clavate, the apex obtuse, narrowly applied to the oogonium....46. *P. debaryanum*
Oospore wall reticulate....47. *P. cystosiphon*
Oogonium echinulate.
Oospore plerotic.
Contiguous sporangia present....48. *P. acanthicum*
Contiguous sporangia absent.
Oogonial protuberances short, conical....49. *P. mamillatum*
Oogonial protuberances long, digitate....50. *P. spinosum*
Oospore aplerotic.
Contiguous sporangia present....51. *P. oligandrum*
Contiguous sporangia absent.
Sporangium proliferous....52. *P. megalacanthum*
Sporangium not proliferous.
Sporangium papillate....53. *P. anandrum*
Sporangium not papillate.
Antheridium typically hypogynous, rarely stalked....54. *P. echinulatum*
Antheridium typically stalked, never hypogynous.
Oogonial protuberances mammiform.
Sporangium regular in contour, devoid of protuberances. 55. *P. mastophorum*
Sporangium irregular in contour, possessing dome-shaped protuberances....56. *P. polymastum*
Oogonial protuberances not mammiform....57. *P. irregulare*
Sexual reproduction absent.
Sporangium proliferous.
Sporangium papillate.
Sporangium prolate ellipsoidal, vesicle sessile....58. *P. undulatum*
Sporangium spherical, vesicle stalked....59. *P. carolinianum*
Sporangium not papillate....60. *P. diacarpum*

Sporangium not proliferous.
Sporangia catenulate ..... 61. *P. intermedium*
Sporangia not catenulate ..... 62. *P. elongatum*
Sporangium unknown.
Oogonium smooth ..... 63. *P. scleroteichum*
Oogonium echinulate.
Antheridium hypogynous, never stalked ..... 64. *P. artotrogus*
Antheridium typically stalked, occasionally hypogynous.
Oogonial protuberances acute ..... 65. *P. echinocarpum*
Oogonial protuberances obtuse ..... 66. *P. acanthophoron*

DESCRIPTION AND DISCUSSION OF THE SPECIES

1. PYTHIUM MONOSPERMUM Pringsheim, Jahrb. Wiss. Bot. 1: 284–306. 1858.

Pythium gracile de Bary, Jahrb. Wiss. Bot. 2: 169–192. 1860.
Pythium reptans de Bary, l.c.
Pythium fecundum Wahrlich, Ber. Deuts. Bot. Ges. 5: 242–246. 1887.
Pythium complens Fischer in Rabenhorst, Kryptogamen-Flora 1: 398–399. 1892.

Hyphae measuring 1.6 to 7.3 μ, mostly 2 to 5 μ, in diameter, frequently including in addition to the cylindrical portion, a number of swollen, bud-like lateral outgrowths. Sporangia filamentous, unbranched or branched, 80 to 170 μ long, 2 to 5 μ in diameter, delimited by a septum. Zoospores few to 60 or more in number, reniform, 3.4 to 6.3 μ wide, 5.3 to 8.8 μ long. Oogonia spherical, thin and smooth-walled, usually intercalary, though frequently terminal or subterminal, 14.2 to 23.7 μ, mostly 15.8 to 21.9 μ in diameter. Antheridia usually one, occasionally two per oogonium, of monoclinous or diclinous origin; antheridial cell slightly inflated, clavate and crook-necked. Oospores single, plerotic, wall inspissate, a single reserve globule and a prolate ellipsoidal refringent body usually embedded in the granular protoplasm.

Originally described as a saprophyte on insect cadavers in water, Germany.

Pythium monospermum and *P. entophytum* were originally described by Pringsheim as the type species of the genus. Since Zopf's transference (1890) of *P. entophytum* to the genus *Lagenidium*, *P. monospermum* remains the type species.

It is indeed curious that *Pythium* spp. with strictly filamentous sporangia like those described by Pringsheim should frequently be referred to as members of the *P. gracile* group, the group name based on a species described a few years later from an asexual stage indistinguishable from that of *P. monospermum*. There may be some reason for this treatment if the division is based on the parasitic behavior of the sundry related forms. Although *P. monospermum* is usually reported as a saprophyte from either insect cadavers or plant debris, the species is occasionally recovered from living plant material. *P. gracile* is more often recorded as a parasite, usually of algae. These two species may be distinguished readily by their sexual stages. In *P. monospermum* the oospores are plerotic, the antheridia both monoclinous and diclinous; in *P. gracile* the oospores are aplerotic, the antheridia diclinous.

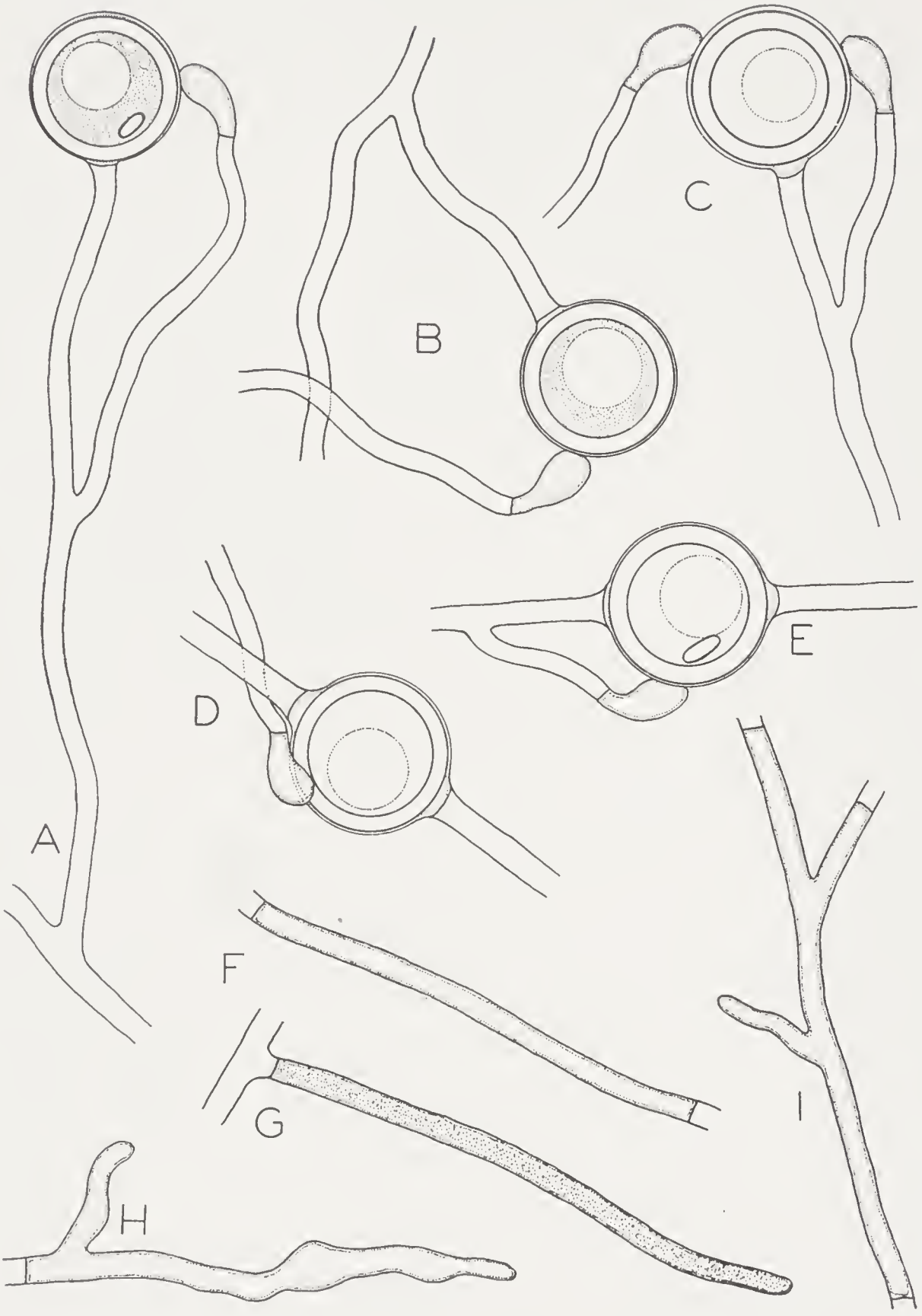

FIG. 1. *Pythium monospermum*. *A–E*, Sexual apparatus. *A*, Acrogenous oogonium with monoclinous antheridium and plerotic oospore. *B*, Acrogenous oogonium with diclinous antheridium. *C*, Acrogenous oogonium with both diclinous and monoclinous antheridia. *D*, Intercalary oogonium with diclinous antheridium. *E*, Intercalary oogonium with monoclinous antheridium. *F–I*, Typical filamentous sporangia.

Pythium monospermum and *P. marinum* both have plerotic, inspissate oospores. They may be distinguished morphologically by the origin and number of antheridia, diclinous and singular in *P. marinum*, monoclinous and diclinous, singular or plural in *P. monospermum*. They also may be easily segregated on the basis of their habitat, marine in *P. marinum* and fresh-water in *P. monospermum*. The sporangia of these species are indistinguishable.

Butler (1907) reports that *Pythium complens* Fischer was described to include *P. gracile, sensu* de Bary, and saprophytic *P. reptans* de Bary; the parasitic form of *P. reptans* de Bary properly belongs in *P. gracile*. Since *P. complens* is indistinguishable from *P. monospermum*, Butler places the saprophytic *P. reptans*, *P. gracile, sensu* de Bary, together with *P. complens* in *P. monospermum*. Butler follows Fischer (1892) in considering *P. fecundum* Wahrlich a synonym of *P. monospermum*. The description of *P. fecundum* presented by Wahrlich is almost identical with that of *P. monospermum*. The salient features of Wahrlich's description are:

Hyphae delicate, 2 to 5 μ, some threads larger, 4 to 5 μ, in diameter. Sporangia filamentous, 2 μ wide by 120 to 160 μ long. Vesicle formed in which 8 to 16 zoospores develop; zoospores are 4 μ broad, 6 μ long, laterally biciliate. Oogonia spherical, smooth and thin-walled, usually intercalary, occasionally subterminal or terminal. Antheridia one or two per oogonium, usually monoclinous when singular, when plural one usually of diclinous origin. Oospores, one to three, aplerotic, 12 to 14 μ in diameter.

Recently Rands and Dopp (1938b) report the isolation of *Pythium complens* from sugarcane roots in Louisiana, U. S. A. In the same year Stevenson and Rands (1938) indicated the synonymity of *P. gracile, sensu* de Bary, with *P. complens*, but failed to recognize the synonymity of *P. complens* with *P. monospermum*. A study of a culture of *P. complens* received by the writer from Rands corroborates the contention of Butler that *P. complens* would be properly embraced in the binomial *P. monospermum* and not in *P. gracile, sensu* de Bary, which is also properly referred to as *P. monospermum*. Fischer recognized the close relationship between *P. complens* and *P. monospermum* when he stated: "Sollte die Bildung einer Sporangienquerwand sich spater herausstellen, so wurde *P. complens* wohl mit *P. monospermum* zu Vereinigen sein." It seems undesirable to maintain two distinct species on the basis of the presence or absence of a delimiting sporangial septum; characteristics of this kind are too variable to be utilized in specific segregation.

Schröter in de Wildeman (1895) reports that the principal difference between *P. complens* and *P. gracile* is that the former is purely saprophytic and unable to attack living algae, whereas *P. gracile* is capable of parasitizing the living cells of algae. The principal difference between these species does not lie in their potentialities as parasites; rather in their distinctly different morphological characteristics, a single, monoclinous antheridium and plerotic oospore in *P. complens* as contrasted with a single, or occasionally two, diclinous anteridium and an aplerotic oospore in *P. gracile*.

The cardinal temperatures for mycelial growth of *Pythium monospermum* are: minimum, approximately 4°, optimum 31° and maximum 37° C.

Pythium monospermum is reported occurring from animal debris in Germany by Pringsheim (1858), de Bary (1860), in France by Dangeard (1890–91); from animal and plant debris in Germany by Wahrlich (1887); from soil in Ireland by Butler (1907), in the United States by Harvey (1925, 1927, 1929) and Raper (1928); from soil and water in U. S. A. by Matthews (1931) and the author; from *Hordeum vulgare* L. in the United States by the author, from *Lepidium sativum* L. in India by Butler and Bisby (1931); from *Nicotiana tabacum* L. in Java by Raciborski (1900); from *Oryza sativa* L. in Japan by Ito and Tokunaga (1933); from *Persea Americana* Mill. in the United States by the author; from *Richardia aethiopica* L. in the Netherlands by Meurs (1928); from *Saccharum officinarum* L. in the United States by Rands and Dopp (1938a, b); from *Spinacia oleracea* L. in the Netherlands by Meurs, and from *Zingiber officinale* Roscoe in India by Butler and Bisby.

2. Pythium marinum Sparrow, Bot. Arkiv 8: 1–24. 1934.

Hyphae measuring 2.5 to 3.5 μ in diameter, sparingly branched, intramatrical. Sporangia strictly filamentous, not inflated, extramatrical; producing 2 to 6 reniform, laterally biciliate zoospores 4.5 μ in width and 7.5 μ in length. Oogonia typically terminal on short lateral branches, spherical to ellipsoidal, smooth-walled, occasionally with a small apical papilla. Antheridia single, diclinous, clavate, 6 to 8 μ wide by 11 to 13 μ long, the apex tapering abruptly. Oospores plerotic, spherical to ellipsoidal, 13 to 20 μ by 15 to 21 μ, with a somewhat inspissate wall measuring 1.5 to 2 μ in thickness; germination not observed.

Saprophytic on dead fronds of *Ceramium rubrum* (Huds.) Ag., Denmark.

Material of this fungus was not available to the writer, the following discussion being based on Sparrow's paper in which he describes the species. As he mentions, this is not the first record of Pythium from Rhodophyceae. De Bary (1860) described *P. reptans* from a fresh water alga, *Bangia atropurpurea* (Roth) Agardh, but as only the asexual stage was seen considerable doubt exists as to the exact identity of the fungus. *P. reptans* is usually considered synonymous with *P. gracile* Schenk, *sensu* Butler.

Pythium marinum is reportedly a member of the *P. gracile* group, though differing considerably from *P. gracile* and closely related forms which not only have aplerotic oospores but frequently polyandrous oogonia and antheridia of monoclinous origin. *P. marinum* is unique at present in being the only member of the genus described from a marine habitat. Of the strictly filamentous sporangial forms, plerotic oospores are common to *P. marinum*, *P. monospermum*, and *P. papillatum*. *P. papillatum* has apandrous, papillate oogonia, being easily distinguishable from *P. marinum* which has monandrous, smooth oogonia. *P. monospermum* exhibits a closer relationship to *P. marinum* than does *P. papillatum*. In both of these species diclinous antheridia are present. Frequently either monoclinous or diclinous or occa-

sionally both types of antheridia are found in *P. monospermum*, while only diclinous antheridia are found in *P. marinum*. Oogonia of *P. monospermum* are more often intercalary than terminal, while those of *P. marinum* are nearly always terminal. There are no outstanding differences in the asexual apparatus and behavior of the latter two species, the development of the vesicle and zoospore formation being typical of the genus.

This species has so far only been reported from *Ceramium rubrum* in Denmark.

3. PYTHIUM PERNICIOSUM Serbinow, Scripta Bot. Hort. Univ. Imp. Petrop. 28: 1–47. 1912.

Hyphae measuring 5 to 9.5 μ in diameter, capable of breaking up into functional units, oidia. Sporangia filamentous, like hyphae, or originating from a swollen, sac-like structure on the hypha. Catenulate spherical reproductive bodies 17.4 to 30 μ in diameter also present, 3 to 5 in a series, which germinate by germ-tubes. Zoospores 8 to 16 in number, 8 to 12 μ in diameter. Oogonia spherical, smooth and thin-walled, acrogenous on short mycelial branches, 18.9 to 30.0 μ in diameter. Antheridia usually one, occasionally two, per oogonium, of monoclinous or diclinous origin; antheridial cell clavate, slightly curved, apical contact with oogonium. Oospores single, aplerotic, wall inspissate, measuring 18.1 to 23.5 μ in diameter; germination unknown.

Originally described as a parasite of tobacco seedlings, Russia.

The writer's observations are confined to the study of a single isolate of the fungus obtained from some rotted poinsettia cuttings, *Euphorbia pulcherrima* Willd., collected at Colma, California. The fungus studied agrees in every detail with the description presented by Serbinow.

Pythium perniciosum produces typical, strictly filamentous sporangia in agar cultures. When such cultures are irrigated with water the sporangia put forth delicate discharge tubes which soon form spherical, thin-walled vesicles at their apices. Serbinow reports seeing 8 to 10 zoospores in a vesicle; in several instances more than 30 zoospores were seen by the author.

Pythium perniciosum is easily distinguished from the other filamentous forms with aplerotic, inspissate oospores in possessing catenulate, spherical, asexual reproductive bodies. These resemble the oogonia, both in size and form. Oogonia, however, have never been observed in chains. It is probable that the catenulate asexual reproductive bodies may be frustrated oogonia. Ward (1883), Wahrlich (1887), Butler (1907) and more recently the writer (Tompkins *et al.*, 1939) have demonstrated that in several species the sexuality of incipient oogonia remains in doubt for a varying period of time. The catenulate bodies germinate under proper environmental conditions by means of germ tubes; germination with the production of zoospores has not been observed.

The occurrence of filamentous and spheroidal elements in the asexual apparatus in the genus *Pythium* is unusual. Drechsler (1929) questions the purity of the culture used by Serbinow as the basis for his description of

P. perniciosum. It is certain that pure cultures were used by the writer; the organism conformed with Serbinow's description in all respects.

The minimum temperature for vegetative growth of *Pythium perniciosum* is 4°, the optimum 28° to 31°, and the maximum 37° C.

Serbinow successfully inoculated the following hosts in addition to *Nicotiana rustica* L. and *N. tabacum* L. from which *Pythium perniciosum* was originally collected: *Amaranthus albus* L., *Atropa belladonna* L., *Barbarea arcuata* Rchb., *Brassica napus* L., *Camelina sativa* L., *Chenopodium* sp., *Datura stramonium* L., *Hyoscyamus niger* L., *Lepidium draba* L., *L. latifolium* L., *Linum usitatissimum* L., *Lycopersicon esculentum* Mill., *Sinapis arvensis* L., *S. orientalis* L., *Solanum dulcamara* L., *Thlapsi arvense* L., *T. montanum* L., and "young fern plants." Eristavi and Mordvintzeff (1930) report the recurrence of the seedling rot of *N. tabacum* caused by *P. perniciosum* in Russia. Van Eek (1938) states that this fungus is associated with a root rot of *Viola tricolor* L. in the Netherlands; Muller and van Eek (1939) report a root rot of *Hibiscus cannabinus* L. and *H. sabdariffa* L. in the Netherland East Indies due to this fungus.

4. Pythium dissotocum Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Pythium oryzae Ito and Tokunaga, Jour. Fac. Agr. Res. Hokkaido Imper. Univ. **32**: 201–233. 1933.

Hyphae measuring 1.5 to 7 μ, mostly 3.5 to 6 μ, in diameter. Sporangia typically undifferentiated hyphal elements, sparingly branched, occasionally including slightly swollen dactyloid laterals. Zoospores usually 10 to 75, though infrequently more than 100 in a vesicle, 8 to 9 μ in diameter when encysted, exhibiting repetitional emergence. Oogonia typically terminal though also intercalay, subspherical, measuring 12 to 33 μ, average 20.7 μ in diameter, smooth-walled, the delimiting septum or septa sometimes inserted beyond the oogonial contour. Antheridia usually monoclinous though also diclinous, 1 to 5 per oogonium, when plural each usually of autonomous origin, often sessile when monoclinous, when diclinous usually originating near the oogonium, measuring 5 to 8 μ in width and 6 to 16 μ in length. Oospores smooth, with a moderately thickened wall, aplerotic though almost filling oogonial cavity, 11 to 27 μ, average 17.6 μ, in diameter, containing a single reserve globule and subspherical or oblate ellipsoidal refringent body.

Isolated from roots of *Saccharum officinarum* L., Louisiana, U. S. A.

The paper in which the species was originally described contained no discussion of the fungus, being limited to the diagnosis. Drechsler recently (1940) discussed the fungus but limited his remarks to amplification of the diagnosis rather than including any comments of the relation of the species to congeneric forms. A culture of this fungus, isolated from sugar cane, was obtained for study. As stated by Drechsler the fungus resembles the general habit of *Pythium ultimum* when cultivated on rather rich substrates, but lacks the density of aerial mycelium encountered in *P. ultimum*. Whereas *P. ultimum* is a rapid growing species at room temperature, *P. dissotocum* grows somewhat more slowly. *P. dissotocum* forms asexual and sexual bodies

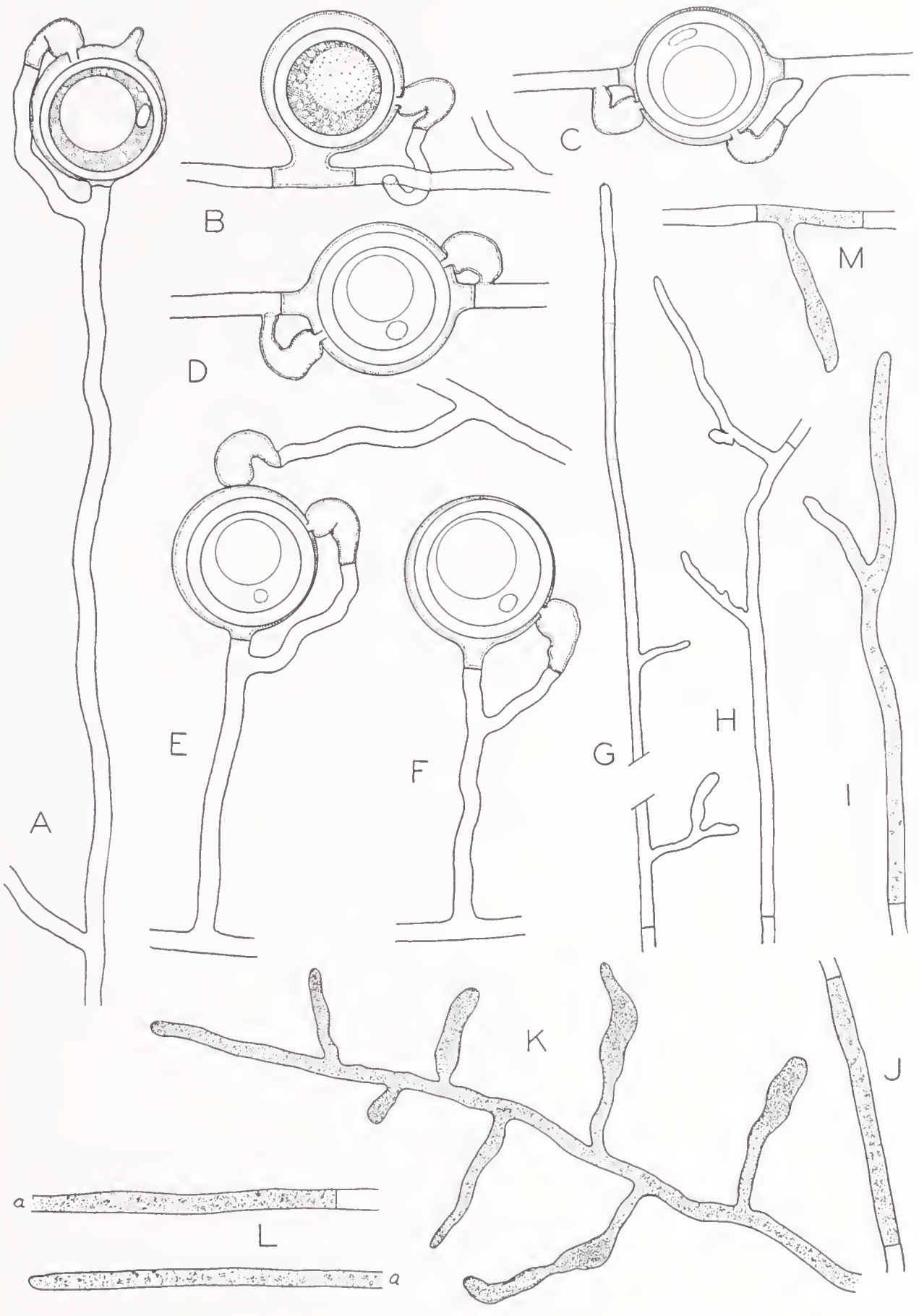

FIG. 2. *Pythium dissotocum*. *A–F*, Sexual apparatus. *G–M*, Filamentous sporangia. *K*, Type of development found in actively growing apical hyphae showing slight inflation of lateral lobes.

readily on suitable substrates. The asexual apparatus is indistinguishable from the vegetative hyphae when grown in a medium containing a paucity of free water. When agar blocks containing the fungus are properly irrigated evacuation tubes are produced at the top of which vesicles bearing zoospores develop. The evacuation tubes arise from the filamentous vegetative structures which may be considered sporangia. Apparently the conversion of a vegetative portion of the thallus into an asexual reproductive unit takes place without any preliminary visible change since evacuation tubes form in many instances from hyphal segments originally believed to be purely vegetative threads. Drechsler reports similar observations in his recent contribution concerning this species.

The sexual reproductive bodies are usually formed intramatrically, rarely on the extramatrical hyphae. The oogonia are more frequently intercalary, though are often terminal or medially disposed as noted in the diagnosis. The antheridia are usually monoclinous, though also diclinous and occasionally both. The antheridial cell is a distinctive feature of the species; it is crook-necked, inflated, breviform, and borne on a relatively short antheridial stalk or sessile. The tip of the antheridial cell is usually flattened where contacting the oogonium, in this respect differing from congeneric forms having short, inflated antheridia. The oospores conform with the description of these bodies in the diagnosis.

A careful study of an authentic culture of *Pythium oryzae* was undertaken in conjunction with the study of *P. dissotocum;* it is concluded that the two organisms must be considered synonymous. Since *P. dissotocum* was described three years prior to *P. oryzae*, *P. dissotocum* is retained as the valid binomial. A comparison of data relative to the two fungi substantiates the above conclusion. According to Drechsler, the sporangia of *P. dissotocum* consist of undifferentiated filaments, occasionally including swollen dactyloid laterals, simple or sparingly branched, 5 to 8 μ in diameter. Ito and Tokunaga (1933) describe the sporangia as "terminalibus, filiformibus, non ramosis, valde longis, 3 μ circiter crassis." Oogonia of *P. dissotocum* are 12 to 33 μ, av. 20.7 μ in diameter, acrogenous or intercalary; oogonia of *P. oryzae* are "terminalibus vel intercalaribus, 15 to 23 μ vel raro 13 μ in diameter." Antheridia of *P. dissotocum* compare favorably with the "antheridiis androgynis vel diclinous, singulis vel binis, abcaudicula per septum sectis, curvata-clavatis vel oblongatis" of *P. oryzae.* Oospores of *P. dissotocum* measure 11 to 27 μ, mostly 15 to 21 μ, average 17.6 μ, in diameter compared with "12 to 20 μ vel raro 10 μ in diameter" in *P. oryzae.*

Further evidence of the synonymity of the species may be found in the data compiled from the study made by the writer. The measurements of *Pythium dissotocum* agree with those given by Drechsler. Some differences, however, were found in the measurements and description of *P. oryzae* as presented by Ito and Tokunaga and observed by the writer. The sporangia of *P. oryzae* are often not exclusively composed of strictly filamentous units

as depicted in the description, but often include a swollen, branched, digitate portion that is indistinguishable from the sporangia of *P. dissotocum*. No doubt the "frequenter multos ramos gemmiformes, breves, turgescentes, usques ad 10 μ crassis formantibus, interdum hyphae grandes gignentibus" described by Ito and Tokunaga as a part of the mycelium is in reality comparable to the companion part of the sporangium found in *P. dissotocum*. The description of the antheridia of *P. oryzae* is somewhat incomplete. Antheridia found in agar media vary from 1 to 5 in number and are borne on short antheridial branches which may be either monoclinous or diclinous in origin. Infrequently the antheridium is sessile; it is arched, inflated, the tip broadly applied to the oogonium, thus possessing a blunt apical aspect. These antheridia are indistinguishable from those of *P. dissotocum*. The oogonia and oospores vary in size more than is indicated in the description: oogonia 12.8 to 31.1 μ, mean 19.6 μ compared with 13 to 23 μ (no mean given) in diameter; oospores 9.7 to 25.6 μ, mean 16.7 μ compared with 10 to 20 μ (no mean given) in diameter.

As an adjunct to the above substantiating evidence for synonymity, reference may be made to the temperature-growth response of these two organisms. The cardinal temperatures for *Pythium dissotocum* are minimum 1°, optimum 28°, and maximum 34° C.; these are identical with those of *P. oryzae*. The growth habits of the two are identical.

Pythium dissotocum has been reported in the United States of America from *Saccharum officinarum* L. by Drechsler (1930a, 1940), Rands (1930), Rands and Abbott (1939) and Rands and Dopp (1938b); from *Beta vulgaris* L., *Pilea pumila* (L.) Gray, *Pisum sativum* L. and *Spinacia oleracea* L. in the U. S. A. by Drechsler (1940); from *Oryza sativa* L. in Japan by Ito and Tokunaga (1933) and Darker (1940).

5. PYTHIUM GRACILE Schenk, Verhandl. Phys.-Med. Ges. Wurzburg **9**: 12–31. 1859.

Pythium sp. Ward, Quart. Jour. Micr. Sci. **23**: 485. 1883.
Pythium diclinum Tokunaga, Trans. Sapporo Nat. Hist. Soc. **14**: 11–33. 1935.

Hyphae fine, measuring 1 to 5.6 μ, mostly 2 to 3.8 μ, in diameter. Sporangia filamentous, indistinguishable from the vegetative hyphae, branched or unbranched. Zoospores from 2 to 30 or more in a vesicle, reniform, 3 to 4 μ wide and 6 to 8 μ long. Oogonia usually formed intramatrically, spherical to ovoid, smooth, thin-walled, mostly acrogenous, occasionally subterminal or intercalary, 14.3 to 27.6 μ, mostly 15.7 to 24.2 μ, in diameter. Antheridia usually one, rarely two per oogonium, of diclinous origin, never monoclinous. Oospores single, aplerotic, wall inspissate, oospore measuring 11.1 to 21.3 μ, mostly 12.2 to 21.1 μ in diameter, containing a single reserve globule usually an ellipsoidal refringent body embedded in the granular protoplasmic content.

Originally described as parasitic on *Spirogyra nitida* (Dillw.) Link, *S. heeriana* Näg., and *Cladophora* sp., Germany.

The description given above is taken in part from Schenk's original work and supplemented with the observation of Fischer (1892), Butler (1907), and the writer.

Considerable confusion exists regarding this species. The fungus was originally described by Schenk in 1859 on the basis of the asexual stage; inasmuch as the sexual stage was not reported it is difficult to be certain of the exact identity of the species. Perhaps the ablest discussion of the species is that published by Butler in 1907 and Butler's scheme is followed here in the discussion of *Pythium gracile.*

A year after Schenk described *Pythium gracile*, de Bary (1860) described an organism indistinguishable from *P. gracile*, calling it *P. reptans.* In 1881 de Bary (1881a) described as *P. gracile, sensu* de Bary, another filamentous Pythium found as a saprophyte on insect cadavers in water. This fungus had originally been included in *P. reptans.* The new *P. gracile* is considered distinct from *P. gracile* Schenk due to its inability to infect living algae. In the same paper de Bary indicates that his *P. reptans* is more than likely *P. gracile* Schenk and suggests that the name *P. reptans* be discarded. Ward in 1883 described and figured a fungus which he considered synonymous with *P. gracile, sensu* de Bary. Differences were reported but were considered of minor importance. In the same paper Ward reports finding a fungus with oospores differing from those of his *P. gracile, sensu* de Bary, and lacking a sexual stage. It has been suggested that these oospores were those which Schenk failed to find in his study of *P. gracile.* Butler prefers to consider the fungus partially studied by Ward and a similar form studied by himself as *P. gracile* Schenk since other filamentous forms have since been provided with specific nomens, such as *P. tenue, P. monospermum* and others. The principal point in favor of Butler's action is expressed in his statement, "This appears to be more satisfactory than to add another to the names of Pythium parasites of algae."

The only morphological difference between *Pythium gracile, sensu* de Bary, and *P. monospermum* is that the sporangium of the former is not delimited by a septum. It is probable that the absence of a septum should not be used as a taxonomic feature. Fischer proposed *P. complens* in 1892 to replace the binomial *P. gracile, sensu* de Bary, since *P. gracile* Schenk takes precedence, *P. complens*, as already discussed, is correctly embraced in *P. monospermum.*

Very little work concerning this species has been done since Schröter's (1897) transference of *Pythium gracile* to *Nematosporangium gracile* and Butler's discussion, except the brief summarization of the species by Sparrow (1927).

Butler included three varieties in his concept of *Pythium gracile;* at present varieties *a.* and *b.* are the only ones considered valid. Variety *b.* was transferred to *P. diclinum* by Ito and Tokunaga; this disposition does not seem warranted. The similarity in size of oogonia and oospores of

P. diclinum as reported by Tokunaga (oogonia 18 to 25.2 μ and oospores 14.4 to 22.8 μ in diameter) and *P. gracile* var. *b.* as reported by Butler (oogonia 15 to 22 μ and oospores 13 to 20 μ, in diameter) is obvious and may indicate that Ito and Tokunaga were dealing with an organism identical with Butler's. The reason for segregating var. *b.* of Butler's *P. gracile* from *P. gracile* as now interpreted and describing it as a new species is not at all clear. Butler considered var. *b.* in conformity with the species despite its saprophytic habit. The oogonia and oospore measurements given by Butler for this variety certainly fall within the limits of the species as presented by Matthews (1931) and the observations of the writer. Specific segregation on a dimensional basis is of doubtful validity as previously indicated. The description of *P. diclinum* is identical with that of *P. gracile* with few exceptions: the hyphae and sporangia are of similar size and description, spherical, thin- and smooth-walled, occasionally subterminal (". . . . interdum prope apicem uno spiculo praeditis" of *P. diclinum*), antheridia strictly diclinous, oospores single and aplerotic with an inspissate wall. The differences in the description of the two species lie in the presence of "gemmiformes formantibus" in *P. diclinum*, which could be almost anything from appresoria to frustrated oogonia, and the generally plural antheridia in *P. diclinum*. There is typically a single antheridium in *P. gracile*, though there are infrequently two or possibly more; in *P. diclinum* there are "antheridiis 1–3 fere 2 quoque oogonia." This discrepancy in the difference in number of antheridia cannot be considered big or valid enough to warrant specific distinction. For these several reasons *P. diclinum* is considered synonymous with *P. gracile.*

Variety *c.* of *Pythium gracile, sensu* Butler, in roots and stems of *Zingiber officinale* Roscoe and in roots of *Ricinus communis* L. in water culture, has been transferred to *P. aphanidermatum;* discussion regarding this transference may be found under *P. aphanidermatum.*

The writer's observations are based on two isolates of the fungus, one from Missouri, the other from California. Both cultures agree with the concept of the species advanced by Butler.

Pythium gracile may be easily cultivated on a variety of nutrient agars. Reproductive structures are produced in moderate abundance. The species is separated from congeneric forms on the points set forth in the key: an aplerotic oospore with a thick wall, 2 to 2.5 μ thick, strictly diclinous antheridia, usually single though often two, the antheridial cell small, inflated-clavate, not greatly curved and narrowly applied to the oogonium, not blunt tipped as in *P. dissotocum.*

Pythium gracile is reported from *Bangia atro-purpurea* (Roth) Ag. (de Bary 1860), *Cladophora* sp. (Schenk 1859) in Germany, *Cladophora* sp. in France and Belgium by Schröter in de Wildeman (1895) and in England (Ward 1883), *Gossypium* sp. in Anglo-Egyptian Sudan (Andrews & Clouston, 1939), *Oryza sativa* L. (Ito & Tokunaga 1935) in Japan, plant debris (Butler

1907) in France and India, (Butler & Bisby 1931) in India, *Ricinus communis* L. (Sydow and Butler, 1907) in India, soil by the author in the United States of America, *Spirogyra* spp. (Sparrow 1933) in the U. S. A., *Spirogyra* sp. (Ward 1883) in England, (de Wildeman 1895) in France and Belgium, (Butler 1907) in India, (Matthews 1931) in the U. S. A., *Spirogyra crassa* Kütz. (Sparrow 1927) in the U. S. A., *S. heeriana* (Dill.) Link and *S. nitida* Näg. (Schenk 1859) in Germany, *S. porticalis* (Vauch.) Cleve (Saccardo *et al.*, 1904) in Alaska, *Triticum aestivum* L. by the author in the U. S. A., *Vaucheria* sp. (de Bary 1860) and *Vaucheria aversa* Hass. (Butler 1907) in Germany, *Xanthosoma sagittifolium* Schott. and *Colocasia antiquorum* Schott. in Gold Coast (Shepherd 1940), *Zingiber officinale* Roscoe (Sydow & Butler 1907) (McRae 1911) in India and (Parham 1935) in Fiji. Petersen (1910) reports *P. gracile* from "different algae" in Denmark. A *Pythium* sp. presumably identical with *P. gracile* is reported from *Triticum aestivum* L. roots in Italy by Petri (1930) and from *Carica papaya* L. in the Union of South Africa by Wager (1932a).

6. Pythium adhaerens Sparrow, Ann. Bot. 65: 257–277. 1931.

Hyphae measuring 5 to 7.2 μ in diameter, intramatrical and extramatrical, forming numerous appressoria, usually 7.2 μ wide by 12 μ long. Sporangia undifferentiated from hyphae, filamentous; zoospores laterally biciliate, 6.5 μ wide by 10.8 μ long, 5.4 to 9 μ, average 7.2 μ, in diameter at encystment, exhibit repetitional emergence. Oogonia spherical, acrogenous or intercalary, smooth-walled, measuring 10.8 to 25.2 μ, average 17.5 μ in diameter. Antheridia diclinous, 1 to 4, typically borne as lateral branches of a single branch which often encircles the oogonium, seldom from several distinct branches, antheridial cell delimited, crook-necked, clavate, 7 μ wide by 15 μ long. Oospores aplerotic, spherical, measuring 7.2 to 21.6 μ, average 14.4 μ, in diameter, with a wall 2 to 2.5 μ thick, containing a single reserve globule; germination either direct or by zoospores.

Described as parasitic on *Rhizoclonium hieroglyphicum* (Ag.) Kütz., Mass., U. S. A.

No isolates of *Pythium adhaerens* were observed by the writer. This fungus lacks the somewhat compact complex of intercommunicating swollen dactyloid elements such as are present in *P. aphanidermatum*, *P. arrhenomanes* and related species. Appressoria are found in *P. adhaerens* which may be at first easily confused with the inflated digital elements of the species mentioned above. If, however, attempts are made to induce zoospore formation in these bodies, they will either produce further vegetative growth or remain unchanged, never producing zoospores.

The sexual apparatus of *Pythium adhaerens* differs somewhat from that of other species of the genus in that the antheridia, of which there are usually two to four per oogonium, arise from a single, diclinous hypha which surrounds the oogonium in a rather characteristic manner. The oogonia are typically intercalary or acrogenous. The oospores are aplerotic, germinating by the production of a branched germ tube; zoospores may also be produced.

Pythium adhaerens is closely related to *P. gracile*, both having aplerotic oospores and strictly diclinous antheridia. They differ decidedly, however, in the origin and disposition of their antheridial cells. In *P. adhaerens* the antheridial cells vary from one to four per oogonium and are all lateral prolongations of a single antheridial branch. *P. gracile*, on the other hand, usually has only one, rarely two, antheridial cells of autonomous origin.

Sparrow, in the same paper describing the species, adds that *Pythium adhaerens* is capable of parasitizing, when artificially inoculated, *Spirogyra crassa* Kütz., *Ulothrix zonata* Kütz., *Tolypothrix* sp., *Lycopersicon esculentum* Mill., *Cucumis sativus* L. and *Cucurbita moschata* Duchesne, thus presenting additional evidence that species described as algal parasites are not necessarily limited as parasites to algal hosts. Sparrow (1932) later added *Beta vulgaris* L., *Cucurbita pepo* L., *Pisum sativum* L., *Synedra* sp. and *Zea mays* L. as susceptible hosts.

7. Pythium angustatum Sparrow, Ann. Bot. **65**: 257–277. 1931.

Hyphae measuring 2 to 3.7 μ in diameter, intramatrical and extramatrical, rarely forming appressoria. Sporangia undifferentiated from hyphae, filamentous; zoospores laterally biciliate, 5 μ wide by 8.5 μ long, average 6.2 μ in diameter at encystment, exhibiting repetitional emergence. Oogonia spherical, rarely sac-like, acrogenous, smooth-walled, measuring 12.6 to 27.0 μ, average 19.8 μ, in diameter. Antheridia typically diclinous, rarely monoclinous, 1 to 5 per oogonium, the antheridial cells terminal on lateral distorted branches, delimited, crook-necked, measuring 5 μ wide and 8.5 μ long, the blunt apex making broad contact with the oogonium. Oospores single, rarely 2 to 4, aplerotic, measuring 10.8 to 25.2 μ, average 18.0 μ, in diameter, wall smooth and not inspissate, 1 to 1.8 μ in thickness, containing a single reserve globule. Oospore germinates by the production of a hypha which later may form sporangia.

Parasitic on *Spirogyra crassa* Kütz., Mass., U. S. A.

No cultures of *Pythium angustatum* were observed by the writer. Sparrow described this species as a companion species to *P. adhaerens*. In contrast to *P. adhaerens*, no appressoria are found in agar cultures of *P. angustatum*. This fact precludes any confusion with inflated filamentous sporangial forms such as *P. graminicolum*, *P. torulosum* and allied species.

Pythium angustatum demonstrates affinities toward *P. apleroticum* and *P. diclinum* from which it is principally distinguished by the disposition and origin of its antheridia. *P. apleroticum* and *P. diclinum* never have monoclinous antheridia, which are frequently displayed in *P. angustatum*. *P. apleroticum* possesses but a single antheridium, whereas *P. angustatum* exhibits a multiplicity. Though *P. angustatum* and *P. adhaerens* are closely related species and demonstrate a distinct relationship to *P. gracile*, they nevertheless retain their individuality.

In addition to parasitizing *Spirogyra crassa*, *Pythium angustatum* is also capable of infecting *Rhizoclonium hieroglyphicum* (Ag.) Kütz., *Oedogonium* sp. and *Zea mays* L. upon inoculation (Sparrow 1932).

8. PYTHIUM APLEROTICUM Tokunaga, Trans. Sapporo Nat. Hist. Soc. **14**: 11–33. 1935.

Hyphae tenuous, about 3 μ in diameter, intramatrical and extramatrical. Sporangia undifferentiated from hyphae, filamentous, usually terminal though sometimes lateral; zoospores reniform, laterally biciliate, 2 to 25 or more found in vesicle, measuring about 4.8 μ wide and 8.4 μ long. Oogonia spherical, acrogenous or intercalary, measuring 11 to 20 μ in diameter. Antheridia single, delimited by basal septum. Oospores single, aplerotic, spherical, measuring 9 to 17 μ in diameter, wall not inspissate, 0.6 to 0.8 μ thick.

Parasitic on *Spirogyra* sp., Sapporo, Japan.

Pythium apleroticum is not a well known species; any discussion of the fungus must necessarily be based exclusively on the works of Ito and Tokunaga in the absence of further information.

Pythium apleroticum is recorded as being allied with *P. gracile* but "differing in the small oogonium and thin oospore wall." A comparison of the measurements of *P. apleroticum* given by Tokunaga with measurements from the writer's collection is presented: *P. apleroticum*, oogonia 11 to 20 μ, oospores 9 to 17 μ in diameter; *P. gracile*, oogonia 14.3 to 27.7 μ, oospores 11.1 to 21.3 μ in diameter. These data substantiate the conclusions of Tokunaga. The difference in thickness of the oospore walls of the two species is perhaps more significant than the oogonial dimensions. The relationship between *P. apleroticum* and *P. angustatum* is probably closer than that between the species and *P. gracile* as stated by Ito and Tokunaga. The algal habit is common to both *P. apleroticum* and *P. angustatum;* perhaps of greater significance is the similarity in oospores, aplerotic ones with exceedingly thin walls being common to both. The inadequate description of the antheridium in *P. apleroticum* is compensated for by the authors in a brief discussion pointing out the striking similarity of their fungus to *P. gracile*, indicating the likeness in kind of antheridium. The single, diclinous antheridium of *P. apleroticum* is clearly distinguishable from the typically plural antheridia of *P. angustatum*, which are borne terminally on lateral, slender distorted branches of a single hypha, though infrequently from several discrete branches. Subsequent study of *P. apleroticum* may afford further distinguishing features not now recognized.

The fungus is only known from an unidentified species of *Spirogyra* collected in Sapporo, Japan.

9. PYTHIUM DICTYOSPORUM Raciborski, Bull. Acad. Int. Sci. Cracovie **1891**: 283–287. 1891; Krakow Nakt. Akad. Umietjet **24**: 1–9. 1892.

Hyphae measuring 1.8 to 2 μ in diameter, isodiametric, intramatrical, and extramatrical, capable of forming appressoria and occasionally irregular hyphal swellings. Sporangia undifferentiated from hyphae, filamentous; zoospores few to 50, reniform, laterally biciliate, 5.4 μ wide and 9 μ long, about 10 μ in diameter at encystment, capable of repeated emergence. Oogonia spherical, typically terminal, smooth- and thin-walled, measuring

12.6 to 28.8 μ, average 21.6 μ, in diameter. Antheridia are monoclinous and diclinous, terminal, 1 to 2 per oogonium, clavate, crook-necked, often 8 μ wide and 20 μ long, making narrow contact with the oogonial wall. Oospores aplerotic, single, with a thick, reticulate wall, the oospore completely sheathed with a gelatinous material, germination by the production of a sporangium which subsequently forms a limited number of zoospores.

Parasitic on *Spirogyra insignis* (Hass.) Kütz. and *S. nitida* (Dillw.) Link, Cracow, Poland.

Pythium dictyosporum was first incompletely described from *Spirogyra insignis* (Hass.) Kütz. in 1891; the next year Raciborski presented an illustrated and more detailed account of the fungus, though failing to give a definite diagnosis of the species, this time describing it from *S. nitida* (Dillw.) Link. De Wildeman (1895) contending that the fungus was presented without adequate diagnosis gives a diagnosis based on material cited by Raciborski and his own observations. That de Wildeman is correct in his contention is questionable, as there were specific descriptions published prior to 1895, though these were presented in a rambling and disconnected style. The description above is taken in part from Sparrow (1931a) and from the writer's observations. Sparrow's diagnosis was chosen in preference to de Wildeman's because it is based on more recent and complete observations of the fungus. The writer's studies are based on preserved algal material containing the fungus.

The predominant features of the species are contained in the above diagnosis. Sparrow (1931a) observes that his isolate of *Pythium dictyosporum* produced only diclinous antheridia whereas Raciborski (1892) reports the production of both diclinous and monoclinous; the isolate studied by the writer produced antheridia of both types. The type of antheridium and its mode of application closely resembles that of *P. monospermum;* the sporangia of these two species are likewise similar. The presence of sculptured oospores in the Pythiaceae is confined to two *Pythium* species, whereas in the two remaining families of the order, Albuginaceae and Peronosporaceae, the presence of this type of oospore is not at all uncommon; this point further emphasizes the interrelationship of the families and may be indicative of their phylogeny. *P. dictyosporum* may readily be distinguished from related species in its unique reticulate-walled oospore. The only other member of the genus with a sculptured oospore wall is *P. cystosiphon*, a not closely related species with spherical sporangia.

Pythium dictyosporum has been observed in *Spirogyra* sp. in France by de Wildeman (1895) in the United States by Matthews (1931) and Sparrow (1933), in *S. insignis* and *S. nitida* by Raciborski in Poland, in *S. crassa* Kütz. in the United States by Sparrow (1931a) and in *Vaucheria* sp. by the author. Sparrow states that this species was capable of infecting *Cucumis sativus* L., *Rhizoclonium hieroglyphicum* (Ag.) Kütz. and *Tolypothrix* sp., he

(1932) later reported that *P. dictyosporum* was capable of infecting *Oedogonium* sp. upon inoculation.

10. PYTHIUM TENUE Gobi, Scrip. Bot. Hort. Univ. Imp. Petropol. **15**: 221–226. 1899–1900.

Hyphae fine, isodiametric, branched, measuring 1 to 3 μ in diameter. Sporangia filamentous, similar to the vegetative hyphae, when intramatrical, vesicle exogenous. Zoospores 2 to 60 in a vesicle, reniform, laterally biciliate. Oogonia spherical, smooth, thin-walled, intramatrical, usually acrogenous, measuring 7.2 to 15.6 μ, mostly 9.8 to 12.2 μ, mean 10.3 μ in diameter. Antheridia usually one, occasionally two, per oogonium, more frequently monoclinous than diclinous, antheridial cell clavate, slightly swollen, terminal, not delimited by septum. Oospores single, aplerotic, globose, smooth, thin-walled, measuring 6.0 to 12.1 μ, usually 7.5 to 9.8 μ, mean 8.4 μ in diameter, containing a single reserve globule and a small ellipsoidal refringent body in the protoplasm.

Originally described as parasitic on *Vaucheria sessilis* DC. and *Mesocarpus* sp., Finland.

Pythium tenue is usually restricted to living algal hosts. The observations of the writer are based upon a study of the fungus in living cells of *Vaucheria* sp. collected near Ashland, Missouri. Attempts to culture the organism on agar substrates were unsuccessful; Matthews (1931) encountered the same difficulty.

Though rather free use is made of the undelimited antheridial cell in the segregation of this species from related forms, a septum has been observed in rare instances. The nature of the antheridium, however, is not the sole criterion for specific identification. *P. tenue* may be separated easily from the aplerotic oospore forms such as *P. dissotocum*, *P. gracile* and *P. adhaerens* on the absence of a thickened oospore wall, as well as on its obligate parasitic character. *P. tenue* may be distinguished from *P. angustatum* by the absence of polyandrous oogonia. *P. aplerotícum* might be confused with *P. tenue* except that its antheridium is always diclinous and never monoclinous as is common in *P. tenue*. *P. papillatum* is easily distinguished from *P. tenue* by its papillate oogonia, absent in *P. tenue*. The unusually small oogonia of *P. tenue* may likewise assist in the identification of the species.

Gobi points out that *P. entophytum* Pringsh., *sensu* Schenk, does not correspond with Pringsheim's description of the organism. He further identifies *P. tenue* as the fungus that Schenk (1859) confused with *P. entophytum*. Since Zopf transferred *P. entophytum* to the genus *Lagenidium* and Schenk failed to realize the exact identity of his fungus, *P. tenue* Gobi remains the valid nomen.

Pythium tenue has been observed in *Mesocarpus* sp. in Finland by Gobi, in *Spirogyra* sp. in the United States by Matthews (1931), in *Vaucheria* sp. in the United States by the author and Matthews, in *Vaucheria sessilis* DC. in Finland by Gobi and in the United States by Sparrow (1933).

11. PYTHIUM PAPILLATUM Matthews, Jour. Elisha Mitchell Soc. 43: 229–232. 1932.

Hyphae irregular in size, main filaments measuring up to 24 μ in diameter at base, usually from 2.3 to 10.1 μ, average 7 μ, in diameter, the apex frequently blunt-tipped; numerous bud-like outgrowths in old cultures. Sporangia filamentous, similar to vegetative hyphae, short or long, branched or unbranched; zoospores few to 20 or more, reniform and laterally biciliate, usually 12 μ long and 7.2 μ wide, when encysted about 12 μ in diameter. Oogonia spherical, subspherical, oval, terminal on short branches, intercalary, often catenulate, 1 to 5, measuring 16.8 to 26.4 μ in diameter, thin-walled, smooth or with one or two papillae. Antheridia not produced. Oospores plerotic except for oogonial neck and papillae, single, occasionally two per oogonium, smooth- and moderately thin-walled. Germination not observed.

Isolated from soil in Chapel Hill, N. C., U. S. A.

Pythium papillatum is unique among the species possessing strictly filamentous sporangia in having papillate oogonia and apandrous, plerotic oospores. Though the oogonial papillae of *P. papillatum* may be confused with the subterminal oogonia of *P. monospermum*, the absence of antheridia alone may separate the species.

In addition to the isolation of *Pythium papillatum* from soil in the U. S. A. by Matthews, a culture was obtained by the writer from rotted barley, *Hordeum vulgare* L. roots collected in Missouri, U. S. A.

12. PYTHIUM AFERTILE Kanouse and Humphrey, Papers Mich. Acad. 8: 129–140. 1927.

Hyphae measuring 3 to 5 μ in diameter, branching, non-septate when young, occasionally septate when old. Sporangia hypha-like, 3 to 5 μ in diameter, 300 μ or more in length, unbranched, quickly collapsing after vesicle formation; zoospores 3 to 30, formed in a terminal, spherical, evanescent vesicle, 9 to 40 μ, average 25 μ, in diameter, the zoospores reniform, laterally biciliate, monoplanetic, measuring 8 to 10 μ in diameter when encysted. Spherical structures resembling chlamydospores are profusely formed, spherical, terminal or intercalary, delimited by septa; by renewed growth forming hyphae or sporangia. Sexual reproduction unknown.

Collected from *Vaucheria* sp. Ann Arbor, Mich., U. S. A.

A culture of this fungus was obtained for study from the Centraalbureau voor Schimmelcultures, Baarn, Holland. The organism received agreed with the description of the species quoted above.

In the absence of a sexual stage it is difficult to identify *Pythium* species. On the basis of the sporangial characters, *Pythium afertile* might be any one of the strictly filamentous species of the genus. Whether the sexual stage will ever be found is doubtful. Its absence may be due to a variety of factors, among them environmental conditions, types of culture media, presence, absence or amount of sex-promoting substances. Although the authors of the species contend that *P. afertile* is a distinct organism, lacking a sexual stage, the disadvantage of giving an organism of this type a binomial can

readily be seen. On the contrary, if a species is to be considered as an entity or a group of like organisms, it is certainly justifiable to describe it and ascribe a binomial to it. If this species is maintained it is understood that *P. monospermum*, *P. adhaerens* and others, lacking sexual development, will automatically become associated with *P. afertile*.

The presence of certain spherical, terminal or intercalary bodies may further distinguish the fungus from allied species. If any significance is to be attached to the presence of chlamydospores, *Pythium afertile* may be considered an ally of *P. perniciosum*. That any degree of significance should be assigned these chlamydospores seems doubtful in the light of our knowledge of the origin and development of oogonia and spherical sporangia which are examined in the discussion following the description of *P. perniciosum*.

P. afertile may either be maintained as a distinct species, lacking a sexual stage, or as a binomial which includes a variety of related species, the sexual stage of which at the time is lacking but which may be encountered upon later observation; transference to another binomial would be mandatory once the sexual stage appeared in a species of the latter category. It seems best to maintain the species pending further study.

Pythium afertile is known from *Gossypium* sp. in the Anglo-Egyptian Sudan by Andrews and Clouston (1938, 1939) and Massey (1936), from *Vaucheria* sp. by Kanouse and Humphrey in the United States and from *Viola tricolor* L. by van Eek (1938) in the Netherlands.

13. Pythium catenulatum Matthews, Studies on the Genus Pythium. 1931.

Hyphae measuring 2 to 4 μ in diameter, branched. Sporangia composed of somewhat inflated hyphae in conjunction with irregularly swollen bud-like lateral outgrowths, forming simple to complex aggregations; zoospores 10 to 20 or more in a vesicle, 6 μ wide by 8 to 11 μ long, reniform and laterally biciliate. Numerous spherical to pyriform asexual reproductive bodies (= conidia, according to Matthews) also present, terminal and intercalary, single or 3 to 8 in a chain, 10 to 20 μ, average 15 μ, in diameter, germinate by production of 1 to 3 germ tubes, never producing sporangia. Oogonia usually spherical, terminal or intercalary, smooth- and thin-walled, measuring 18 to 38 μ in diameter. Antheridia of monoclinous or diclinous origin, from 1 to 12, usually 5 to 6, per oogonium, clavate, crook-necked, making narrow apical contact with the oogonial wall. Oospores plerotic, single, smooth- and thick-walled, containing a single reserve globule and refringent body in the granular protoplasm, oospores measuring 16 to 32 μ in diameter; germination not observed.

Originally described from water containing vegetable debris collected in N. C., U. S. A.

Asexual reproduction in *Pythium catenulatum* is very similar to that found in *P. perniciosum;* there are, however, certain differences, which separate the species. Both species have catenulate spherical, asexual reproductive bodies which germinate only by the production of one to five germ tubes when a suitable environmental condition prevails. In *P. catenulatum*

these spherical bodies range in size from 10 to 20 μ, usually 15 μ, in diameter, the interval between bodies varying from 1 to 35 μ in length; the comparable structures in *P. perniciosum* range in size from 17.3 to 30 μ, usually 24 μ in diameter, with less interval irregularity, usually measuring from 20 to 25 μ between structures. In general habit the asexual reproductive bodies of *P. catenulatum* resemble those of *P. intermedium*, a species lacking filamentous sporangia and a sexual stage. The sporangia of *P. catenulatum* differ from those of *P. perniciosum* in being inflated and having a disjuncted moriform aspect.

The sexual stage of *Pythium catenulatum* is quite like that of *P. arrhenomanes*, both species possessing plerotic oospores and an abundance of functional antheridia. The antheridia of *P. catenulatum* are both monoclinous and diclinous whereas those of *P. arrhenomanes* are strictly diclinous.

The sexual stages of *Pythium perniciosum* and *P. catenulatum* cannot be confused. As stated by Matthews and observed by the writer, *P. perniciosum* possesses aplerotic oospores and one, rarely two, antheridia; *P. catenulatum* possesses plerotic oospores and one to twelve, usually five to six, antheridia. There are no unique or distinguishing macroscopic features evident when *P. catenulatum* is cultivated on sundry nutrient agar substrates, growth being quite like that of *P. debaryanum*, showing copious, loose, aerial, mycelial wefts which are haphazardly disposed.

Pythium catenulatum was originally isolated from water containing plant debris in N. C., U. S. A.; the writer has isolated this species from plant debris collected from the margin of a lake in Mo., U. S. A.

14. PYTHIUM TORULOSUM Coker and Patterson, Jour. Elisha Mitchell Soc. **42**: 247–250. 1927.

Hyphae measuring 2.5 to 4 μ in diameter, irregularly branched. Sporangia consisting of few to numerous irregularly branched, inflated toruloid members, forming simple or complex sporangial complexes; zoospores few to 20, 7 to 8 μ in diameter when encysted, reniform and laterally biciliate. Oogonia spherical to subspherical, terminal either on long filaments or short lateral branches, intercalary, measuring 12 to 19 μ, mostly 17 to 18 μ in diameter, smooth- and thin-walled. Antheridia of monoclinous origin, usually from the oogonial stalk, occasionally from the parent filament, usually 1, sometimes 2, per oogonium, borne on a short stalk, rarely branched, antheridial cell not greatly inflated, allantoid-clavate, the apex contacting the oogonial wall. Oospores plerotic, single, with a moderately thin wall.

Isolated from a water culture, *Teleranea nematodes* (Gottsche) Howe and *Thuidium delicatulum* (L.) Mitt., Chapel Hill, N. C., U. S. A.

Though this species is described from Bryophyta, it has been isolated from Phanerogams and proven pathogenic by van Luijk (1934a), Vanterpool (1938), the writer and possibly others.

Various isolates of the fungus exhibit some peculiarities, particularly in the growth habit on agar substrates. The isolates from wheat roots procured

through Vanterpool are typical of the species, although the antheridium usually arises some distance, 25 μ, below the oogonium on the oogonial stalk, whereas Coker and Patterson figure the antheridium arising in close proximity, 5 to 10 μ, to the oogonium. Such a difference cannot be deemed sufficient to separate Vanterpool's strain from the type species. Van Luijk's strain from grass roots conforms more closely to the delineations of the species than Vanterpool's in that the antheridium arises in close proximity to the oogonium on the oogonial stalk. A culture from barley isolated by the writer is typical of the species and agrees with van Luijk's fungus. A fungus described by Petri (1930) as being a member of the *Pythium gracile* group is in all probability *P. torulosum;* this has also been suggested by Vanterpool (1938).

There are certain macroscopic differences in the mycelial habit of isolates of this fungus. Vanterpool reports that his strain produces a vigorous mycelial growth with a tendency toward aerial development. Van Luijk's strain grows less vigorously and exhibits a very definite rosette habit on solid substrates with only meagre aerial development. The writer's strain shows a definite tendency toward a radiate habit and only a slight tendency toward rosetting. Vanterpool separates his strain from van Luijk's on the basis of the growth habit and position of the antheridium, but retains both in the species. The writer's experience has shown that too much emphasis should not be placed on the exact nature of the growth habit of *Pythium* spp., that some variation is often observed in forms morphologically indistinguishable. This is particularly well exemplified in *P. vexans*.

The asexual stage of *Pythium torulosum* is more or less typical of all the species possessing lobulate sporangia. *P. torulosum* may be distinguished readily from *P. graminicolum*, to which it is closely allied, by the morphology of the antheridial cell. In *P. torulosum* there is usually only one antheridium, rarely two, which is only slightly inflated and is typically allantoid-clavate, usually sessile but sometimes borne on a short antheridial stalk. The antheridia of *P. graminicolum* are more numerous and are inflated, crook-necked, clavate, borne on a longer antheridial stalk, rarely sessile. There is also a difference in the size of the oogonia of the two species: 12.0 to 19.0 μ in diameter in *P. torulosum* compared with 16.5 to 28.6 μ in diameter according to Subramaniam (1928), often larger, 24 to 36 μ in diameter. *P. torulosum* is easily separated from other plerotic oospore species with inflated filamentous sporangia, *P. inflatum*, *P. arrhenomanes*, and *P. periilum*, in possessing monoclinous antheridia.

The temperature-growth response of *P. torulosum* may conveniently be used as an adjunct to specific identification.

Originally described from water, *Teleranea nematodes* (Gottsche) Howe and *Thuidium delicatulum* (L.) Mitt. by Coker and Patterson, Matthews (1931) later reports *Pythium torulosum* again from water and ". . . a collection of leafy liverworts and mosses . . . ," further stating that Coker and Patterson obtained this fungus from *Trichocolea tomentella* (Ehr.) Dum., all

from N. C., U. S. A.; the writer obtained an isolate of the fungus from rotted roots of *Hordeum vulgare* L. in Mo., U. S. A. Van Luijk (1934) records *P. torulosum* from *Agrostis stolonifera* L. and *Festuca duriuscula* L. from the Netherlands and Vanterpool (1938) from *Triticum aestivum* L. roots in England. The fungus reported by Petri (1930) from wheat in Italy may possibly be *P. torulosum*. Ten Houten (1939) reports what is believed to be this species from *Abies grandis* Lindl. and *Pinus sylvestris* L. in the Netherlands.

15. Pythium graminicolum Subramaniam. Agr. Res. Inst. Pusa (India). Bull. 177: 1–7. 1928.

Hyphae measuring 3 to 7 μ in diameter, irregularly branched, hyaline and granular. Sporangia inflated filamentous, simple or irregularly branched, usually forming irregular toruloid, digitate complexes, terminal or intercalary; zoospores 15 to 48 in number, 8 to 11 μ in diameter when encysted, reniform and laterally biciliate, capable of repeated emergence. Oogonia spherical, smooth and thin walled, terminal, intercalary, measuring 16.5 to 36.2 μ, mean 25.0 μ, in diameter. Antheridia are of monoclinous origin, borne on stalks of varied length, 1 to 6 in number, the antheridial cell clavate with flattened apex. Oospores plerotic, single, smooth- and moderately thick-walled, hyaline or somewhat brownish, 15.4 to 35.3 μ, mean 23.0 μ, in diameter; germination not observed.

Originally described from *Triticum aestivum* L., India.

Since Subramaniam presented the description of *Pythium graminicolum* considerable controversy has arisen as to the exact identity of certain root-infesting *Pythium* spp. possessing an inflated filamentous asexual stage. Several years following Subramaniam's presentation, Drechsler (1936) partially resolved the problem in a treatise on *P. graminicolum* and *P. arrhenomanes*. Prior to this, the two species were considered synonymous by some students of the genus.

Type cultures of *Pythium graminicolum* are not available. The writer was fortunate in receiving from Subramaniam a number of cultures isolated from sugarcane; a brief report concerning the occurrence of this organism on sugarcane has been published (Subramaniam 1936a). A study of the sugarcane isolates revealed them to be identical with the description of the species.

The antheridia are monoclinous either from the oogonial stalk or its parent filament and originate in moderate proximity to the oogonium, usually not over 60 μ below it. The number of antheridia varies from 1 to 6 per oogonium. Usually the antheridia originate on separate stalks, but occasionally the stalk may be branched, bearing two antheridial cells. In two instances sessile antheridia were observed; Subramaniam gives illustrations showing this condition in his original work on the species. The antheridial cell is usually curved, clavate and bluntly tipped, making apical contact with the oogonial wall. The antheridia, because of their origin, are not radially arranged about the oogonium; this is a distinguishing factor in the separation of *P. graminicolum* from *P. arrhenomanes*. Drechsler reports similar observations concerning the antheridia.

The oogonia of *Pythium graminicolum* are invariably acrogenous, spherical, smooth and thin-walled and range in size from 16.5 to 36.2 μ, mean 24.8 μ, in diameter. Oospores are plerotic, smooth and thick-walled, ranging in size from 15.4 to 35.3 μ, mean 23.7 μ, in diameter. For convenience, a comparison of data on the size of sexual reproductive bodies is tabulated below:

Author	*Host*	*Oogonia* (μ)	*Oospores* (μ)
Subramaniam (1928)	Wheat	16.5–28.6	15.4–26.4
Subramaniam (1936)	Sugarcane	16.9–33.0 av. 25.0	16.3–30.8 av. 23.0
Rands and Dopp (1938)	Sugarcane	19.0–28.0 m. 23.0	———
Matthews (1931)	Sarracenia	24.0–36.0 av. 30.0	21.6–32.0 av. 28.0
Writer	Sugarcane	16.5–36.2 m. 24.8	15.4–35.3 m. 23.7
Writer	Barley	18.7–29.3 m. 24.1	18.0–29.9 m. 23.2
Writer	Wheat	17.3–31.2 m. 25.1	16.1–30.0 m. 24.0
Writer	Corn	17.1–30.8 m. 24.9	18.8–29.8 m. 23.3

Only one isolate each of a series from a given host is presented from the writer's collection. Those listed are considered typical of the series. Occasionally in old cultures spherical bodies are observed which resemble oogonia. Under suitable environmental conditions these structures germinate by the production of a germ tube; production of zoospores has not been observed. The term conidia has been used for these structures. Inasmuch as they are found singly and are not deciduous, application of this term would seem unfortunate. The structures appear to be analogous to those found in *Pythium ultimum* in which species they have been demonstrated to be unfertilized oogonia apparently functioning as food reservoirs.

Carpenter (1921) first ascribed the binomial *Pythium butleri* to the Hawaiian sugarcane root parasite. Seven years later (1928b) he referred the fungus to *P. aphanidermatum* and then later (1934) identified the organism as *P. graminicolum*, considering *P. arrhenomanes* synonymous with *P. graminicolum*. Drechsler in 1936 assigned the binomial *P. arrhenomanes* to the sugarcane parasite, referring Carpenter's fungus to this species; Rands and Dopp (1938b) concur with him in this.

A study of four isolates of a Hawaiian sugarcane fungus received from Carpenter were identified by the writer as *Pythium graminicolum*. If the cultures received are typical of the sugarcane series, this fungus is incorrectly transferred to *Pythium arrhenomanes* and it should be maintained under the binomial *P. graminicolum*.

Following Drechsler's scheme (1936), with which the writer is in agreement, *Pythium graminicolum* may be distinguished from *P. arrhenomanes*, its closest ally, on the basis of antheridial habit and certain cultural peculiarities. In *P. graminicolum* the antheridia are monoclinous, arising in rather close proximity, within 60 μ, to the oogonium, antheridial cells few,

1 to 6, usually 2 to 4, and irregularly arranged about the oogonium. In *P. arrhenomanes*, on the other hand, the antheridia are diclinous and have no discernible connection with the oogonial stalk, the antheridial cells numerous, 1 to 25, usually in excess of 8, and fairly regularly arranged about the oogonium. *P. graminicolum* usually fruits in culture in moderate abundance on ordinary culture media, the antheridia and oogonia retaining their identity many weeks after fecundation has occurred; *P. arrhenomanes* fruits sparingly on ordinary culture media, the antheridia and oogonia losing their identity soon after fecundation has taken place.

Macroscopically and in their temperature-growth relations, *P. graminicolum* and *P. arrhenomanes* are indistinguishable.

Ho, Meredith, and Melhus (1941) have preferred to use the binomial *Pythium graminicola* instead of the binomial *P. graminicolum* originally established by Subramaniam. Subramaniam apparently took the liberty of turning the indeclinable noun, graminicola, into an adjective agreeing with the genus name, and since this is the original usage, it is the form which the writer retains.

Hosts for *Pythium graminicolum* are cited in the following table:

PLANTS REPORTED BY VARIOUS INVESTIGATORS AS SUSCEPTIBLE TO PYTHIUM GRAMINICOLUM

Agropyron cristatum (L.) Gaertn. UNITED STATES: Buchholtz (1942b).
Ananas comosus Merr. HAWAII: Parris (1940).
Avena sativa L. INDIA: Subramaniam (1936). UNITED STATES: Ho and Melhus (1938); Author.
Curcuma longa L. CEYLON: Anonymous (1936a).
Gossypium sp. ANGLO-EGYPTIAN SUDAN: Massey (1936).
Hordeum vulgare L. INDIA: Subramaniam (1936). UNITED STATES: Ho and Melhus (1938); Ho, Meredith, Melhus (1941); Author.
Oryza sativa L. HAWAII: Parris (1940).
Panicum miliaceum L. UNITED STATES: Ho and Melhus (1938).
Phleum pratense L. UNITED STATES: Ho and Melhus (1938).
Pisum arvense L. UNITED STATES: Weimer (1940).
Saccharum officinarum L. HAWAII: Carpenter (1934); Lyon (1936); Martin (1941); Martin and Carpenter (1935); Parris (1940). INDIA: Subramaniam (1936). MAURITIUS: Shepherd (1933). PHILIPPINE IS.: Ocfemia (1939). PUERTO RICO: Seaver *et al.* (1932). UNITED STATES: Rands (1930); Rands and Abbott (1939); Rands and Dopp (1934, 1938a, 1938b); Stevenson and Rands (1938).
Secale cereale L. UNITED STATES: Ho and Melhus (1938).
Setaria glauca Beauv. UNITED STATES: Ho, Meredith and Melhus (1941).
Soil. UNITED STATES: Matthews (1931); Meredith (1938).
Sorghum vulgare Pers. INDIA: Subramaniam (1936a). ITALY: Goidanich (1939). UNITED STATES: Ho, Meredith and Melhus (1941); Author.
S. vulgare var. *sudanense* (Piper) Hitchc. HAWAII: Lyon (1936).
Triticum aestivum L. INDIA: Subramaniam (1928, 1936a); Butler and Bisby (1931). CANADA: Vanterpool (1938); Vanterpool and Truscott (1932). ENGLAND: Vanterpool (1938). UNITED STATES: Ho and Melhus (1938, 1940); Ho, Meredith and Melhus (1941); Author.
Vicia sp. UNITED STATES: Weimer (1940).
Zea mays L. INDIA: Subramaniam (1936a). MALAYA: Milsum and Grist (1941). UNITED STATES: Buchanan (1938); Ho (1941); Ho and Koepper (1942); Ho and Melhus (1938, 1940); Melhus (1940); Melhus *et al.* (1939, 1940); Author.
Zingiber officinale Roscoe. CEYLON: Anonymous (1936a); Park (1935).

16. PYTHIUM INFLATUM Matthews, Studies on the Genus Pythium. 1931.

Hyphae well developed on corn meal agar, narrow, measuring 1 to 4 μ in diameter. Sporangia irregular to spherical toruloid outgrowths which measure 8 to 20 μ in diameter; zoospores unknown. Oogonia rarely found, spherical and smooth, acrogenous or intercalary, measuring 18 to 26 μ in diameter. Antheridia of diclinous origin, 1 to 2 per oogonium. Oospores plerotic, single, with a thick wall, containing a reserve globule and a refringent body embedded in the granular protoplasm.

Isolated from a decaying seedling in a culture dish of *Vaucheria*, Chapel Hill, N. C., U. S. A.

Because material of this fungus was unavailable to the writer, discussion of the species must be confined to the diagnosis presented by Matthews and the remarks made in the original article by her.

Morphologically, *Pythium inflatum* exhibits affinities to *P. torulosum*, *P. graminicolum*, *P. arrhenomanes*, and *P. periilum*. Matthews states, "This plant is similar to *P. monospermum* but differs from it in the larger toruloid outgrowths on the mycelium." Since the sporangia of *P. inflatum* and *P. monospermum* are not comparable, it seems advisable to compare *P. inflatum* with the more closely related forms. The antheridia of *P. torulosum* and *P. graminicolum* are monoclinous, origin not proximal to the oogonium. Matthews separates *P. inflatum* from *P. graminicolum* in noting ". . . the smaller hyphae, the absence of large spherical conidia, slowness of growth, absence of zoospores, rareness of oogonia and the type and number of of antheridia." The antheridial character is sufficient for specific segregation in this case. *P. arrhenomanes* and *P. periilum*, like *P. inflatum*, have diclinous antheridia. The antheridial cells arise from separate branches in both *P. arrhenomanes* and *P. inflatum*. Whereas in *P. arrhenomanes* the antheridial branches are frequently divided and the antheridial cells are many, up to 25 per oogonium, in *P. inflatum* the antheridial branches are simple and the antheridial cells few, one to two per oogonium. *P. periilum*, though possessing diclinous antheridia, is distinctly different from *P. inflatum* in having the antheridial cells arising from a single antheridial branch.

The oogonia and oospores are similar in *Pythium inflatum*, *P. torulosum*, *P. graminicolum*, *P. arrhenomanes*, and *P. periilum*.

Pythium inflatum is not known from any substrate other than the vegetable debris from which it was originally described.

17. PYTHIUM ARRHENOMANES Drechsler, Phytopathology **18**: 873–875. 1928.

Nematosporangium arrhenomanes (Drechs.) Sideris, Mycologia **23**: 252–295. 1931.
N. arrhenomanes var. *hawaiianensis* Sideris l.c.
N. epiphanosporon Sideris l.c.
N. hyphalosticton Sideris l.c.
N. leiohyphon Sideris l.c.
N. leucosticton Sideris l.c.
N. polyandron Sideris l.c.
N. rhizophthoron Sideris l.c.

N. spaniogamon Sideris l.c.
N. thysanohyphalon Sideris l.c.
Pythium arrhenomanes var. *canadensis* Vanterpool & Truscott, Canad. Jour. Res. **6**: 68–93. 1932.
Pythium arrhenomanes var. *philippinensis* Roldan, Philipp. Agr. **21**: 165–176. 1932.

Mycelium intra- and intercellular, hyphae measuring 2.0 to 5.5 μ in diameter. Sporangia inflated, filamentous, lobulate, forming complexes of elements up to 20 μ or more in diameter, zoospores 20 to 50 or more in a vesicle borne on an evacuation tube usually 3 to 4 μ in diameter and of variable length, up to 75 μ, biciliate, measuring approximately 12 μ in diameter upon encystment. Oogonia subspherical to spherical, acrogenous, occasionally intercalary, smooth- and thin-walled, measuring 17.3 to 56.3 μ, mean 30.1 μ, in diameter. Antheridia of diclinous origin, numerous, 15 to 25 often visible, usually borne terminally though occasionally laterally on 4 to 8 branches each usually contributing up to 4 antheridia, antheridial cells are crook-necked, 6 to 9 μ wide and 12 to 25 μ long, making narrow contact with the oogonium. Oospores plerotic, 15.5 to 54.2 μ, mean 28.2 μ, in diameter, wall 1.2 to 2.0 μ, usually 1.6 μ thick, containing a single reserve globule.
Originally described from *Zea mays* L. roots in Wisconsin, U. S. A.

The diagnosis given concerns the identity of the *Pythium* sp. that Johann, Holbert and Dickson (1926, 1928) report as the cause of a seedling disease of dent corn.

Rands and Dopp (1934) have published the most extensive study of *Pythium arrhenomanes;* a subsequent paper by these authors (1938b) deals with this and associated species.

Sideris (1931) in his study of *Nematosporangium* spp. (*Pythium* spp.) lists and described eight new species and three new varieties. Rands and Dopp (1934) have since combined these organisms in the binomial *P. arrhenomanes*. A study of Sideris' fungi and a series of isolates of *P. arrhenomanes*, including the varieties *P. arrhenomanes canadensis*, *P. arrhenomanes hawaiiensis* and *P. arrhenomanes philippinensis* was undertaken by the writer.

The various isolates of *Pythium arrhenomanes*, including those of Sideris, were observed on a series of nutrient substrates. The morphology of the organisms was noted in each case and measurements of the oogonia made. Oospore measurements were not taken because the oospores are plerotic, less abundant than oogonia and frequently aborted. Oogonial measurements of representatives of each series are presented:

Organism	*Host*	*Range* (μ)	*Mean* (μ)
P. arrhenomanes	Sugarcane	28.6–37.3	33.1
Do.	Do.	17.3–29.1	23.5
Do.	Do.	28.0–43.6	34.7
Do.	Do.	26.3–44.2	33.2
Do.	Do.	19.0–31.0	24.2
Do.	Do.	32.1–56.3	42.0
Do.	Corn	25.1–36.8	30.1
Do.	Do.	31.0–50.1	39.0
Do.	Do.	18.3–28.7	23.1
P. arrhenomanes canadensis	Wheat	27.7–44.6	34.9

Organism	*Host*	*Range* (μ)	*Mean* (μ)
P. arrhenomanes hawaiianensis	Pineapple	23.0–37.4	30.3
P. arrhenomanes philippinensis	Corn	22.1–39.4	30.1
P. spaniogamon	Pineapple	23.0–34.6	30.2
P. hyphalosticton	Do.	30.0–46.3	36.1
P. polyandron	Do.	26.5–47.0	38.4
P. thysanohyphalon	Do.	27.1–48.1	35.8
P. rhizophthoron	Do.	26.3–38.0	30.8
P. leucosticton	Bilbergia	27.8–49.0	36.4
P. leiohyphon	Pineapple	29.1–52.0	37.5
P. epiphanosporon	Do.	23.8–48.3	34.3

The antheridia of each isolate studied were identical in that the antheridial stalks were diclinous with no demonstrable connection with the oogonial stalk. Each antheridial stalk bore one, occasionally two, narrow, clavate and crook-necked antheridial cell which in the aggregate varies in number from one to twenty-five; these were apically applied to the oogonial wall and were more or less radiately arranged about the oogonium, with an approximately equal interval between cells.

Though individual differences may be noted in the oogonial size of certain isolates, there exist a sufficient number of intermediate or overlapping groups to integrate the series into a single natural unit. The uniform antheridial character, despite the variation in numbers, is additional evidence that the organisms listed above represent a single, natural group. Those listed as other than *Pythium arrhenomanes* must be considered synonymous with *P. arrhenomanes;* their similarity in growth habit and temperature-growth relations is presented to further attest to their synonymity.

The observations of the writer corroborate the investigation of Rands and Dopp.

Pythium arrhenomanes differs from *P. inflatum* in possessing antheridial branches which are frequently divided and in having many antheridial cells, up to twenty-five, usually more than ten, per oogonium. The antheridial stalks are simple and the antheridial cells few, one to two per oogonium, in *P. inflatum*. *P. periilum* is readily separated from *P. arrhenomanes* in having the two to five antheridial cells originating from a single antheridial stalk that is characteristically adnate to the oogonium. The distinction between *P. arrhenomanes* and *P. graminicolum* is discussed under *P. graminicolum*.

Pythium arrhenomanes is reported from the hosts listed below:

Aegilops triuncialis L. United States: Fischer *et al.* (1942); Vanterpool and Sprague (1942).

Agropyron amurense Drob. United States: Fischer *et al.* (1942) Vanterpool and Sprague (1942).

A. caninum (L.) Beauv. United States: Vanterpool and Sprague (1942).

A. ciliare (Trin.) Franch. United States: Fischer *et al.* (1942); Vanterpool and Sprague (1942).

A. cristatum (L.) Gaertn. Canada: Bisby *et al.* (1938); Conners (1935); Vanterpool (1940b). United States: Fischer *et al.* (1942); Sprague and Atkinson (1942); Vanterpool and Sprague (1942).

A. dasystachyum (Fisch.) Scribn. United States: Fischer *et al.* (1942); Vanterpool and Sprague (1942).

A. desertorum (Fisch.) Schult. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
A. elongatum (Host) Beauv. UNITED STATES: Fischer *et al.* (1942).
A. inerme (Scribn. and Sm.) Rydb. UNITED STATES: Sprague.
A. intermedium (Host) Beauv. UNITED STATES: Fischer *et al.* (1942): Vanterpool and Sprague (1942).
A. michnoi Roshev. UNITED STATES: Sprague.
A. pauciflorum Hitchc. CANADA: Vanterpool (1940b).
A. pungens (Pers.) Roem. and Schult. UNITED STATES: Vanterpool and Sprague (1942).
A. repens (L.) Beauv. CANADA: Bisby *et al.* (1938); Conners (1935); Vanterpool (1940b). UNITED STATES: Fischer *et al.* (1942); Sprague and Atkinson (1942); Vanterpool and Sprague (1942).
A. riparium Scribn. and Sm. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
A. sibiricum (Willd.) Beauv. UNITED STATES: Fischer *et al.* (1942).
A. smithii Rydb. CANADA: Vanterpool. UNITED STATES: Sprague.
A. spicatum (Pursh) Scribn. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
A. tenerum Vasey. CANADA: Bisby *et al.* (1938); Conners (1935).
A. trachycaulum (Link) Malte. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
A. trichophorum (Link) Richt. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
A. ugamicum Drobov. UNITED STATES: Sprague.
Ammophila arenaria (L.) Link. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
Ananas comosus Merr. HAWAII: Johnson (1935); Parris (1940); Sideris (1931).
Andropogon furcatus Muhl. UNITED STATES: Fischer *et al.* (1942).
Arrhenatherum elatius (L.) Mert. and Koch. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
Avena fatua L. CANADA: Bisby *et al.* (1938); Conners (1935); Vanterpool (1940b). UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
A. sativa L. CANADA: Bisby *et al.* (1938); Conners (1935); Vanterpool and Truscott (1932). UNITED STATES: Sprague and Atkinson (1942); Vanterpool and Sprague (1942); Author.
Bilbergia sp. HAWAII: Parris (1940); Sideris (1931).
Bouteloua curtipendula (Michx.) Torr. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
B. gracilis (H.B.K.) Lag. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
Brachypodium sylvaticum (Huds.) Beauv. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
Bromus carinatus Hook and Arn. UNITED STATES: Vanterpool and Sprague (1942).
B. erectus Huds. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
B. inermis Leyss. CANADA: Bisby *et al.* (1938); Conners (1935); Vanterpool (1940b). UNITED STATES: Fischer *et al.* (1942); Sprague and Atkinson (1942); Vanterpool and Sprague (1942).
B. marginatus Nees. UNITED STATES: Fischer *et al.* (1942).
Cajanus cajan Millsp. HAWAII: Parris (1940); Sideris (1931).
Chaetochloa viridis Scribn. CANADA: Conners (1935).
Commelina nudiflora L. HAWAII: Parris (1940); Sideris (1931).
Echinochloa crus-galli (L.) Beauv. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
Elymus canadensis L. UNITED STATES: Sprague.
E. condensatus Presl. UNITED STATES: Sprague.
E. giganteus Vahl. UNITED STATES: Sprague.
E. glaucus Buckl. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
E. interruptus Buckl. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
E. junceus Fisch. UNITED STATES: Fischer *et al.* (1942); Sprague and Atkinson (1942); Vanterpool and Sprague (1942).

E. macounii Vasey. UNITED STATES: Fischer *et al.* (1942).
E. sibiricus L. UNITED STATES: Sprague.
Festuca elatior L. UNITED STATES: Fischer *et al.* (1942).
F. elatior var. *arundinacea* (Screb.) Wimm. UNITED STATES: Fischer *et al.* (1942).
F. octoflora Walt. UNITED STATES: Sprague.
F. rubra L. var. *commutata* Gaud. UNITED STATES: Vanterpool and Sprague (1942).
Hordeum distichon L. UNITED STATES: Sprague.
H. jubatum L. UNITED STATES: Fischer *et al.* (1942).
H. vulgare L. CANADA: Bisby *et al.* (1938); Vanterpool and Truscott (1932). UNITED STATES: Vanterpool and Sprague (1942); Wood and Nance (1938); Author.
Ipomoea batatas Lam. HAWAII: Parris (1940); Sideris (1931).
Muhlenbergia racemosa (Michx.) B.S.P. UNITED STATES: Fischer *et al.* (1942).
Musa cavendishii Lamb. HAWAII: Parris (1940); Sideris (1931).
Oryzopsis hymenoides (Roem. and Schult.) Rick. UNITED STATES: Fischer *et al.* (1942); Sprague and Atkinson (1942).
Panicum barbinode Trin. HAWAII: Sideris (1931).
P. capillare L. UNITED STATES: Sprague.
P. miliaceum L. UNITED STATES: Fischer *et al.* (1942); Sprague and Atkinson (1942); Vanterpool and Sprague (1942).
P. subvillosum Ashe. UNITED STATES: Sprague.
P. virgatum L. UNITED STATES: Sprague and Atkinson (1942).
Phalaris arundinacea L. CANADA: Bisby *et al.* (1938); Conners (1935); Vanterpool (1940b).
Phaseolus aureus Roxb. HAWAII: Parris (1940); Sideris (1931).
Phleum pratense L. CANADA: Bisby *et al.* (1938); Conners (1935); Vanterpool (1940b). UNITED STATES: Sprague and Atkinson (1942).
Saccharum barberi Jesweit. UNITED STATES: Rands and Dopp (1938b).
S. officinarum L. HAWAII: Martin (1942); Parris (1940); Sideris (1931); Stevenson and Rands (1938). MAURITIUS: Stevenson and Rands (1938); Wiehe (1940a, b). PHILIPPINE ISLANDS: Roldan (1932). UNITED STATES: Brandes, Dowell and Baker (1936); Edson and Wood (1936); Flor (1930a, 1930b); Rands (1930); Rands and Abbott (1937, 1939); Rands, Abbott and Summers (1936); Rands and Dopp (1934, 1938a, 1938b); Stevenson and Rands (1938); Tims (1932b); Wood and Nance (1938).
S. robustum Jesweit. UNITED STATES: Rands and Dopp (1938b).
S. sinense Roxb. UNITED STATES: Rands and Dopp (1938b).
S. spontaneum L. UNITED STATES: Rands and Dopp (1938b).
Schedonnardus paniculatus (Nutt.) Trel. UNITED STATES: Sprague.
Secale cereale L. CANADA: Bisby *et al.* (1938); Vanterpool and Truscott (1932). UNITED STATES: Sprague and Atkinson (1942); Vanterpool and Sprague (1942); Author.
Setaria italica (L.) Beauv. UNITED STATES: Fischer *et al.* (1942); Sprague and Atkinson (1942); Vanterpool and Sprague (1942).
S. lutescens (Weigel) F. T. Hubb. UNITED STATES: Sprague.
S. viridis (L.) Beauv. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942). CANADA: Bisby *et al.* (1938); Vanterpool (1940b).
Solanum tuberosum L. HAWAII: Parris (1940); Sideris (1931).
Sorghastrum nutans (L.) Nash. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
Sorghum sp. UNITED STATES: Altstatt (1942); Brown (1940). CANADA: Vanterpool (1940).
S. vulgare Pers. ITALY: Goidanich (1939). UNITED STATES: Bowman *et al.* (1937); Brown (1940); Cushing *et al.* (1940); Edson and Wood (1937); Elliott *et al.* (1936, 1937); Ezekiel (1938); Fischer *et al.* (1942); Jackson (1938); Johnston (1942); Jones and Gaines (1938); Kendrick and Briggs (1939); Melchers (1940); Middleton (1938); Myers (1934); Nance (1939, 1940); Sprague and Atkinson (1942); Vanterpool and Sprague (1942); Wagner (1936).
S. vulgare var. *sudanense* (Piper) Hitchc. UNITED STATES: Fischer *et al.* (1942); Sprague and Atkinson (1942); Vanterpool and Sprague (1942); Author.
Sphenopholis obtusata (Michx.) Scribn. UNITED STATES: Sprague.
Stipa comata Trin. UNITED STATES: Fischer *et al.* (1942); Vanterpool and Sprague (1942).
S. spartea Trin. UNITED STATES: Fischer *et al.* (1942).

S. viridula Trin. UNITED STATES: Sprague and Atkinson (1942).
Triticum aestivum L. CANADA: Bisby *et al.* (1938); Conners (1935); Garrett (1934); Graham and Greenberg (1939); Simmonds (1939a); Vanterpool and Truscott (1932). ENGLAND: Vanterpool (1938). HAWAII: Parris (1940); Sideris (1931). UNITED STATES: Maneval (1940); Sprague and Atkinson (1942); Vanterpool and Sprague (1942); Author.
T. dicoccum Schrank. UNITED STATES: Sprague and Atkinson (1942); Vanterpool and Sprague (1942).
T. durum Dest. UNITED STATES: Sprague and Atkinson (1942); Vanterpool and Sprague (1942).
Uniola latifolia Mich. UNITED STATES: Author.
Vicia faba L. HAWAII: Parris (1940); Sideris (1931).
Zea mays L. CANADA: Bisby *et al.* (1938); Vanterpool and Truscott (1932). HAWAII: Parris (1940); Sideris (1931). PHILIPPINE ISLANDS: Roldan (1932). UNITED STATES: Branstetter (1927); Drechsler (1928); Johann, Holbert and Dickson (1926, 1928); Koehler and Holbert (1930); Maneval (1937); Mumford (1930); Mumford and Shirkey (1937); Rands and Dopp (1934, 1938a, 1938b); Sprague and Atkinson (1942); Valleau, Karraker and Johnson (1926); Vanterpool and Sprague (1942); Author.

18. PYTHIUM PERILUM Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 1.5 to 6 μ, usually 3.5 to 6 μ, in diameter. Appressoria acrogenous, knob-like, often 7 μ wide and 7 to 10 μ long. Sporangia composed of both undifferentiated and inflated, dactyloid mycelial elements, measuring up to 12 μ in diameter, forming relatively simple aggregates; zoospores up to 75 in number formed in a vesicle borne on an evacuation tube up to 0.5 mm. in length and 3 to 4 μ wide, reniform, laterally bicilliate, 8 to 9 μ in diameter at encystment, capable of repeated emergence. Oogonia subspherical, smooth- and thin-walled, acrogenous or intercalary, the oogonium often including a portion of the supporting filament, up to 4 μ in length, oogonia measuring 16 to 22 μ, average 18.8 μ, in diameter. Antheridia diclinous, infrequently from the oogonial stalk some distance from the oogonium, varying from 2 to 5 in number, acrogenous or infrequently lateral, all on branching prolongations of a single hypha, usually including several similar vegetative prolongations wrapped about the oogonium and oogonial stalk, the antheridial cells crook-necked, 4 to 5 μ wide and 7 to 14 μ long, the bluntly rounded apex making narrow oogonial contact. Oospores plerotic, single, 14 to 20 μ, average 17.3 μ, in diameter, with a wall 1.1 to 1.8 μ, average 1.5 μ thick, containing a single reserve globule and an oblate ellipsoidal or subspherical refringent body.

Isolated from roots of *Saccharum officinarum* L., Louisiana, U. S. A.

A more complete understanding of the species may be afforded by a discussion of the interrelationships of this species and allied forms.

Drechsler recently (1940) elaborated upon the original diagnosis but did not present any remarks concerning the systematic position of the fungus. Stevenson and Rands (1938) present an abbreviated description of the fungus in their discussion of the fungi and bacteria associated with sugarcane and its products.

Observations made from a study of the type culture indicate that on the basis of its sporangial habit this species is intermediate between *Pythium monospermum* and *P. myriotylum*. In the former the sporangial elements appear as entirely undifferentiated vegetative elements, whereas in the latter the sporangia may consist of outwardly undifferentiated filaments, but more

often include swollen, dactyloid elements which may or may not be arranged in complex units. *P. periilum* differs considerably however, from *P. monospermum* and *P. myriotylum* in its sexual phase. A plerotic oospore is common to both *P. periilum* and *P. monospermum*, while *P. myriotylum* possesses aplerotic oospores. The antheridial cells of *P. monospermum* arise from separate antheridial branches while those of *P. periilum* arise from a single antheridial branch. The antheridial habit of *P. periilum* resembles that of *P. adhaerens*, previously discussed. In *P. adhaerens* the antheridial branch is not closely associated with the oogonium, always remaining distinctly discernible and lacking vegetative prolongations. The antheridial stalk of *P. periilum* is usually intimately associated with the oogonium, applying itself to the oogonial wall and frequently encircling the oogonium. Considerable difficulty is often encountered in tracing the origin of the individual antheridial cells because of the application of the parent filament and the frequent presence of vegetative prolongations comparable to the individual antheridial stalks but lacking the acrogenous antheridium.

The antheridial habit of *Pythium periilum* is sufficient to distinguish it from its congeners.

Pythium periilum has been reported from roots of sugarcane, *Saccharum officinarum* L., by Drechsler (1930a, 1940), Rands (1930), Rands and Dopp (1938b), Stevenson and Rands (1938), and from *Agropyron repens* (L.) Beauv. by Fischer *et al.* (1942) in the United States.

19. Pythium indigoferae Butler, Mem. Dept. Agr. India Bot. 1[5]: 1–160. 1907.

Nematosporangium indigoferae (Butler) Sideris, Mycologia **23**: 252–295. 1931.

Hyphae very irregular in size, measuring up to 8 μ in diameter, frequently forming large lateral, spherical to cylindrical outgrowths up to 12 μ in diameter, similar to sporangia. Sporangia inflated filamentous, usually sparingly formed, composed of swollen digitate simple or branched elements which may form simple or complex aggregations; zoospores 4 to 20. Oogonia spherical, acrogenous on short lateral branches or borne laterally or tangentially in the sporangial complexes, measuring 18 to 20 μ in diameter. Antheridia monoclinous, occasionally diclinous, single, usually arising in close proximity to the oogonium, antheridial cell more or less allantoid, clavate, the antheridial stalk and cell nearly straight, the oogonial stalk and oogonium strongly curved toward the antheridium. Oospore aplerotic, single moderately thick-walled, oospores measuring 13 to 18 μ in diameter; germination prompt by the production of a hypha.

Epiphytic on leaves of *Indigofera arrecta* Hochst., Calcutta, India.

The diagnosis given for *Pythium indigoferae* is taken from the original work of Butler (1907). This fungus has a peculiar habitat, namely in the waxy covering of leaves of *Indigofera arrecta* Hochst. It is decidedly uncommon to find a *Pythium* species that may be considered a true epiphyte. A culture of this fungus was obtained from Baarn for study. The organism cor-

responds in all details with the description of the species and may be considered a type. Butler states that *P. indigoferae* ". . . is related to *P. gracile* in the structure of the sexual organs, but differs in the origin of the antheridium from the oogonial stalk." It differs further in possessing sporangia which are lobulate, usually occurring in the simple to complex aggregations of toruloid, dactyloid elements. The sexual apparatus is a distinctive feature of the species. In contrast to the majority of *Pythium* spp. in which the oogonial stalk is straight and the antheridium is curved toward it, the oogonial stalk of *P. indigoferae* is conspicuously curved toward the antheridium which consists of a straight branch. Additional distinction lies in the shape of the antheridial cell which is filamentous and only rarely somewhat inflated at its apex. There is only a delicate union between the apex of the antheridium and oogonial wall, the remaining portion of the antheridium being free. The antheridia of *P. indigoferae* are very similar to those of *P. torulosum* and further similarity with *P. torulosum* is evidenced in the asexual stage. *P. indigoferae* differs from *P. torulosum* in its oogonial disposition and aplerotic oospores.

As stated by Butler, the size of the oogonia in *Pythium indigoferae* is remarkably constant. Butler lists the oogonia as being 18 to 20 μ in diameter. Measurement of 200 oogonia made by the writer gave a range of 16.3 to 22.4 μ in diameter and a mean diameter of 19.2 μ. Oospore measurements agree with those reported by Butler.

Pythium deliense is the only other species of *Pythium* in which the oogonium is directed toward the antheridium. *P. deliense* may be distinguished morphologically from *P. indigoferae* by the absence of sexual organs in conjunction with sporangial complexes. The difference in the orientation of the sporangial emission tube of *P. deliense* and *P. indigoferae* as pointed out by Meurs (1934) is not a satisfactory criterion for specific identification. The emission tube of *P. indigoferae* is usually laterally directed, though occasional instances of terminal disposition have been observed. The writer concurs with the opinion of Meurs that the emission tube in *P. deliense* is usually terminal and rarely lateral; however, the use of variable morphologic features as criteria for the separation of closely related species should be avoided.

Both *Pythium indigoferae* and *P. deliense* exhibit a rosette habit of growth on solid nutrient media; the rate of growth at room temperature is also similar. A comparison of the respective optima and maxima temperatures for growth, however, reveals that they are very dissimilar; the maximum temperature for mycelial growth of *P. indigoferae* is 37° C. while that of *P. deliense* is 43° C. The temperature-growth relation of the two fungi constitutes a valuable factor in specific segregation.

Pythium indigoferae is listed from leaves of *Indigofera arrecta* Hochst. in India by Butler (1907), Butler and Bisby (1931) and Sydow and Butler (1907), from *Ananas comosus* Merr. by Sideris (1931) and according to Sideris obtained from roots of *Cucumis sativus* L. by McRae in India.

20. PYTHIUM DELIENSE Meurs, Phytopath. Zeit. 7: 169–185. 1934.

Hyphae abundantly and irregularly branched, measuring from 2.6 to 8.6 μ in diameter. Sporangia terminal, rarely intercalary, inflated filamentous, up to 210 μ long and of variable diameter but always larger than parent hypha, usually provided with inflated lateral branches, usually not forming intricate complexes; zoospores 3 to 25 or more, reniform, laterally biciliate, measuring 8 to 12 μ in diameter when encysted; the evacuation tube of variable length, usually of terminal origin though occasionally lateral. Oogonia spherical, terminal, smooth- and thin-walled, measuring 15 to 23.1 μ, mostly 16.1 to 20.0 μ, av. 18.2 μ, in diameter. Antheridia typically monoclinous, occasionally diclinous, usually single, rarely two per oogonium, the antheridial stalk usually straight, the oogonial branch and oogonium bent toward the antheridial cell, infrequently the two stalks are parallel branches of the same hypha, the antheridial cell is filamentous-clavate, terminal or intercalary, 12.8 to 27.0 μ, mostly 14.1 to 20.3 μ, long and 4.7 to 15.5 μ, mostly 8.1 to 11.4 μ, wide making apical contact with the oogonial wall. Oospores aplerotic, provided with a wall 0.9 to 2.2 μ, mostly 1.3 to 1.8 μ, average 1.62 μ thick, containing a single reserve globule and refringent body, oospore measuring 12.5 to 17.5 μ, mostly 13.7 to 16.2 μ, average 14.8 μ, in diameter.

Originally described as one of the causal agents of stemburn of *Nicotiana tabacum* L., Sumatra, N. E. I.

A study of the type culture was undertaken. As the work progressed, it became increasingly apparent that *Pythium deliense* and *P. indigoferae* are closely related species. The oogonia and oospores of *P. deliense* vary in size more than is indicated in Meurs' diagnosis: oogonia range from 12.1 to 31.3 μ, mean 18.9 μ, in diameter; oospores range from 10.2 to 23.2 μ, mean 15.3 μ, in diameter. The antheridia are appropriately described in the diagnosis.

No sexual organs were formed in connection with the sporangial complexes, substantiating the observation of Meurs.

As indicated in the discussion of *Pythium indigoferae*, *P. deliense* exhibits a sexual stage which is common to both species, namely the bending of the oogonial stalk and appended oogonium toward the antheridium. The antheridial stalk remains more or less straight; this phenomenon is in sharp contrast to the behavior of the other *Pythium* spp. in which the converse is the rule.

The emission tube in *Pythium deliense* is reported to arise most frequently in a terminal position, rarely laterally. The emission tube in *P. indigoferae* is, according to Meurs, laterally oriented. The writer has occasionally observed the emission tube to be acrogenous in *P. indigoferae*. As stated for *P. indigoferae*, it seems best to avoid the use of variable characteristics in the separation of closely allied species; contrary to Meurs' opinion, it is not necessary to rely upon this characteristic for specific identification.

Pythium deliense is also closely allied to *Pythium aphanidermatum*. The oogonia and oospores are very similar in size and shape and in instances where the oogonial stalk and oogonium are not directed toward the straight

antheridial stalk in *P. deliense*, as instances of parallel branches or diclinous antheridia, some difficulty in separation might be incurred were it not for differences in the character of the antheridial cells of the two species. Intercalary antheridia are common to both species but usually more frequent in *P. aphanidermatum* than in *P. deliense*. In *P. aphanidermatum*, these are usually formed in a parent filament rather than on a monoclinous lateral branch derived from the oogonial stalk; in any event the antheridial cell in *P. aphanidermatum* is typically broad and stout, barrel-shaped or suborbicular and usually sessile. Antheridial cells of *P. deliense* are much less broad and stout, are more slender and clavate, are not produced in the same manner as those of *P. aphanidermatum* and are typically stalked. These two species are further distinguished by their asexual reproductive structures, simple and sparingly branched and elongate in *P. deliense*, complex, much branched and forming lobulate aggregations in *P. aphanidermatum*.

Utilization of the temperature relations and the absence of any sexual apparatus in association with the asexual structures will suffice as a basis for segregating *Pythium deliense* from *P. indigoferae*. The oogonial and antheridial habit of *P. deliense* makes it impossible to confuse the species with other aplerotic forms such as *P. aphanidermatum*, *P. tardicrescens*, *P. volutum*, *P. aristosporum*, and *P. myriotylum*.

Pythium deliense, together with *P. aphanidermatum* and *P. myriotylum*, is one of the causal agents of tobacco stemburn, occurring in Sumatra. Heretofore the tobacco pathogens had been referred to as *Pythium* spp. or *Pythium debaryanum* by Jochems (1926, 1927a, b). In addition to the original description by Meurs, *P. deliense* has been reported from tobacco in Sumatra by Jochems (1934) and van der Weij (1935). Meurs believes that the fungus discussed by van Breda de Haan (1896) as *Phytophthora nicotianae* and subsequently believed to be derived from a mixed culture, contained as a component part the present described *Pythium deliense*.

21. Pythium aphanidermatum (Edson) Fitzpatrick, Jour. Agr. Res. 4: 279–292. 1915.

Nematosporangium aphanidermatum (Edson) Fitzp. Mycologia **15**: 166–173. 1923.
N. aphanidermatum (Edson) Sideris, Mycologia **23**: 252–295. 1931.
N. aphanidermatum var. *hawaiiensis* Sideris, l.c.
Pythium butleri Subramaniam, Mem. Dept. Agr. India Bot. **10**: 181–194. 1919.
Rheosporangium aphanidermatus Edson, Jour. Agr. Res. **4**: 279–292. 1915; Mycologia **15**: 166–173. 1923.

Hyphae measuring 2.8 to 7.3 μ, largely 4 to 6 μ, in diameter, hyaline and nonseptate except in fructification. Sporangia (presporangia according to Edson) inflated filamentous, branched or unbranched, of varying length from less than 50 μ to more than 1000 μ and 4 to 20 μ wide, usually forming complexes; zoospores reniform, laterally biciliate, measuring about 12 μ long and 7.5 μ wide. Oogonia spherical, terminal, rarely intercalary, measuring 22 to 27 μ in diameter. Antheridia usually monoclinous but also diclinous, typically intercalary though often terminal, 1 to 2 per oogonium, barrel- or

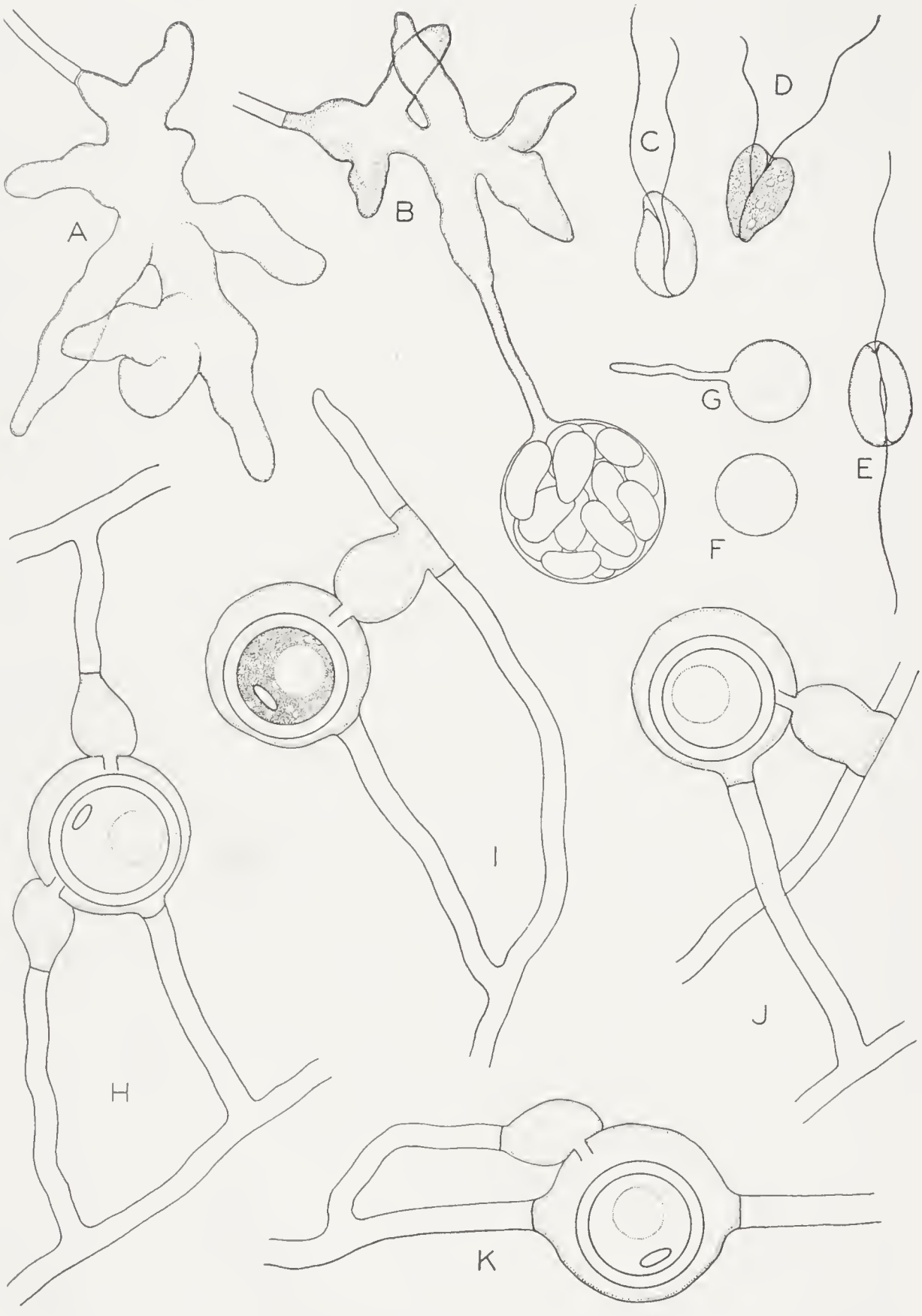

FIG. 3. *Pythium aphanidermatum*. *A–G*, Asexual stages. *A*, Inflated filamentous sporangium. *B*, Sporangium with vesicle containing zoospores. *C–E*, Zoospores. *F*, Encysted zoospore. *G*, Encysted zoospore producing germ tube. *H–K*, Sexual stages. *H*, *K*, Oogonia with terminal antheridia. *I*, *J*, Oogonia with intercalary antheridia.

dome-shaped, suborbicular, becoming cylindric or broadly clavate, usually measuring 9 to 11 μ by 10 to 14 μ, producing a conspicuous penetration tube. Oospores aplerotic, single, with a moderately thick wall, measuring 17 to 19 μ in diameter; germination by the production of a germ tube.

Originally described as pathogenic on *Beta vulgaris* L., U. S. A.

The diagnosis given above is taken in large part from Edson's paper in which he describes *Rheosporangium aphanidermatus* as a new genus and species of the Saprolegniaceae.

Carpenter (1921) working with pure cultures of the sugarcane *Pythium*, noted that his fungus agreed very closely in both the asexual and the sexual stages with the sugar beet root rot fungus *Rheosporangium aphanidermatus*. Carpenter states that ". . . it seems more natural to include this fungus (*R. aphanidermatus*) under the genus Pythium of the Pythiaceae. . . ." He also noted a rather close agreement of his fungus with *P. butleri*, considered a new species by Subramaniam (1919). In summarizing his work Carpenter says that he ". . . considers the cane fungus morphologically the same as *R. aphanidermatus* and *P. butleri*."

Coker (1923) in his monograph of the Saprolegniaceae concludes that there is no apparent reason for retention of the genus *Rheosporangium* in the Saprolegniaceae and that it is best placed in the genus *Pythium* in the Pythiaceae.

It was not until Fitzpatrick (1923) published his comments on generic concepts in the Pythiaceae that *Rheosporangium aphanidermatus* was transferred to the genus *Pythium* as *P. aphanidermatum* (Eds.) Fitzp.

Sporangia of *Pythium aphanidermatum* are typically lobulate, branched, inflated and rather freely produced in most culture media. Zoospore production occurs as described in the description above. Oogonia are usually terminal though occasionally intercalary, spherical, smooth, thin-walled. The antheridium is typically intercalary, barrel-shaped, dome-shaped or suborbicular, usually sessile on a supporting hypha. Acrogenous antheridia may also be present but are not as typical of the species as the intercalary antheridia. Oospores are aplerotic, smooth and moderately thick-walled, 1.0 to 2.0 μ in thickness.

A comparison of some measurements of the diameters of oogonia and oospores is tabulated below:

Author	*Host*	*Oogonia* (μ)	*Oospores* (μ)
Edson (1915)	Sugar beet	22–27	17–19
Meurs (1934)	Tobacco	16.7–28.7 av. 22.9	15.6–26.2 av. 20.5
Tasugi and Takatuzi (1935)	Bean	16.2–30.5 av. 23.3	12.4–24.8 av. 18.8
Writer	Bean	15.1–32.3 m. 26.0	12.1–28.4 m. 19.8
Writer	Sugar beet	16.3–29.9 m. 24.0	13.1–25.6 m. 19.0
Writer	Cucumber	16.8–34.2 m. 26.8	13.8–28.4 m. 21.3

Subramaniam (1919) described *Pythium butleri* as a new species of *Pythium* parasitic on ginger, tobacco, and papaya in India. This species was designed to replace type *c.* of *P. gracile*, described by Butler (1907) as a parasite of ginger and castor bean in India. In 1927 Mitra (1927) indicated that *P. butleri* and *P. aphanidermatum* are closely related but cannot be considered identical. A year later Mitra and Subramaniam (1928), as a result of a series of cross inoculations of fruits of various Cucurbitaceae with *P. butleri* and *P. aphanidermatum* consider the former merely a strain of the latter. Fitzpatrick, Ramakrishna-Ayyar (1929) and Butler and Bisby (1931) consider these two names synonymous. A comparative study of isolates of *P. aphanidermatum* and *P. butleri* conducted by the writer substantiates the conclusions of Fitzpatrick and the others mentioned above.

Drechsler (1934a) maintains that *Pythium butleri* and *P. aphanidermatum* should be kept as separate species; the writer is not in agreement with this. Drechsler's basis for separation is presented below: Sporangia of *P. aphanidermatum* are smaller, less extensive and less branched than those of *P. butleri;* the zoospores are likewise smaller in *P. aphanidermatum*, 9 μ opposed to 11 μ in diameter for *P. butleri*. The average diameter of oogonia and oospores of *P. aphanidermatum* is 22 μ and 17.5 μ respectively, compared with 27 μ and 22.5 μ respectively for *P. butleri*.

The values cited by Drechsler for *Pythium butleri* are not greatly different from those for *P. aphanidermatum* and cannot be considered significantly divergent. The morphology of cultures received by the writer as *P. butleri* is identical with that of the morphology of *P. aphanidermatum* cultures. Similarly, the growth-temperature relations of the two are identical.

Since *Pythium aphanidermatum* was described prior to *P. butleri*, the former is maintained as the valid binomial. This is in agreement with the work of Fitzpatrick and Matthews.

In addition to the morphologic peculiarities of *Pythium aphanidermatum* which distinguish this fungus from related species, the general growth habit and temperature relations may be utilized in some instances as an aid to specific identification. In contrast to the rosette growth habit of *P. indigoferae* and *P. deliense*, the mycelial development of *P. aphanidermatum* is haphazardly disposed, forming a copious amount of loosely arranged aerial hyphae. *P. tardicrescens*, *P. volutum*, and *P. aristosporum* have an appressed aerial mycelium which develops slowly in comparison with the rapidly produced, abundant aerial mycelium of *P. aphanidermatum*. *P. myriotylum* and *P. aphanidermatum* are indistinguishable macroscopically. The growth-temperature values for *P. aphanidermatum*, *P. deliense*, and *P. myriotylum* are very similar and distinctly different from those of *P. tardicrescens*, *P. volutum*, and *P. aristosporum*.

The host range of *Pythium aphanidermatum* includes the following listed plants:

Agave rigida var. *sisalana* Perr. KENYA: McDonald (1932).
Agrostis stolonifera L. UNITED STATES: Monteith (1933).
Amaranthus sp. MALAYA: Milsum and Grist (1941); Sharples (1929).
A. gangeticus L. INDIA: Butler and Bisby (1931).
Amorphophallus sp. INDIA: Butler and Bisby (1931); Sundaraman (1926–27).
Ananas comosus Merr. HAWAII: Martin (1933); Parris (1940); Sideris (1931).
Armoracia rusticana Gaertn. UNITED STATES: Nance (1940).
Basella sp. INDIA: Butler and Bisby (1931); Sundaraman (1926–27).
Benincasa hispida Cogn. CHINA: Yu (1934, 1940).
Beta vulgaris var. *macrorhiza* Stev. AUSTRALIA: Simmonds (1933). AUSTRIA: Fischer (1931); Miestinger *et al.* (1932). CHINA: Yu (1934, 1940). UNITED STATES: Edson (1915a, 1915b); Durrell (1939); Kreutzer and Durrell (1938); Middleton (1938); Nance (1939).
B. vulgaris var. *cicla* L. UNITED STATES: Matthews (1931).
Brassica chinensis L. CHINA: Yu (1934).
B. oleracea var. *capitata* L. CHINA: Yu (1934). INDIA: Ramakrishna-Ayyar (1929). UNITED STATES: Drechsler (1925b).
Cannabis sativa L. INDIA: Galloway (1937).
Capsicum annuum L. CHINA: Yu (1934). INDIA: Butler and Bisby (1931); McRae (1928); Mitra (1925); Mitra and Subramaniam (1928); Subramaniam (1919). UNITED STATES: Author.
Carica papaya L. HAWAII: Jones *et al.* (1941); Parris (1940); Sideris (1931, 1935). INDIA: Bhargava (1941); Butler and Bisby (1931); Mitra (1925); Mitra and Subramaniam (1928); Subramaniam (1919). PUERTO RICO: Seaver *et al.* (1932). UNION SOUTH AFRICA: Doidge and Bottomley (1931); Wager (1931, 1932a, 1933, 1940). UNITED STATES: Author.
Citrullus vulgaris Schrad. BALUCHISTAN: Kheswalla (1936). CHINA: Teng (1935); Yu (1934, 1940). UNITED STATES: Drechsler (1925c); Gottlieb and Butler (1939); Author.
Colocasia antiquorum Schott. GOLD COAST: Wright (1931).
Cucumis melo L. CHINA: Yu (1934). UNITED STATES: Drechsler (1925c); Gottlieb and Butler (1939); Author.
C. melo var. *inodoratus* Naud. UNITED STATES: Drechsler (1925c); Author.
C. melo var. *momordica* Roxb. INDIA: Mitra (1927); Mitra and Subramaniam (1928).
C. melo var. *reticulatus* Naud. UNITED STATES: Gottlieb and Butler (1939); Author.
C. sativus L. CHINA: Yu (1934, 1940). INDIA: Butler and Bisby (1931); McRae (1923, 1924); Mitra (1927); Mitra and Subramaniam (1928). ITALY: Petri (1931). JAPAN: Darker (1940); Tasugi and Takatuzi (1935). PALESTINE: Reichert (1939). POLAND: Garbowske and Juraszkówna (1933); Siemaszko (1931). PUERTO RICO: Alvarez (1941). UNITED STATES: Drechsler (1925c); Gottlieb and Butler (1939); Nance (1939); Ramsey (1939); Author.
Cucurbita sp. POLAND: Siemaszko (1931).
C. moschata Duchesne. CHINA: Yu (1934).
C. pepo L. UNION SOUTH AFRICA: Wager (1932a, 1940). UNITED STATES: Drechsler (1925c); Author.
C. pepo var. *condensa* Bailey. UNITED STATES: Drechsler (1925c); Gottlieb and Butler (1939); Author.
Curcuma longa L. CEYLON: Park (1934).
Datura sp. INDIA: Butler and Bisby (1931); Sundaraman (1926–27).
D. fastuosa L. JAPAN: Tasugi and Takatuzi (1935).
Daucus carota L. UNITED STATES: Gottlieb and Butler (1939).
Euphorbia antiquorum L. INDIA: Ramakrishna-Ayyar (1929).
Festuca sp. CYPRUS: Nattrass (1936).
Ficus carica L. JAPAN: Tasugi and Takatuzi (1935).
Gaillardia sp. MALAYA: Thompson (1939).
Gossypium sp. ANGLO-EGYPTIAN SUDAN: Andrews and Clouston (1938); Massey (1936).
G. hirsutum L. CHINA: Teng (1935).
Ipomoea batatas Lam. UNION SOUTH AFRICA: Wager (1931). UNITED STATES: Gottlieb and Butler (1939); Harter and Whitney (1927b); Lauritzen, Harter and Whitney (1933); Nance (1940); Author.

Lactuca sativa var. *angustana* Irish. CHINA: Teng (1937).
Lagenaria leucantha Rusby. CHINA: Yu (1934). INDIA: Butler and Bisby (1931); McRae (1923); Mitra (1927); Mitra and Subramaniam (1928).
Lepidium sativum L. MALAYA: Milsum and Grist (1941).
Linum usitatissimum L. NETHERLANDS: Diddens (1932).
Luffa acutangula Roxb. INDIA: Butler and Bisby (1931); McRae (1923); Mitra (1927); Mitra and Subramaniam (1928).
L. aegyptiaca Mill. INDIA: Butler and Bisby (1931); McRae (1923); Mitra (1927); Mitra and Subramaniam (1928).
L. cylindrica Roem. CHINA Yu (1934, 1940).
Lycopersicon esculentum Mill. CHINA: Yu (1934). INDIA: Ramakrishna-Ayyar (1929). MALAYA: Tempany (1934). PALESTINE: Reichert (1939). UNION SOUTH AFRICA: Wager (1931, 1932a, 1933, 1940). UNITED STATES: Brown (1940); Gottlieb and Butler (1939); Author.
Momordica balsamina L. CHINA: Yu (1934).
M. charantia L. INDIA: Mitra (1927); Mitra and Subramaniam (1928).
Nicotiana tabacum L. CHINA: Yu (1934). GOLD COAST: Bunting and Dade (1924); Symond (1939). INDIA: Butler and Bisby (1931); Galloway (1935); Mitra (1925); Mitra and Subramaniam (1928); Subramaniam (1919); Venkatarayan (1937). NYASALAND: Hornby (1933). RHODESIA: Hopkins (1939a). SUMATRA: Drechsler (1926); Jochems (1926, 1927); Meurs (1934); van der Goot (1935, 1937); van der Weij (1935); van Hall (1925). TOGOLAND: Bunting (1924). UNION SOUTH AFRICA: Wager (1931, 1932a, 1933, 1940). UNITED STATES: Anderson *et al.* (1939).
Opuntia dillenii Haw. INDIA: Butler and Bisby (1931); Ramakrishna-Ayyar (1929); Sundaraman (1926–27).
Phaseolus aureus Roxb. UNITED STATES: Author.
P. limensis Macf. UNITED STATES: Author.
P. vulgaris L. JAPAN: Asuyama (1935); Tasugi and Takatuzi (1935). UNITED STATES: Harter and Zaumeyer (1931a, 1931b); Lauritzen, Harter and Whitney (1933); McLaughlin (1942); Zaumeyer (1930).
Physalis sp. INDIA: Butler and Bisby (1931); Sundaraman (1926–27).
Picea engelmanni Engelm. UNITED STATES: Rathbun (1923); Rathbun-Gravatt (1925, 1931); Gravatt (1925).
Pinus banksiana Lamb. UNITED STATES: Hartley (1921); Hartley, Merrill and Rhoads (1918); Rathbun (1922, 1923); Rathbun-Gravatt (1925, 1931).
P. resinosa Ait. UNITED STATES: Gravatt (1925); Rathbun (1922, 1923); Rathbun-Gravatt (1925, 1931).
Poa sp. UNITED STATES: Monteith (1933).
Pseudotsuga taxifolia Britt. UNITED STATES: Eliason (1928).
Pteridium aquilinum Kuhn. UNION SOUTH AFRICA: Wager (1933, 1940).
Pyrus malus L. UNITED STATES: Gottlieb and Butler (1939).
Raphanus sativus L. CHINA: Yu (1934). UNITED STATES: Brown (1940); Edson (1915b); Edson and Wood (1936, 1937); Gardner (1925); Nance (1939); Vaughn (1924).
R. sativus var. *longipinnatus* Bailey. UNITED STATES: Gardner (1925); Gregory (1925).
Ricinus communis L. INDIA: Butler (1907); Butler and Bisby (1931); Subramaniam (1919).
Saccharum officinarum L. HAWAII: Agee (1932); Bourne (1924); Carpenter (1921, 1928a, c, 1930); Heck (1934); Martin (1930, 1931, 1934); Sideris (1935). PHILIPPINE ISLANDS: Agati (1931). PUERTO RICO: Bourne (1924); Cook (1939). UNITED STATES: Drechsler (1934a); Rands (1930); Rands and Dopp (1938b); Stevenson and Rands (1938); Tims (1932b).
Secale cereale L. CHINA: Teng (1935).
Sechium edule Sw. UNION SOUTH AFRICA: Wager (1932a, 1933, 1940).
Solanum sp. INDIA: Butler and Bisby (1931); Sundaraman (1926–27).
S. melongena L. CHINA: Yu (1940). INDIA: Ramakrishna-Ayyar (1929). UNION SOUTH AFRICA: Wager (1933, 1940). UNITED STATES: Drechsler (1926); Gottlieb and Butler (1939); Author.
S. tuberosum L. CYPRUS: Nattrass (1936, 1937). INDIA: Butler and Bisby (1931); Ramakrishna-Ayyar (1929); Subramaniam (1919). UNION SOUTH AFRICA: Doidge and Bot-

tomley (1931); Wager (1931, 1932a, 1932b, 1933, 1940). UNITED STATES: Nance (1940); Author.

Sorghum vulgare Pers. var. *sudanense* (Piper) Hitchc. HAWAII: Martin (1934).

Spinacea oleracea L. UNITED STATES: Drechsler (1938).

Tephrosia toxicaria Pers. MALAYA: Sharples (1930).

Trichosanthes anguina L. INDIA: Butler and Bisby (1931); McRae (1923, 1924); Mitra (1927); Mitra and Subramaniam (1928).

T. dioica Roxb. INDIA: Butler and Bisby (1931); McRae (1924); Mitra (1927); Mitra and Subramaniam (1928).

Viola tricolor L. NETHERLANDS: van Eek (1938).

Vitis vinifera L. UNITED STATES: Gottlieb and Butler (1939).

Xanthosoma sagittifolium Schott. GOLD COAST: Wright (1931).

Zea mays L. PHILIPPINE ISLANDS: Agati (1931). UNITED STATES: Altstatt (1942); Nance (1939, 1940); Author.

Zingiber officinale Roscoe. CEYLON: Park (1934). INDIA: Butler (1907); Butler and Bisby (1931); Mitra and Subramaniam (1928); Sen (1930); Subramaniam (1919); Thomas (1938).

Zinnia sp. MALAYA: Sharples (1929); Thompson (1931).

The occurrence of *Pythium aphanidermatum* on radish is questioned by Kendrick (1927), who states the disease to be due to *Aphanomyces raphani* Kendrick.

22. PYTHIUM TARDICRESCENS Vanterpool, Ann. Appl. Biol. **25**: 528–543. 1938.

Hyphae measuring mostly 2.5 to 5 μ in diameter, irregularly branched, frequently forming knob-like appressoria. Sporangia inflated filamentous, toruloid or moderately lobed, never producing complex lobulations; zoospores not observed, the sporangium germinating by means of germ tubes which may terminate in a spherical body (conidium, according to Vanterpool). Oogonia spherical, terminal on short branches, rarely intercalary, measuring 17 to 30 μ, average 24.1 μ, in diameter, containing several oil globules. Antheridia commonly monoclinous though also diclinous, 1 to 6, usually 2 to 3, per oogonium, clavate or crook-necked, 6 to 8.5 μ wide and 16 μ long, making narrow or moderate oogonial contact. Oospores aplerotic, single, with a moderately thick wall, 1.25 to 2 μ thick, containing a central reserve globule embedded in a finely granular matrix, oospores measuring 16 to 26 μ, average 20.3 μ, in diameter; germination not observed.

Originally isolated from diseased roots of *Triticum aestivum* L., Canada.

Pythium tardicrescens is described by Vanterpool as a new member of the lobulate sporangial congeners causing browning root rot of graminicolous hosts; the diagnosis given above is taken from the original work.

The description of the fungus is sufficiently complete and further discussion of the morphological features is unnecessary. The observations of the writer on isolates of this species obtained from Vanterpool concur with the remarks made by him. The fungus has an appressed, radiate, non-lustrous growth on nutrient agars. The rate of growth is slower than in any other lobulate diverticulate form in the writer's collection; the maximum temperature value is likewise the lowest of the group.

Pythium tardicrescens may be separated from congenerics which possess monoclinous, occasionally diclinous, antheridia in that the oogonial branch

is not curved toward the antheridium as in *P. indigoferae* and *P. deliense*. *P. tardicrescens* may be distinguished from *P. aphanidermatum* by its antheridial cells which are typically terminal, not greatly inflated, varying in number from one to six, usually two to three, per oogonium. These are in contrast to the typically intercalary, though occasionally acrogenous, antheridial cells which are inflated and vary from one to two, usually one, per oogonium in *P. aphanidermatum*. *P. indigoferae*, *P. deliense*, *P. aphanidermatum*, and *P. tardicrescens* all possess aplerotic oospores.

The temperature-growth relations of this species may be used as an additional basis for specific segregation.

This species is known to cause a root rot of wheat, *Triticum aestivum* L., in Canada and England and is stated to be ". . . pathogenic to other cereals and certain grasses when artificially inoculated . . ." according to Vanterpool (1938); this fungus is further associated with the browning root rot of wheat by Vanterpool in subsequent papers (1940a, 1940b).

23. Pythium volutum Vanterpool and Truscott, Canad. Jour. Res. 6: 68–93. 1932.

Hyphae measuring 2.0 to 5.5 μ in diameter. Sporangia inflated filamentous consisting of small lobulations or toruloid outgrowths; zoospores produced in meagre to moderate numbers, about 10 to 14 μ in diameter. Oogonia spherical to subspherical, smooth- and thin-walled, terminal on short lateral branches, rarely intercalary, measuring 19.5 to 40.2 μ, average 30 μ, in diameter. Antheridia typically diclinous, rarely monoclinous, producing 3 to 10 antheridia per oogonium, a single stalk producing as many as four antheridial cells which are straight or crook-necked, clavate, making narrow apical contact with the oogonium. Oospores aplerotic, single with a smooth, thickened wall about 2 μ thick, containing 1, and occasionally 2, reserve globule, and a single refringent body, the oospore measuring 14.7 to 36.4 μ average 27.7 μ, in diameter; germination not observed.

Originally isolated from roots of *Triticum aestivum* L., and *Avena sativa* L., Canada.

Cultures of *Pythium volutum* were secured from Vanterpool; this species is unique among the present known congeners with inflated diverticula. The species may be distinguished from others on the antheridial character alone. The antheridial branch is usually diclinous in origin and is helicoidally disposed about the oogonial stalk. The antheridial cells per antheridial stalk vary from one to four in number. When plural antheridia are present, rarely more than two of the antheridial stalks are entwined about the stalk of the oogone; usually only a single antheridial branch coils about the supporting oogonial branch.

Pythium volutum is easily cultivated on nutrient agar substrates with the production of moderately abundant aerial development and a more or less radially disposed mycelium. Like *P. tardicrescens*, *P. volutum* is a moderately slow growing species. The macroscopic aspect of mycelial development of

P. volutum resembles that of many strains of *P. arrhenomanes*, though *P. volutum* is quite distinct morphologically from *P. arrhenomanes*. The difference between the two species is further borne out by their temperature-growth responses as reported recently by Vanterpool (1938) and confirmed by the writer.

Pythium volutum, in addition to attacking *Triticum aestivum* L. and *Avena sativa* L. in Canada, attacks *T. aestivum* in England (Vanterpool, 1938); *P. volutum* can infect *Hordeum vulgare* L., *Secale cereale* L. and *Zea mays* L., when artificially inoculated (Bisby *et al.* 1938) and (Vanterpool and Truscott, 1932). van Luijk (1934a, 1938a) isolated this species from *Agrostis stolonifera* L., *Festuca duriuscula* L., and *Lolium annuum* var. *westerwoldicum* in the Netherlands.

24. PYTHIUM ARISTOSPORUM Vanterpool, Ann. Appl. Biol. **25**: 528–543. 1938.

Hyphae measuring 2.5 to 6.5 μ in diameter, producing numerous appressoria. Sporangia inflated filamentous, digitate, lobulate, simple or complex, usually germinating by production of germ tubes, though rarely producing reniform, biciliate zoospores, about 10 to 12 μ in diameter when encysted. Oogonia subspherical, smooth- and thin-walled, usually acrogenous on short lateral branches, intercalary, measuring 21 to 36 μ, average 28.8 μ, in diameter. Antheridia diclinous and monoclinous, usually 3 to 6, though as many as 8 or more, clavate, crook-necked, 6.8 μ wide and 17.4 μ long, making narrow to moderate apical contact with the oogonial wall; a single antheridial branch may supply as many as 4 antheridial cells, the whole forming entanglements about the oogonium, though vegetative prolongations are rare. Oospores aplerotic, single with a smooth, dark wall 1.5 to 2.0 μ thick, containing a single reserve globule and refringent body, oospore measuring 13 to 30 μ, average 24.2 μ, in diameter.

Originally isolated from roots of *Triticum aestivum* L., Canada.

Pythium aristosporum was reported by Vanterpool as a companion fungus to *P. tardicrescens* in causing the browning root rot of graminicolous hosts. The diagnosis presented above is taken from Vanterpool. As in *P. tardicrescens*, the writer's observations are limited to a study of isolates received from Vanterpool, and the observations of the writer agree with his description.

Pythium aristosporum differs from *P. tardicrescens* not only in its macroscopic appearance and temperature-growth response, but in certain morphologic details. Slight aerial development on nutrient agar substrates is common to both species. In *P. aristosporum*, however, there is no definite radial disposition of hyphae, the whole developing a granular, creamy-yellow appearance after several days' growth. The writer concurs with Vanterpool's observation that the rate of growth of *P. aristosporum* exceeds that of *P. tardicrescens;* further, that the maximum temperature for growth is higher. As stated by Vanterpool, *P. aristosporum* shows affinity to *P. myriotylum* in its antheridial disposition; in addition, *P. aristosporum* is also closely allied to *P. volutum* in possessing diclinous, and occasional mono-

clinous, antheridia. *P. aristosporum* differs from *P. volutum* in the non-helicoid antheridial branch. *P. myriotylum* and *P. aristosporum* may be distinguished morphologically on the basis of their antheridia. In *P. myriotylum* the antheridial branch does not originate in close proximity to the oogonium; the antheridial cells are crook-necked, not fully laterally adnate, rather making only basal and apical contact with the oogonium; vegetative prolongations of the anteridial branch are quite common. *P. aristosporum*, on the contrary, has an antheridial branch which arises in close proximity to the oogonium, possesses antheridial cells which are also crook-necked but which make only apical contact with the oogonium; vegetative prolongations of the antheridial branch are quite rare.

Further differences between *P. aristosporum* and *P. myriotylum* are found in their temperature-growth responses and growth habits.

Pythium aristosporum is known to parasitize roots of *Triticum aestivum* L. in Canada, and to be pathogenic to *Avena sativa* L., *Hordeum vulgare* L., and *Secale cereale* L. upon inoculation. Fischer *et al.* (1942) report this species from *Agropyron trachycaulum* (Link) Malte and *Echinochloa crus-galli* (L.) Beauv., Vanterpool and Sprague (1942) report the fungus from *Hordeum vulgare* L. and *Triticum aestivum* L. and Sprague adds *Dactylis glomerata* L., all from the United States.

25. Pythium myriotylum Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 2.5 to 8.5 μ in diameter, when young mostly 3 to 4 μ, when old mostly 7 to 8.5 μ in diameter; forming numerous clavate or knob-like appressoria measuring 7 to 11 μ in diameter. Sporangia terminal or intercalary, sometimes undifferentiated though generally including an inflated filamentous lobulate or digitate lateral element, the undifferentiated portions measuring 0.1 to 0.3 mm. in length and 3 to 7 μ in diameter, the inflated portions of variable size, 10 to 175 μ in length and 12 μ in diameter; evacuation tube arising either from the undifferentiated or inflated portion, 10 to 100 μ long and 2 to 3.5 μ wide; 3 to 40 zoospores formed in the apical vesicle, reniform and laterally biciliate, 9 to 16 μ, average 11 μ, in diameter, germinating by a single germ tube. Oogonia spherical to subspherical, smooth- and thin-walled, terminal or intercalary, measuring 15 to 44 μ, average 26.5 μ, in diameter. Antheridia typically diclinous, when monoclinous usually originating in excess of 100 μ from the oogonium, 1 to 10, usually 3 to 6, per oogonium, the antheridial cell terminally expanded, clavate, crook-necked, arched, measuring 8 to 30 μ long and 4 to 8 μ wide, the apex making narrow apical contact with the oogonial wall, the middle arched upward and the proximal end frequently contacting the oogonial wall. Oospores aplerotic, single, with a wall 1 to 2 μ thick, containing a single reserve globule and a subspherical or strongly flattened refringent body, oospores measuring 12 to 37 μ, average 20.8 μ, in diameter.

Cause of fruit rot of *Citrullus vulgaris* Schrad., *Cucumis sativus* L. and *Solanum melongena* L., and decaying rootlets of *Lycopersicon esculentum* Mill. in U. S. A.

A study of the type culture, an isolate from *Nicotiana tabacum* L. in Sumatra and a culture from *Carica papaya* L. from South Africa was made.

The observations of the writer agree in all respects with the delimitations of the species as given by Drechsler. The Sumatran isolate, as stated by Meurs (1934), has oogonia which are slightly larger than those cited by Drechsler, 21.2 to 38.1 μ, mostly 26.1 to 32.1 μ, mean 28.6 μ in diameter. The South African isolate measured 19 to 38.5 μ, mostly 22.1 to 29.6 μ, mean 25.7 μ in diameter. The sporangia of the tobacco and papaya strains conformed with those of the type culture. The antheridia of all three isolates were comparable, varying from 1 to 10, usually 3 to 6, in number, of diclinous origin, occasionally monoclinous. The antheridial cell is uniquely applied to the oogonium, making basal and apical contact, the middle part arched outward and free from the oogonium. Vegetative prolongations on the antheridial branches are commonly observed.

The fungus grows rapidly at room temperature on suitable agar substrates. The high maximum temperature for growth dismisses any confusion with congeners with aplerotic oospores, such as *Pythium aristosporum*, *P. volutum*, *P. tardicrescens*, and *P. indigoferae*.

Pythium myriotylum, *P. deliense*, and *P. aphanidermatum* all have high maxima growth temperatures and cannot be segregated on this basis alone; morphological differences must be employed to separate the three species. The oogonium in *P. myriotylum* is not inclined toward the oogonium as in *P. deliense*. The typically intercalary antheridia of *P. aphanidermatum* are absent in *P. myriotylum*..

So far as the writer is aware, *Pythium myriotylum* has been encountered only in the warmer climates; these areas and the hosts from which the fungus is known are listed below: *Carica papaya* L. by Wager (1932a, 1940) in South Africa; *Citrullus vulgaris* Schrad., *Cucumis sativus* L., and *Lycopersicon esculentum* Mill. by Drechsler (1930a) in southeastern United States; *Nicotiana tabacum* L. by Jochems (1934), Meurs (1934) and van der Weij (1935) in Sumatra; *Phasoleus vulgaris* L. (inoculation) by Harter and Whitney (1927a) in the United States; probably this species by Deighton (1936) in Sierra Leone; seedling root rot of *Robinia pseudo-acacia* L. by Weiss (1942) in the United States; *Solanum melongena* L. by Drechsler (1930a) in the United States; *Zingiber officinale* Roscoe by Park (1937, 1941) in Ceylon, by Uppal (1940) in India.

26. PYTHIUM PERIPLOCUM Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 1.5 to 9 μ, mostly 1.8 to 4.2 μ, in diameter, irregularly and abundantly branched. Sporangia inflated filamentous, usually intercalary though often acrogenous, digitate, lobulate elements 10 to 30 μ long and 8 to 20 μ wide, simple, or 20 to 40 elements grouped to form intricate moriform aggregations; zoospores few to 125 in a vesicle, broadly reniform, laterally biciliate, measuring 8 to 11 μ in diameter upon encystment. Oogonia spherical to subspherical, provided with an echinulate wall, the spines 2 to 4 μ, average 2.8 μ, in length and 1.4 to 3 μ, average 1.8 μ, at the base, tapering slightly to a rounded apex, the oogonia acrogenous or intercalary, measuring

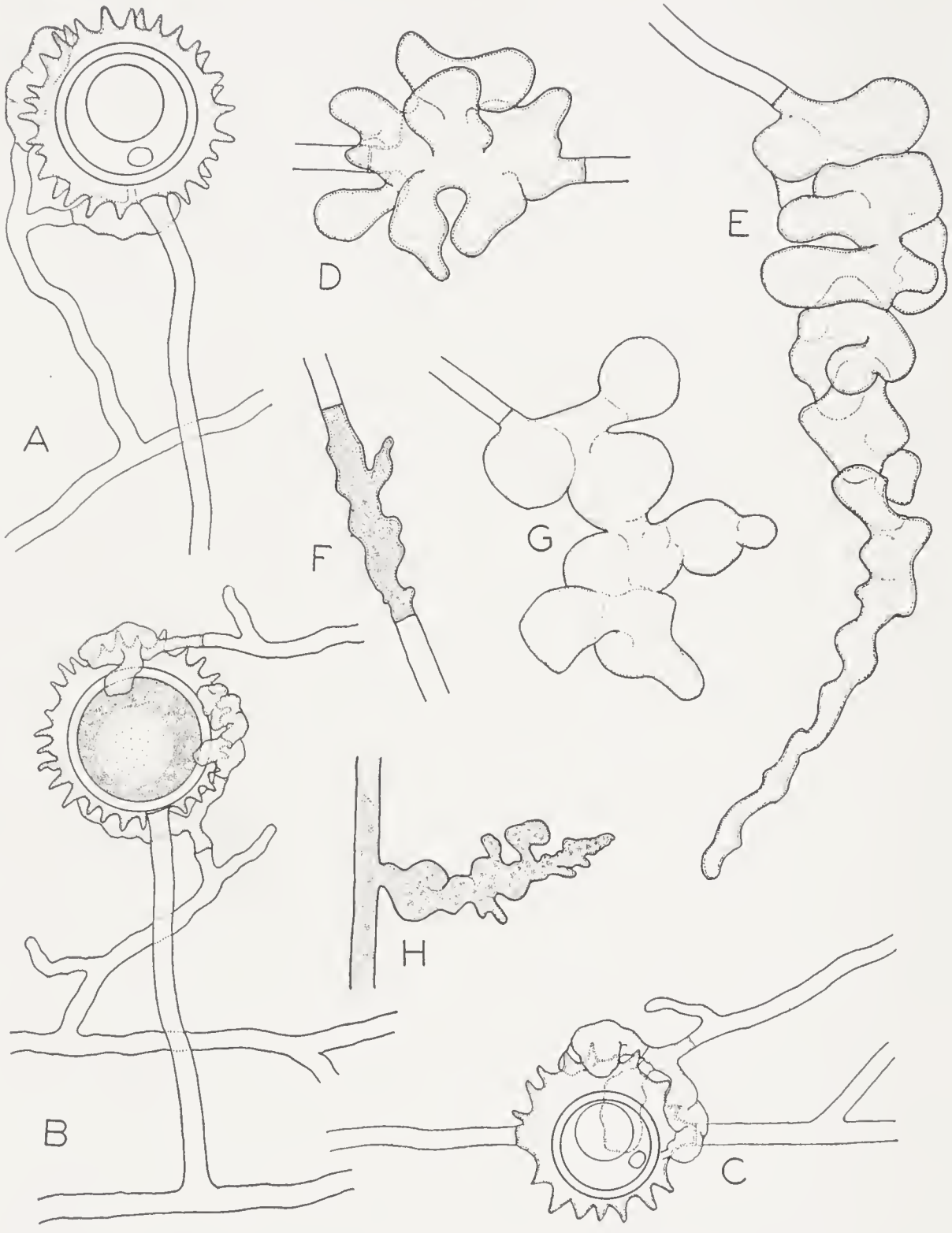

FIG. 4. *Pythium periplocum*. *A–C*, Sexual apparatus. *A*, *B*, Acrogenous oogonia with lobate diclinous antheridia. *C*, Intercalary oogonium with 3 antheridial cells from a single diclinous stalk. *D–H*, Sporangia. *D*, Intercalary sporangium. *E*, *G*, Terminal moriform type sporangia. *F*, immature intercalary sporangium. *H*, Immature acrogenous sporangium.

13 to 32 μ, average 24.6 μ, in diameter, not including the spiny protuberances. Antheridia diclinous, 1 to 4 in number, usually originating from 1 to 2 stalks, the antheridial cell sometimes clavate, more often long, 15 to 30 μ, and markedly lobed, the lobes 5 to 10 μ long and 5 to 8 μ wide, making ventral contact with the oogonial wall, antheridia and distal portion of the antheridial stalks intricately and extensively wrapped about the oogonium. Oospores aplerotic, single, spherical with smooth, thin wall measuring 0.7 to 1.9 μ, average 1.4 μ, in thickness and containing a single reserve globule and subspherical or flattened refringent body, the oospore measuring 11.5 to 27 μ, average 21.2 μ, in diameter.

Originally described from decayed fruits of *Citrullus vulgaris* Schrad. in the United States.

Pythium periplocum is at present the only known species of *Pythium* possessing filamentous sporangia that also possess echinulate oogonia.

In addition to the paper in which the species is described, Drechsler (1939) gives a more recent and elaborated account of the morphology and habit of the fungus.

The observations of the writer are in agreement with the detailed diagnosis of Drechsler. In culture on solid nutrient agar substrates, *Pythium periplocum* produces a moderate amount of aerial mycelium which has a peculiar cumulous aspect; small tufts of the aerial mycelium are aggregated to form a spherical, raised, hyphal mass. No other filamentous species of *Pythium* exhibits this habit in culture. The identical habit is expressed, however, in cultures of *P. acanthicum*, a member of the group of species possessing spherical sporangia, but is unknown in any other species of the group.

The hosts from which this species is known are: *Citrullus vulgaris* Schrad., Drechsler (1930a, 1939), *Cucumis melo* L. var. *reticulatus* Naud. and *C. melo* var. *inodoratus* Naud., author, *Prunus serotina* Erhr., Drechsler (1939) in the United States, and from *Gossypium* sp. in the Anglo-Egyptian Sudan by Andrews and Clouston (1938).

27. Pythium salpingophorum Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 1.5 to 7.0 μ, mostly 2 to 5.5 μ, in diameter; appressoria are clavate, 7 to 8.5 μ at their apex, frequently forming connected systems of falcate elements. Sporangia spherical to subspherical, usually formed in abundance, terminal, subterminal or intercalary, measuring 17 to 33 μ, average 24 μ, in diameter, usually proliferous, the secondary sporangium borne within the primary one; zoospores 15 to 40, longitudinally grooved and biciliate, broadly reniform, measuring 7.5 to 9.2 μ, average 8.5 μ, in diameter upon encystment. Oogonia spherical to subspherical, terminal, intercalary or tangentially intercalary, often forming a series of 2 to 5, smooth- and thin-walled, often including a portion of the supporting stalk, measuring 11 to 22 μ, average 15.8 μ, in diameter, excluding the attached plugs; approximately 75 percent of the oogonia develop parthenogenetically. Antheridia typically monoclinous, though also diclinous, 1 rarely 2, per

oogonium, when diclinous often lateral, sessile and straight, when monoclinous, proximal to the oogonium and strongly crook-necked, either type measuring 10 to 20 μ long and 3.5 to 6 μ wide, either delimited or undelimited by a septum; antheridia often lacking or non-functional. Oospores plerotic, the oospore wall usually fused with the oogonial wall, single, measuring 10 to 19 μ, average 14.6 μ, in diameter, with a thin wall, measuring 0.8 to 1.5 μ, average 1.2 μ, thick, containing a single reserve globule and refringent body.

Originally isolated from decaying roots of *Pisum sativum* L., United States.

The observations of the writer are confined to the study of two isolates which agree with the description of the fungus as given above.

Pythium salpingophorum presents many peculiarities which assist in its ready identification. It is the only species possessing plerotic oospores which has proliferous sporangia. The intercalary oogonia resemble those of *P. debaryanum*, having a short filamentous portion of the hypha attached to the oogonium, the whole being more or less prolate-limoniform. The oospores of *P. salpingophorum*, however, are quite different from those of *P. debaryanum*, being plerotic and usually fusing indistinguishably with the wall of the spherical part of the oogonium whereas those of *P. debaryanum* are aplerotic and not fused with the oogonial wall.

A large number of the oospores of *Pythium salpingophorum* are apandrous while others are parthenogenetic despite the presence of an antheridium; the antheridia present in the latter case are usually not delimited by a septum and lack a fertilization tube. The antheridia present closely resemble those of *P. ultimum;* the species may be distinguished from *P. ultimum* by its plerotic oospores and proliferous sporangia.

Further relationship of *Pythium salpingophorum* and *P. debaryanum* is evident in their temperature-growth relationships and growth habits.

In addition to the isolation of the fungus from roots of *Pisum sativum* L. by Drechsler, the author has obtained it from roots of *Ceanothus cyaneus* Eastw. and *Lycopersicon esculentum* Mill.

28. Pythium conidiophorum Jokl, Öst. Bot. Zeits. 67: 33–37. 1918.

Hyphae non-septate, measuring 2 to 6.3 μ in diameter. Sporangia are spherical, terminal on long branches up to 100 μ in length, varying in size from 8 to 20 μ in diameter, germinating by germ tubes. Oogonia are spherical, terminal, usually borne on short lateral branches, 6.3 to 15.9 μ in diameter. Oospores are plerotic and apandrous.

Parasitic on *Spirogyra dubia* Kütz., *S. communis* (Haas.) Kütz., and *S. varians* Kütz., Albania.

The diagnosis given above was condensed from the original German text published by Jokl.

Pythium conidiophorum is readily distinguished from its congeners *P. hypogynum*, *P. rostratum*, and *P. iwayamai* in possessing apandrous oospores. The sporangia are reported to be frequently deciduous, always germinating

by means of germ tubes; these structures are termed conidia by Jokl and they are without doubt the most analagous reproductive bodies to conidia in the genus. Just what disposition should be made regarding these structures and further identity of the species is questionable due to the rather brief discussion of the organism and the fact that this species has not been observed subsequent to its description.

No hosts other than those from which the species was originally described have been reported.

29. PYTHIUM HYPOGYNUM Middleton, sp. nov.

Hyphae non-septate when young, irregularly septate when old, measuring 1.5 to 8.3 μ, average 5.1 μ, in diameter. Sporangia terminal, rarely intercalary, spherical to subspherical, smooth- and thin-walled, measuring 6.5 to 34.6 μ, average 22.1 μ, in diameter, sporangia germinating by means of germ tubes or zoospores. Oogonia terminal, spherical to subspherical, smooth- and thin-walled, measuring 10.2 to 35.1 μ, average 22.0 μ, in diameter. Antheridia hypogynous, single, delimited within the oogonial stalk at a distance varying from 5 to 30 μ below the oogonium; antheridial cell small, 2.8 to 8.3 μ, average 5.4 μ, wide, 3.0 to 11.1 μ, average 6.6 μ, long, supplied with a tenuous fertilization tube that just penetrates the oogonial wall. Oospores plerotic, smooth-walled, containing a single reserve globule and a prolate ellipsoidal or flattened refringent body; oospore germination not observed.

Isolated from decayed roots of *Hordeum vulgare* L., Missouri, U. S. A.

Hyphae juvenes non septatae, veteres irregulariter septatae, 1.5–8.3 μ diam.; sporangia terminalia, rare interposita, globosa vel subglobosa, cuticula levi tenuique, 6.5–34.6 μ diam., tubulis vel zoosporis germinantia. Oogonia terminalia, globosa vel subglobosa, membrana levi tenuique, 10.2–35.1 μ diam. Antheridia hypogyna, solitaria, sub oogonio 5–30 μ in stipite eiusdem delimitata; antheridii cellula parva, 2.8–8.3 μ lata, 3.0–11.1 μ longa, oogonii membranam appendicula brevi tantum penetrans. Oosporae pleroticae, cuticulis levibus, unicam globulam et corpus prolatum ellipsoidale vel complanatum habentes; germinatio non visa.[2]

Pythium hypogynum exhibits similarities to *P. rostratum* from which it may be distinguished by the character of the antheridium, the predominance of acrogenous sporangia and its strictly terminal oogonia. The antheridial character also serves to separate the species from *P. iwayamai* which has either mono- or diclinous, never hypogynous antheridia.

Pythium hypogynum represents one of a series of species with analagous or related types of antheridia, such as found in *P. rostratum*, *P. proliferum*, *P. pulchrum*, *P. paroecandrum*, *P. ultimum*, and perhaps others. Though the antheridia of these several species are similar, the species remain quite distinct and in some instances not at all closely related. It is interesting to consider the possible origin and evolution of the types embraced in this group of species. Assuming the premise of evolutionary advancement through reduction and simplification, species such as *P. hypogynum* and *P. pulchrum* would be derived from organisms such as *P. ultimum* and *P. paroecandrum*, cases in which the sessile, monoclinous antheridium originating immediately

[2] Thanks are extended to Dr. H. W. Rickett for preparation of the Latin diagnosis.

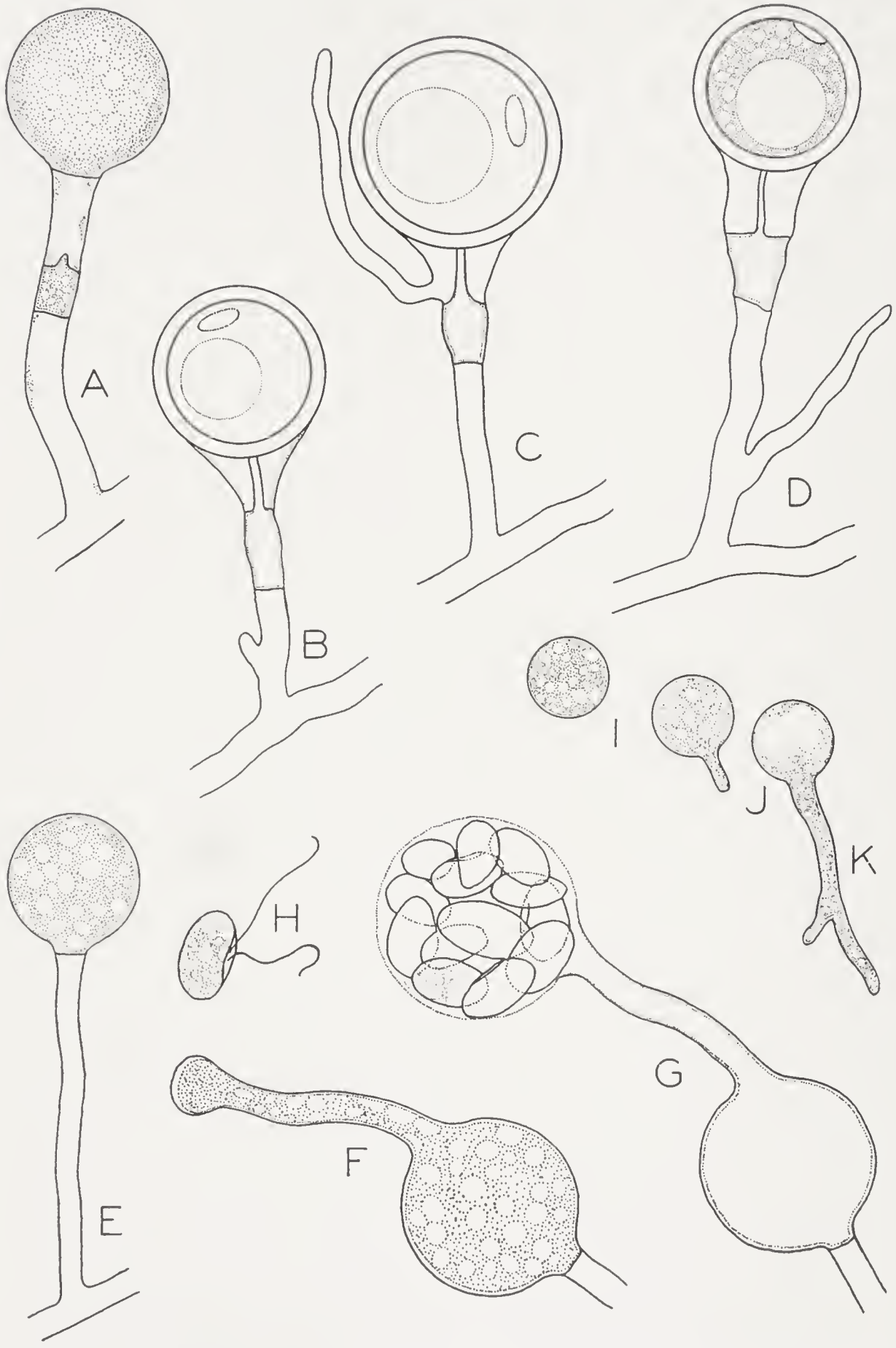

FIG. 5. *Pythium hypogynum*. *A–D*, Sexual apparatus. *A*, Unfertilized oogonium with differentiated periplasm with hypogynous antheridium forming a fertilization tube. *B–D*, mature oospores with empty antheridial cell. *E–K*, Asexual apparatus. *E*, Typical sporangium. *F*, *G*, Development of evacuation tube and vesicle containing zoospores. *H*, active zoospore. *I–K*, Three phases of zoospore germination.

adjacent to the oogonium and frequently not delimited from the oogonial stalk by a septum, has been further reduced to a mere lateral protuberance or hypogynal cell. *P. rostratum* and *P. proliferum* can be considered as intermediate types, either species showing partial reduction of the antheridium or complete reduction to the hypogynal cell; both of these extremes and their consequent intermediates are frequently encountered in a single isolate of either species. In fact, a morphologically distinguishable antheridium is often unidentifiable in *P. proliferum*. On rare occasion this absence of a visible antheridium has been observed in *P. hypogynum;* perhaps this condition reflects relationship to species completely lacking antheridia, such as *P. anandrum*, representing an additional advancement in evolutionary development. In several instances in which the antheridium was lacking in *P. hypogynum* the writer was able to demonstrate the migration of a single, moderately sized nucleus in the oogonial stalk upwards toward the oogonial cavity; this movement originated in the region where the antheridial cell is normally located. Subsequently an oospore developed. Whether this phenomenon occurs in all instances in apandrous oospore development is questionable. In any event it may account for fertilization of oogonia normally fecundated by functional antheridia which are upon occasion lacking. This observation certainly should be extended in the study of other species of this genus and may suggest the necessity of further cytological investigations in related genera and opening the question of fertilization in apandrous species and parthenogenesis in the fungi. Parthenogenesis in certain species of *Pythium* may be quite lacking, being so named for lack of cytological information.

The possible evolutionary trend indicated above solely on the basis of certain antheridial characteristics is not necessarily suggested when the asexual stage is considered. Sporangia of *Pythium ultimum* indicate the reverse relationship, these bodies having lost their ability to produce zoospores, germinating by means of germ tubes; the sporangia of *P. hypogynum* and *P. pulchrum* commonly germinate by the production of zoospores, a process generally accepted as more primitive. Whether or not a developmental trend has been established either through the antheridial or sporangial habits, a certain analogy exists among species possessing the antheridial types discussed.

The growth habit of *Pythium hypogynum* is radiate while that of *P. rostratum* is rosette. The temperature-growth responses of the two species are likewise dissimilar; the cardinal temperatures for *P. hypogynum* are 1°, 31° to 34°, and 37° C, while those for *P. rostratum* are 4°, 22° to 25°, and 34° C.

This species has been observed on *Heuchera hispida* Pursh. and *Hordeum vulgare* L. in the United States.

30. PYTHIUM ROSTRATUM Butler, Mem. Dept. Agr. India Bot. 1[5]: 1–160. 1907.

Pythium diameson Sideris, Mycologia 24: 14-61. 1932.

Hyphae often large, measuring up to 6 to 8 μ in diameter. Sporangia are spherical to oval, terminal or intercalary, measuring 23 to 34 μ, average 28 μ, in diameter, usually appearing before sexual reproductive bodies; zoospores few to 40, 8 to 11 μ, average 9.6 μ, in diameter at encystment, formed in a vesicle arising from a large, stout evacuation tube usually equal to the diameter of the sporangium, frequently of lateral origin. Oogonia spherical to subspherical, typically intercalary, occasionally terminal measuring 13 to 29.4 μ, average 21 μ, in diameter. Antheridia typically monoclinous, infrequently hypogynal, single, rarely 2 per oogonium, the antheridial cell often extremely short, reduced to a lateral swelling immediately adjacent to the oogonium, then 2 to 5 μ long and 3 to 5 μ wide, or strongly crook-necked, in either case arising from a cell cut off immediately below the oogonium, the antheridial cell sessile and not otherwise delimited, the whole forming the antheridium. Oospore plerotic, single, with a moderately thickened wall, measuring 12 to 27 μ, average 20 μ, in diameter; germination not observed.

Originally described as saprophytic in garden soil, France.

The most distinctive characteristic of *Pythium rostratum* is the antheridium; this readily distinguishes the species from its congeners *P. conidiophorum*, *P. hypogynum*, and *P. iwayamai*. *P. rostratum* is more closely related to *P. hypogynum* than to any other species, though differing from it not only in antheridia but also in the position of the oogonia which are principally intercalary in *P. rostratum* and acrogenous in *P. hypogynum*.

Sideris described *Pythium diameson* and *P. plerosporon* as new species. *P. diameson* is considered by the writer to be synonymous with *P. rostratum*. Until additional cultures of *P. plerosporon* are available for study, it seems advisable to retain this species as of doubtful validity and possibly as synonymous with *P. rostratum*.

The sporangia of *Pythium diameson* compare favorably with those of *P. rostratum*, being both acrogenous and intercalary, spherical to subspherical, though Sideris' measurements are somewhat smaller, 15 to 20 μ in diameter compared with 23 to 34 μ in diameter for *P. rostratum*. Measurements of sporangia of *P. diameson* made by the writer are slightly larger, 14.1 to 28.3 μ in diameter; similar differences were found in the size of the oogonia, Sideris reporting 15 to 17 μ in diameter, the writer 12.2 to 29.3 μ in diameter. Although the oogonia of *P. diameson* are reportedly mostly acrogenous, large numbers of intercalary ones can be found. The antheridia of both species are similar, perhaps more frequently longer in *P. diameson* than in *P. rostratum*, nevertheless exclusively monoclinous, usually sessile and oriented as those in *P. rostratum*. The oospores of both species are plerotic and fairly thick walled. The growth habits and temperature-growth responses of the two species are identical.

Pythium rostratum and *P. hypogynum* may be further distinguished by their mycelial type of growth and temperature responses.

Pythium rostratum is listed from the following substrates:

Ananas comosus Merr. HAWAII: Sideris and Paxton (1931); Sideris (1932); Parris (1940)
Antirrhinum majus L. UNITED STATES: Author.

Avena sativa L. UNITED STATES: Author.
Cajanus indicus Spreng. HAWAII: Sideris (1932); Parris (1940).
Canavalia ensiformis DC. HAWAII: Sideris (1932); Parris (1940).
Citrus aurantium L. UNITED STATES: Wager (1942b).
C. limonia Osbeck. UNITED STATES: Wager (1942b).
Erica regerminans L. UNITED STATES: Author.
Fuchsia sp. UNITED STATES: Author.
Hordeum vulgare L. UNITED STATES: Author.
Ipomoea batatas Lam. HAWAII: Sideris (1932); Parris (1940).
Lactuca sativa L. UNITED STATES: Author.
Lathyrus odoratus L. UNITED STATES: Author.
Lupinus angustifolius L. GERMANY: Schultz (1939).
Lupinus arboreus Sims. UNITED STATES: Author.
Medicago sativa L. UNITED STATES: Buchholtz and Meredith (1938); Author.
Nemophila menziesii H. and A. UNITED STATES: Author.
Oryza sativa L. UNITED STATES: Author.
Pennisetum barbinode.[3] HAWAII: Sideris (1932); Parris (1940).
Persea americana Mill. UNITED STATES: Author.
Phaseolus aureus Roxb. UNITED STATES: Author.
P. vulgaris L. UNITED STATES: Author.
Saccharum officinarum L. HAWAII: Sideris (1932); Parris (1940).
Soil. FRANCE: Butler (1907). UNITED STATES: Meredith (1938, 1940); Author.
Solanum tuberosum L. HAWAII: Sideris (1932); Parris (1940).
Spinacea oleracea L. UNITED STATES: Author.
Triticum aestivum L. UNITED STATES: Author.
Vicia faba L. HAWAII: Sideris (1932); Parris (1940). UNITED STATES: Author.
Zea mays L. HAWAII: Sideris (1932); Parris (1940).

31. PYTHIUM IWAYAMAI Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 11–33. 1935.

Pythium sp. Iwayama, Toyama Agr. Expt. Sta. Publ. 1933.

Hyphae intra- and extramatrical, hyaline to olive-yellow, branched, measuring 2.9 to 8.9 μ, average 6.6 μ, in diameter. Sporangia spherical to prolate ellipsoidal, ovoid or citriform, acrogenous, wall thin and light olive-yellow, measuring 28 to 48 μ long by 44 μ wide; zoospores 8 to 12, when encysted measuring 9 to 15 μ in diameter. Chlamydospores intercalary, ellipsoidal, pale olive in color, 36 to 48 μ long and 24 μ wide with a more or less thick wall. Oogonia terminal or intercalary, spherical, smooth. Antheridia monoclinous or diclinous, usually single, sometimes two, clavate. Oospores plerotic, pale olive-yellow in color, measuring 19 to 24 μ in diameter.

Originally described as parasitic on *Hordeum vulgare* L. and *Triticum aestivum* L., Japan.

Cultures of this fungus were not available to study and inasmuch as no other reports of the species exist at present, the discussion is confined to the translated description given above, apparently prepared from Iwayama's original report.

The usually elliptical or limoniform sporangia are somewhat larger than those of any of its congeners, *Pythium conidiophorum*, *P. hypogynum*, and *P. rostratum*, about 40 μ in diameter compared with 14 μ, 23 μ and 28 μ in diameter, respectively.

Pythium iwayamai is the only species of the group with nonproliferous sporangia and plerotic oospores which has stalked antheridia that are either monoclinous or diclinous and never hypogynous.

[3] This probably stands for *Pennisetum purpurascens* var. *barbinode* Kuntze (*Panicum barbinode* Trin.).

Iwayama described a rot of cereals occurring under snow, calling it the snow-rot disease. An unidentified species of *Pythium* was isolated from the material and proven pathogenic by inoculation. The fungus was described but not given a specific name until Ito reported it to be a new species in 1935. Iwayama states that the optimum temperature for growth is between 15° and 18° C.

The fungus is unknown from any other hosts than those from which it was originally described. In Ito's description it is stated to be limited to graminicolous hosts, ". . . necnon implantis plurimorum speciorum gramineorum," though no data are presented to substantiate the statement.

32. PYTHIUM PROLIFERUM de Bary, Jahr. Wiss. Bot. **2**: 169–192. 1860.

Hyphae freely branched and well developed on most culture media, measuring 2 to 5 μ in diameter. Sporangia acrogenous, spherical to ovoid, measuring 24 to 36.6 μ in diameter, proliferous, the secondary and tertiary sporangia formed within the walls of the primary ones; zoospores 3 to 40, reniform and laterally biciliate, formed in a vesicle borne on a short, stout evacuation tube, zoospores measuring 9 to 16 μ in diameter upon encystment. Oogonia spherical, smooth or with a short apical papilla, terminal or intercalary, frequently catenulate, measuring 15 to 28 μ in diameter. Antheridia hypogynous, monoclinous or diclinous, 1 to 3 per oogonium, usually single and hypogynous, when mono- or diclinous, either sessile or stalked, only slightly inflated, clavate, making moderate apical contact with the oogonial wall. Oospores aplerotic, thick-walled, measuring 14 to 24 μ in diameter, containing a single reserve globule and refringent body; germination not observed.

Originally described from insect cadavers in Germany.

De Bary originally described this species from the asexual stage only; it was not until later that he reported the sexual phase (1881a, 1881b).

This species has received considerable attention from various mycologists, probably because it is easily isolated and readily produces proliferous sporangia. Butler (1907), Crooks (1937), Dissmann (1927) and Ward (1883) all present similar accounts of the fungus. Büsgen (1882) includes a discussion of this species in his study of the development of the sporangium.

The sporangium is usually papillate with the evacuation tube arising from the papilla. The secondary or tertiary sporangia are usually formed within the primary but sometimes beyond it. Occasionally sporangia may germinate by the production of germ tubes rather than zoospores.

Although the oogonium does not exhibit any unique peculiarities, the oospore differs from that of the other proliferous sporangial species, *Pythium polytylum*, *P. helicoides*, *P. oedochilum*, and *P. paligenes*, in containing a single reserve globule and refringent body whereas the others contain several. The number of reserve globules contained in an oospore is considered a specific feature in the segregation and identification of these species. This feature, together with the occurrence of a hypogynal antheridium serves to distinguish *P. proliferum* from its allies.

Pythium proliferum has been isolated from *Fragaria chiloensis* Duchesne in Scotland by Wardlaw (1927); from *Gossypium* sp. in the Anglo-Egyptian Sudan by Andrews and Clouston (1939); from insect cadavers in England by Ward (1883), in France by Butler (1907), in Germany by de Bary (1881a) and Schroeter (1889), in India by Butler (1907), Butler and Bisby (1931) and by Sydow and Butler (1907); from *Medicago sativa* L. in the United States by the author; from *Nymphaea* sp. and *Nuphar* sp. in Denmark by Petersen (1909, 1910); *Nymphaea candida* Presl. in Bohemia by Dissmann (1927); *Ricinus communis* L. seeds in India by Butler and Bisby (1931); soil in the United States by Harvey (1927), Höhnk (1933), Matthews (1931), Raper (1928) and the author; vegetable debris in France and India by Butler (1907) and in the United States by the author; water in Australia by Crooks (1937) and in the United States by Matthews (1931). A fungus which may be this species has been isolated from *Bromus inermis* Leyss. and *B. tectorum* L. by Fischer *et al.* (1942) in the United States.

33. Pythium marsipium Drechsler, Phytopathology, **31**: 478–507. 1941.

Hyphae branched, measuring 2 to 7.5 μ in diameter. Sporangia spherical or asymmetrically utriform, papillate, broadly rounded at one end and skewly beaked at the other, proliferous, generally by production of the secondary sporangium within the primary, when subspherical measuring 20 to 70 μ in diameter, largely intercalary in broad hyphae but sometimes acrogenous, when utriform measuring 25 to 70 μ in length by 20 to 45 μ in width, largely acrogenous on sporangiophores 20 to 100 μ long and 3 to 4 μ wide with the long axis of the sporangium transverse or oblique to the supporting stalk; zoospores sometimes produced in a vesicle sessile on the papilla, more frequently the vesicle is formed at the apex of an evacuation tube 2 to 8 μ wide and up to 100 μ in length which may or may not arise from the papilla, zoospores reniform, laterally biciliate from the longitudinal groove, from 2 to 125 in number, measuring 9 to 12 μ in diameter when encysted. Oogonia subspherical, measuring 23 to 29 μ, average 30.9 μ, in diameter, acrogenous, subterminal, mostly intercalary, infrequently catenulate. Antheridia diclinous, 1 to 4 per oogonium, usually 1 to 3, irregularly expanded, 10 to 20 μ in length by 8 to 12 μ in width, broad oogonial contact with conspicuous fertilization tube. Oospores aplerotic, subspherical, measuring 19 to 33 μ, average 26.2 μ, in diameter, wall moderately thick, 1.3 to 2.8 μ, average 1.95 μ, containing a single reserve globule and refringent body.

Occurring in decaying leaves of *Nymphaea tuberosa* Paine, in the United States.

Cultures of this newly described species were not available for observation.

The sporangia resemble those found in *Pythiogeton* in being utriform, the long axis generally attached transversely or obliquely to the sporangiophore rather than parallel to it. The resemblance to *Pythiogeton* is only superficial, the organism resembling other *Pythium* species in all other respects.

Pythium marsipium should prove easily distinguishable from its congeners exhibiting proliferous sporangia and aplerotic oospores. *P. nagaii* differs from

this group in possessing a thin-walled oospore. *P. helicoides*, *P. oedochilum*, *P. palingenes*, and *P. polytylum* all differ from *P. marsipium* in their plurality of reserve globules and refringent bodies in their oospores; a single reserve globule and refringent body is present in *P. marsipium*. Drechsler (1941) suggests the term unitary for oospores of this latter type. *P. proliferum*, the only other species in this group possessing unitary oospores with an inspissate wall, exhibits sporangia and antheridia of quite different appearance. The utriform sporangia of *P. marsipium* alone would serve to separate this species from all the congeners listed above, including *P. nagaii* and *P. proliferum*. Whereas the antheridia of *P. proliferum* are either monoclinous, diclinous or hypogynal, those of *P. marsipium* are typically diclinous, rarely monoclinous and never hypogynal. The antheridial cell of *P. marsipium* more closely resembles that of *P. dissotocum* than any other, being inflated, short, crooked, with a broad apex which is closely appressed to the oogonial wall, the longitudinal axis of the antheridium coincident with the radius of the oogonium. The mono- and diclinous antheridial cells of *P. proliferum* are not as broad and stout, usually crooked, more clavate and make moderate to narrow apical contact with the oogonial wall.

Pythium marsipium is at present known only from decayed leaves of *Nymphaea tuberosa* Paine, collected in Wisconsin, U. S. A.

34. Pythium polytylum Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 1.9 to 8.0 μ; appressoria usually abundant, curved, swollen, clavate, 6 to 8 μ in diameter at the apex. Sporangia subspherical, usually 28 to 33 μ in diameter, exclusive of the papilla present during resting stage, typically acrogenous, though also intercalary, and laterally intercalary, proliferous; sometimes the apical sporangium assumes a lateral position by the resumption of growth of the supporting filament; zoospores 10 to 35, longitudinally grooved, reniform, laterally biciliate, upon encystment measuring 9.5 to 11.5 μ in diameter. Oogonia subspherical, sometimes irregularly so due to protruding toward antheridium, smooth- and thin-walled, acrogenous, sometimes lateral and sessile on parent hypha, measuring 26 to 40 μ, average 32.6 μ, in diameter. Antheridia typically diclinous, sometimes monoclinous, and then originating at least 60 μ below the oogonium, 1 to 4, mostly 1 or 2 per oogonium, lateral and sessile, but typically terminal on branches 5 to 80 μ, average 25 μ, long, antheridial stalk rarely exhibiting helicoid involvements, antheridial cells curved, elongate—cylindrical, frequently wavy in profile, 15 to 40 μ, average 30 μ long and 5 to 7.5 μ, average 6 μ, wide, the entire length usually intimately applied to the oogonium, the ferlization tube arising from the navel position. Oospores aplerotic, single, measuring 25 to 33 μ, average 28.8 μ, in diameter, with a wall 2.1 to 3.4 μ, average 2.6 μ, thick, containing 6 to 20 reserve globules and 4 to 8 or more refringent bodies; germination not observed.

Originally isolated from *Prunella vulgaris* L., in the United States.

A single isolation obtained by the writer from roots of spinach, *Spinacea oleracea* L., is referable to this species.

Pythium polytylum is one of four species having several characteristics in common, namely oospores containing several reserve globules, thick oospore walls, cylindrical, inflated antheridia, and papillate sporangia.

The morphological details of *Pythium polytylum* are adequately presented above.

Pythium polytylum is distinguished from its congeners, *P. helicoides*, *P. oedochilum*, and *P. palingenes* in possessing a spherical, papillate sporangium, whereas the other species have ovoid to obovoid papillate sporangia. The sporangia of *P. polytylum* are proliferous though they do not proliferate as readily as those of *P. palingenes* and *P. helicoides*.

The antheridia of *Pythium polytylum* are usually diclinous, the antheridial cell typically elongate, cylindrical and irregular in outline; the navel position of the fertilization tube is common to this species and *P. helicoides*, *P. oedochilum*, and *P. palingenes*. The antheridial stalk is rarely helicoid about the oogonial stalk, not as in *P. helicoides*. The antheridium, oogonium and oospore are of little value in segregating the species from related forms.

The fungus is known only from roots of *Prunella vulgaris* L. and *Spinacea oleracea* L. in the United States.

35. PYTHIUM HELICOIDES Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 4 to 9.5 μ in diameter, appressoria usually present, clavate, measuring 6 to 8 μ near the apex. Sporangia typically obovoid, sometimes subspherical, papillate, acrogenous, on long hyphae, often in racemose or cymoid arrangement, the sporangia sometimes lateral from prolongation of the supporting element, proliferous, measuring 9 to 40 μ, average 28 μ, in width, and 17 to 45 μ, average 31 μ, long, excluding the papilla which is about 4 μ long and 6 μ at the base; zoospores 2 to 40, longitudinally grooved, reniform, laterally biciliate, measuring 10 to 15 μ, average 12.3 μ, in diameter upon encystment, capable of repeated emergence. Oogonia subspherical, sometimes broadly protruding toward the antheridium, smooth-walled, typically acrogenous though often laterally sessile, measuring 26 to 40 μ, average 33 μ, in diameter. Antheridia diclinous, 1 to 4, occasionally 2 antheridia borne on a single branch, the antheridial cell elongated, curved, cylindrical, regular in contour, measuring 20 to 42 μ long and 6 to 9 μ wide, intimately applied to the oogonial wall and producing a fertilization tube from the navel position; a part of the antheridial branch or branches or a lateral prolongation of the branch wound about the oogonial stalk in 2 to 4 close helical turns. Oospores aplerotic, measuring 21 to 32 μ, average 27.5 μ, in diameter, provided with a thick wall measuring 2.5 to 3.2 μ, containing 6 to 20 reserve globules and 2 to 4 refringent bodies.

Originally described from *Phaseolus vulgaris* L. in the United States.

Four isolates of this fungus were obtained by the writer; these cultures were used as the basis for the following discussion.

The sporangia of *Pythium helicoides* are very similar to those of *P. oedochilum* and *P. palingenes* and only serve to distinguish this group of three species from *P. polytylum*.

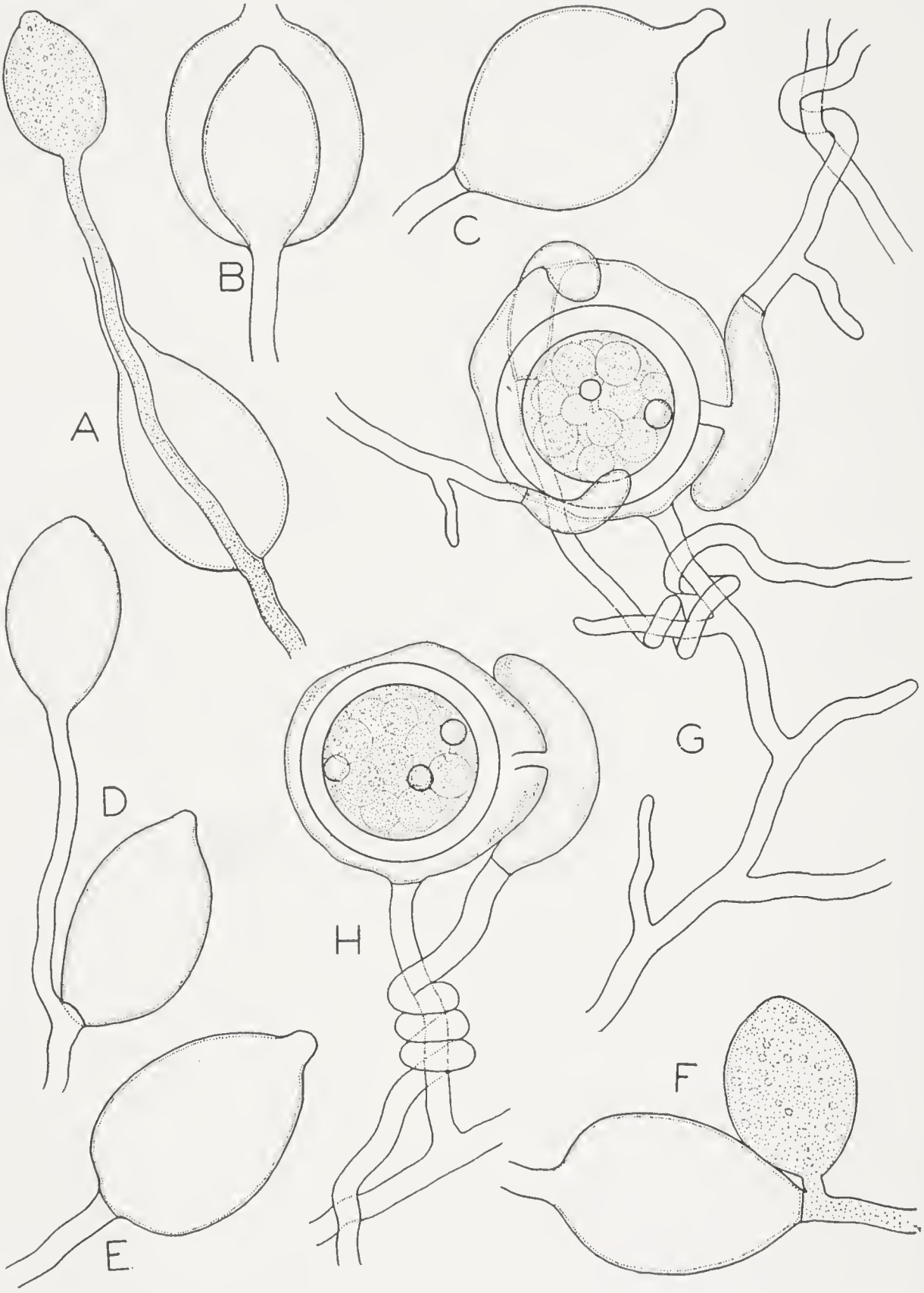

FIG. 6. *Pythium helicoides*. *A–F*, Sporangia. *A*, *B*, Two types of proliferation with production of secondary sporangia. *C*, Sporangium with normal papilla. *E*, Sporangium with elongated and distorted papilla. *D*, *F*, Production of secondary sporangia from the sporangiophore at the base of the primary sporangium. *G*, *H*, Sexual apparatus. Oospores with plural reserve globules and refringent bodies. Note the navel position of the antheridial fertilization tube.

Drechsler (1939) recently has given an account of *Pythium helicoides* which is supplementary to the original description of the species. He points out the similarity of the sexual stages of *P. helicoides*, *P. oedochilum*, *P. palingenes*, and *P. polytylum* but does not indicate the differences. The distinctive features by which this species may be identified, in the writer's opinion, are given below.

The antheridium of *Pythium helicoides* is like that of *P. volutum* in being regularly applied about the oogonial stalk in several helicoid turns. The antheridial cell is cylindrical, elongate, curved and regular in contour, the fertilization tube arising in a navel position. The regular, smooth and unfurrowed character of the antheridial cell distinguishes this species from *P. oedochilum* and *P. palingenes*. Although the coiling of the antheridial branch about the oogonial stalk is commonly observed in *P. helicoides*, it cannot be used as a valid taxonomic feature, for helicoidally disposed antheridial branches are occasionally observed in *P. oedochilum* and *P. palingenes*.

The repetitional emergence of zoospores reported in this species by Drechsler is also occasionally observed in *Pythium palingenes* and *P. aphanidermatum*.

The maximum temperature for mycelial development of *Pythium helicoides* is higher than that of *P. oedochilum;* temperature values for *P. palingenes* are lacking.

Pythium helicoides has been isolated from *Citrullus vulgaris* Schrad. by Drechsler (1939), from *Phaseolus vulgaris* L., by Drechsler (1930a) and the author, from *Pisum sativum* L. and *Spinacea oleracea* L. by the author, all in the United States.

36. PYTHIUM OEDOCHILUM Drechsler, Jour. Wash. Acad. Sci. **20**: 398–418. 1930.

Hyphae measuring 1.8 to 6.5 μ; appressoria sparingly produced as swollen, clavate, curved bodies measuring 5 to 7 μ at their apices. Sporangia ovoid to obovoid, subspherical, typically acrogenous, sometimes later in a lateral position due to continued growth of the sporangiophore, rarely intercalary, proliferous, smooth-walled, papillate, the papilla 6 to 8 μ long and same at base, sporangia measuring 17 to 42 μ, average 30 μ, in width and 25 to 48 μ, average 35 μ, in length; zoospores 10 to 35, longitudinally grooved, reniform and laterally biciliate, measuring 11 to 15 μ in diameter upon encystment. Oogonia subspherical, often with protruding wall toward antheridium, typically terminal, occasionally intercalary or laterally sessile, measuring 19 to 39 μ, average 31.5 μ, in diameter, provided with a stout, smooth wall. Antheridia usually diclinous, when monoclinous arising in excess of 40 μ from the oogonium, 1 to 4, usually 1 to 2, per oogonium, the curved, elongated, cylindrical, wavy contoured antheridial cells, usually borne terminally on branches not over 50 μ long; infrequently lateral and sessile, involvement of the oogonial stalk by the antheridium rare, though the oogonium is intimately attached by the antheridium usually over its entire length and producing a fertilization tube a bit forward of the navel position. Oospores aplerotic, single, measuring 16 to 34μ, average 28.1 μ, in diameter, with a

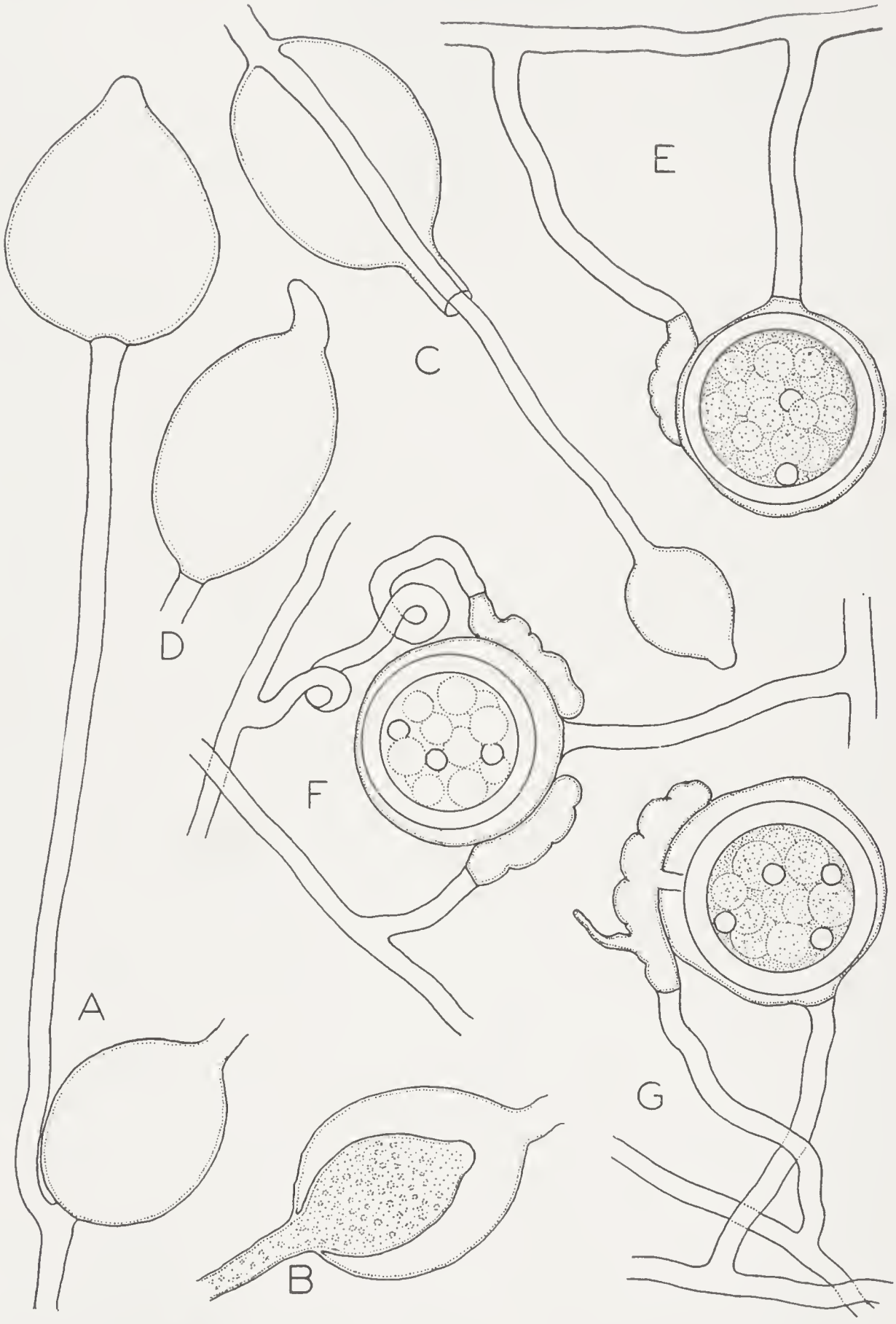

FIG. 7. *Pythium oedochilum*. *A–D*, Sporangia. *A*, Secondary sporangium borne on sporangiophore originating from base of primary sporangium. *B*, Secondary sporangium produced within the primary. *C*, Secondary sporangium produced outside the primary. *D*, Sporangium with skewed papilla. *E–G*, Sexual apparatus.

thick, smooth wall measuring 1.8 to 3.6 μ, average 2.5 μ, and containing 5 to 20 reserve globules and 3 to 20 refringent bodies.

Originally isolated from roots of *Dahlia* sp., United States.

Pythium oedochilum exhibits similarities to *P. polytylum*, *P. helicoides*, and *P. palingenes*, both in its asexual and sexual stages. The similarity is most marked in the structure of the oogonium, antheridium, and oospore. However, there are certain differences which permit segregation of the species. *P. oedochilum* differs from *P. polytylum* in having an ovoid to obovoid, papillate sporangium while that of *P. polytylum* is spherical and papillate. The elongate, cylindrical, curved antheridium of irregular, wavy contour of *P. oedochilum* is common to *P. polytylum* and *P. palingenes* but distinguishes *P. oedochilum* from *P. helicoides*, in which the antheridium is obviously regular and unfurrowed in outline. The oogonia of *P. oedochilum* are acrogenous and very rarely laterally sessile while those of *P. palingenes* are typically laterally and tangentially sessile, very rarely acrogenous. Although both *P. oedochilum* and *P. palingenes* have proliferous sporangia, they are tardily proliferous in the former and promptly in the latter.

The temperature values for mycelial development of *Pythium oedochilum* differ from those of *P. helicoides* and may be used as an adjunct to their differentiation: maximum temperature for growth of *P. oedochilum* is 40° C. and for *P. helicoides* is 43° C.

Pythium oedochilum has been isolated from *Bidens aristosa* (Michx.) Britton by Drechsler (1941), from *Citrullus vulgaris* Schrad. fruits by Drechsler (1939) and the author, from *Dahlia* sp., later said to be *D. rosea* Cav. (1941), by Drechsler (1930a), from roots of *Daphne odora* Thunb. and *Matthiola incana* R Br. by the author, all in the United States.

37. PYTHIUM PALINGENES Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 2 to 7 μ in diameter; appressoria produced in moderate abundance, measuring 5.5 to 7.5 μ at the apex. Sporangia usually ovoid, sometimes subspherical, typically terminal, occasionally lateral due to prolongation of sporangiophore, rarely intercalary, proliferous, measuring 24 to 42 μ, average 33 μ, in length and 18 to 36 μ, average 29 μ, in width, excluding the sessile apical papilla approximately 6 μ wide at base and 4 μ long; zoospores 6 to 30, longitudinally grooved, laterally biciliate, reniform, measuring 11 to 17 μ, average 14 μ, in diameter upon encystment, exhibiting repetitional development. Oogonia subspherical, frequently protruding toward the antheridium, typically terminal on short lateral branches or sessile, laterally and tangentially intercalary, occasionally terminal on branches up to 75 μ or more, measuring 19 to 41, average 34 μ, in diameter. Antheridia typically diclinous, rarely monoclinous, 1 to 4, usually 2, per oogonium, the curved, elongated, cylindrical, irregularly contoured antheridial cells borne terminally on branches 5 to 50 μ long, sometimes lateral and sessile, often a portion of the antheridial branch coiled 2 or 3 times about the oogonial stalk, the antheridial cells measuring 20 to 28 μ long and 6 to 8 μ wide, usually closely applied their entire length to the oogonium, the fertilization tube originating forward of the navel position. Oospores aplerotic, measuring 18

to 37 μ, average 31.3 μ, in diameter, with a thick wall, 1.5 to 3.5 μ, average 2.6 μ and containing 5 to 30 reserve globules and 3 to 5 refringent bodies.

Originally described from roots of *Ambrosia trifida* L., in the United States.

No cultures of *Pythium palingenes* were observed by the writer, the following brief discussion being based on the original description and a subsequent paper by Drechsler (1941). *P. palingenes* is very closely related to *P. oedochilum* and its allies, *P. helicoides* and *P. polytylum*.

The ovoid to obovoid papillate sporangia of *Pythium palingenes* readily separates this species from *P. polytylum*, which has spherical papillate sporangia. The antheridial character, antheridial cell elongate, cylindrical, curved and irregular in outline, distinguishes *P. palingenes* from *P. helicoides* but not from *P. oedochilum*. The presence of predominantly acrogenous oogonia rather than laterally and tangentially intercalary oogonia segregates *P. oedochilum* from *P. palingenes*.

The numerous proliferous sporangia of *Pythium palingenes* are sharply contrasted with the only occasional proliferous sporangia of *P. oedochilum*. The repetitional emergence of zoospores without an intervening vegetative phase further distinguishes *P. palingenes* from *P. oedochilum*.

The presence of a single reserve globule in the oospore of *Pythium proliferum* in contrast with a plurality of reserve globules in *P. palingenes* makes confusion of the two species impossible. *P. palingenes* is distinguishable from *P. nagaii* in the character of the oogonial wall, inspissate in the former and not inspissate in the latter.

This species is only known through the two reports of Drechsler (1930a, 1941) stating its isolation from *Ambrosia trifida* L. in the United States.

38. Pythium nagaii Ito & Tokunaga, Jour. Fac. Agr. Hokkaido Imper. Univ. **32**: 201–233. 1933.

Hyphae measuring 1.5 to 4.0 μ in diameter. Sporangia spherical, ovoid or pyriform, proliferous, measuring 24 to 36 μ long by 20 to 26 μ wide; zoospores few to 25, formed in a spherical vesicle borne on short, apical evacuation tube, measuring 8.2 to 9.6 μ in diameter upon encystment, germinating by a tenuous hypha. Oogonia usually spherical, occasionally irregular in contour, measuring 14 to 22 μ in diameter. Antheridia monoclinous, single, delimited from the oogonial stalk by a septum, ovoid, round or clavate, more or less curved. Oospore aplerotic, typically single, spherical, measuring 12 to 19 μ in diameter, with a very thin oospore wall, approximately 0.8 μ thick containing a single reserve globule; germination not observed.

Originally described from seedlings of *Oryza sativa* L., Japan.

Two cultures of this fungus were observed by the writer, one from Baarn, the other from E. C. Tullis, Arkansas, and identified by V. D. Matthews. The cultures agreed with the delineation of the fungus by Ito and Tokunaga.

The only similarity of *Pythium nagaii* to *P. helicoides*, *P. marsipium*, *P. oedochilum*, *P. palingenes*, *P. polytylum*, and *P. proliferum* is the proliferous

nature of the sporangium and the aplerotic oospore. In the absence of the sexual apparatus positive identification cannot be assured although distinction between *P. nagaii* and all but *P. proliferum* is possible due to the difference in size of sporangia. The presence of utriform sporangia would preclude confusion with *P. marsipium*. As suggested by Drechsler (1941), instances in which proliferous sporangial species have been observed and referred to *P. proliferum* in the absence of any observations on the sexual phase may be of doubtful validity, inasmuch as there are a number of proliferous sporangial species very similar in the asexual stage but dissimilar in the sexual stage. The very thin oospore wall of *P. nagaii* immediately enables distinction from *P. proliferum* and all the other aplerotic oospore species with proliferous sporangia.

Though *Pythium nagaii* is morphologically separable from *P. helicoides* and *P. oedochilum*, the temperature-growth relation may be used as an adjunct to specific segregation.

This species is reported from *Oryza sativa* L. in Japan by Ito and Tokunaga, Darker (1940) and in the United States by Edson and Wood (1937).

39. Pythium anguillulae-aceti Sadebeck, Bot. Centralb. **29**: 318. 1887.

Hyphae fine. Sporangia, conidia and oogonia formed simultaneously. Sporangia rare, conidia numerous, produced in chains or in clusters of 4 to 5, spherical, about 6 μ in diameter, often deciduous; zoospores rarely formed. Oogonia numerous, very small, up to 6 μ in diameter. Oospores spherical, small, largest about 6 μ in diameter, germinating by germ tubes.

Parasitic on vinegar eelworm, *Anguillula aceti*, Germany.

This is a little known species, apparently not collected since its description.

The species may be identified by its extremely small apandrous oospores and oogonia. The conidia referred to in the description may be considered sporangia which germinate by the production of germ tubes rather than zoospores. The species is further distinguished by the catenulate arrangement of its sporangia.

40. Pythium pulchrum von Minden, Mykol. Untersuch. Ber. R. Falck **1**: 146–255. 1916.

Pythium epigynum Höhnk, Mycologia **24**: 489–507. 1932.

Hyphae measuring 1.5 to 7.5 μ in diameter. Sporangia spherical, elliptical or pyriform, terminal or intercalary, measuring 24 to 48 μ, average 38.2 μ, in diameter, occasionally catenulate, 2 to 4 in a series; zoospores few to 30 or more, formed in vesicle arising from an evacuation tube mesially or occasionally laterally situated, reniform, laterally biciliate, measuring 11 to 16 μ in diameter upon encystment. Oogonia more or less spherical, terminal or intercalary, sometimes 2 to 5 in a series, measuring 19.6 to 37.8 μ, average 28.3 μ, in diameter. Antheridia hypogynous, monoclinous or diclinous, 1 to 2 per oogonium, when monoclinous often sessile or a short lateral protrusion

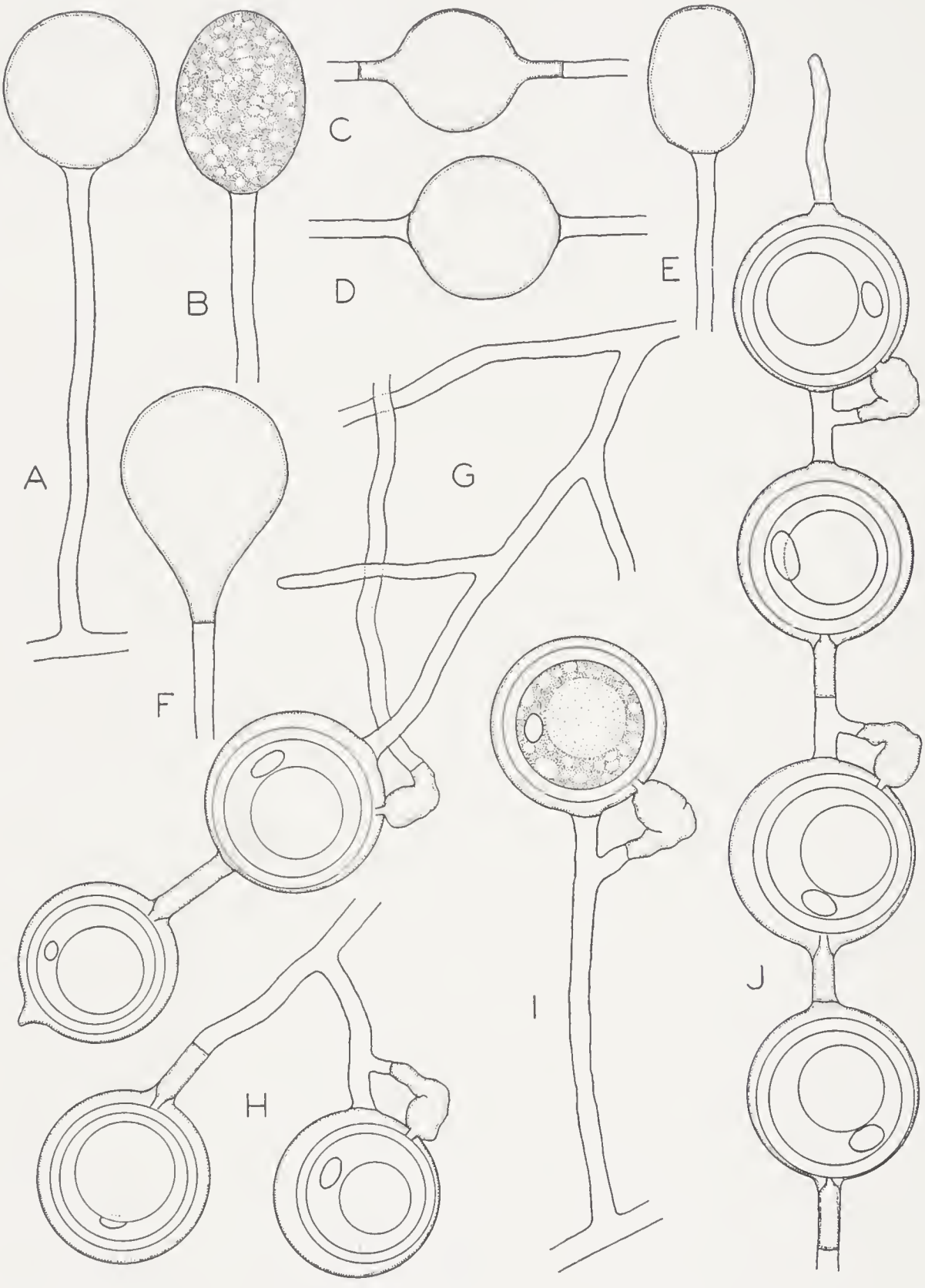

FIG. 8. *Pythium pulchrum*. *A–F*, Sporangia. *G–J*, Sexual apparatus. *G*, Two oogonia, one fertilized by a hypogynous antheridium, the other by a diclinous one. *J*, Catenulate oogonia with hypogynous and monoclinous antheridia.

originating immediately below the oogonium and delimited by a basal septum, then usually as long as it is broad, sometimes on a somewhat longer branch, the antheridial cell only slightly swollen, clavate, making moderate oogonial contact. Oospore aplerotic, single, measuring 13.8 to 31.1 μ, average 24.6 μ, in diameter, with a moderately thick wall, containing a single reserve globule and refringent body.

Originally described as saprophytic on ant eggs, Germany.

Pythium pulchrum is unique among non-proliferous sporangial types with aplerotic oospores in having a hypogynous antheridial cell. Von Minden reports that the species possesses monoclinous as well as hypogynous antheridia and that the hypogynous type is the more common. The observations of the writer are in agreement with those of von Minden.

Additional distinction is afforded *Pythium pulchrum* by its sporangia which are frequently arranged in chains of 2 to 5; no catenulate sporangia were observed in *P. polymorphon*, *P. ultimum*, *P. paroecandrum*, *P. splendens*, and *P. debaryanum*.

Pythium epigynum cannot be considered distinct from *P. pulchrum* despite the remarks of its author. The difference in the sizes of the sporangia, oogonia, and oospores of these two species are insufficiently different to permit specific segregation; both organisms have similarly shaped asexual and sexual bodies. The sporangia of these species are both borne in chains; both have aplerotic oospores. The absence of monoclinous or diclinous antheridia in *P. epigynum* cannot be used as a basis for separation from *P. pulchrum* for certain strains of *P. pulchrum* have been observed which failed to produce other than hypogynous antheridia.

Pythium pulchrum is known from animal debris in Germany by von Minden, from *Antirrhinum majus* L., *Callistephus chinensis* Nees and *Persea americana* Mill. by the author, from *Phaseolus vulgaris* L., *Pisum sativum* L., *Zea mays* L. and grass (unidentified) through inoculation by Höhnk (1932), from *Medicago sativa* L., by Buchholtz and Meredith (1938), from soil by Höhnk (1932, 1933), Matthews (1931) and Meredith (1938), all in the United States.

41. PYTHIUM POLYMORPHON Sideris, Mycologia **24**: 14–61. 1932.

Hyphae more or less uniform, measuring 4 to 8 μ in diameter. Sporangia spherical, subspherical or elliptical, terminal or intercalary, 20 to 40 μ, average 29.3 μ, in diameter, zoospores infrequently produced, the sporangia usually germinating by means of germ tubes. Oogonia spherical, terminal or intercalary, smooth to almost papillate, and thin-walled, measuring 13.2 to 29.8 μ (Sideris = 20 to 22 μ), average 22.1 μ, in diameter. Antheridia mono- or diclinous, 1 to 3 per oogonium, the antheridial stalk of variable length but typically falcate or sigmoid, the antheridial cell clavate, not greatly swollen nor curved, 8 to 12 μ long by 6 to 7.5 μ wide. Oospores aplerotic, single, measuring 10.3 to 25.1 μ (Sideris = 17 to 20 μ), average 18.2 μ, in diameter, with a smooth, unthickened wall.

Originally described as parasitic on *Ananas comosus* Merr., Hawaii.

Pythium polymorphon is one of the few species of *Pythium* which may be readily identified solely by the character of the antheridial branch, which is relatively long, originating either from the oogonial stalk or from a dissociated hypha, varying in length from approximately 50 to 180 μ, usually about 90 μ, and is falcate or sometimes sigmoid in shape. The antheridial cell is allantoid-clavate, the apex narrowly applied to the oogonium in the upper or lower equatorial plane.

The oogonium is similar to that of *Pythium debaryanum* in both size and shape. Occasionally, however, a few oogonia were observed which were of somewhat irregular contour, the irregularities more or less papillate; the latter type of oogonium resembles that infrequently observed in *P. irregulare*. Sideris reports that the oospores are sometimes echinulate. All observations made by the writer in an attempt to find echinulate oospores were unsuccessful. It is concluded that the echinulation mentioned by Sideris is more correctly referred to the oogonium than to the oospores.

In addition to the host from which the species was originally described, Sideris reports receiving a culture of the fungus from *Nicotiana tabacum* L. from the United States.

42. Pythium ultimum Trow, Ann. Bot. **15**: 269–312. 1901.

Hyphae long and slender, 1.7 to 6.5 μ, average 3.8 μ, in diameter, branched, septate only in old cultures, aerial growth luxuriant. Sporangia chiefly terminal and spherical, from 12 to 28 μ, average 20 μ, in diameter, occasionally intercalary and barrel-shaped, 14 to 17 μ, to 22.9 by 27.8 μ, in size, germinating only by germ tubes. Oogonia smooth, terminal, spherical, rarely intercalary, 19.6 to 22.9 μ, average 20.6 μ, in diameter. Antheridia usually 1 per oogonium, monoclinous from immediately below the oogonium, curved, sometimes 2 per oogonium, then often of diclinous origin and straight. Oospores aplerotic, single, spherical, 14.7 to 18.3 μ, average 16.3 μ, in diameter, with a smooth, thick wall, containing a single central reserve globule and refringent body.

Saprophytic on boiled potato tubers, house flies, cabbage leaves and other vegetable and animal substrata, England.

Despite the frequent occurrence of *Pythium ultimum* as a parasite on a large variety of hosts and the frequent isolation of this pathogen, few reports are available concerning the morphology of the species.

The report of van Luijk (1934a) is fairly typical of the treatment by many mycologists who prefer to consider *P. ultimum* a synonym of *P. debaryanum*. Van Luijk contends the differences between the two species are insufficient to warrant their retention as valid species. On the contrary Drechsler (1927) presents arguments in favor of the maintenance of the two binomials as distinct species. The writer concurs with Drechsler's opinion.

Pythium ultimum is distinguished from *P. debaryanum* by its typically monoclinous antheridia. The antheridium of *P. ultimum* originates immediately adjacent to the oogonium, is swollen, sharply curved upwards,

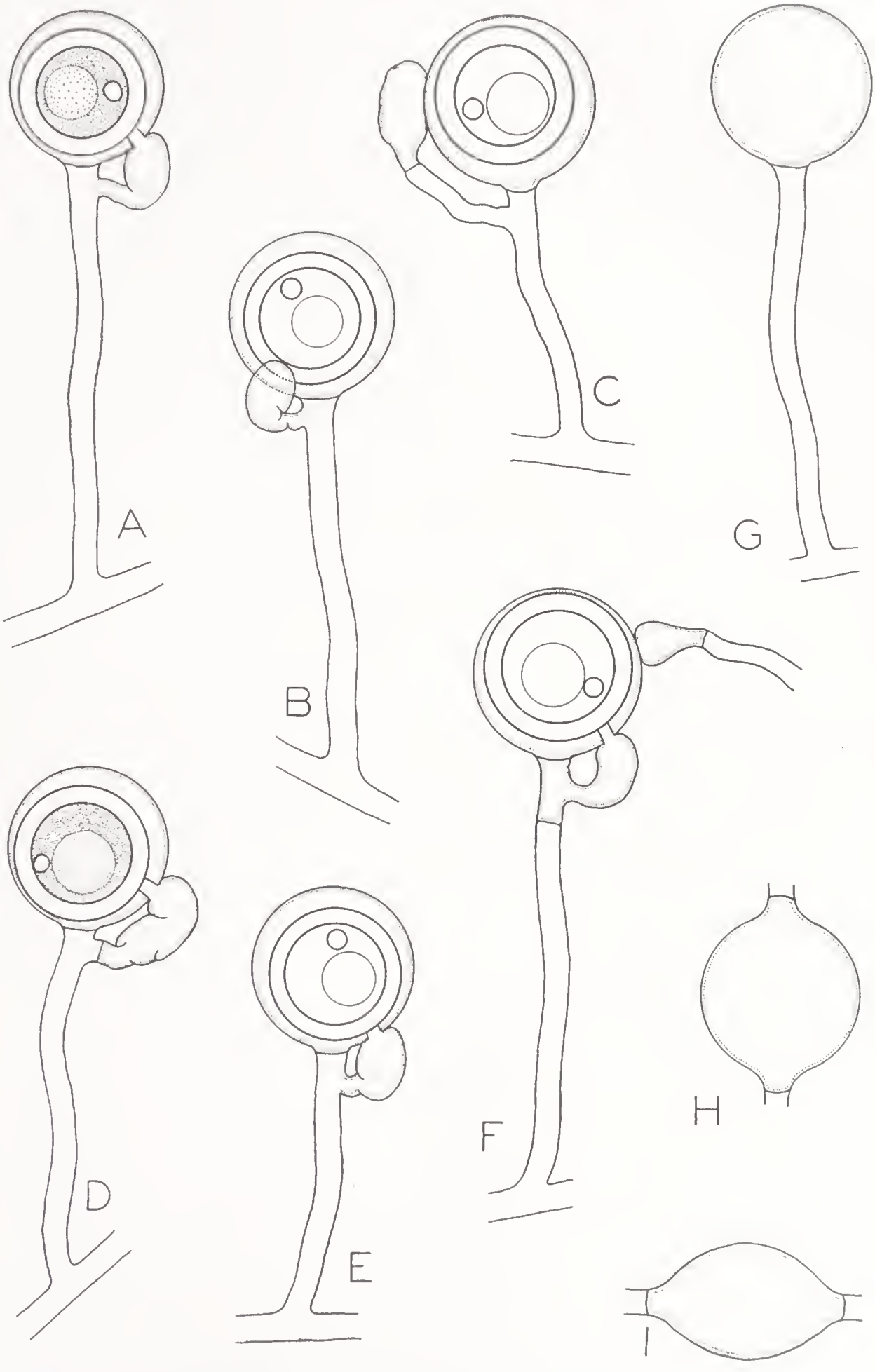

FIG. 9. *Pythium ultimum*. *A–F*, Sexual apparatus. *A–E*, Typical oogonia and oospores with monoclinous antheridium. *F*, Oogonium with both monoclinous and diclinous antheridia. *G–I*, Sporangia. *G*, Typical acrogenous sporangium. *H*, *I*, Intercalary sporangia.

making narrow apical contact with the basal portion of the oogonial wall, rarely delimited by a septum. The infrequent diclinous antheridia are similar to a variety of species and are not distinctive enough to be utilized in the identification of this species. The antheridium of *P. debaryanum*, although frequently monoclinous, never originates immediately adjacent to the oogonium and is never swollen or sharply upcurved. There is usually only a single antheridium in *P. ultimum*, while 2 to 6 are not uncommon in *P. debaryanum*, and the majority are diclinous.

There are also some differences in the oospores of *Pythium ultimum* and *P. debaryanum*. In *P. ultimum* the oospore wall is usually at least 2.0 μ thick, while the wall of *P. debaryanum* is somewhat thinner, not exceeding 1.5 μ in thickness. Drechsler (1927) reports that the reserve globule is smaller and that the refringent body is subspherical in *P. ultimum*, and that the reserve globule is larger and the refringent body oblate ellipsoidal in *P. debaryanum*. The absence of zoospores in *P. ultimum* may also distinguish the species, though no great degree of significance should be attached to this point.

Pythium ultimum may be segregated from *P. paroecandrum* on the basis of its antheridia. (Refer to *P. paroecandrum*.)

The growth habit and temperature values for *Pythium ultimum* are of no value in distinguishing this species from *P. debaryanum*.

Pythium ultimum is perhaps the most common member of the genus in the United States and frequently reported throughout the world; the following table presents the hosts and distribution of the species:

Achimines grandiflora DC. United States: Author.
Agropyron caninum (L.) Beauv. United States: Fischer *et al.* (1942).
A. cristatum (L.) Gaertn. United States: Fischer *et al.* (1942).
A. desertorum (Fisch.) Schult. United States: Fischer *et al.* (1942).
A. repens (L.) Beauv. United States: Fischer *et al.* (1942).
A. semicostatum (Steud.) Nees. United States: Sprague.
A. smithii Rydb. United States: Sprague.
Agrostis alba L. United States: Fischer *et al.* (1942).
Allium cepa L. United States: Tompkins *et al.* (1939).
Aloe variegata L. Union South Africa: Wager (1940). United States: Author.
Andropogon furcatus Muhl. United States: Fischer *et al.* (1942).
Antirrhinum majus L. United States: Middleton (1938); Nance (1939).
Apium graveolens L. var. *dulce* DC. United States: Alexander, Young and Kiger (1931); Author.
Arachis hypogea L. Union South Africa: Wager (1931, 1932a, 1940). United States: Wood and Nance (1938).
Arctotis stoechadifolia Berg. United States: Author.
Atropa belladonna L. United States: Middleton (1941a).
Azalea obtusum Planch. United States: Middleton.
Begonia lloydii (Hort. name). United States: Middleton (1938, 1942); Nance (1939).
B. semperflorens Link and Otto. United States: Maneval (1940); Middleton (1938); Middleton, Tucker and Tompkins (1938); Nance (1939).
B. tuberhybrida Voss. United States: Middleton (1938, 1942); Nance (1939).
Bellis perennis L. United States: Author.
Beta vulgaris L. United States: Poole (1934b); Wood and Nance (1938); Author.
B. vulgaris var. *crassa* Alef. Canada: Jones (1935).
B. vulgaris var. *macrorhiza*, Stev. Canada: Jones (1935). United States: Alexander, Young and Kiger (1931); Leach (1939); Author.

Boronia megastigma Ness. UNITED STATES: Author.
Bouteloua curtipendula (Michx.) Torr. UNITED STATES: Author.
Brassica campestris L. var. *napo-brassica*. UNITED STATES: Tompkins *et al.* (1939).
B. oleracea L. ENGLAND: Trow (1901). UNION SOUTH AFRICA: Wager (1932a, 1940). UNITED STATES: Drechsler (1925b, 1927); Harter and Whitney (1927a); Walker (1927); Author.
B. rapa L. UNITED STATES: Poole (1934b); Tompkins *et al.* (1939).
Bromus carinatus Hook and Arn. UNITED STATES: Sprague.
B. erectus Huds. UNITED STATES: Sprague.
B. inermis Leyss. UNITED STATES: Fischer *et al.* (1942).
Calceolaria crenatiflora Cav. UNITED STATES: Author.
Calendula officinalis L. UNITED STATES: Author.
Callistephus chinensis Nees. UNION SOUTH AFRICA: Wager (1931, 1932a, 1932c, 1940). UNITED STATES: Middleton (1938); Nance (1939).
Campanula medium L. UNITED STATES: Author.
Capsicum annuum L. UNITED STATES: Tompkins *et al.* (1939); Author.
Carludovica palmata Ruiz and Pav. PHILIPPINE ISLANDS: Roldan (1939).
Carica papaya L. AUSTRALIA: Simmonds (1937). RHODESIA: Hopkins (1939b). UNION SOUTH AFRICA: Doidge and Bottomley (1931); Wager (1931, 1932a, 1940). UNITED STATES: Author.
Chamaecyparis lawsoniana Parl. UNITED STATES: Author.
Cicer arietinum L. UNITED STATES: Middleton (1938); Nance (1939).
Citrullus vulgaris Schrad. UNITED STATES: Drechsler (1939); Middleton (1938); Nance (1939); Tompkins *et al.* (1939).
Citrus aurantium L. UNITED STATES: Author.
C. limonia Osbeck. UNITED STATES: Tompkins *et al.* (1939); Author.
C. sinensis Osbeck. UNION SOUTH AFRICA: Wager (1940). UNITED STATES: Tompkins *et al.* (1939); Author.
Clarkia elegans Dougl. RHODESIA: Hopkins (1939b).
Colchicum byzantium Ker-Gawl. ENGLAND: Moore (1940b).
C. speciosum Stev. var. *album*. ENGLAND: Moore (1940b).
Coleus sp. RHODESIA: Hopkins (1939b).
Cucumis melo L. UNITED STATES: Author.
C. melo var. *inodoratus* Naud. UNITED STATES: Tompkins *et al.* (1939).
C. melo var. *reticulatus* Naud. UNITED STATES: Tompkins *et al.* (1939).
Cucumis sativus L. UNITED STATES: Tompkins *et al.* (1939); Author.
Cucurbita maxima Duchesne. UNITED STATES: Tompkins *et al.* (1939).
C. pepo L. UNITED STATES: Tompkins *et al.* (1939).
C. pepo var. *condensa* Bailey. UNITED STATES: Middleton (1938); Nance (1939); Tompkins *et al.* (1939).
Cupressus sp. RHODESIA: Hopkins (1931, 1939b).
Cyphomandra betacea Sendt. UNITED STATES: Author.
Dahlia sp. UNION SOUTH AFRICA: Wager (1932a, 1940).
Daucus carota L. var. *sativa* DC. UNITED STATES: Tompkins *et al.* (1939); Author.
Delosperma sp. UNION SOUTH AFRICA: Wager (1940).
Delphinium sp. UNION SOUTH AFRICA: Wager (1940). UNITED STATES: Author.
D. ajacis L. UNITED STATES: Author.
D. cardinale Hook. UNITED STATES: Author.
D. consolida L. UNITED STATES: Author.
D. cultorum Voss. UNITED STATES: Author.
Dianthus barbatus L. UNITED STATES: Author.
D. carophyllus L. UNITED STATES: Author.
D. plumarius L. UNION SOUTH AFRICA: Wager (1940). UNITED STATES: Author.
Dimorphotheca aurantiaca DC. UNITED STATES: Author.
Dinteranthus microspermis. UNION SOUTH AFRICA: Wager (1940).
Dioscorea batatas Decne. FRANCE: Foex, Dufrenoy and Labrousse (1931); Labrousse (1931b).
Duvalia parviflora. UNION SOUTH AFRICA: Wager (1940).
Echinochloa crus-galli (L.) Beauv. UNITED STATES: Sprague.
Elymus virginicus L. UNITED STATES: Sprague.
Euphorbia pulcherrima Willd. UNITED STATES: Middleton (1938); Nance (1939).

Fenestraria aurantiaca N. E. Brown. UNION SOUTH AFRICA: Wager (1940).
Fragaria chiloensis Duchesne. UNITED STATES: Author.
Fuchsia sp. UNITED STATES: Author.
Gaillardia aristata Pursh. UNITED STATES: Author.
Gilia sp. UNION SOUTH AFRICA: Wager (1940).
Gossypium sp. UNITED STATES: Arndt (1935).
Helipterum roseum Benth. UNITED STATES: Author.
Heuchera hispida Pursh. UNITED STATES: Author.
Hordeum vulgare L. UNITED STATES: Sprague; Author.
Ipomoea batatas Lam. UNION SOUTH AFRICA: Doidge and Bottomley (1931); Wager (1931, 1932a, 1940). UNITED STATES: Drechsler (1934); Edson and Wood (1936, 1937); Harter and Weimer (1929); Harter and Whitney (1927a, b); Nance (1939, 1940); Poole (1934a, b); Tompkins *et al.* (1939); Author.
Lactuca sativa L. UNITED STATES: Alexander, Young and Kiger (1931); Nance (1939, 1940); Owens (1939); Author.
Lathyrus odoratus L. UNION SOUTH AFRICA: Wager (1940). UNITED STATES: Guba (1936); Author.
Lepidium sativum L. ENGLAND: Trow (1901).
Lilium longiflorum Thunb. UNITED STATES: Author.
Lupinus sp. UNITED STATES: Author.
Lycopersicon esculentum Mill. ENGLAND: Vanterpool. NEW ZEALAND: Brien and Chamberlain (1936); Chamberlain and Brien (1937); Brien (1939). UNION SOUTH AFRICA: Wager (1940). UNITED STATES: Alexander, Young and Kiger (1931); Horsfall (1932a, b); Tompkins *et al.* (1939); Wilson and Tilford (1933); Author.
Malus sylvestris Mill. UNITED STATES: Tompkins *et al.* (1939).
Medicago sativa L. UNITED STATES: Buchholtz and Meredith (1938); Middleton (1938); Nance (1939).
Mesembryanthemum sp. UNION SOUTH AFRICA: Wager (1940).
Nasturtium sp. UNION SOUTH AFRICA: Wager (1932a, 1940).
Nicotiana biglovii S. Wats. var. *quadrivalvis*. UNITED STATES: Author.
N. tabacum L. RHODESIA: Hopkins (1939a). UNION SOUTH AFRICA: Wager (1940). UNITED STATES: Alexander, Young and Kiger (1931); Author.
N. trigonophylla Dun. UNITED STATES: Author.
Papaver nudicaule L. UNION SOUTH AFRICA: Doidge and Bottomley (1931); Wager (1931, 1932a, 1940). UNITED STATES: Author.
Pastinaca sativa L. UNITED STATES: Tompkins *et al.* (1939).
Pelargonium domesticum Bailey. UNITED STATES: Gill (1936).
P. graveolens L'Hér. UNITED STATES: Gill (1936).
P. hortorum Bailey. UNITED STATES: Gill (1936).
P. zonale L'Hér. CANADA: Bisby *et al.* (1938).
Persea americana Mill. UNITED STATES: Wager (1942a); Author.
Phaseolus aureus Roxb. UNITED STATES: Author.
P. vulgaris L. UNION SOUTH AFRICA: Wager (1932a, 1940). UNITED STATES: Harter and Whitney (1927a, 1927b); Middleton (1938); Nance (1939); Poole (1934b); Tompkins *et al.* (1939).
Phragmites communis Trin. UNITED STATES: Fischer *et al.* (1942).
Picea engelmanni Engelm. UNITED STATES: Rathbun-Gravatt (1931).
Pinus sp. PHILIPPINE ISLANDS: Roldan (1939). RHODESIA: Hopkins (1939a). UNION SOUTH AFRICA: Wager (1940).
P. aristata Engelm. UNITED STATES: Jackson (1940).
P. banksiana Lamb. UNITED STATES: Fischer (1941); Rathbun-Gravatt (1931).
P. resinosa Ait. UNITED STATES: Rathbun-Gravatt (1931); Author.
P. sylvestris L. UNITED STATES: Author.
Pisum sativum L. UNION SOUTH AFRICA: Wager (1932a, 1940). ENGLAND: Ogilvie, Croxall and Hickman (1940); Ogilvie and Hickman (1937); Baylis (1941). UNITED STATES: Parrot (1940); Author.
Poa ampla Merr. UNITED STATES: Fischer *et al.* (1942).
P. canbyi (Scribn.) Piper. UNITED STATES: Sprague.
P. secunda Presl. UNITED STATES: Fischer *et al.* (1942).

Primula malachoides Franch. UNITED STATES: Author.
P. obconica Hance. UNITED STATES: Author.
P. sinensis Lindl. UNITED STATES: Author.
Pseudotsuga taxifolia Britt. UNITED STATES: Jackson (1940); Weiss (1942).
Ranunculus asiaticus L. UNITED STATES: Tompkins and Middleton (1939, 1942).
Raphanus sativus L. UNITED STATES: Poole (1934b).
Rheum rhaponticum L. UNION SOUTH AFRICA: Wager (1931, 1932a, 1940). UNITED STATES: Middleton (1938, 1941b).
Ricinus communis L. UNITED STATES: Author.
Saccharum officinarum L. UNITED STATES: Rands (1930); Rands and Dopp (1938b); Stevenson and Rands (1938).
Saintpaulia ioanthe Wendl. UNITED STATES: Author.
Senecio cruentus DC. UNITED STATES: Author.
Sinningia speciosa Benth. and Hook. UNITED STATES: Author.
Soil. UNITED STATES: Meredith (1938, 1940).
Solanum melongena L. UNITED STATES: Tompkins *et al.* (1939).
S. tuberosum L. CANADA: Jones (1935). ENGLAND: Pethybridge and Smith (1930); Trow (1901). NEW ZEALAND: Brien (1939, 1940). UNION SOUTH AFRICA: Wager (1940). UNITED STATES: Poole (1934b); Tompkins *et al.* (1939); Author.
Sorghum vulgare Pers. UNITED STATES: Author.
Spinacea oleracea L. FRANCE: Dufrenoy (1931); Foex (1931, 1935); Foex, Dufrenoy and Labrousse (1931); Labrousse (1931a, 1931b, 1933). GERMANY: Fabel (1931). UNITED STATES: Nance (1940); Pirone *et al.* (1933); Author.
Stapelia sp. UNION SOUTH AFRICA: Wager (1940).
Streptocarpus sp. UNITED STATES: Author.
Striga lutea Lour. UNION SOUTH AFRICA: Wager (1931, 1940).
Tagetes erecta L. UNITED STATES: Author.
T. patula L. UNITED STATES: Middleton (1938); Nance (1939).
Tavaresia sp. UNION SOUTH AFRICA: Wager (1940).
Triticum aestivum L. UNITED STATES: Sprague; Author.
Tulipa gesneriana L. DENMARK: Moore and Buddin (1937). HOLLAND: Moore and Buddin (1937).
Ulmus americana L. UNITED STATES: Wright (1941).
U. pumila L. UNITED STATES: Author.
Vigna sinensis Endl. UNITED STATES: Middleton (1938); Nance (1939, 1940).
Viola tricolor L. UNITED STATES: Author.
Zea mays L. UNITED STATES: Sprague; Author.

Numerous other plants are reported as susceptible when grown in naturally infested soil inhabited by other soil fungi as well as by *Pythium ultimum;* for this reason these hosts are not listed. These hosts may be found by referring to the contributions of Haenseler (1935), Heuberger and Horsfall (1939), Horsfall (1932a, 1932b, 1934, 1935, 1938), Horsfall, Newhall and Guterman (1934), Kadow and Anderson (1937), Ogilvie and Hickman (1938), Pirone, Newhall, Stuart, Horsfall and Harrison (1933) and Seaver *et al.* (1932).

43. PYTHIUM PAROECANDRUM Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 2.7 to 9 μ in diameter; appressoria moderately abundant, apices curved and clavate, measuring 8 to 11 μ wide. Sporangia subspherical to prolate ellipsoidal, typically intercalary, occasionally terminal on either long or short laterals, when subspherical measuring 12 to 33 μ, average 22.8 μ in diameter, when ellipsoidal measuring from 16 μ long and 12 μ wide to 41 μ long and 36 μ wide, average 30.1 μ long and 23.0 μ wide;

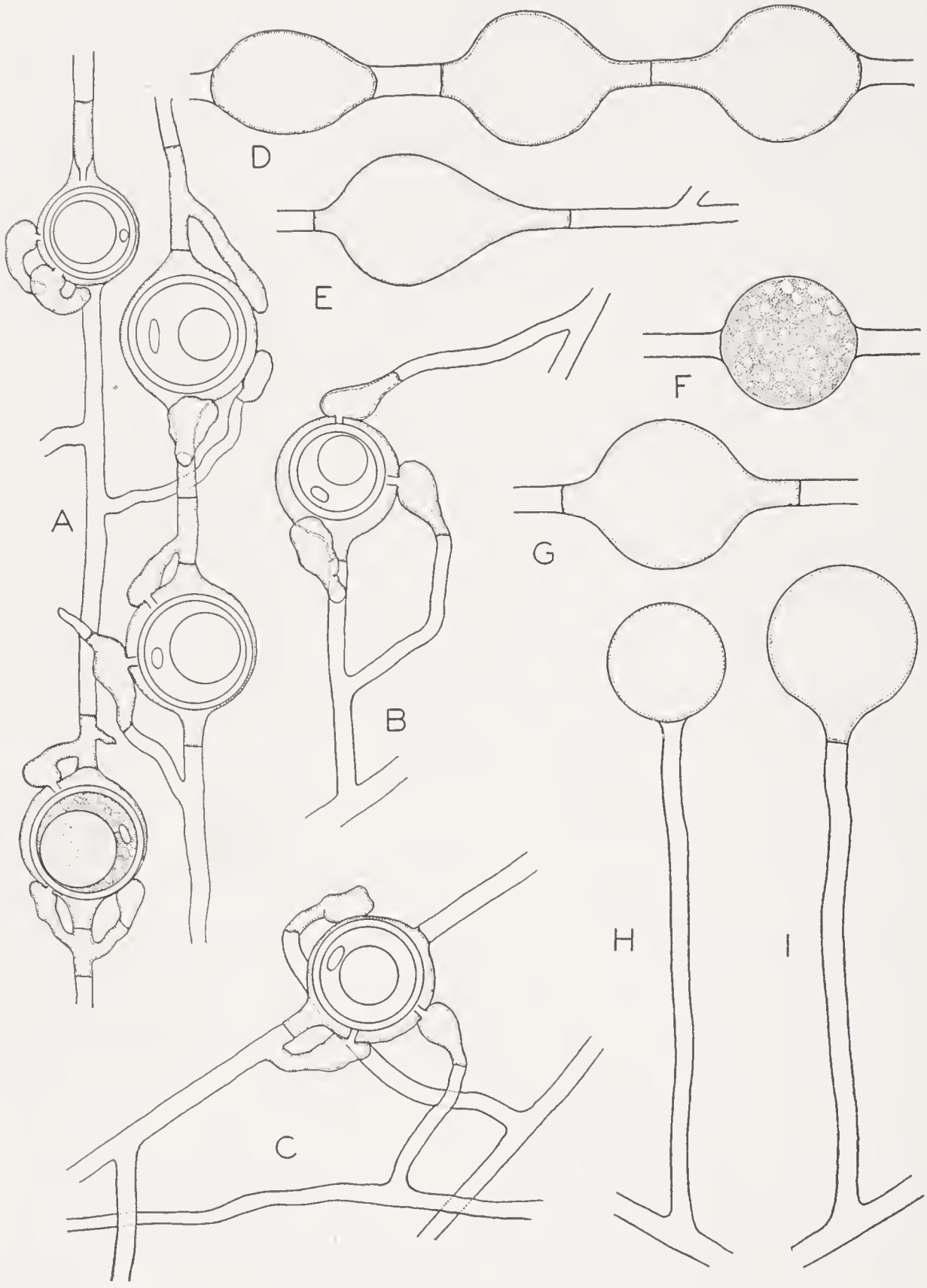

FIG. 10. *Pythium paroecandrum*. *A–C*, Sexual apparatus. *D–J*, Sporangia.

zoospores 3 to 25, biciliate, reniform, measuring 9 to 11 μ upon encystment. Oogonia subspherical, often including a portion of the supporting element on one end if acrogenous, both if intercalary, typically intercalary, occasionally acrogenous, measuring 11 to 28 μ, average 21.4 μ, in diameter, smooth- and thin-walled. Antheridia mono- and diclinous, 1 to 5 per oogonium, when monoclinous often undifferentiated and adjacent to oogonium, 7 to 15 μ long and 4 to 7 μ wide, sometimes swollen or together with a bulbous lateral outgrowth, sessile, and pouch-like or crook-necked, measuring 7 to 15 μ long and 6 to 8 μ wide, sometimes including an intercalary segment adjacent to the oogonium together with a proximal or distal cylindrical portion, sometimes two antheridia comprising one functional unit; when diclinous usually terminal, rarely intercalary, inflated, crook-necked, the antheridial cell 10 to 20 μ long and 6 to 8 μ wide. Oospores aplerotic, with a moderately thin wall, 1.1 to 1.5 μ, average 1.3 μ, thick, containing a single reserve globule and refringent body, oospore measuring 10 to 22 μ, average 18.3 μ, in diameter.

Originally described from roots of *Allium vineale* L., in the United States.

The asexual stage of *Pythium paroecandrum* is analogous with those of *P. debaryanum*, *P. irregulare*, *P. mamillatum*, and *P. ultimum*. The sexual stage of *P. paroecandrum* is, however, quite different from the species cited, with the exception of *P. ultimum*. Drechsler (1940) reports that the sexual stage of *P. paroecandrum* is similar to and yet distinct from that of *P. rostratum*, *P. pulchrum*, and *P. piperinum*. These species may be related, but the differences are sufficiently great to obviate any confusion among them. The oospore of *P. rostratum* is plerotic in contrast with the aplerotic condition in *P. paroecandrum*. The typical hypogynous antheridium readily separates *P. pulchrum* from the monoclinous antheridium of *P. paroecandrum*. *P. piperinum* is considered synonymous with *P. vexans* and exhibits antheridia strikingly different than those of *P. paroecandrum*.

Pythium paroecandrum and *P. ultimum* are quite similar and are most likely to be confused. The antheridium in both species is typically sessile, rarely stalked, and originates from the oogonial stalk immediately below the oogonium. Both species may have antheridia either mono- or diclinous but they are predominantly monoclinous. The typically single, monoclinous antheridium of *P. ultimum* is short, swollen, sharply hooked upward with the apex applied rather narrowly to the lower equatorial plane of the oogonium, usually in close proximity to the oogonial stalk. The antheridia of *P. paroecandrum* are also monoclinous but usually plural, with 2 to 5, usually 3, applied to an oogonium. The antheridial cell is of various shapes, usually either outwardly undifferentiated or somewhat saccate or swollen, neither uniformly inflated nor sharply hooked upwards and, though usually applied to the lower equatorial plane of the oogonium, not regularly placed in close proximity to the oogonial stalk.

The non-inspissate oospore wall of *Pythium paroecandrum* approximately 1.3 μ in thickness, further distinguishes this species from *P. ultimum* which has an inspissate wall about 2.2 μ thick.

Drechsler originally reported this species in the United States from *Allium vineale* L.; subsequently he reported it from *Impatiens pallida* Nutt. and *Sanguinaria canadensis* L. (1940); the author has obtained this fungus from *Aloe ciliaris* Haw. and *A. variegata* L., in the United States.

44. PYTHIUM SPLENDENS Braun, Jour. Agr. Res. **30**: 1043–1062. 1925.

Pythium splendens var. *hawaiianum* Sideris, Mycologia **24**: 14–63. 1932.

Hyphae measuring 3.5 to 9.2 μ, average 6.4 μ in diameter; falcate appressoria sometimes present. Sporangia spherical, acrogenous, smooth- and thin-walled, measuring 21.7 μ to 48.9 μ, average 36.2 μ, in diameter, usually dark in color on most nutrient substrates, usually displaying 1 to 2 light colored globules; zoospores rarely formed, germination usually through production of 1 to 6 germ tubes. Oogonia spherical, terminal, smooth- and thin-walled, measuring 25.5 to 34.7 μ, average 31.7 μ, in diameter. Antheridia mono- and diclinous, 1 to 8 per oogonium, clavate and crook-necked, making moderate oogonial contact. Oospores aplerotic, measuring 21.3 to 29.8 μ, average 26.6 μ, in diameter, supplied with a thick wall.

Originally described as parasitic on cuttings of *Pelargonium* sp., United States.

Pythium splendens is one of the few species of *Pythium* that may be identified solely on the basis of its sporangial characteristics. This is fortunate because the sexual stage is rarely produced in the standard culture media employed in this study.

The sporangia are spherical, acrogenous and large, measuring 22 to 49 μ, average 36 μ, in diameter, with a thin, smooth wall. The contents of the sporangium are characteristically dense, finely granular and dark in color, usually containing 1 to 3 small, hyaline globules. Sideris (1932) reports that the sporangia are spherical, subspherical and ellipsoid, mostly terminal and occasionally intercalary. Spherical sporangia were the only kind commonly observed by the writer and intercalary sporangia were seen only very rarely.

The writer concurs with Braun's description of the sexual stage and remarks concerning the temperature relations of the species.

A variety of *Pythium splendens* described by Sideris as *P. splendens* var. *hawaiianum* because it differed from the species ". . . in the stronger aerial mycelial development and the size of the conidia, which measure on an average of about 5 μ more in diameter" . . . is considered insufficiently distinct from the species to warrant its establishment as a variety. The temperature-growth response and growth habit are considered additional evidence of its synonymity with the species.

The hosts affected by *Pythium splendens* and their geographic distribution are given below:

Ananas comosus Merr. HAWAII: Parris (1940); Sideris (1932); Sideris and Paxton (1929, 1931).

Begonia sp. MALAYA: Sharples (1930). UNITED STATES: Braun (1925).

B. semperflorens Link and Otto. UNITED STATES: Maneval (1940); Middleton, Tucker and Tompkins (1938, 1942).

Cajanus cajan Millsp. HAWAII: Parris (1940); Sideris (1932).
Canavalia ensiformis DC. HAWAII: Parris (1940); Sideris (1932).
Carica papaya L. UNION SOUTH AFRICA: Wager (1931, 1932a, 1940).
Chrysanthemum sp. NETHERLANDS: Buisman (1927).
Citrus aurantium L. UNITED STATES: Author.
Coleus sp. UNITED STATES: Braun (1925); Author.
Cucumis sativus L. UNITED STATES: Braun (1925).
Geranium sp. NETHERLANDS: Buisman (1927).
Helianthus annuus L. HAWAII: Parris (1940; Sideris (1932).
Hordeum vulgare L. UNITED STATES: Author.
Ipomoea batatas Lam. HAWAII: Parris (1940); Sideris (1932). UNITED STATES: Harter and Whitney (1927b).
Linum usitatissimum L. NETHERLANDS: Diddens (1932).
Medicago sativa L. UNITED STATES: Buchholtz and Meredith (1938).
Nicotiana tabacum L. NETHERLANDS: Meurs (1928).
Pelargonium sp. UNITED STATES: Braun (1925).
P. zonale L'Hér. UNITED STATES: Dodge and Swift (1932).
Phaseolus aureus Roxb. HAWAII: Parris (1940); Sideris (1932).
P. vulgaris L. UNITED STATES: Harter and Whitney (1927a).
Piper betle L. MALAYA: Thompson (1939).
Raphanus sativus L. UNITED STATES: Braun (1925).
Saccharum officinarum L. HAWAII: Parris (1940); Sideris (1932); Stevenson and Rands (1938).
Triticum aestivum L. HAWAII: Parris (1940); Sideris (1932).
Vicia faba L. HAWAII: Parris (1940); Sideris (1932).
Vigna sinensis Endl. HAWAII: Parris (1940); Sideris (1932).

45. PYTHIUM VEXANS de Bary, Jour. Bot. 14: 105–126. 1876.

Pythium complectens Braun, Jour. Agr. Res. 29: 399–419. 1924.
Pythium allantocladon Sideris, Mycologia 24: 14–63. 1932.
Pythium ascophallon Sideris, l.c.
Pythium euthyhyphon Sideris, l.c.
Pythium polycladon Sideris, l.c.
Pythium piperinum Dastur, Proc. Indian Acad. Sci. 1: 778–815. 1935.

Mycelium fine, branched, with the hyphae tapering at the tip. Sporangia terminal or intercalary, pyriform, ovoid, subspherical to spherical, 17 to 24 μ, average 21 μ, in diameter, usually germinating by germ tubes, more rarely by zoospores. Oogonia spherical, smooth usually terminal on short lateral branches, occasionally inserted on a broad base, varying from 15 to 28 μ, average about 22 μ, in diameter. Antheridia usually 1, rarely 2, monoclinous, rarely diclinous and never hypogynous. Oospores aplerotic, smooth measuring 11 to 23 μ, average about 19 μ, in diameter, germinating by germ tubes or zoospores.

Originally described as saprophytic on tubers of *Solanum tuberosum* L., Germany.

Pythium vexans is readily distinguished from its congeners possessing aplerotic oospores by its typically monoclinous, stalked antheridium which arises in close proximity to the oogonium and by the clavate, swollen antheridial cell with the apex bell-shaped and broadly applied, sometimes fused with the oogonial wall.

A number of species are considered by the writer to be synonymous with *Pythium vexans*. Drechsler (1938) recently indicated the possible synonymity

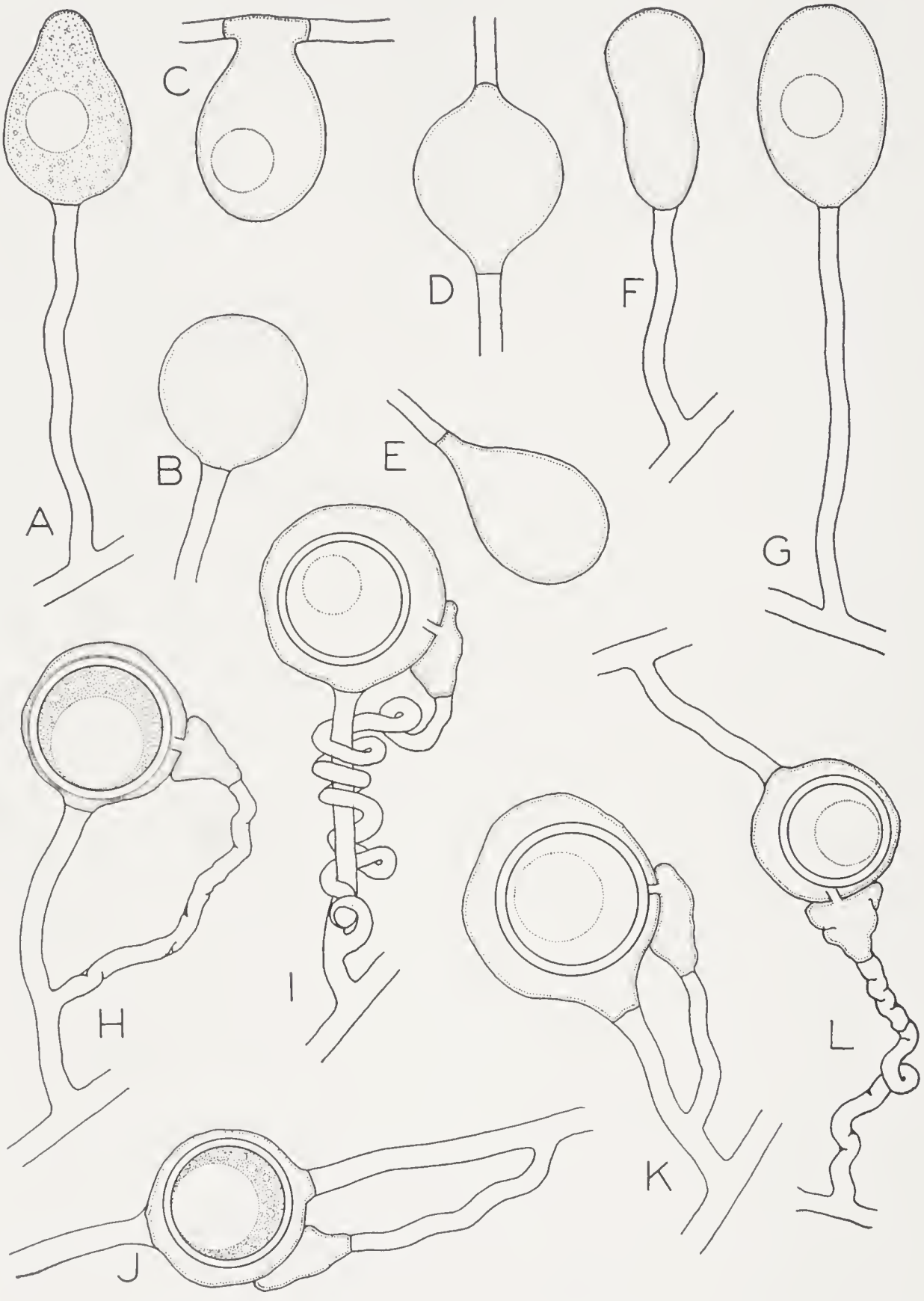

FIG. 11. *Pythium vexans*. *A–G*, Sporangia. *H–L*, Sexual apparatus. *H*, *J*, *K*, Typical structures. *I*, *L*, Structures with coiled or irregularly oriented antheridial branches occasionally observed.

of *P. complectens* and *P. vexans* but did not present evidence in support of his contention. His observation, however, is in agreement with that of the writer.

The species which are believed to be synonymous with *Pythium vexans* are *P. complectens*, *P. allantocladon*, *P. ascophallon*, *P. euthyhyphon*, *P. polycladon*, and *P. piperinum*. The oogonia and oospores of these species are similar in size, shape and position on the mycelium. A comparison of range and average diameters of their oogonia and oospores is presented below:

Name	*Oogonia* (μ)	*Oospores* (μ)
P. vexans	15–28 av. 22	11–23 av. 19
P. complectens	13–23 av. 19	11–21 av. 16
P. allantocladon	18–24 av. 21	10–20 av. 16
P. ascophallon	16–25 av. 21	12–18 av. 16
P. euthyhyphon	18–26 av. 20	12–21 av. 18
P. polycladon	av. 20	av. 20
P. piperinum	15–28 av. 21	13–20 av. 18

The sporangia of these strains are likewise indistinguishable. The antheridia of all except *Pythium piperinum* are similar and identical with those of *P. vexans*. The antheridium of *P. piperinum* resembles the type of antheridium described by Butler (1907) for his *P. vexans*. In this case the antheridial branch is multicellular and, though originating from the oogonial stalk, follows an irregular course. The supposedly multiple antheridia of *P. piperinum* are none other than the cells of the antheridial stalk which are intimately associated with the oogonium and were confused with the true antheridium.

The growth habits and temperature-growth relations of the several species considered synonymous with *Pythium vexans* are similar and are given here as additional evidence of their similarity to and synonymity with *P. vexans*.

Hosts for *Pythium vexans* are given below:

Agropyron inerme (Scribn. and Sm.) Rydb. UNITED STATES: Sprague.
A. smithii Rydb. UNITED STATES: Sprague.
Ananas comosus Merr. HAWAII: Parris (1940); Sideris (1932).
Antirrhinum majus L. UNITED STATES: Author.
Armeria sp. UNION SOUTH AFRICA: Wager (1940).
Arrhenatherum elatius (L.) Mert. and Koch. UNITED STATES: Sprague.
Avena sativa L. UNITED STATES: Sprague.
Bromus inermis Leyss. UNITED STATES: Sprague.
Carica papaya L. HAWAII: Parris (1940); Sideris (1932). UNION SOUTH AFRICA: Wager (1932a, 1940).
Citrus aurantium L. UNITED STATES: Wager (1942b); Author.
C. limonia Osbeck. UNITED STATES: Wager (1942b); Author.
Coleus sp. UNITED STATES: Braun (1924).
Delphinium ajacis L. UNITED STATES: Middleton (1938); Nance (1939).
Dianthus caryophyllus L. MALAYA: Thompson (1939). UNITED STATES: Author.

Durio zebethinus. MALAYA: Thompson (1938, 1939).
Echinochloa crus-galli (L.) Beauv. UNITED STATES: Sprague.
Elymus canadensis L. UNITED STATES: Sprague.
E. glaucus Buckl. UNITED STATES: Sprague.
Festuca ovina L. UNITED STATES: Sprague.
Geum chiloense Balb. UNITED STATES: Author.
Godetia grandiflora Lindl. UNITED STATES: Author.
Hevea brasiliensis Muell. INDIO-CHINA: Anonymous (1941). MALAYA: Thompson (1929).
Hordeum bulbosum L. UNITED STATES: Sprague.
Linum usitatissimum L. NETHERLANDS: Diddens (1932).
Lupinus albus L. GERMANY: Schultz (1939).
Matthiola incana R. Br. UNITED STATES: Middleton (1938); Nance (1939).
Medicago sativa L. GERMANY: Schultz (1939).
Nemesia strumosa Benth. UNITED STATES: Author.
Nicotiana tabacum L. (?) JAVA: Raciborski (1900).
Pelargonium sp. UGANDA: Hansford (1938). UNITED STATES: Braun (1924); Author.
Persea americana Mill. UNITED STATES: Wager (1942a); Author.
Phragmites communis Trin. UNITED STATES: Sprague.
Piper betle L. INDIA: Dastur (1935).
P. longa INDIA: Dastur (1935).
Poa nevadensis Vasey. UNITED STATES: Sprague.
P. palustris L. UNITED STATES: Sprague.
Ricinus communis L. HAWAII: Parris (1940); Sideris (1932).
Saccharum officinarum L. UNITED STATES: Rands (1930); Rands and Dopp (1938b); Stevenson and Rands (1938).
Soil. ENGLAND: Butler (1907). IRELAND: Butler (1907). FRANCE: Butler (1907). UNITED STATES: Meredith (1940); Author.
Solanum tuberosum L. GERMANY: de Bary (1876, 1881b); Frank (1881). INDIA (?): Cunningham (1897).
Thea sinensis L. MALAYA: Thompson (1936).

46. PYTHIUM DEBARYANUM Hesse, Inaugr. Dissert. Halle. 1874.

Pythium equiseti Sadebeck, Verhandl. Bot. Ver. Prov. Brand. **16**: 116–124. 1874.
Lucidium pythiodes Lohde, Tagebl. Versamm. Deuts. Naturf. Aerzte Breslau **47**: 203. 1874.
Pythium autumnale Sadebeck, Tagebl. Versamm. Deutsch. Naturf. Aerzte Breslau **49**: 100. 1876.
Artotrogus debaryanus Atkinson, Cornell Agr. Exp. Sta. Bull. **94**: 233–272. 1895.
Pythium haplomitrii Lilienfeld, Bull. Intern. Acad. Sci. Cracovie **1911**: 336. 1911.
Pythium debaryanum var. *pelargonii* Braun, Jour. Agr. Res. **30**: 1043–1062. 1925.
Pythium marchantiae Nicolas, Compt. Rend. Acad. Sci. **182**: 82–83. 1926.
Pythium fabae Cheney, Aust. Jour. Exper. Biol. Med. Sci. **10**: 143–155. 1932.
Pythium araiosporon Sideris, Mycologia **24**: 14–61. 1932.
Pythium cactacearum Preti, Riv. Pat. Veg. **26**: 331–353. 1936.

Hyphae branched, usually 5 μ in diameter, septate in old cultures. Sporangia spherical to oval, terminal or intercalary, 15 to 26 μ, average 19 μ, in diameter, germinating either by germ tubes or zoospores. Oogonia smooth, terminal or intercalary, usually spherical, 15 to 28 μ, average 21 μ, in diameter. Antheridia 1 to 6 per oogonium, monoclinous and diclinous, when monoclinous arising some distance below the oogonium, not adjacent to it. Oospores smooth, aplerotic, 12 to 20 μ, average 17 μ, in diameter, germinating by means of germ tubes.

Parasitic and saprophytic on a variety of plants.

Pythium debaryanum and *P. ultimum* are the species most often encountered. *P. debaryanum* is distinguished from *P. ultimum* by its plurality of

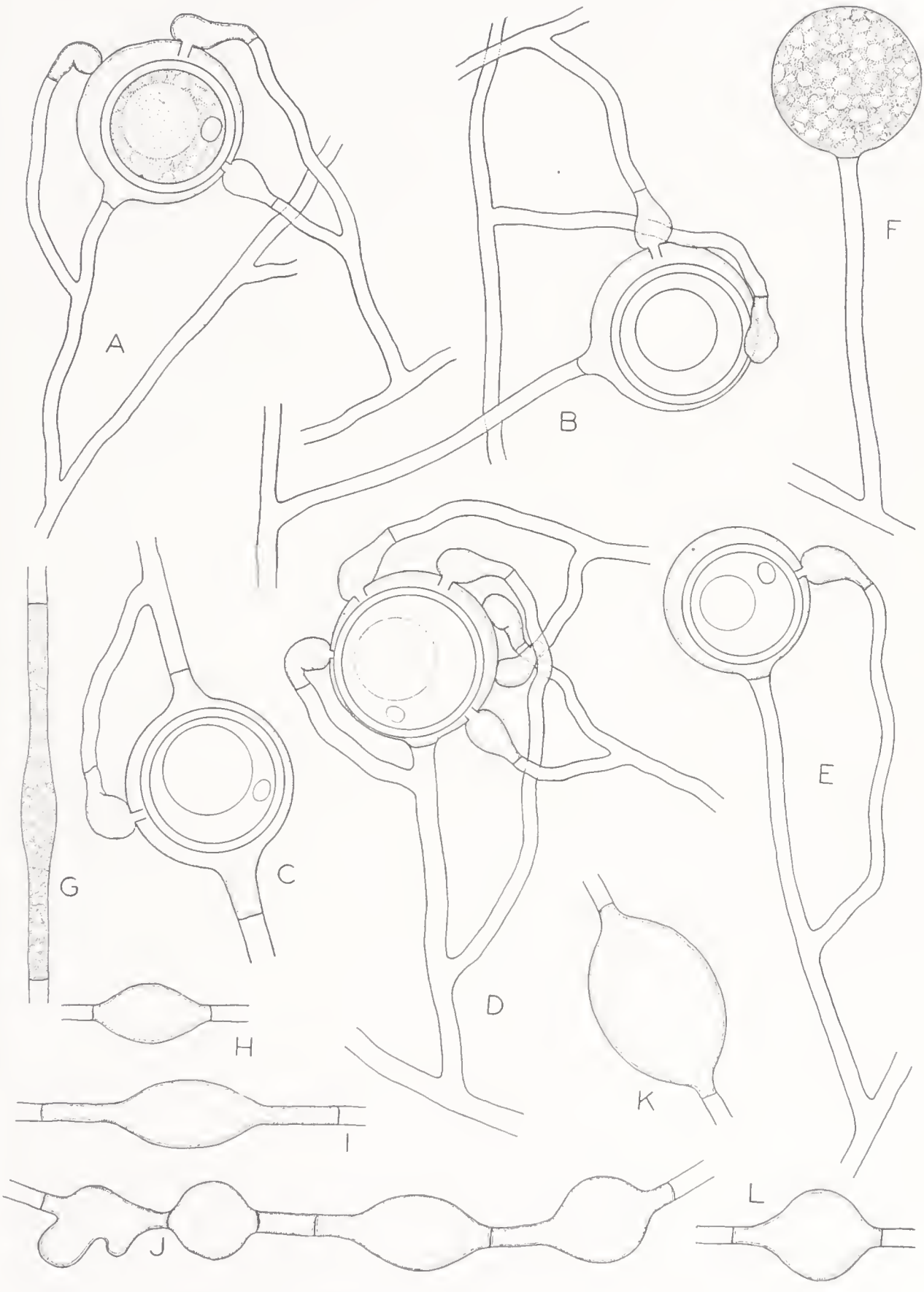

FIG. 12. *Pythium debaryanum*. *A–E*, Typical sexual stages. *F–L*, Sporangial types.

antheridia which are mono- or diclinous; when monoclinous they are stalked and originate some distance from the oogonium. *P. ultimum* usually has only a single antheridium which characteristically arises adjacent to the oogonium, is sessile and peculiarly shaped. Butler (1913) and Drechsler (1927) report similar observations on the antheridia of these two species. Van Luijk (1934b) believes the antheridial character insufficient for specific segregation, preferring to consider *P. ultimum* synonymous with *P. debaryanum.*

Pythium debaryanum may also be separated from *P. vexans* on the basis of antheridial differences. The antheridium of *P. debaryanum* has a narrow apex which is lightly applied to the oogonial wall, whereas the antheridium of *P. vexans* has a bell-shaped apex which is closely appressed and frequently fused with the oogonial wall. These two species differ in growth habits but not in temperature-growth relations.

Pythium araiosporon resembles *P. debaryanum* morphologically and in its growth habit and temperature values; it is considered synonymous with *P. debaryanum.*

Pythium fabae is indistinguishable morphologically from *P. debaryanum* and also resembles.it in its habit of growth and responses to cardinal temperatures.

Pythium debaryanum var. *pelargonii* is reported by Braun (1925) to differ from *P. debaryanum* in the occurrence of sporangia with greater variation in size, in the position of the antheridial branch which adheres to the oogonium, and the presence of irregular swollen intercalary bodies. None of these points is sufficiently distinctive to segregate this variety from the species; additional evidence of their similarity is supplied by their temperature-growth relations. This variety is considered synonymous with *P. debaryanum.*

Although *Pythium marchantiae* and *P. cactacearum* are not well known species, the descriptions indicate that they are very similar to *P. debaryanum* and should be considered synonymous.

The writer follows Butler (1907) and Matthews (1931) in listing the other synonymous species.

The hosts from which *Pythium debaryanum* is known are presented below:

Acer sp. BULGARIA: Christoff (1933).
Agropyron caninum (L.) Beauv. UNITED STATES: Fischer *et al.* (1942).
A. ciliare (Trin.) Franch. UNITED STATES: Fischer *et al.* (1942).
A. cristatum (L.) Gaertn. UNITED STATES: Buchholtz (1942b); Fischer *et al.* (1942).
A. desertorum (Fisch.) Schult. UNITED STATES: Fischer *et al.* (1942).
A. inerme (Scribn. and Sm.) Rydb. UNITED STATES: Sprague.
A. intermedium (Host.) Beauv. UNITED STATES: Sprague.
A. michnoi Roshev. UNITED STATES: Sprague.
A. repens (L.) Beauv. UNITED STATES: Fischer *et al.* (1942).
A. riparium Scribn. and Sm. UNITED STATES: Fischer *et al.* (1942).
A. semicostatum (Steud.) Nees UNITED STATES: Sprague.
A. sibiricum (Willd.) Beauv. UNITED STATES: Fischer *et al.* (1942).
A. smithii Rydb. UNITED STATES: Fischer *et al.* (1942).

A. trachycaulum (Lk.) Malte UNITED STATES: Sprague.
A. trichophorum (Link) Richt. UNITED STATES: Fischer *et al.* (1942).
Agrostis alba L. UNITED STATES: Fischer *et al.* (1942).
A. stolonifera L. NETHERLANDS: van Luijk (1934a).
Amaranthus sp. GERMANY: de Bary (1881b).
A. caudatus L. UNITED STATES: Edson and Wood (1937).
Ananas comosus Merr. HAWAII: Carpenter (1919); Parris (1940); Sideris (1932).
Andropogon furcatus Muhl. UNITED STATES: Fischer *et al.* (1942).
A. halli Hack. UNITED STATES: Fischer *et al.* (1942).
A. scoparius Michx. UNITED STATES: Fischer *et al.* (1942).
Antirrhinum majus L. RHODESIA: Hopkins (1941). UNITED STATES: Sprague (1942a).
Apium graveolens L. var. *dulce* DC. UNITED STATES: Edson and Wood (1936); Foster and Weber (1924). SWEDEN: Hammarlund (1933).
Aquilegia vulgaris L. NETHERLANDS: Meurs (1928).
Arabis alpina L. NETHERLANDS: Meurs (1928).
Arrhenatherum elatius (L.) Mert. UNITED STATES: Fischer *et al.* (1942).
Aster sp. (?) UNITED STATES: Sprague (1942a).
Atropa belladonna L. UNITED STATES: Middleton (1941a).
Avena nuda L. UNITED STATES: Sprague.
A. sativa L. DENMARK: Gram and Rostrup (1922a, 1924). NETHERLANDS: Brandenburg (1931a). UNITED STATES: Melhus, Reddy and Buchholtz (1939); Melhus *et al.* (1940); Nance (1940); Sprague (1942a); Welch (1940, 1942); Author.
Begonia sp. GERMANY: Flachs (1931); Pape (1927b, 1933). UNITED STATES: Braun (1925).
B. semperflorens Link and Otto. UNITED STATES: Maneval (1940); Middleton, Tucker and Tompkins (1938, 1942); Swift (1932).
Berberis gracilis Hartw. UNITED STATES: Middleton (1938); Nance (1939).
Beta vulgaris L.: AUSTRIA: Fischer (1931); Miestinger *et al.* (1932). BELGIUM: Decoux, Vanderwaeren and Roland (1935, 1936, 1938); Marchal (1929). BULGARIA: Savoff (1924). CZECHOSLOVAKIA: Stehlik (1934); Stehlik and Neuwirth (1929, 1931). DENMARK: Anonymous (1926a); Gram (1926, 1927a, 1927b, 1928, 1937); Gram and Rostrup (1922b, 1923, 1924); Rostrup (1895–6). ENGLAND: Beaumont (1937); Beaumont and Hodson (1931); Petherbridge and Stirrup (1935); Staniland and Beaumont (1938); Woodward and Dillon-Weston (1929). GERMANY: Anonymous (1924b, 1926c, 1927a, 1927b, 1927c, 1930, 1932, 1936c); Appel (1929); Barkhoff (1928); Busse, Peters and Ulrich (1911); Esmarch (1925); Gehring and Brothuhn (1922, 1924); Greis (1939); Gropp (1929); Hecke (1923); Korff and Böning (1930); Kuster (1927); Laske (1936); Lhode (1874); Molz (1927, 1929); Neuwirth (1933); Oberdorfer (1930, 1931); Otto (1930); Peters (1906, 1911, 1924); Plaut (1929); Schaffnit and Meyer-Hermann (1930); Schille (1939); Schmidt (1934); Stocklasa (1898); Voelkel and Klemm (1932). IRELAND: Gallagher (1929), Greeves and Muskett (1936); Hughes (1935); Murphy (1927). ITALY: Campanile (1923). NETHERLANDS: de Haan (1935); Meijer (1934); Meurs (1928); Verhoeven (1927). SCOTLAND: Foister (1941). SWEDEN: Arrhenius (1923); Hammarlund (1933). SWITZERLAND: Gaümann (1928). UNITED STATES: Brandes and Coons (1934); Buchanan (1935, 1938); Buchholtz (1938); Campbell (1939); Clinton (1916); Coons (1924); Coons and Stewart (1927); Doran (1936); Edson (1913, 1915a, 1915b); Edson and Wood (1936); Hartwell and Damon (1914); Johnson (1914a); La Clerg (1937, 1939); Nance (1940); Rumbold (1924); Stewart (1931); Young (1940); Author.
Bouteloua curtipendula (Michx.) Torr. UNITED STATES: Fischer *et al.* (1942).
B. gracilis (H.B.K.) Lag. UNITED STATES: Fischer *et al.* (1942).
Brassica caulorapa Pasq. UNITED STATES: Johnson (1914a).
B. juncea (L.) Coss. PHILIPPINE ISLANDS: Ramos (1926).
B. napus L. GERMANY: Klemm (1938).
B. oleracea L. var. *botrytis* L. DENMARK: Jorgenson (1933). GERMANY: Kryopoulous (1916). RUSSIA: Bubentzoff (1935). UNITED STATES: Johnson (1914a); Author.
B. oleracea var. *capitata* L. DENMARK: Ferdinandsen (1923). GERMANY: Nicolaisen (1925). PHILIPPINE ISLANDS: Ramos (1926). PUERTO RICO: Cook (1939). SWEDEN: Hammarlund (1933). UNITED STATES: Drechsler (1926); Hoffman (1912); Johnson (1914a); Walker (1927); Author.

B. oleracea var. *gemmifera* Zenker. Canada: Godbout (1930).
B. pekinensis Rupr. Philippine Islands: Ramos (1926).
B. rapa L. Germany: Klemm (1938). Philippine Islands: Ramos (1926). United States: Johnson (1914a).
Bromus arvensis L. United States: Fischer *et al.* (1942).
B. carinatus Hesse. United States: Sprague.
B. erectus Huds. United States: Sprague.
B. inermis Leyss. United States: Fischer *et al.* (1942).
B. japonicus Thurb. United States: Sprague.
Cajanus cajan Millsp. Hawaii: Parris (1940); Sideris (1932).
Camelina sativa L. Germany: Frank (1881); Hesse (1874); Zopf (1890).
Canavalia ensiformis DC. Hawaii: Parris (1940); Sideris (1932).
Cannabis sativa L. Germany: Schultz (1939).
Capsella bursa-pastoris L. Germany: de Bary (1881b); Zopf (1890).
Capsicum annuum L. India: Narasimhan (1934). Italy: Petri (1935). Philippine Islands: Ramos (1926). United States: Edson and Wood (1936); Erwin (1932); Lehman (1921); Nance (1940); Author.
Carathamus tinctorius L. Philippine Islands: Ramos (1926).
Carica papaya L. Hawaii: Parris (1940); Sideris (1932). Philippine Islands: Ramos (1926); Reinking (1918).
Celosia cristata L. Philippine Islands: Ramos (1926).
Centaurea margaritacea Tenore. Italy: Voglino (1908).
Cereus sp. Italy: Flachs (1935).
C. grandiflorus. Italy: Preti (1932).
C. marginatus. Italy: Preti (1932).
C. spachianus. Italy: Preti (1932).
Chrysanthemum sp. Netherlands: Buisman (1927). United States: Sprague (1942a).
Cichorium endiva L. United States: Johnson (1914a).
Citrullus vulgaris Schrad. United States: Walker and Weber (1931).
Citrus sinensis Osbeck. Italy: Petri (1935).
Clarkia sp. India: Butler and Bisby (1931).
C. elegans Dougl. United States: Lewis (1937).
Coleus sp. United States: Braun (1925).
Colocasia esculenta Schott. Hawaii: Parris (1940). United States: Nance (1940).
Coriandrum sativum L. Philippine Islands: Ramos (1926).
Crotalaria anagyroides L. Philippine Islands: Ramos (1926).
C. toxicaria. Sumatra: Palm (1926).
Cucumis melo L. var. *reticulatus* Naud. Canada: Godbout (1930). United States: Edson and Wood (1936).
C. sativus L. Canada: Godbout (1930). England: Smith (1900). Czechoslovakia: Anonymous (1931). Norway: Schoyen (1897). United States: Allen and Haenseler (1935). Anonymous (1929); Braun (1925); Edson and Wood (1936); Garman (1901); Haenseler (1930); Humphrey (1892); Johnson (1914a); Author.
Cucurbita maxima Duchesne. United States: Johnson (1914a).
C. pepo L. United States: Johnson (1914a).
Dahlia sp. England: Ward (1883). Netherlands: van Poeteren (1935).
Daucus carota L. England: Ward (1883). United States: Author.
Dianthus caryophyllus L. Germany: Pape (1925). United States: Johnson (1914a).
Echinochloa crus-galli (L.) Beauv. United States: Fischer *et al.* (1942).
Elymus canadensis L. United States: Sprague.
E. dahuricus Turcz. United States: Sprague.
E. glaucus Buckl. United States: Sprague.
E. junceus Fisch. United States: Fischer *et al.* (1942).
E. macounii Vasey. United States: Fischer *et al.* (1942).
E. sibiricus L. United States: Sprague.
Equisetum sp. Germany: Frank (1881).
E. arvense. England: Smith (1876). Germany: Sadebeck (1874, 1875).
E. limosum. Germany: Sadebeck (1876, 1881)

E. palustre. GERMANY: Sadebeck (1874, 1875, 1876, 1881).
Eschscholzia californica Cham. NETHERLANDS: Meurs (1928).
Euphorbia pulcherrima Willd. UNITED STATES: Author.
Fern. UNITED STATES: Matthews (1931).
Festuca duriuscula L. NETHERLANDS: van Luijk (1934a).
F. elatior L. UNITED STATES: Fischer *et al.* (1942).
F. elatior var. *arundinacea* (Schren.) Wimm. UNITED STATES: Fischer *et al.* (1942).
F. octoflora Walt. UNITED STATES: Sprague.
F. rubra L. var. *commutata* Gaud. UNITED STATES: Fischer *et al.* (1942).
Fragaria chiloensis Duchesne. SCOTLAND: Wardlaw (1928).
Geranium sp. NETHERLANDS: Buisman (1927).
Gilia sp. INDIA: Butler and Bisby (1931). UNITED STATES: Atkinson (1895); Galloway (1891).
Gloxinia sp. NETHERLANDS: Meurs (1928).
Godetia willdenowiana Spach. NETHERLANDS: Meurs (1928).
Gossypium sp. BARBADOS: Lewton (1903). ANGLO-EGYPTIAN SUDAN (?): Andrews and Clouston (1938).
Gypsophila alba. UNITED STATES: Edson and Wood (1936).
Haplomitrium hookeri Nees. POLAND: Lilienfield (1911).
Hedera helix L. UNITED STATES: Maneval (1937).
Hibiscus esculentus L. PHILIPPINE ISLANDS: Ramos (1926).
Hordeum bulbosum L. UNITED STATES: Fischer *et al.* (1942).
H. distichon L. UNITED STATES: Sprague.
H. jubatum L. UNITED STATES: Sprague.
H. vulgare L. DENMARK: Gram and Rostrup (1922a, 1924).
Impatiens balsamina L. UNITED STATES: Johnson (1914a).
I. sultani Hook. ENGLAND: Pim (1888).
Insect cadavers. DENMARK: Petersen (1910).
Ipomoea batatas Lam. HAWAII: Parris (1940); Sideris (1932). UNITED STATES: Harter (1924); Author.
Iris pseudacorus L. SWEDEN: Palm (1935).
Koeleria cristata (L.) Pers. UNITED STATES: Sprague.
Lactuca sativa L. ENGLAND: Abdel-Salam (1933). PHILIPPINE ISLANDS: Ramos (1926). UNITED STATES: Byars and Gilbert (1920); Johnson (1914a); Matthews (1931); Weber and Foster (1928).
Lens esculenta Mnsh. NETHERLANDS: Meurs (1928).
Lepidium sativum L. GERMANY: de Bary (1881b); Lhode (1874); Zopf (1890). INDIA: Butler (1907, 1913); Butler and Bisby (1931). IRELAND: Muskett, Carrothers and Cairns (1932). UNITED STATES: Doran (1936); Hemmi (1923); Johnson (1914a).
Lilium longiflorum Thunb. UNITED STATES: Author.
L. regale Wils. UNITED STATES: van Hook (1930).
Linum usitatissimum L. CANADA: Bisby *et al.* (1938); Vanterpool (1935b). NETHERLANDS: Diddens (1932). RUSSIA: Kletschetow (1924). UNITED STATES: Buchanan (1938); Edson and Wood (1936, 1937); Wood and Nance (1938).
Lobelia sp. UNITED STATES: Atkinson (1895); Galloway (1891).
Luffa acutangula Roxb. PHILIPPINE ISLANDS: Ramos (1926).
Lupinus sp. GERMANY: Pape (1927a). UNITED STATES: Gould (1940); Middleton (1938); Nance (1939).
L. angustifolius. GERMANY: Noll (1939); Schultz (1939).
L. luteus L. GERMANY: Schultz (1939).
L. polyphyllus Lindl. NETHERLANDS: Meurs (1928).
L. texensis Hook. UNITED STATES: Taubenhaus and Ezekiel (1932).
Lycopersicon esculentum Mill. CANADA: Berkeley (1925). DENMARK: Weber (1922, 1924). PHILIPPINE ISLANDS: Reinking (1919); Ramos (1926). PUERTO RICO: Cook (1939). SWEDEN: Hammarlund (1933). UNITED STATES: Byars and Gilbert (1920). Horne, Essig, and Herms (1923); Johnson (1914a); Middleton (1938); Mumford (1934, 1935); Nance (1939); Weber and Ramsey (1926).
Marchantia polymorpha L. FRANCE: Nicolas (1926, 1927).

Medicago sativa L. MAURITIUS: Anonymous (1925a). NETHERLANDS: van Luijk (1938a, 1938b). UNITED STATES: Buchholtz (1942a); Buchholtz and Meredith (1938); Maneval (1937); Melhus, Reddy and Buchholtz (1939); Middleton (1938); Nance (1939).

Musa cavendishii Lamb. HAWAII: Carpenter (1919).

Nicotiana rustica L. RUSSIA: Kuprianoff and Gorelnko (1930); Grushevoy (1938).

N. tabacum L. CANADA: Anonymous (1927d); Nelson, Major and McRae (1931). GERMANY: Böning (1928, 1931, 1935); Kotte (1929). INDIA: Hector (1931). ITALY: Trotter (1938). MAURITIUS: Corbett (1937). PHILIPPINE ISLANDS: Clara (1925); David and Roldan (1926); Ramos (1926); Reinking (1919). PUERTO RICO: Cook (1931, 1939); Edson and Wood (1937); Gage (1939); Nolla (1932). RHODESIA: Hopkins (1927, 1930). ROUMANIA: Banu and Constantinescu (1935); Ghimpu (1939); Knechtel (1914); Savulescu (1930). RUSSIA: Grushevoy (1938); Kuprianoff and Gorelnko (1930); Zaprometoff (1926). SUMATRA: Jochems (1926, 1927a, b); Van der Goot (1928); Van Hall (1925). TURKEY: Perrin and Osman (1928). UNION SOUTH AFRICA: Moore and Smith (1933). UNITED STATES: Anderson and Clinton (1926); Anderson, Swanback and Street (1929); Clinton and Anderson (1927); Edson and Wood (1936, 1937); Johnson (1914a, b, 1924); Sideris (1932).

Opuntia sp. UNITED STATES: Author.

Oryza sativa L. HAWAII: Carpenter (1919). PHILIPPINE ISLANDS: Ramos (1926).

Oryzopsis hymenoides (Roem. and Schult.) Rick. UNITED STATES: Fischer *et al.* (1942).

O. miliacea (L.) Benth. and Hook. UNITED STATES: Fischer *et al.* (1942).

Pachyrrhizus angulatus Rich. PHILIPPINE ISLANDS: Ramos (1926).

Panicum capillare L. UNITED STATES: Fischer *et al.* (1942).

P. miliaceum L. GERMANY: Frank (1881); Hesse (1874); Zopf (1890). UNITED STATES: Sprague (1942a).

P. virgatum L. UNITED STATES: Fischer *et al.* (1942).

Pelargonium sp. ENGLAND: Buddin and Wakefield (1924); Ward (1883). GERMANY: Pape (1931); Peters (1910). NETHERLANDS: van Poeteren (1922). UNITED STATES: Braun (1925); Johnson (1914a).

P. domesticum Bailey: UNITED STATES: Gill (1936).

P. graveolens L'Hér. UNITED STATES: Gill (1936).

P. hortorum Bailey. UNITED STATES: Gill (1936).

P. zonale L'Hér. CANADA: Bisby *et al.* (1938).

Phalaris arundinacea L. UNITED STATES: Fischer *et al.* (1942).

Phaseolus vulgaris L. NETHERLANDS: Meurs (1928). PHILIPPINE ISLANDS: Ramos (1926). PUERTO RICO: Cook (1939). SWEDEN: Hammarlund (1933). UNION SOUTH AFRICA: Wager (1940). UNITED STATES: Harter and Whitney (1927a); Johnson (1914a); Moore (1938); Author.

Phragmites communis Trin. UNITED STATES: Sprague.

Phyllocactus phyllanthoides Link. ITALY: Preti (1936).

Picea sp. BULGARIA: Christoff (1933). UNITED STATES: Anonymous (1928); Edson and Wood (1936, 1937).

P. canadensis Link. UNITED STATES: Hoffman (1912). Toumey and Li (1924).

P. engelmanni Engelm. UNITED STATES: Gravatt (1925); Hartley (1921); Hartley and Pierce (1917); Rathbun (1923); Rathbun-Gravatt (1925); Spaulding (1914).

P. excelsa Link. SWITZERLAND: Roth (1935a, 1935b).

P. parryana (Andre) Parry. UNITED STATES: Hartley (1921); Hartley and Pierce (1917); Spaulding (1914).

P. sitchensis (Bong.) Carr. UNITED STATES: Hartley (1921); Hartley and Pierce (1917); Spaulding (1914).

Pinus sp. BULGARIA: Dimitroff and Bioltscheff (1936). UNITED STATES: Pierce and Hartley (1919); Hartley and Merrill (1914); Anonymous (1928); Hartley (1912a).

P. banksiana Ait. UNITED STATES: Fischer, P. L. (1941); Gravatt (1925); Hartley, Merrill and Rhoads (1918); Rathbun (1922, 1923); Rathbun-Gravatt (1925).

P. insularis Endl. PHILIPPINE ISLANDS: Roldan (1933).

P. massoniana Lamb. PHILIPPINE ISLANDS: Roldan (1933).

P. nigra Arn. var. *austriaca*. ITALY: Sibilia (1928). UNITED STATES: Wilde and White (1939).

P. ponderosa Dougl. UNITED STATES: Fischer, P. L. (1941); Hartley, Merrill and Rhoads (1918); Hoffman (1912).
P. resinosa Ait. UNITED STATES: Eliason (1928); Fischer P. L. (1941); Gravatt (1925); May and Young (1927); Rathbun (1922, 1923); Rathbun-Gravatt (1925); Tilford (1938); Wilde and White (1939).
P. strobus L. BULGARIA: Christoff (1933). UNITED STATES: Toumey and Li (1924); Wilde and White (1939).
P. sylvestris L. BULGARIA: Christoff (1933). NETHERLANDS: Ten Houten (1939).
Pisum arvense L. SWEDEN: Hammarlund (1933).
P. sativum L. CANADA: Stone (1924). ENGLAND: Baylis (1941); Carruthers (1899). GERMANY: Noll (1939). NETHERLANDS: Meurs (1928). SWEDEN: Hammarlund (1933). UNITED STATES: Anonymous (1923); Drechsler (1925); Harter and Whitney (1927a); Matthews (1931); Sturgis (1898); Vaughn (1924).
Poa ampla Merr. UNITED STATES: Fischer *et al.* (1942).
P. bulbosa L. UNITED STATES: Fischer *et al.* (1942).
P. palustris L. UNITED STATES: Fischer *et al.* (1942).
P. pratensis L. UNITED STATES: Sprague.
P. secunda Presl. UNITED STATES: Fischer *et al.* (1942).
Polianthes tuberosa L. UNITED STATES: Edson and Wood (1936).
Pseudotsuga taxifolia Britt. UNITED STATES: Eliason (1928); Hartley (1921); Hartley and Pierce (1917); Pierce and Hartley (1919); Spaulding (1914).
Psophocarpus tetragonolobus DC. PHILIPPINE ISLANDS: Ramos (1926).
Pyrus communis L. BELGIUM: Marchal and Marchal (1921).
Ranunculus asiaticus L. UNITED STATES: Tompkins and Middleton (1939, 1942).
Raphanus sativus L. PHILIPPINE ISLANDS: Ramos (1926). SWEDEN: Hammarlund (1933). UNITED STATES: Braun (1925); Hoffman (1912).
Ricinus communis L. INDIA: Butler (1913).
Robinia sp. GERMANY: Hiltner (1902).
Saccharum officinarum L. HAWAII: Carpenter (1919, 1921); Parris (1940); Sideris (1932). UNITED STATES: Rands (1930); Rands and Dopp (1938b); Stevenson and Rands (1938).
Salsola tragus L. UNITED STATES: Hoffman (1912).
Salvia sclarea L. ITALY: Voglino (1923).
Satureia hortensis L. ITALY: Voglino (1923).
Schedonnardus paniculatus (Nutt.) Trel. UNITED STATES: Sprague.
Secale cereale L. UNITED STATES: Sprague.
S. montanum Guss. UNITED STATES: Sprague.
Selaginella sp. NETHERLANDS: van Poeteren (1923).
Setaria italica (L.) Beauv. UNITED STATES: Fischer *et al.* (1942).
S. viridis (L.) Beauv. UNITED STATES: Fischer *et al.* (1942).
Sinapis sp. GERMANY: Lhode (1874).
Soil. GERMANY: Butler (1907). UNITED STATES: Harvey (1925, 1927, 1929); Matthews (1931); Meredith (1938, 1940); Raper (1928); Author.
Solanum melongena L. PHILIPPINE ISLANDS: Ramos (1926). PUERTO RICO: Cook (1939); UNITED STATES: Drechsler (1926).
S. tuberosum L. AUSTRALIA: Bald (1941); Fish, Pugsley and Tyland (1937). CANADA: Dickson (1922); Sanford (1924). ENGLAND: Chona (1932); Pethybridge and Smith (1930); Ward (1883). GERMANY: de Bary (1881); Sadebeck (1875); Zopf (1890). HAWAII: Parris (1940); Sideris (1932). SWEDEN: Hammarlund (1933). TASMANIA: Bald (1941); Dowson (1931). UNITED STATES: Anonymous (1925b); Hawkins (1916, 1917); Hawkins and Harvey (1919); Orton (1909); Porter and Schneider (1939); Schade (1936); Shapovalov and Link. (1924).
Sorghum vulgare L. MANCHURIA: Miuri (1921). UNITED STATES: Fischer *et al.* (1942).
S. vulgare var. *sudanense* (Piper) Hitchc. UNITED STATES: Fischer *et al.* (1942).
Soya max L. UNITED STATES: Lehman and Wolfe (1926); Morse and Cartter (1939); Sprague (1942b); Wolf and Lehman (1924).
Sparganium simplex Huds. SWEDEN: Palm (1935).
Spergula arvensis L. GERMANY: Frank (1881); Hesse (1874); Zopf (1890).

Spinacea oleracea L. CANADA: Godbout (1930). NETHERLANDS: Meurs (1928). UNITED STATES: Author.
Stanhopea saccata Batem. GERMANY: Lhode (1874).
Stapelia sp. UNION SOUTH AFRICA: Wager (1940).
Stipa viridula Trin. UNITED STATES: Fischer *et al.* (1942).
Tecoma stans L. PHILIPPINE ISLANDS: Ramos (1926).
Tilia europaea L. UNITED STATES: Spaulding (1914).
T. ulmifolia Scop. UNITED STATES: Spaulding (1914).
Trifolium hybridum L. GERMANY: Zopf (1890).
T. pratense L. UNITED STATES: Melhus, Reddy and Buchholtz (1939).
T. repens L. GERMANY: Frank (1881); Hesse (1874); Zopf (1890).
Trigonella foenum-graceum L. UNITED STATES: Hartley (1921).
Tsuga canadensis Carr. UNITED STATES: Toumey and Li (1924); Hartley (1921).
T. mertensiana (Bong.) Sarg. UNITED STATES: Hartley and Pierce (1917); Pierce and Hartley (1919); Spaulding (1914).
Tulipa gesneriana L. (?). ENGLAND: Moore and Buddin (1937).
Vicia faba L. AUSTRALIA: Cheney (1932). GERMANY: Noll (1939). HAWAII: Parris (1940); Sideris (1932). UNITED STATES: Author.
Vigna sp. UNITED STATES: Hartley (1921).
V. sinensis Endl. HAWAII: Parris (1940); Sideris (1932). PHILIPPINE ISLANDS: Ramos (1926). UNITED STATES: Author.
Viola cornuta L. NETHERLANDS: Meurs (1928).
V. tricolor. GERMANY: Kaven (1934); Flachs (1931). NETHERLANDS: van Eek (1938). NORWAY: Solberg (1927). UNITED STATES: Author.
Viscaria sp. UNITED STATES: Atkinson (1895); Galloway (1891).
Vitis vinifera L. GREECE: Sarejanni (1936).
Zea mays L. GERMANY: Frank (1881); Hesse (1874); Zopf (1890). HAWAII: Parris (1940); Sideris (1932). ITALY: Curzi (1929). MANCHURIA: Miuri (1921). PHILIPPINE ISLANDS: Ramos (1926). RUSSIA: Speschneff (1896–97). UNITED STATES: Buchanan (1938); Ho (1941); Ho and Melhus (1940); Melhus (1940); Sprague; Author.

Pythium debaryanum is reported from seedlings in Canada (Bisby *et al.*, 1938) in Denmark (Jørstad, 1922), in Scotland (Anonymous, 1926b), in the United States (Marsh, 1937; Raeder, 1921), and in Puerto Rico and the Virgin Islands (Seaver *et al.*, 1932). *P. debaryanum* is reported from conifers in Denmark (Jørstad, 1925), and in the United States (Hartley 1910, 1912b; Hartley and Hahn, 1919) and from broad leaf tree seeds in Russia (Sokoloff, 1940).

47. PYTHIUM CYSTOSIPHON (Roze and Cornu) Lindstedt, Synopsis der Saprolegniaceen und Beobactungen uber einige Arten. 1872.

Cystosiphon pythioides Roze and Cornu, Ann. Sci. Nat. V. **11**: 72–91. 1869.
Pythium pythioides Ramsbottom, Trans. Brit. Mycol. Soc. **5**: 304. 1916.

Hyphae endophytic, rarely septate, perforating the cells of the host. Sporangia usually spherical, never prolate ellipsoidal, formed in the peripheral host cells, producing a single tube which bears the exogenous vesicle; zoospores 1 to 49, elongate, 8 to 16 μ long, more or less reniform, biciliate. Oogonia spherical, terminal or intercalary. Antheridia diclinous, 1 to 3 per oogonium, borne terminally on branches of varying length. Oospores aplerotic, single, provided with a thick, reticulately sculptured wall.

Parasitic in *Lemna arrhiza* L., *L. gibba* L., *L. minor* L., and *Riccia fluitans* L., France.

Roze and Cornu created *Cystosiphon* as a new genus intermediate between the Saprolegniaceae and Peronosporaceae. Lindstedt pointed out the synonymity of the genera *Cystosiphon* and *Pythium* transferring *C. pythiodes* to *P. cystosiphon*.

The species is unique among spheroidal sporangial forms in possessing an oospore with a reticulate wall.

Pythium cystosiphon apparently has not been reported since the original description.

48. Pythium acanthicum Drechsler, Jour. Wash. Acad. Sci. **20**: 398–418. 1930.

Hyphae measuring 1.3 to 5.6 μ in diameter. Sporangia typically intercalary though sometimes acrogenous, then subspherical measuring 12 to 43 μ in diameter, when intercalary usually composed of a subspherical part and a contiguous portion of the hypha, short or up to 75 μ long, sometimes composed of 2 or more subspherical portions associated by means of a communicating hypha; zoospores 5 to 50, biciliate, reniform and longitudinally grooved, measuring 8 to 9.5 μ in diameter upon encystment. Oogonia acrogenous but typically intercalary or laterally or tangentially intercalary, subspherical with an echinulate wall, measuring 13 to 30 μ, average 23.7 μ, in diameter excluding the spines which are 1.5 to 5 μ, average 2.7 μ, in length and average 1.9 μ in basal diameter, tapering slightly to a rounded apex. Antheridia typically monoclinous, occasionally diclinous, 1 to 2, usually 1 per oogonium, borne terminally on branches 6 to 25 μ, usually 10 to 15 μ, long, the antheridial cell inflated clavate, straight or crook-necked, 8 to 17 μ long and 5 to 9 μ wide, the larger cells frequently lobate due to 1 or 2 transverse constrictions, the apex broadly applied, sometimes the entire cell longitudinally applied to the oogonium. Oospores plerotic though usually free from the oogonial wall, single, measuring 12 to 27 μ, average 21.7 μ, in diameter, provided with a moderately thickened wall, measuring 1.3 to 2 μ, average 1.6 μ, thick and containing a single reserve globule and refringent body; germination by means of germ tubes or zoospores.

Originally described from fruits of *Citrullus vulgaris* Schrad., United States.

A number of isolates of this species were available for study. The sporangia of *Pythium acanthicum* are very distinctive and readily separate the species from all other echinulate oogonial forms with plerotic oospores. Similar sporangia are found in *P. oligandrum*, a species possessing echinulate oogonia and aplerotic oospores. This type of sporangium is termed contiguous because of the intimate association of the subspherical part with an attached hyphal element.

The oogonial protuberances of *Pythium acanthicum* are peculiar to the species, though those of *P. mamillatum* resemble them but differ in being longer and usually curved. The spines of *P. spinosum* differ strikingly from those of both the other species in their length and digitate character.

The antheridia of *Pythium acanthicum* are typically monoclinous, the antheridial stalk frequently bearing a branched, vegetative prolongation of

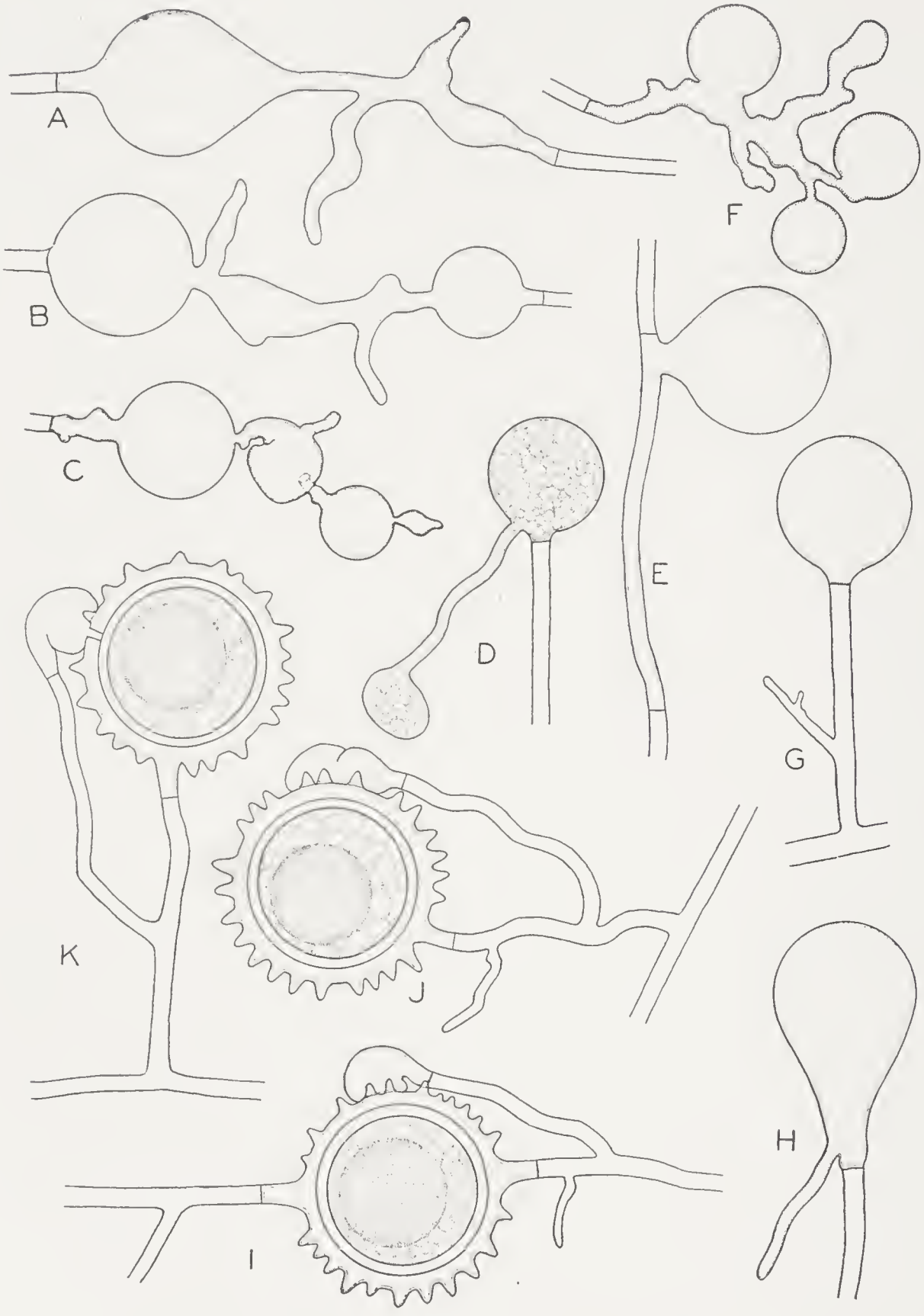

FIG. 13. *Pythium acanthicum*. *A–H*, Sporangia of typical morphology. *A*, *B*, *C*, *F*, Exemplify the typical contiguous sporangial habit. *I–K*, Oogonia, antheridia, and mature oospores.

varying length. No vegetative prolongations occur on the monoclinous antheridia of *P. mamillatum* and *P. spinosum*. The antheridial cell of *P. acanthicum* is inflated and frequently lobate with one or several transverse constrictions, differing decidedly from the less inflated, clavate, smooth, crook-necked antheridia of *P. mamillatum* and *P. spinosum*.

The mode of oospore germination in this species is twofold, by germ tubes and zoospores; the production of zoospore is the more common. Zoospore formation is usually accomplished in the same manner as displayed when originating from sporangia. Occasionally a saccate or irregularly defined bladder-like structure is produced in place of the normally spherical vesicle. Still other instances have been observed in which the germ tube gives rise to a spherical or saccate structure which in turn gives rise to an evacuation tube capped by a vesicle in which the zoospores are delineated; these observations are corroborated by the recent report and illustrations of Wager (1940).

The cumulous mycelial growth habit and the temperature-growth relations of *Pythium acanthicum* are useful adjuncts for the identification of this species.

Pythium acanthicum is known from *Citrullus vulgaris* Schrad. from the original report and a subsequent one by Drechsler (1939), and by the author in the United States, from *Dahlia* sp. and *Pisum sativum* L., by Wager (1940) in South Africa and by the author from *Phaseolus vulgaris* L. and *Solanum melongena* L. in the United States.

49. Pythium mamillatum Meurs, Wortelrot, Veroorzaakt door Schimmels uit de Geslachten *Pythium* Pringsheim en *Aphanomyces* de Bary. 1928.

Hyphae measuring 4 to 9.3 μ in diameter. Sporangia spherical, 14.3 to 20.7 μ, average 16.3 μ, in diameter, acrogenous, or intercalary; zoospores 5 to 14, reniform and laterally biciliate. Oogonia spherical, typically acrogenous and rarely intercalary, with an echinulate wall, the spines are conical and obtusely tipped, measuring 2.7 to 6.0 μ, average 4.4 μ, long, the oogonia measuring 13 to 19.3 μ, average 16.4 μ, in diameter, excluding the protuberances. Antheridia monoclinous, usually single and arising in close proximity to the oogonium, the stalk short, the cell clavate with the apex moderately applied to the oogonial wall. Oospores plerotic, single, with a moderately thick wall; germination not observed.

Originally described as causing damping-off of *Beta vulgaris* L. seedlings, Netherlands.

The sporangia of *Pythium mamillatum* are quite similar to those of *P. spinosum*, spherical to subspherical, acrogenous and intercalary. Zoospores are freely produced in *P. mamillatum* and rarely in *P. spinosum*, the sporangia of the latter germinating by means of 1 to 3 germ tubes. The presence of contiguous sporangia serves in distinguishing *P. acanthicum* from *P. mamillatum*.

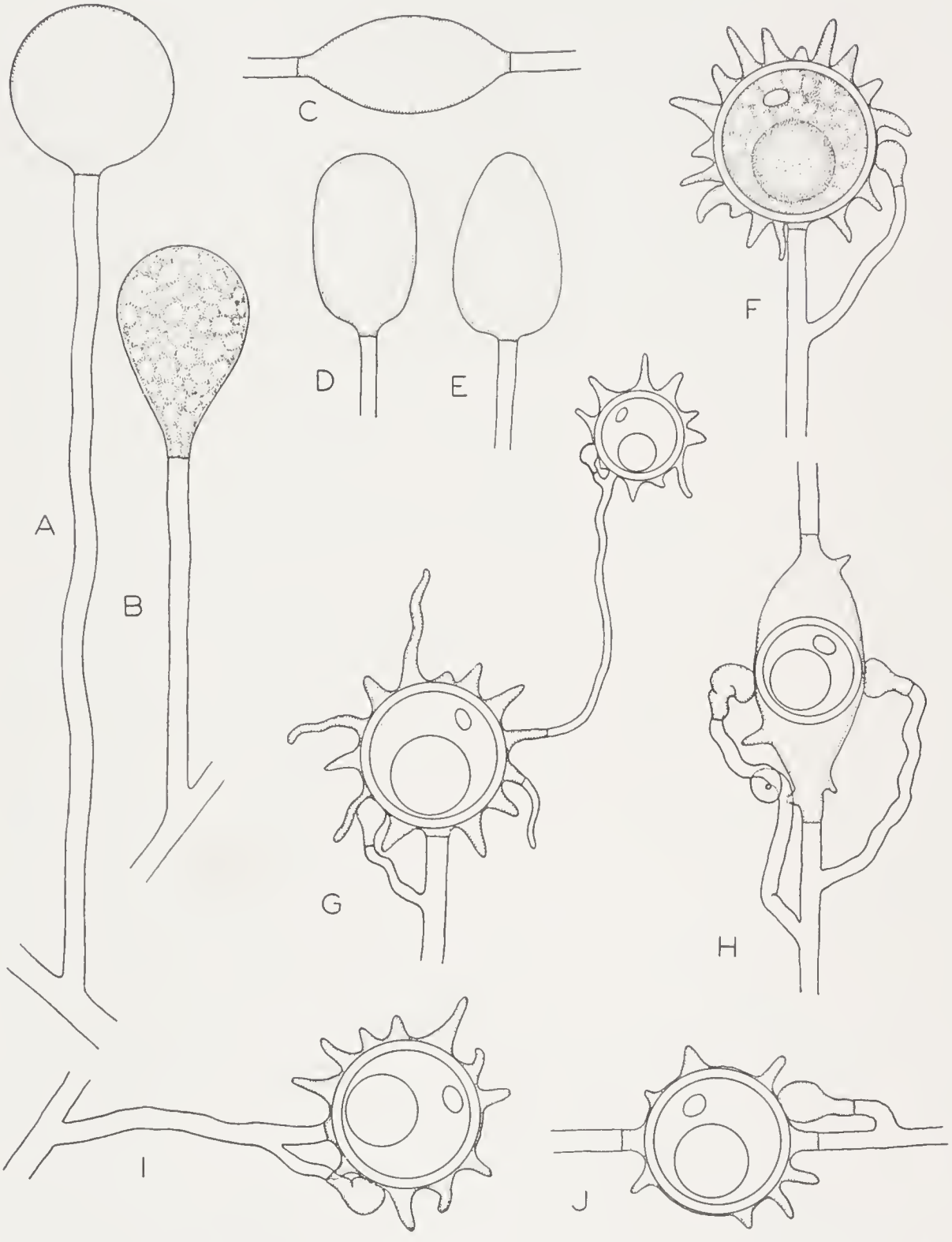

FIG. 14. *Pythium mamillatum*. *A–E*, Sporangia of usual occurrence. *A*, *B*, *C*, Most typical of the sporangial habits. *F–J*, The sexual apparatus. *F*, *I*, *J*, Demonstrate the typical monoclinous type of antheridia and the common types of oogonial echinulation. *H*, Infrequently produced intercalary oogonium with few spines, usually produced in aquatic substrate. *G*, Oogonium demonstrating the occasional proliferation of spines which may bear an oogonium which frequently contains an oospore.

The spherical, echinulate oogonia of *Pythium mamillatum* and *P. spinosum* are more often acrogenous than intercalary. The oogonial protuberances, however, are very different; they are conical, obtuse and about 4.4 μ long in *P. mamillatum*, while in *P. spinosum* they are digitate, obtuse and about 7 μ long. Occasionally the writer has observed spines of *P. mamillatum* to continue growth and give rise to a short filament which would infrequently give rise to an oogonium; mature oospores have been observed in such oogonia.

The antheridia of *Pythium mamillatum* are monoclinous, originating in close proximity to the oogonium; the antheridial stalk is short and upcurved, bearing an inflated, smooth, clavate, crook-necked antheridial cell which is narrowly applied to the oogonium. The antheridia of *P. spinosum* are more or less analogous, while those of *P. acanthicum* are decidedly different. The antheridia of *P. spinosum* are both monoclinous and diclinous, and do not originate as close to the oogonium as in *P. mamillatum;* the antheridial cell of *P. spinosum* is allantoid-clavate, slightly curved and narrowly applied to the oogonium.

Pythium mamillatum and *P. spinosum* may be distinguished also by their differences in growth habit and temperature-growth relationship.

This fungus has been observed on the following listed plants:

Ageratum houstonianum Mill. UNITED STATES: Author.
Agrostis stolonifera L. NETHERLANDS: van Luijk (1934a, 1938a).
Ananas comosus Merr. HAWAII: Parris (1940); Sideris (1932).
Antirrhinum majus L. UNITED STATES: Author.
Aquilegia caerulea James. UNITED STATES: Author.
Avena sativa L. NETHERLANDS: Brandenburg (1931a).
Beta vulgaris L. NETHERLANDS: Meurs (1928, 1929); van Poeteren (1930).
Cladophora sp. SWEDEN: Palm (1932).
Collinsia bicolor Benth. UNITED STATES: Author.
Festuca duriuscula L. NETHERLANDS: van Luijk (1934a).
Geum chiloense Balb. UNITED STATES: Author.
Gleocapsa sp. SWEDEN: Palm (1932).
Linum usitatissimum L. NETHERLANDS: Diddens (1932).
Medicago sativa L. UNITED STATES: Author.
Pelargonium domesticum Bailey. UNITED STATES: Gill (1936).
P. graveolens L'Hér. UNITED STATES: Gill (1936).
P. hortorum Bailey. UNITED STATES: Gill (1936).
Saccharum officinarum L. UNITED STATES: Rands and Dopp (1938b); Stevenson and Rands (1938).
Soil. UNITED STATES: Matthews (1931).
Triticum aestivum L. UNITED STATES: Author.
Viola tricolor L. ENGLAND: Chesters. UNITED STATES: Author.

50. PYTHIUM SPINOSUM Sawada, Jour. Nat. Hist. Soc. Formosa **16**: 199–212. 1926.

Hyphae measuring 2.5 to 5.0 μ in diameter. Sporangia are spherical to subspherical when acrogenous, spherical to spindle-shaped when intercalary, measuring 14 to 33 μ, average 22.4 μ, in diameter, wall usually smooth but occasionally may bear digitate or blunt conical spines; zoospores rarely formed, germination usually accomplished by means of germ tubes. Oogonia

spherical to subspherical when acrogenous, sometimes limoniform when intercalary, measuring 13.2 to 27.4 μ, average 18.2 μ, in diameter, excluding the spines which are conical with obtuse apices or more commonly digitate, 5 to 8 μ long and 1.5 to 2 μ broad at the base. Antheridia terminal, typically monoclinous though sometimes diclinous, 1, rarely 2, per oogonium, originating close to the oogonium and measuring 12 to 32 μ long and 3 to 5 μ wide. Oospores plerotic, single, smooth-walled, measuring 10.1 to 25.3 μ, average 16.7 μ, in diameter; germination not observed.

Parasitic on seedlings of *Antirrhinum majus* L., Formosa.

The description given above is taken in large part from a translation of the original Japanese obtained through the courtesy of Dr. V. A. Wager. Supplementary information obtained from the writer's observations is also included. In the original description the oogonia are said to be 17 to 24 μ, average 19.7 μ, in diameter, including the spines which are 5 to 8 μ long; just how a plerotic oospore measuring 12 to 21 μ, average 16.6 μ, in diameter would be oriented in such an oogonium allowing for a minimum spine length and thickness of oogonial wall is not quite clear. The measurements given in the description presented above are taken from the author's study of the species.

Supplementary literature describing the species and giving its host range are found in an anonymous abstract in the Japanese Journal of Botany, **3**: 108–109, 1927, and Sawada (1927, 1931).

Pythium spinosum is distinguished from other echinulate oogonial forms with plerotic oospores by the characteristic long, digitate, obtuse oogonial protuberances.

The antheridium of *Pythium spinosum* differs from those of its congeners, *P. acanthicum* and *P. mamillatum*, in being rather long, narrow, allantoid, the apical portion not inflated or lobate. The antheridial stalk is devoid of the vegetative prolongations commonly observed in *P. acanthicum* and is longer and straighter than in *P. mamillatum*. Cook and Collins (1937) report that the antheridium of *P. spinosum* is stalked, androgynous (monoclinous) and rarely arises from the oogonial stalk. Apparently these authors have interpreted erroneously the meaning of androgynous (monoclinous) antheridia, for these are customarily defined as arising from the oogonial stalk.

The growth habit and temperature-growth relations of *Pythium spinosum* may be used to distinguish it from related species.

This species has been observed on the following plants:

Allium cepa L. United States: Author.
A. fistulosum L. Formosa: Sawada (1931); Sawada and Chen (1926).
A. schaenoprasum L. Formosa: Sawada (1931); Sawada and Chen (1926); Darker (1940).
Antirrhinum majus L. Formosa: Sawada (1931); Sawada and Chen (1926).
Arctium lappa L. Formosa: Sawada (1931).
Brassica campestris L. Formosa: Sawada (1931); Sawada and Chen (1926).
B. oleracea L. var. *capitata* L. Japan: Tasugi and Siino (1940).
Calendula officinalis L. Formosa: Sawada (1931). Japan: Tasugi and Siino (1940).
Callistephus chinensis Nees. Japan: Tasugi and Siino (1940).
Campanula medium L. Formosa: Sawada (1931).

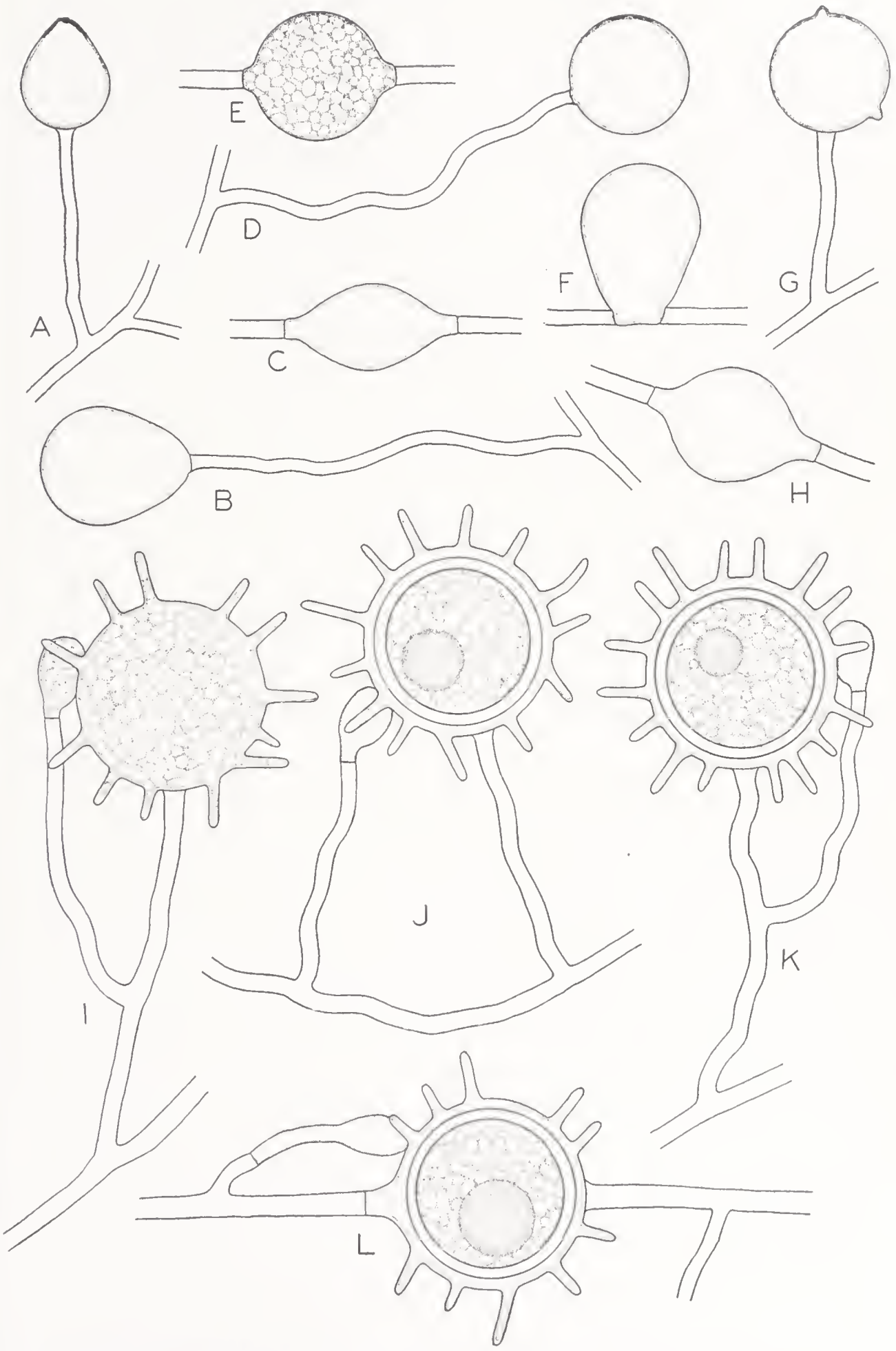

FIG. 15. *Pythium spinosum*. *A–H*, Sporangia showing typical morphology. *I–L*, Various stages in the development of the oospores. *I*, Immature oogonium with typical monoclinous antheridium. *J*, Antheridia of this type are of infrequent occurrence. *L*, Typical occasional intercalary sexual apparatus.

Carica papaya L. UNION SOUTH AFRICA: Wager (1931, 1932a, 1940).
Chrysanthemum coronarium L. FORMOSA: Sawada (1931); Sawada and Chen (1926).
Citrullus vulgaris Schrad. UNITED STATES: Author.
Coriandrum sativum L. FORMOSA: Sawada (1931).
Cucumis sativus L. FORMOSA: Sawada (1931); Sawada and Chen (1926). JAPAN: Tasugi and Siino (1940).
Daucus carota L. FORMOSA: Sawada (1931); Sawada and Chen (1926).
Dianthus chinensis L. FORMOSA: Sawada (1931); Sawada and Chen (1926).
Lactuca sativa L. FORMOSA: Sawada (1931); Sawada and Chen (1926). JAPAN: Tasugi and Siino (1940).
Lycopersicon esculentum Mill. FORMOSA: Sawada (1931). JAPAN: Tasugi and Siino (1940).
Papaver rhoeas L. JAPAN: Tasugi and Siino (1940).
Primula sinensis Lindl. ENGLAND: Cook and Collins (1937).
Raphanus sativus L. FORMOSA: Sawada (1931); Sawada and Chen (1926).
Soil. BOHEMIA: Cejp (1931).
Solanum melongena L. FORMOSA: Sawada and Chen (1926). JAPAN: Tasugi and Siino (1940).
Zinnia elegans Jacq. FORMOSA: Sawada (1931). JAPAN: Tasugi and Siino (1940).

51. PYTHIUM OLIGANDRUM Drechsler, Jour. Wash. Acad. Sci. **20**: 398–418. 1930.

Hyphae measuring 1.5 to 6.8 μ in diameter. Sporangia acrogenous, typically intercalary, subspherical, measuring 25 to 45 μ in diameter, usually consisting of 1 to 5 subspherical bodies together with a contiguous portion of a filament of varying length, 5 to 75 μ long, sometimes the filament irregularly swollen or branched; zoospores 20 to 50, laterally biciliate, broadly reniform, measuring 9 to 10 μ in diameter upon encystment. Oogonia subspherical, echinulate, terminal or subterminal, also laterally or tangentially intercalary, 17 to 35 μ, average 26.4 μ, in diameter exclusive of the spines which are numerous, conical and acutely tipped, 3 to 7 μ, average 3.9 μ, long, and 1.5 to 3.5 μ, average 2.2 μ, in basal diameter; oogonia developing parthenogenetically in about 4 out of 5 cases. Antheridia lacking in many instances, when present usually diclinous, infrequently monoclinous, the terminal antheridial cell 12 to 25 μ, average 18 μ, long, and 5.5 to 8 μ wide, clavate and slightly crook-necked, often lobed by 2 to 3 transverse constrictions. Oospores aplerotic, single, 15 to 30 μ, average 23.1 μ, in diameter with a wall approximately 1.5 μ thick and containing a single reserve globule and refringent body.

Originally described from roots of *Pisum sativum* L., United States.

The contiguous sporangia of *Pythium oligandrum* are a distinctive feature of the species and readily distinguish it from other echinulate oogonial types with aplerotic oospores.

The oogonia of *Pythium oligandrum* are very similar to those of *P. anandrum;* both have conical, acute spines. The spines of *P. oligandrum* are from 3 to 7 μ, average 3.9 μ, in length and 1.5 to 3.5 μ, average 2.2 μ, in basal diameter, whereas the spines of *P. anandrum* are from 3 to 11 μ, average 7.1 μ, in length and 2 to 4 μ, average 2.8 μ, in basal diameter. The oogonial protuberances are more or less regularly arranged over the oogonium, differing considerably from the irregular placement of acantha in *P. irregulare.*

The antheridium of *Pythium oligandrum* is ordinarily diclinous with an inflated saccate antheridial cell which is closely applied to the oogonium. A

large percentage of the oogonia develop oospores in the absence of a visible antheridium; the species is, in this respect, analagous to *P. anandrum* in which there is no antheridium.

Pythium oligandrum and *P. anandrum* are quite different in their growth habits and temperature-growth relations; the maximum temperature for growth of *P. oligandrum* is 37° C. and between 31° and 34° C. for *P. anandrum*.

In addition to the original isolation of this species from *Pisum sativum* L. in the United States by Drechsler, it has been found on *Daucus carota* L., *Euphorbia pulcherrima* Willd., *Rheum rhaponticum* L., *Stipa* sp. and *Triticum aestivum* L. in the United States by the author, from *Viola tricolor* L. in England by Chesters, from *Prunus amygdalus* Stokes fruits in Cyprus by Nattrass (1937), from *Antirrhinum majus* L., *Cucurbita pepo* L., *Brassica oleracea* L. var. *capitata* L., and *Papaver rhoeas* L. in South Africa by Wager (1940); snapdragon and shirley poppy were originally reported by Wager (1931) as hosts for *Pythium* sp. cf. *artotrogus*, but transferred by him in 1940 to *P. oligandrum*.

52. PYTHIUM MEGALACANTHUM de Bary, Abhandl. Senckenb. Naturf. Ges. **12**: 19. 1881; Bŏt. Zeit. **39**: 539–544. 1881.

Pythium megalacanthum var. *callistephi* Tasugi and Siino, Ann. Phytopath. Soc. Japan **10**: 278–293. 1940.

Mycelium fine, branched, intracellular and intercellular. Sporangia formed in the epidermal cells of the host, or exogenously, acrogenous and intercalary, spherical to subspherical, frequently proliferous through continued growth of the sporangiophore, forming the secondary sporangium above the primary. Zoospores large, 4 to 5 μ to 18 to 20 μ, usually 12 to 15 μ, in size. Oogonia acrogenous or intercalary, spherical at first with a smooth surface which later became echinulate, exclusive of spines measuring 36 to 45 μ in diameter; spines 6 to 9 μ long, conical, usually acutely tipped. Antheridia diclinous, 1 or more to an oogonium. Oospores aplerotic, smooth and thick-walled, about 27 μ in diameter.

Saprophytic on vegetable debris, Germany.

Pythium megalacanthum differs from all other echinulate oogonial forms in possessing proliferous sporangia. The reports of Buisman (1927) and Diddens (1932) concerning a disease of flax, presumably caused by *P. megalacanthum*, state that the sporangia of the flax fungus are similar to those described by de Bary for *P. megalacanthum*. All attempts to induce zoospore formation and sporangial proliferation failed. Drechsler (1939), in a discussion of the flax parasite states that he also was unable to induce zoospore formation in this fungus. All attempts by the writer to secure zoospore formation and sporangial proliferation of the flax fungus likewise were unsuccessful. Nicolas and Aggery (1928) and Petri (1935) report observations concerning a fungus which was believed to be *P. megalacanthum* but do not mention whether they saw sporangial proliferation. Cejp (1931),

working with a culture of *P. megalacanthum* isolated from soil, describes the sporangia of his fungus as proliferous. An isolate identified by the writer as *P. megalacanthum* exhibited proliferous sporangia when agar blocks containing the fungus were irrigated with water.

Drechsler (1939) points out that the exact identity of the flax fungus is questionable, that until proliferous sporangia are observed specific assignment of the organism to *P. megalacanthum* is not possible. The writer concurs with this opinion, but believes that tentative identification of the fungus as *P. megalacanthum* is justifiable in view of the similarity of the sexual stage of the flax fungus to that of the referred species.

The reports of *Pythium megalacanthum* published subsequent to de Bary's description of the species, with the possible exception of Cejp's, do not present sufficient evidence to prove that the fungus referred to that binomial is actually identical with the species originally studied by de Bary.

The oogonia of *Pythium megalacanthum* resemble those of *P. mastophorum* and *P. polymastum;* however, the spines of these species are typically mammiform while those of *P. megalacanthum* are more or less conical, straight or slightly curved, with an obtuse to almost acute tip.

Pythium mastophorum and *P. polymastum* lack proliferous sporangia and are thus readily distinguished from *P. megalacanthum;* if proliferous sporangia fail to develop the species may usually be distinguished by the type of echinulation present.

Pythium megalacanthum is reported from the plants listed below:

Ananas comosus Merr. HAWAII: Sideris (1932).
Antirrhinum majus L. JAPAN: Tasugi and Siino (1940).
Calendula officinalis L. JAPAN: Tasugi and Siino (1940).
Callistephus chinensis Nees. JAPAN: Tasugi and Siino (1940).
Chrysanthemum sp. NETHERLANDS: Van Poeteren (1938).
Citrus sinensis Osbeck. ITALY: Petri (1935).
Erica regerminans L. UNITED STATES: Author.
Euphorbia pulcherrima Willd. UNITED STATES: Author.
Lactuca sativa L. JAPAN: Tasugi and Siino (1940).
Lepidium sativum L. ENGLAND: Salmon and Ware (1938). GERMANY: de Bary (1881b).
Linum usitatissimum L. GERMANY: Schilling (1928). NETHERLANDS: Buisman (1927); De Jonge (1933); Diddens (1932); Dorst (1938); Van der Meer (1928).
Lycopersicon esculentum Mill. JAPAN: Tasugi and Siino (1940). UNITED STATES: Author.
Papaver nudicaule L. UNITED STATES: Author.
P. rhoeas L. JAPAN: Tasugi and Siino (1940).
Pelargonium sp. NETHERLANDS: Van Poeteren (1938).
Petroselinum hortense Hoffm. FRANCE: Nicolas and Aggery (1928).
Primula sp. NETHERLANDS: Van Poeteren (1938).
P. obconica Hance: UNITED STATES: Author.
Soil. BOHEMIA: Cejp (1931). GERMANY: de Bary (1881b).
Todea africana Willd. GERMANY: de Bary (1881b).
Veronica hederaefolia L. GERMANY: Schröter (1889).
Zinnia elegans Jacq. JAPAN: Tasugi and Siino (1940).
Melon. FRANCE: Labrousse and Marcel (1934).

53. PYTHIUM ANANDRUM Drechsler, Jour. Wash. Acad. 20: 398–418. 1930.

Hyphae measuring 2 to 8 μ in diameter. Sporangia terminal, usually on long sporangiophores, sometimes later occupying a lateral position due to continued growth of the supporting branch, elongate, prolate ellipsoidal, 32 to 85 μ, average 50.4 μ, long and 18 to 40 μ, average 25.3 μ, wide, papillate, the apical papilla 3 to 5 μ long and 6 to 8 μ broad at base, occasionally proliferous, the subsequent sporangia usually produced beyond the primary; zoospores usually produced in sessile vesicle, 8 to 30, laterally biciliate, reniform, measuring 12 to 14 μ in diameter upon encystment. Oogonia subspherical, terminal, measuring 12 to 33 μ, average 28.3 μ, in diameter, exclusive of the numerous conical, acutely tipped spines which measure 2 to 11 μ, average 7.1 μ, in length and 1 to 4 μ, average 2.8 μ, in basal width; oogonia are constantly parthenogenetic. Antheridia lacking. Oospore aplerotic and apandrous, single, measuring 11 to 28 μ, average 24.4 μ, in diameter, provided with a wall approximately 1.6 μ thick and containing a single reserve globule and refringent body.

Originally described from *Rheum rhaponticum* L., United States.

Pythium anandrum is readily identified by its non-proliferous papillate sporangia, unique among echinulate oogonial congeners. The oogonia of *P. anandrum* and *P. oligandrum* are very similar, both having conical, narrow, acute spines; the contiguous sporangia of *P. oligandrum* are in sharp contrast with the prolate ellipsoidal sporangia of *P. anandrum*. The absence of antheridia also serves in segregating this species from *P. oligandrum*.

The minimum temperature for mycelial development of *Pythium anandrum* is low, 1° C., compared with 10° C. for *P. oligandrum*.

Pythium anandrum is known only in the United States from *Citrullus vulgaris* Schrad. fruits by Drechsler (1939), fruits of *Cucumis sativus* L. and roots of *Phaseolus vulgaris* L. by the author, *Rheum rhaponticum* L. crowns by Drechsler (1930a) and the author (1941b); and roots of *Spinacea oleracea* L. by the author.

54. PYTHIUM ECHINULATUM Matthews, Studies on the genus Pythium. 1931.

Hyphae measuring 2 to 8 μ in diameter. Sporangia spherical to cylindrical, terminal or intercalary, often catenulate, 3 or 4 in a series, measuring 10 to 30 μ, average about 20 μ, in diameter; zoospores or germ tubes are produced. Oogonia spherical to cylindrical, terminal or intercalary, measuring 14 to 30 μ, average about 24 μ, in diameter exclusive of the many spines 2 to 8 μ in length. Antheridia monoclinous, typically hypogynal, 1 to 4 per oogonium, usually 1. Oospores aplerotic, 14 to 24 μ, average about 20 μ, in diameter, 1 to 2 per oogonium, possessing a thick wall enclosing a single reserve globule and refringent body.

Originally described from soil in the United States.

There are several distinctive features in this species, particularly in its asexual stage. The sporangia are largely intercalary and very frequently catenulate, whereas in all other species possessing spiny oogonia spherical, intercalary, sporangia may be present but never arranged in a chain.

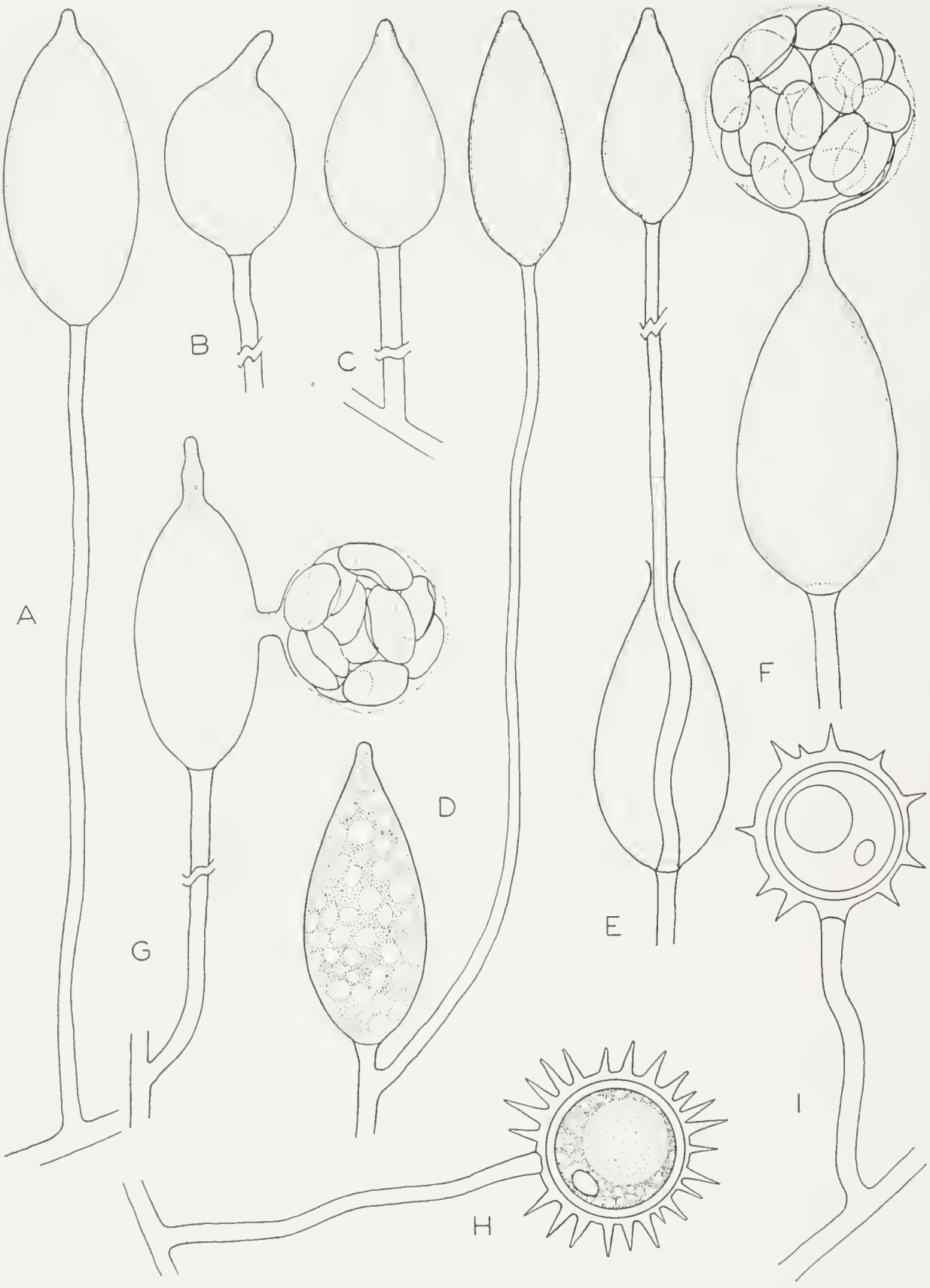

FIG. 16. *Pythium anandrum*. *A–E*, Various sporangial types. *A*, *C*, Typical shape. *B*, Sporangium of unusual occurrence, possessing a skewly beaked papilla. *D*, Secondary sporangium produced at the tip of a sporangiophore originating at the base of the primary sporangium. *E*, Typical sporangial proliferation. *F*, *G*, Zoospore formation. *G*, The apical emission tube has become nonfunctional, the secondary emission tube being formed in a lateral position. *F*, vesicle formation of the usual type. *H*, *I*, The sexual apparatus.

The oogonia of *Pythium echinulatum* resemble those of *P. oligandrum* and *P. anandrum* more than any other species, but differ in having spines with a relatively large basal diameter and an acuminate form rather than with a relatively small basal diameter and a conical form with an acute apex.

Hypogynal antheridia are usually present in *Pythium echinulatum;* they resemble those observed in *P. artotrogus* and *P. echinocarpum*, species lacking an asexual reproductive phase. This type of antheridium is not found in congeners possessing echinulate oogonia and an asexual reproductive phase.

A culture received as *Pythium echinulatum* proved to be more closely related to *P. acanthicum*. The growth habit and temperature-growth relations of this particular isolate are identical with those of *P. acanthicum*.

The species is known only from the original description and a single report from rotting *Viola tricolor* L. plants in the Netherlands by van Eek (1938).

55. Pythium mastophorum Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 2 to 7.8 μ in diameter; appressoria moderately produced, knob-like, 8 to 12 μ in diameter at the expanded apex. Sporangia subspherical, smooth-walled, typically terminal, occasionally subterminal or apically intercalary, 17 to 38 μ, average 29.3 μ, in diameter; zoospores 3 to 14, reniform and laterally biciliate, measuring 12 to 14 μ in diameter upon encystment. Oogonia subspherical, typically terminal on branches 5 to 25 μ long, measuring 24 to 48 μ, average 35 μ, in diameter exclusive of the spiny protuberances 2 to 8 μ, average 5.2 μ, long and 2 to 6 μ, average 4.3 μ, wide at the base, conical or more typically mammiform, the apex usually attenuated and papillate. Antheridia diclinous, usually terminal but infrequently intercalary, usually somewhat lobate, 7 to 15 μ wide and 16 to 28 μ long, making broad apical contact with the oogonium, frequently the antheridial stalk becomes intimately associated with the oogonial stalk, usually 1 per oogonium. Oospores aplerotic, 20 to 44 μ, average 28.9 μ, in diameter, provided with a wall 1.4 to 2.3 μ, average 1.8 μ, thick and containing a single reserve globule and refringent body; germination by means of zoospores or germ tubes.

Originally described from *Bellis perennis* L., United States.

The description given above adequately delineates the species but does not indicate its relationships or distinguishing characteristics.

The oogonia of *Pythium mastophorum*, *P. polymastum*, and *P. megalacanthum* are very similar. Those of *P. megalacanthum* have conical, obtuse or slightly pointed spines, while the spines of *P. mastophorum* and *P. polymastum* are mammiform. The average diameter of the oogonia of *P. mastophorum* is 35 μ compared with an average of 45 μ in *P. polymastum;* the oospores are proportionally smaller, 29 μ and 35 μ in diameter, respectively.

Pythium mastophorum and *P. polymastum* are readily distinguished by their sporangia and type of evacuation tube. The sporangia of *P. mastophorum* are largely terminal, sometimes intercalary, spherical and regular in outline, and develop an evacuation tube that is contorted, bearing one or

several branched, vegetative prolongations; the sporangia of *P. polymastum* are largely intercalary, irregular in contour due to the presence of dome-like protuberances, or composed of 2 or 3 lobed communicating parts, and develop a straight unbranched evacuation tube.

The mammiform spines distinguish *Pythium mastophorum* from *P. irregulare*, *P. echinulatum*, and *P. anandrum*. The typically diclinous antheridium of *P. mastophorum* differs decidedly from the typically monoclinous antheridia of *P. irregulare*, hypogynous antheridium of *P. echinulatum* and the absence of antheridia on *P. anandrum*.

This species is known from *Bellis perennis* L. by Drechsler (1930a, 1939) and from *Calceolaria crenatiflora* Cav. by the author, both in the United States.

56. PYTHIUM POLYMASTUM Drechsler, Jour. Wash. Acad. **20**: 398–418. 1930.

Hyphae measuring 2.5 to 9.5 μ in diameter; appressoria usually lacking. Sporangia usually subspherical, often noticeably oblate, usually intercalary, or infrequently terminal and then later becoming lateral due to prolongation of the sporangiophore, measuring 20 to 36 μ, average 30 μ, in diameter, sometimes irregular in shape due to 1 or more dome-shaped protuberances or even composed of 2 or 3 subspherical parts fused into a lobate structure; zoospores 2 to 12, formed in a vesicle arising indiscriminately from the sporangium, reniform and laterally biciliate, measuring 14 to 17 μ in diameter upon encystment. Oogonia subspherical, typically terminal on short lateral branches, also lateral and sessile, measuring 29 to 67 μ, average 45.6 μ, in diameter exclusive of the conical or mammiform spines 1 to 10 μ, average 5.5 μ, long, and 2.5 to 6.5, average 4.5 μ, wide at base. Antheridia diclinous, 1 to 4 per oogonium, usually terminal, sometimes intercalary, lateral or sessile on the parent filament, the antheridial cell broadly saccate, cylindrical or barrel-shaped, lobate or with diverticulate protuberances, 20 to 43 μ, average 27 μ, long and 12 to 21 μ, average 15 μ, wide, making broad apical oogonial contact. Oospores aplerotic, 25 to 42 μ, average 35.3 μ, in diameter, with a wall about 1.6 μ thick containing a single reserve globule and refringent body; germination not observed.

Originally described from *Lactuca sativa* L., United States.

The observations of the writer concur with Drechsler's description of the fungus.

Pythium polymastum exhibits resemblances to *P. mastophorum* and *P. megalacanthum;* all possess fairly large echinulate oogonia and aplerotic oospores. The spines of *P. polymastum* and *P. mastophorum* are typically mammiform, while those of *P. megalacanthum* are conical and obtuse or papillate.

The sporangium of *P. polymastum* is non-proliferous and non-papillate, distinguishing this species from *P. megalacanthum* and *P. anandrum*. A peculiar characteristic of the sporangium of *P. polymastum* is its tendency to become irregular in contour and often even lobate composed of two or three subspherical parts fused together. The sporangium of *P. mastophorum*

is regular in contour and devoid of protuberances. The evacuation tube of *P. polymastum* is fairly short, straight and unbranched, further distinguishing the species from *P. mastophorum* in which the evacuation tube is longer, more or less contorted and frequently bears several branched vegetative prolongations. The size of the oogonia may be used here to advantage, those of *P. mastophorum* averaging 35 μ in diameter while the oogonia of *P. polymastum* average 45.6 μ in diameter.

In addition to the reports of Drechsler (1930a, 1939) isolating this fungus from *Lactuca sativa* L., the author has obtained it from roots of *Bellis perennis* L., *Daucus carota* L. and *Euphorbia pulcherrima* Willd., all in the United States.

57. PYTHIUM IRREGULARE Buisman, Med. Phytopath. Lab. 11: 1–51. 1927.

Hyphae measuring 2.6 to 7.9 μ in diameter. Sporangia of various shapes, mostly spherical to obovate, but sometimes ellipsoidal, pyriform and truncate, terminal or intercalary, measuring 10 to 26.7 μ, average 18.0 μ, in diameter; zoospores seldom produced, about 8 μ in diameter upon encystment. Oogonia spherical to limoniform, measuring 9.6 to 28.3 μ, average 17.8 μ, in diameter, usually intercalary though also terminal, of irregular contour, fairly smooth or undulant to definitely irregularly echinulate, the spines varying in shape but usually of broad base and acuminate apex, straight or contorted. Antheridia typically monoclinous, occasionally diclinous, 1 to 4, usually 1 to 2, per oogonium, stalked, the antheridial cell short, clavate and slightly crook-necked, making apical contact with the oogonium. Oospores aplerotic, measuring 8.1 to 25.2 μ, average 15.4 μ, in diameter, wall about 1.4 μ thick and containing a single reserve globule and refringent body.

Originally described from *Cucumis sativa* L., *Lupinus* sp. and *Pisum sativum* L., the Netherlands.

The sporangia of *Pythium irregulare* vary in shape from spherical to subspherical, elliptical to ovoid, pyriform to truncate, and are either acrogenous or intercalary, probably of more diverse shape than any other species of the genus.

The sexual stage of *Pythium irregulare* is analogous to that of *P. debaryanum*, differing principally in the presence of oogonial protuberances in the former. The spines of *P. irregulare* are few to numerous, always of irregular lengths and irregularly arranged on the oogonium. The spines may be dome-shaped, conical, papillate or curved-digitate, septate or non-septate but always with a blunt tip. The oogonia are both intercalary and acrogenous. The antheridia are usually monoclinous, occasionally diclinous; the antheridial cell is stalked, small in relation to the oogonium, slightly inflated, clavate, crook-necked, the apex slightly applied to the oogonial wall. The antheridia vary from 1 to 5 in number, usually 2 to 3, per oogonium.

The oospore is aplerotic, thin-walled, filled with dense, granular protoplasm containing a single, small, reserve globule and a prolate ellipsoidal refringent body.

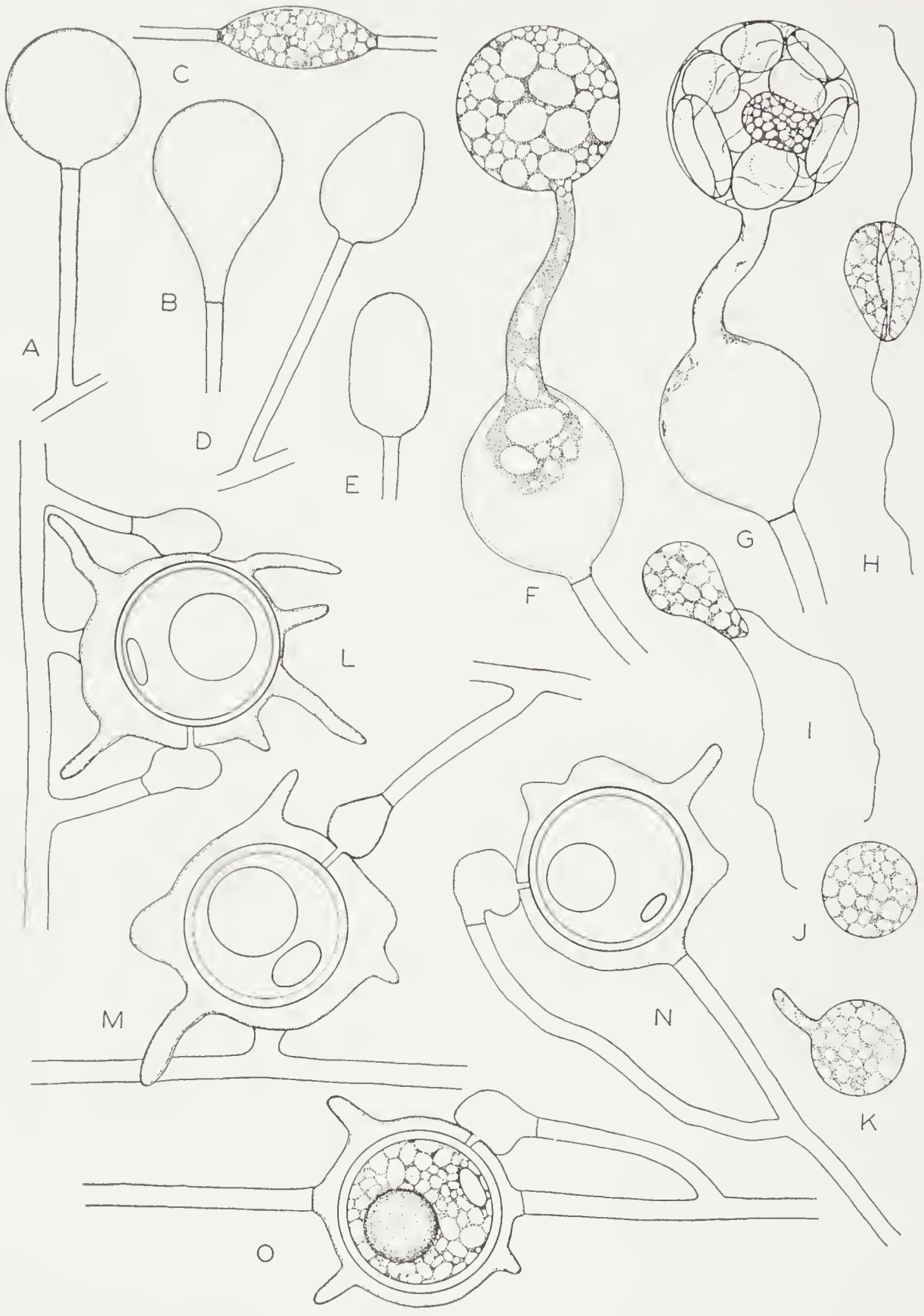

FIG. 17. *Pythium irregulare*. *A–E*, Sporangial types of usual occurrence. *F*, *G*, Zoospore formation. *F*, The contents of the sporangia flow through the emission tube into the apical, rounded vesicle; the vesicle contents are as yet undifferentiated. *G*, The vesicle contents have become differentiated into zoospores. These will be liberated by the subsequent dissolution of the vesicle wall. *H*, Typical motile zoospores. *I*, Zoospores beginning quiescence. *J*, Zoospores at encystment. *K*, Zoospores initiate germination. *L–O*, Various orientations of the sexual apparatus.

The growth habit and temperature-growth relations of *Pythium irregulare* are very similar to those of *P. debaryanum*, *P. ultimum*, and many other species and cannot be used as adjuncts in identifying it.

Agropyron cristatum (L.) Gaertn. UNITED STATES: Fischer *et al.*, (1942).
Agrostis stolonifera L. NETHERLANDS: Van Luijk (1934a, 1938a).
Ananas comosus Merr. HAWAII: Parris (1940); Sideris (1932); Sideris and Paxton (1931).
Antirrhinum majus L. ENGLAND: Vanterpool. UNITED STATES: Middleton (1938); Nance (1939).
Anthoceros sp. UNITED STATES: Matthews (1931).
Atropa belladonna L. UNITED STATES: Middleton (1941a).
Avena sativa L. UNITED STATES: Melhus, Reddy and Buchholtz (1939); Sprague.
Azalea obtusum Planch. UNITED STATES: Author.
Bouteloua gracilis (H.B.K.) Lag. UNITED STATES: Sprague.
Bromus inermis Leyss. UNITED STATES: Fischer *et al.* (1942).
B. tectorum L. UNITED STATES: Fischer *et al.* (1942).
Cajanus cajan Millsp. HAWAII: Parris (1940). Sideris (1932).
Canavalia ensiformis DC. HAWAII: Parris (1940). Sideris (1932).
Carica papaya L. UNION SOUTH AFRICA: Wager (1931, 1932a, 1940).
Chamaecyperis lawsoniana Parl. UNITED STATES: Author.
Citrullus vulgaris Schrad. UNITED STATES: Younkin (1938); Younkin and Melhus (1939, 1940); Author.
Citrus sp. UNION SOUTH AFRICA: Wager (1931, 1940).
Cucumis sativus L. NETHERLANDS: Buisman (1927).
Elymus junceus Fisch. UNITED STATES: Fischer *et al.* (1942).
Festuca duriuscula L. NETHERLANDS: Van Luijk (1934a, 1938a).
F. elatior L. UNITED STATES: Fischer *et al.* (1942).
Helianthus annuus L. HAWAII: Parris (1940); Sideris (1932).
Hordeum bulbosum L. UNITED STATES: Sprague.
Ipomoea batatas Lam. HAWAII: Parris (1940); Sideris (1932).
Linum usitatissimum L. NETHERLANDS: Diddens (1932).
Lolium annuum L. var. *westerwoldicum*. NETHERLANDS: Van Luijk (1938a).
Lupinus sp. NETHERLANDS: Buisman (1927).
Phaseolus aureus Roxb. HAWAII: Parris (1940); Sideris (1932).
Picea abies Karst. UNITED STATES: Author.
Pimelea ferruginea Labill. UNITED STATES: Author.
Pinus resinosa Ait. UNITED STATES: Roth and Riker (1942); Author.
P. rigida Mill. UNITED STATES: Author.
Pisum arvense L. UNITED STATES: Weimer (1940).
P. sativum L. NETHERLANDS: Buisman (1927).
Primula obconica Nance. UNITED STATES: Middleton (1938); Nance (1939).
P. sinensis Lindl. UNITED STATES: Author.
Ranunculus asiaticus L. UNITED STATES: Tompkins and Middleton (1939, 1942).
Saccharum officinarum L. UNITED STATES: Rands and Dopp (1938b); Stevenson and Rands (1938).
Spinacea oleracea L. UNITED STATES: Nance (1940).
Triticum aestivum L. UNITED STATES: Sprague.
Tritonia sp. UNITED STATES: Author.
Vicia sp. UNITED STATES: Weimer (1940).
V. faba L. HAWAII: Parris (1940); Sideris (1932).

58. PYTHIUM UNDULATUM Petersen, Bot. Tidssk. **29**: 345–440. 1909; Ann. Mycol. **8**: 494–560. 1910.

Mycelium within the substrate difficult to distinguish, extramatrical mycelium rarely branched, more or less undulating, long, measuring 3 to 6 μ in diameter. Sporangia acrogenous, ellipsoidal, sometimes with a small apical papilla, measuring 130 μ long and 50 μ wide, proliferous, the subse-

quent sporangia usually formed within the primary one; zoospores laterally biciliate, reniform, measuring 15 to 20 μ in diameter upon encystment, formed in a sessile, apical vesicle. Sexual reproduction not observed.

Originally described from leaves of *Nymphaea alba* L. and *Nuphar luteum* Sibth. and Sm., Denmark.

The sporangia are proliferous, a new sporangium being formed within or beyond the old one. Secondary sporangia formed exogenously are borne on relatively short stalks. Occasionally sporangia are formed laterally at the base of an acrogenous primary sporangium. Regardless of position of sporangium, the vesicle is sessile, arising from the papilla.

Pythium undulatum may be distinguished from *P. intermedium* and *P. elongatum* by its proliferous habit which is typical also in *P. diacarpum*, and *P. carolinianum*. *P. undulatum* differs from these species in its vesicle which is sessile upon the sporangium, while those of *P. diacarpum* and *P. carolinianum* are stalked.

In addition to *Nymphaea alba* L. and *Nuphar luteum*, Petersen lists this species from old fruits of *Iris* sp. and vegetable debris (". . . branches of trees . . .") in Denmark, Dissmann (1927) isolated *Pythium undulatum* from leaves of *Nymphaea candida* Presl. in Bohemia and Matthews (1931) from soil in the United States.

59. Pythium carolinianum Matthews, Studies on the genus Pythium. 1931.

Hyphae fine, measuring 1 to 4 μ in diameter. Sporangia typically acrogenous, intercalary, spherical to elongate, measuring 20 to 30 μ, average 25 μ, in diameter, with a well developed apical papilla, proliferous with the secondary sporangia formed inside or out of the primary ones; zoospores numerous, formed in a stalked vesicle, laterally biciliate and reniform, measuring 8 to 10 μ in length. Sporangia sometimes germinate by the production of germ tubes.

Originally described from *Spirogyra* sp., United States.

A single isolate of this species was observed by the writer; *Pythium carolinianum* is readily identified. The shape of the sporangium and the position of the vesicle separate *P. carolinianum* from *P. undulatum*. The sporangium of *P. carolinianum* is spherical to elliptical with the vesicle borne on a short stalk, while the sporangium of *P. undulatum* is prolate ellipsoidal and bears a sessile vesicle. The sporangia of both species are proliferous and papillate, distinguishing them from *P. diacarpum* which is a proliferous form without papillae. *P. intermedium* and *P. elongatum* are not proliferous species and cannot be confused with *P. carolinianum*.

Pythium carolinianum is known only from *Spirogyra* sp. (Matthews) and vegetable debris (Author) in the United States.

60. Pythium diacarpum Butler, Mem. Dept. Agr. India 1^5: 1–160. 1907.

Hyphae very slender, less than 1.5 μ in diameter, sparingly branched. Sporangia acrogenous and spherical, approximately 30 μ in diameter, capable

of proliferation. Evacuation tube long, several times the length of the sporangium, often wavy or helicoid, about 2 to 3 μ in diameter; zoospores infrequently formed exogenously though typically within the vesicle, capable of repeated emergence. Sexual reproduction not observed.

Saprophytic on vegetable debris, Germany.

The non-papillate sporangium of *Pythium diacarpum* readily distinguishes the species from the allied proliferous forms, *P. undulatum* and *P. carolinianum*. The sporangium of *P. diacarpum* is spherical and borne on a fine hypha, 1.5 μ in diameter. The evacuation tube produced at the initiation of zoospore formation is characteristically twice the diameter of the sporangiophore. At the tip of the evacuation tube an urn-shaped vesicle forms which remains distinguishable for some time; none of the allied species, *P. undulatum*, *P. carolinianum*, *P. intermedium*, or *P. elongatum* exhibit a long evacuation tube of this type. The proliferous habit of *P. diacarpum* further separates it from *P. intermedium* and *P. elongatum*.

This species is known only from the original report.

61. PYTHIUM INTERMEDIUM de Bary, Bot. Zeit. **39**: 553–558. 1881.

Artotrogus intermedius Atkinson, Cornell Agr. Exp. Sta. Bull. 94: 233. 1895.

Mycelium branched, 2 to 6 μ in diameter, coenocytic when young, septate when mature. Sporangia spherical, 18 to 24 μ in diameter, catenulate, often up to 13 in a series, the sporangia sessile or separated by short stalks. Sexual reproduction unknown.

Parasitic on prothallia of *Ceratopteris*, *Equisetum* and *Todea* and saprophytic on *Amaranthus* and *Lepidium* seedlings.

Pythium intermedium is readily identified by its catenulate sporangia. These are usually spherical when stalked and frequently pyriform when sessile, the tapered part arising from the sporangium below. The terminal sporangia are occasionally deciduous when more than 10 are arranged in a series. Germination is usually accomplished by the production of zoospores in a vesicle on a short evacuation tube; germ tubes are occasionally formed.

The intercalary sporangia of *Pythium carolinianum* are sometimes regarded as catenulate and may possibly be mistaken for those of *P. intermedium;* however, *P. carolinianum* is distinguished from *P. intermedium* by its proliferous, papillate sporangia.

Pythium intermedium is known from the following list of plants: *Agrostis stolonifera* L. in the Netherlands by van Luijk (1938a); *Amaranthus* sp. in Germany by de Bary (1881); *Aquilegia vulgaris* L. and *Arabis alpina* L. in the Netherlands by Meurs (1928); *Begonia lloydii* (Hort. name) and *B. tuberhybrida* Voss in the United States by Middleton (1938, 1942) and Nance (1939); *Ceratopteris* sp. in Germany by de Bary (1881); *Chrysanthemum morifolium* Ram. in the Netherlands by Buisman (1927); *Equisetum* sp. in Germany by de Bary (1881); Fern prothallia in the United States by Atkinson (1895); *Festuca duriuscula* L. in the Netherlands by van Luijk (1938a);

Fragaria vesca L., *Godetia willdenowiana* Spach. and *Hesperis matronalis* L. in the Netherlands by Meurs (1928); *Lepidium* sp. in Germany by de Bary (1881); *Linum usitatissimum* L. in the Netherlands by Diddens (1932); *Lolium annuum* L. var. *westerwoldicum* in the Netherlands by van Luijk (1938a); *Lupinus angustifolius* L. in Germany by Schultz (1939); *Pelargonium* sp. in the Netherlands by Buisman (1927); *Phaseolus vulgaris* L. and *Pisum sativum* L. in the Netherlands by Meurs (1928); *Pyrus communis* L. in Belgium by Marchal and Marchal (1921), *P. Malus* L. in Germany by Schultz (1939); *Ricinus communis* L. in the United States by the author. Soil in England, France and Germany by Butler (1907); *Todea* sp. in Germany by de Bary (1881); *Tulipa gesneriana* L. in the United States by the author; *Ulmus campestris* L. var. *latifolia* in the Netherlands by Westerdijk and Buisman (1929) and Westerdijk, Ledeboer and Went (1931); *Viola tricolor* L. in the Netherlands by Meurs (1928) and van Eek (1938).

62. Pythium elongatum Matthews, Studies on the genus Pythium. 1931.

Hyphae measuring 2 to 4 μ in diameter, branched. Sporangia terminal or intercalary, spherical, pyriform, cylindrical or curved, when spherical measuring 12 to 50 μ, when cylindrical measuring up to 65 μ long; zoospores produced in a vesicle borne on a very long evacuation tube, laterally biciliate, reniform, about 6 μ wide and 10 to 12 μ long. Sporangia also germinate by means of germ tubes. Sexual reproduction not observed.

Originally isolated from soil, United States.

Pythium elongatum is one of two species lacking sexual reproduction that does not have a proliferous or catenulate sporangial habit. The absence of sporangial proliferation distinguishes the species from *P. undulatum*, *P. carolinianum*, and *P. diacarpum;* the absence of catenulate sporangia separates the species from *P. intermedium*.

The fungus is known only from the original report.

63. Pythium scleroteichum Drechsler, Jour. Agr. Res. **49**: 881–890. 1934.

Hyphae branched, measuring 2.5 to 7 μ in diameter; terminal, clavate, appressoria present measuring 5 to 12 μ in diameter. Sporangia unknown. Oogonia spherical, typically acrogenous though sometimes intercalary, measuring 16 to 32 μ, average 23.8 μ, in diameter, the wall sturdy and smooth, measuring 0.5 to 1.2 μ, average 0.9 μ, thick, the septum or septa usually inserted beyond the oogonial contour. Antheridia monoclinous or diclinous, terminal, 1 to 5, usually 3, per oogonium frequently 2 cells to a stalk, the cell clavate, crook-necked, 9 to 16 μ long and 4 to 7 μ wide, the blunt apical end making narrow contact or broadly appressed to the oogonial wall, the antheridial branches sometimes containing a number of septa and constricted at intervals by relatively deep, transverse, dorsal furrows, the branches together with their frequent vegetative prolongations extensively and intimately wrapped about the oogonium. Oospore aplerotic, distinctly yellowish, measuring 11 to 26 μ, average 18.7 μ, in diameter, the wall thin, 0.8 to 1.4 μ, average 1.1 μ, thick and containing a single reserve globule and refringent body.

Originally described from roots of *Ipomoea batatas* Lam., United States.

Pythium scleroteichum is one of a group of species ascribed to the genus *Pythium* despite the absence of sporangia. The type and method of sporangial germination is a generic characteristic, and there may be objections to the inclusion of a species not exhibiting sporangial habits typical of *Pythium*. However, this species and other members of the group are included in the genus on the basis of their mycelial character, type of oogonium, antheridium, and oospores.

An oogonium devoid of echinulations readily distinguishes *Pythium scleroteichum* from *P. acanthophoron*, *P. artotrogus*, and *P. echinocarpum*. The antheridia resemble somewhat those of *P. adhaereus* and *P. periilum* in that the several antheridial cells arise from a single antheridial branch. The antheridia differ, however, in not being restricted in origin to a single monoclinous or diclinous branch, often arising from separate parent hyphae without close mycelial connection. As in *P. periilum*, the antheridia and vegetative ramifications of the antheridial stalk frequently envelop the oogonium intimately and extensively. The antheridial branches are sometimes constricted by fairly deep, transverse fissures.

As pointed out by Drechsler (1934b) the oospores of this species degenerate in culture unless the pH of the nutrient agar substrate ranges between 4.5 and 5.0.

The temperature-growth relations of this fungus may be used as an adjunct to its morphological identification.

This species is known only from sweet-potato, *Ipomoea batatas* Lam., in the United States, originally from a study of mottle necrosis by Harter and Whitney (1927b); it was not until 1934 that a description of the fungus was given by Drechsler.

64. Pythium artotrogus (Mont.) de Bary, Abhandl. Senckenb. Naturf. Ges. **12**: 225–340. 1881.

Artotrogus hydnosporus Montagne, Gard. Chron. **1845**: 640. 1845; Jour. Roy. Hort. Soc. **1**: 9–34. 1846.
Pythium hydnosporum (Mont.) Schröter in Engl. & Prantl, Nat. Pfl. **1**: 104–105. 1897.
Pythium artotrogus var. *macracanthum* Sideris, Mycologia **24**: 14–61. 1932.

Hyphae branched, measuring 2.6 to 7.8 μ in diameter. Sporangia lacking. Oogonia spherical, usually intercalary though also acrogenous, measuring 18 to 27 μ in diameter, wall conspicuously echinulate. Antheridia typically hypogynous. Oospores aplerotic, 15 to 24 μ in diameter.

Originally described from *Solanum tuberosum* L., France.

The first description of the species by Montagne was incorporated in an editorial by Berkeley in 1845. A year later, Montagne gave the fungus a Latin diagnosis which was included in an article on potato blight by Berkeley. De Bary (1876) observed *Pythium artotrogus* in a diseased potato tuber and noted the similarity of *Artotrogus* and *Pythium* in his study of the potato blight fungus, *Phytophthora infestans*. It was not until later (1881)

that he concluded that the two genera were synonymous and transferred *Artotrogus hydnosporus* to the genus *Pythium* as *P. artotrogus*. Fischer (1892) in his treatment of the genus concurs with de Bary on this point. Schröter (1897), however, though recognizing the synonymity of the two, transferred *A. hydnosporus* to *P. hydnosporum*, disregarding the usual practice of transferring the invalid generic nomen to specific rank in the valid genus.

A study of the species more complete than that by Montagne is presented by Butler (1907). Sporangia are unknown. Oogonia are spherical, 18 to 27 μ, average 22.4 μ, in diameter, acrogenous or intercalary. Oogonial protuberances are conical, 2.7 to 8.3 μ long and about 1.8 μ wide in basal diameter, tapering to an acute apex. The antheridium is hypogynous, rarely sessile adjacent to the oogonium. Oospores are aplerotic and smooth walled. The oospore frequently germinates by the production of a branched hypha.

Acuminate spines and the hypogynous antheridium distinguish *Pythium artotrogus* from *P. acanthophoron*. The absence of monoclinous and diclinous antheridia distinguish *P. artotrogus* from *P. echinocarpum*.

Sideris described *Pythium artotrogus* var. *macracanthum* as a new variety stating, ". . . it differs from *P. artotrogus* in having some epigynous antheridia, in the greater length of the spines of its oospores and the greater amount of aerial mycelium produced in culture media." Epigynous antheridia, monoclinous and adjacent to the oogonium as sessile, inflated, clavate structures, are not unique to the new variety. Its spines are conical, acuminate, 7.5 to 10 μ long and 1.0 to 1.5 μ in basal diameter compared with the conical, acuminate, 2.7 to 8.3 μ long and about 1.8 μ basal diameter of the species. Further, Sideris states erroneously that the oospores are echinulate; the oogonia are echinulate and the oospores smooth. The differences distinguishing the variety from the species do not seem adequate to maintain it as a valid nomen.

Pythium artotrogus is known from the following hosts:

Ananas comosus Merr. HAWAII: Parris (1940); Sideris (1932); Sideris and Paxton (1931).
Apium graveolens L. var. *dulce* DC. ENGLAND: Stirrup and Ewan (1931).
Arabis alpina L. NETHERLANDS: Meurs (1928).
Brassica oleracea L. var. *botrytis* L. GERMANY: Kryopoulus (1916).
Cajanus cajan Millsp. HAWAII: Parris (1940); Sideris (1932).
Canavalia ensiformis DC. HAWAII: Parris (1940); Sideris (1932).
Ipomoea batatas Lam. HAWAII: Parris (1940); Sideris (1932).
Lycopersicon esculentum Mill. HUNGARY: Moesz (1938).
Nymphaea candida Presl. BOHEMIA: Dissmann (1927).
Panicum purpurascens Raddi. HAWAII: Parris (1940); Sideris (1932).
Phaseolus vulgaris L. HAWAII: Parris (1940); Sideris (1932).
Pinus banksiana Lamb. UNITED STATES: Gravatt (1925); Hartley (1921).
P. nigra Arn. var. *austriaca* Aschers. and Graebn. NETHERLANDS: Ten Houten (1939).
P. ponderosa Dougl. UNITED STATES: Hartley (1921).
P. resinosa Ait. UNITED STATES: Gravatt (1925); Hartley (1921).
Pseudotsuga taxifolia Britt. NETHERLANDS: Ten Houten (1939).
Raphanus sativus L. SWEDEN: Palm (1934).
Saccharum officinarum L. HAWAII: Parris (1940); Sideris (1932). UNITED STATES: Edgerton and Moreland (1921); Stevenson and Rands (1938).

Solanum tuberosum L. ENGLAND: Smith (1876). FRANCE: Montagne (1845, 1846). GERMANY: de Bary (1876, 1881b). INDIA: Butler (1907); Butler and Bisby (1931); Sydow and Butler (1907).
Spinacea oleracea L. NETHERLANDS: Meurs (1928).
Vigna sinensis Endl. HAWAII: Parris (1940).
Viola tricolor L. NORWAY: Solberg (1926).
Water. UNITED STATES: Matthews (1931).

Wager (1931) refers an isolate from *Antirrhinum majus* L. and *Papaver rhoeas* L. to this species, later (1940) placing the fungus under the binomial *Pythium oligandrum*.

65. PYTHIUM ECHINOCARPUM Ito & Tokunaga, Jour. Fac. Agr. Hokkaido Imper. Univ. **32**: 201–233. 1933.

Hyphae slender, 1.2 to 4.8 μ in diameter. Sporangia unknown. Oogonia terminal or intercalary, spherical, measuring 15 to 24 μ in diameter exclusive of the pointed, conical spines 4 to 9 μ long and 1.5 μ in basal diameter. Antheridia monoclinous, or diclinous, rarely hypogynous, single or rarely 2 per oogonium, when not hypogynous, clavate elongate and usually crook-necked, when hypogynous cylindrical and rarely inflated. Oospores aplerotic, single, 13 to 21 μ in diameter, the wall thin, 0.8 to 1.2 μ thick; germination not observed.

Originally described from seedlings of *Oryza sativa* L., Japan.

Cultures of this species were not available for study; the discussion is based on the material presented in the original article from which the description is taken.

The oogonia of *Pythium echinocarpum* are very much like those of *P. artotrogus;* they are both spherical, either terminal or intercalary, the spines of similar dimension and both conical and acutely tipped. The pointed spines of *P. echinocarpum* distinguish it from *P. acanthophoron* which possesses obtusely tipped spines. The oospores of the three species are all aplerotic. The commonly monoclinous or diclinous antheridia of *P. echinocarpum* separates the species from *P. artotrogus* which possesses hypogynous antheridia. Further study may prove the species to be synonymous with *P. artotrogus*.

This species is known only from the original description and the report of Darker (1940) stating its occurrence on *Oryza sativa* L. seedlings in Japan.

66. PYTHIUM ACANTHOPHORON Sideris, Mycologia **24**: 14–61. 1932.

Hyphae irregular, slightly dendroid, measuring 3 to 7 μ in diameter. Sporangia unknown. Oogonia spherical, acrogenous or intercalary, measuring 20 to 30 μ in diameter exclusive of the obtusely tipped spines (Sideris = 5 to 7 μ long). Antheridia monoclinous or diclinous, slightly curved, clavate, making broad oogonial contact, 10 to 20 μ long and 4 to 8 μ wide. Oospore aplerotic, 15 to 25 μ in diameter, the wall 1.5 μ thick.

Originally described from *Ananas comosus* Merr., Hawaii.

Sideris erroneously described *Pythium acanthophoron* as having smooth oogonia and spiny oospores. An authentic culture of this fungus studied

had spiny oogonia and smooth oospores. The oogonial protuberances are conical, usually 2.5 μ long and 1.7 μ in basal diameter, with an obtuse or blunt tip. The antheridia are mostly monoclinous, rarely diclinous, borne on a relatively short antheridial stalk. The antheridial cell is somewhat saccate and appressed to the oogonium.

Pythium acanthophoron and *P. acanthicum* are similar in size, shape, character of spines and type of antheridia, though these are more clavate and crook-necked in *P. acanthicum;* they differ decidedly, however, in the type of oospores, aplerotic in *P. acanthophoron*, plerotic in *P. acanthicum*. The conical, obtuse spines of *P. acanthophoron* separate this species from *P. artotrogus* and *P. echinulatum* both of which have acute spines.

The pulvinate growth habit of *P. acanthophoron* readily distinguishes it from *P. acanthicum*, which has a cumulous growth habit. *P. acanthophoron* is unique among the species studied in its failure to grow at temperatures below 13° C.

This species is known only from Hawaii on *Ananas comosus* Merr.

DOUBTFUL AND INVALID SPECIES

A number of the *Pythium* spp. reported here have previously been designated as doubtful by other authors, particularly Butler (1907) and Matthews (1931). A few are newly presented and the reasons given for their inclusion here. With the exception of *nomina nuda*, the larger number of entries are designated doubtful in the absence of adequate description and observation since their original presentation.

PYTHIUM ACTINOSPHAERII Brandt, Monatsb. K. Preuss. Akad. Wiss., Berlin, **1881**: 399. 1881.

Fischer (1892) believes this organism not to be a *Pythium;* Butler (1907) believes it to be a *Saprolegnia* sp. Zopf (1890) states that *P. actinosphaerii* was incorrectly described as a new species and should now be referred to *Actinosphaerium Eichhornii* Brandt; Zopf gives no morphologic discussion of the organism.

PYTHIUM AKANENSE Tokunaga, Trans. Sapporo Nat. Hist. Soc. **12**: 119–123. 1932.

The description of this fungus would indicate that it probably should be referred to *Pythium monospermum* rather than be maintained as a new and valid species. Information regarding the disposition of the antheridium is not given. The only differences between *P. akanense* and *P. monospermum* seems to be in the thickness of the oospore wall, a criterion which should not be used as the sole factor in specific segregation. Until the fungus has been observed and further studied it seems desirable to consider it a doubtful species, possibly synonymous with *P. monospermum*. This fungus is recorded from *Aegagropila sauteri* (Nees) Kütz. in Japan by Tokunaga, Ito and Tokunaga (1935) and Darker (1940); Tokunaga also reports the fungus able to infect *Cladophora* sp. upon inoculation.

PYTHIUM CHAMAIHYPHON Sideris, Mycologia **24**: 14–61. 1932.

This species was presumably transferred to *Pythium polycladon* as a variety of it. Since the inclusion of *P. polycladon* in *P. vexans* the taxonomic status of *P. chamaihyphon* has been doubtful.

PYTHIUM CHARACEARUM de Wildeman, Ann. Soc. Belg. Micros. **20**: 107–136. 1896.

Described as a new species on oogonia of *Chara* sp. collected in Switzerland. Acrogenous oogonia of unknown size, one to four antheridia of unknown origin and aplerotic oospores 20 to 30 μ in diameter are described; the asexual apparatus was not observed. The fungus has not been observed subsequent to its description. Until a more detailed diagnosis of this species is presented it must be considered of doubtful validity.

PYTHIUM CHLOROCOCCI Lohde, Tagebl. Versamm. Deutsch. Naturf. Aerzte, Breslau, **47**: 203. 1874.

The description presented is too meager and incomplete to permit identification of the species.

PYTHIUM CIRCUMDANS (Lohde) Fischer, Tagebl. Versamm. Deutsch. Naturf. Aerzte, Breslau, **47**: 203. 1874.

This species, although referred to as *Lucidium circumdans*, is probably a species of *Pythium*, Fischer (1892) transferring it to this genus and including it under *P. debaryanum*. Butler (1907) does not agree with Fischer's interpretation. On the basis of the imperfect description it would appear best to consider the species of doubtful validity.

PYTHIUM DACTYLIFERUM Drechsler, Proc. Cong. Internat. Soc. Sugarcane Tech. **3**: 122. 1930.

This species was enumerated together with others associated with root rot and seed piece decay of sugarcane in the United States by Rands. A later report by Rands and Dopp (1938b) state that this organism originally isolated in 1927 and thought to be an undescribed species and named *P. dactyliferum* by Drechsler has been subsequently identified by him as *P. irregulare*. Inasmuch as no description of the organism in question was presented the name becomes a *nomen nudum*.

PYTHIUM DAPHNIDARUM Petersen, Bot. Tidssk. **29**: 345–420. 1909.

This species is difficult to identify from the description given, particularly in the absence of information regarding the nature and origin of the antheridium. Petersen indicates that this species may be of doubtful status and that it may prove to be referable to *Pythium gracile* or *P. complens* upon further study. It is reported as parasitic on *Daphnia hyalina*, *D. cucullata* and *Bosmina coregoni* in Denmark.

PYTHIUM DICHOTOMUM Dangeard, Ann. Sci. Nat. VII. **4**: 313. 1886.

This species was imperfectly observed and described and probably is not a *Pythium*.

PYTHIUM FERAX de Bary, Bot. Zeit. **39**: 553–563. 1881.

Despite the description presented by de Bary, in the absence of figures and measurements it is difficult to ascertain the exact nature of the fungus with which he worked. *P. ferax* resembles *P. proliferum* and may be identical with it, though de Bary states that his fungus may be separated from *P. proliferum* on the basis of its possessing smaller hyphae, nonvacuolated sporangia and extramatrical oogonia. This fungus has not been observed since its original description.

PYTHIUM FIMBRIATUM de la Rue, Bull. Soc. Imp. Natur. Moscow **42**: 469. 1869.

This is a *nomen nudum*, inasmuch as no description of the fungus was presented.

PYTHIUM GIBBOSUM de Wildeman, Ann. Soc. Belg. Micros. **20**: 107–136. 1896.

De Wildeman described this fungus as a doubtful species of the genus, remarking that it resembled *Aphanomyces* very closely. No description is given as the fungus was not well understood; the species justifiably becomes a *nomen nudum*.

PYTHIUM GLOBOSUM Schenk, Verh. Phys.-Med. Gesell. Würzburg **9**: 12–31. 1859.

According to Matthews (1931) this species and one described by Schenk as *Pythium proliferum* are identical and both of them improperly assigned to this genus. According to Zopf (1890) and Butler (1907), these two fungi are probably *Myzoctium* and *Lagenidium*.

PYTHIUM GLOBOSUM Walz, Bot. Zeit. **28**: 553. 1870.

This fungus is presumably a mixture of *Pythium proliferum* of Schenk and *P. globosum* of the same author. Since these two organisms are thought to be *Myzoctium* and *Lagenidium*, Walz's fungus cannot be considered a member of the genus *Pythium*.

PYTHIUM HYDRODICTYORUM de Wildeman, Ann. Soc. Belg. Micros. **21**: 22. 1897.

This species is reported parasitic on *Hydrodictyon*. Since the description is incomplete and only the sexual stage was observed this species is of questionable validity.

PYTHIUM IMPERFECTUM Cornu, Ann. Sci. Nat. VI. **15**: 13. 1872.

The description given is not adequate to identify the species.

PYTHIUM INCERTUM Renny, Jour. Bot. **14**: 156. 1876.

Fischer (1892) does not consider this fungus to be a *Pythium* and Butler (1907) indicates that the identity of this species is very uncertain. Matthews (1931) concurs in this opinion.

PYTHIUM LATERALE Pringsheim, Jahrb. Wiss. Bot. 9: 191–234. 1873–1874.

No detailed description of this fungus was given by Pringsheim, the fungus presented in an incidental manner when the author was comparing a few features in common with the imperfectly described *Pythium utriforme* of Cornu. *P. laterale* was said to differ from *P. utriforme* principally in the lateral orientation of the evacuation tube. The features presented are too meager to permit consideration of *P. laterale* as a valid species.

PYTHIUM MUSCAE Dangeard, Botaniste 22: 325–490. 1931.

Only the asexual stage of this fungus was observed. Dangeard states that zoospores were formed in typical *Pythium* fashion. The following comment of Dangeard is worth mention: "Nous reconnaissons volontiers qu'une observation isolée n'a pas grande valeur: toutefois, comme le champignon en lui-même est intérressant, il nous a paru qu'on pouvait sans inconvenient le décrire sous le nom proposé plus haut." No host or locality are given.

PYTHIUM NICOTIANAE Van Hall, Med. Inst. Plantenzkt. 67: 1–53. 1925.

This is a *nomen nudum*, no description of the fungus being given. It is possible that reference may have been intended to be for *Phytophthora nicotianae* Breda de Haan.

PYTHIUM PALMIVORUM Butler, Mem. Dept. Agric. India Bot. 1^5: 1–160. 1907.

Butler (1924) subsequently transferred this species to the genus *Phytophthora*.

PYTHIUM PLEROSPORON Sideris, Mycologia 24: 14–61. 1932.

The description of this species is inadequate to identify it; in the writer's opinion it may prove to be synonymous with *Pythium rostratum*.

PYTHIUM POLYANDRUM Van Hall, Med. Inst. Plantenzkt. 67: 1–53. 1925.

This is a *nomen nudum*, no description of the fungus being given.

PYTHIUM POLYSPORUM Sorokin, Trudy Obshch. Estestv. Imp. Kazan Univ. 2: 23. 1872.

Butler (1907) excludes this species from the genus *Pythium* on the basis of its asexual and sexual stages; the writer concurs in this opinion. Matthews (1931) does not believe it to be a *Pythium*, but suggests that it may be a new genus of the Pythiaceae.

PYTHIUM PROLIFERUM Schenk, Verh. Phys.-Med. Gesell. Würzburg 9: 12–31. 1859.

This fungus is not a *Pythium* species. It is thought to be a species of *Myzoctium* by Zopf (1890).

PYTHIUM SADEBECKIANUM Wittmack, Mitt. Ver. Ford. Moorkultur **10**: 83. 1892.

The inadequate description does not permit positive identification of the organism. It is possible that it may be a species of *Aphanomyces;* Jones and Drechsler (1925) and Matthews (1931) are of this opinion.

PYTHIUM TERATOSPORON Sideris, Mycologia **24**: 14–61. 1932.

Originally described as a *Pythium*, Sideris in a footnote transfers it to the genus *Phytophthora*. The writer considers this species to be identical with *Phytophthora drechsleri* Tucker; this observation has been confirmed by Tucker.

PYTHIUM TRACHEOPHILUM.

This name has been proposed for a pathogen causing stunt of lettuce. The fungus is treated in an unpublished thesis; consideration of it must await publication.

PYTHIUM UTRIFORME Cornu, Ann. Sci. Nat. VI. **15**: 13. 1872.

This fungus cannot be identified definitely from the description.

SUMMARY

A collection of over 2000 cultures of *Pythium* from various parts of the world and representing most of the known species was used in the investigation reported here.

The various isolates were grown on a number of different nutrient substrates for comparison of their growth habits and types of reproduction.

A detailed investigation of the morphology of the species indicated that the character of the sporangium, oogonium, antheridium and oospore could be used as criteria for the segregation of the species.

Isolates of all the species were grown on corn meal agar at temperatures ranging from 1° to 46° C. at 3° intervals. It was observed that different species varied considerably in their temperature relations. Different isolations of the same species behaved similarly indicating that the response to temperature is a specific feature. The temperature-growth relations are used as an adjunct to morphologic characters for identification of the species.

A description and discussion is given for each of the 66 species believed valid. Species reported and inadequately described or lacking description are listed as doubtful. Several species are shown to be synonymous with others, and the reasons for their elimination are included in the discussion of valid species. One new species, *Pythium hypogynum*, is presented.

A key based on certain morphologic features considered valid is provided for the identification of the species.

An attempt has been made to enumerate all the hosts on which the species are known to occur and their geographic distribution; this information is presented following the discussion of each species.

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GLOSSARY

acantha, spine.
acrogenous, originating or borne at the tip.
adnate, broadly and closely attached.
allantoid, sausage-shaped.
amphigynous, a basal type of antheridium which envelopes the oogonial stalk.
androgynous, antheridia and oogonia borne together or originating from the same hyphae.
apandrous, oospores formed in the absence of antheridia.
aplerotic, not filling.
appressorium, the flattened, thickened, or turflike tip of a hyphal branch by which the fungus is attached to the substrate.
arachnoid, resembling a spider's web.
catenulate, having a chainlike form.
clavate, club-shaped.
contiguous, an inflated portion in contact with another inflated portion, or near in succession through an intercommunicating filamentous portion.
dactyloid, finger-shaped.
diclinous, oogonia and antheridia arising from different hyphae, the antheridium not originating from the oogonial stalk.
digitate, finger-shaped.
diplanetism, the succession of two morphologically different phases of the swarm period always in the same sequence, separated by a rest period.
diverticulum, a pocket or closed branch opening off a main passage.
echinulate, spiny.
encystment, period of rest when a spore rounds-up and presents a visible wall.
endogenous, originating in the inside.
exogenous, originating on the outside.
falcate, sickle-shaped, hooked or curved like a sickle.
hyaline, colorless.
helicoid, spiral-shaped.
hypogynous, antheridium beneath the oogonium, specifically in the Pythiaceae either within the oogonial stalk or composed of a portion of the oogonial stalk adjacent to the oogonium.
inspissate, thickened.
intercalary, growth or development which is not apical but between the apex and the base.
monandrous, oospores formed in the presence of a single functional antheridium.
monoclinous, oogonia and antheridia arising from the same hyphae, the antheridium originating from the oogonial stalk.
monoplanetism, the condition of having one swarm phase, with no rest period.
moriform, mulberrylike culsters.
papilla, a nipple-shaped projection.
paragynous, the antheridium in lateral association with the oogonium, as opposed to *amphigynous*.
parthenogenesis, the process by which a new individual arises from haploid cells in the absence of fertilization.
plerotic, filling.
polyandrous, oospores formed in the presence of several functional antheridia.
pulvinate, cushion-shaped or strongly convexed.
refringent, refractive.
reticulate, netlike.
sigmoid, curved as in the letter *C* or doubly curved in different directions as in the letter *S*.
toruloid, resembling the genus *Torula*, chainlike; cylindrical portions with swollen intervening sections at varying intervals.

ADDITIONAL REFERENCES

There are a large number of references to hosts from which unidentified species of *Pythium* have been obtained. In the absence of any discussion of the fungus it is difficult to know just what disposition should be made of the

record. In order that the list of *Pythium* references may be made as complete as possible these entries are compiled and presented:

Agrostis sp. UNITED STATES: Nance (1940).
A. dispar Michx. ALSACE: Anonymous (1936b).
A. stolonifera L. ALSACE: Anonymous (1936b).
Albizzia sp. SUMATRA: Kuyper (1928).
Allium cepa L. UNITED STATES: Anderson (1924); Edson and Wood (1936); Newhall (1939).
Anacardium occidentale L. PALESTINE: Reichert (1939).
Ananas comosus Merr. HAWAII: Johnson (1935); Sideris and Paxton (1929).
Anona squamosa L. PALESTINE: Reichert (1939).
Antirrhinum majus L. UNITED STATES: Altstatt (1942); Nance (1940).
Apium graveolens L. var. *dulce* DC. BERMUDA: Whetzel (1922). CHINA: Yu (1940).
Arachis hypogea L. UNITED STATES: Edson and Wood (1937); Shaw (1936).
Avena sativa L. UNITED STATES: Edgerton, Tims and Mills (1929); Melhus (1938); Melhus, Reddy and Buchholtz (1939). CANADA: Robertson (1931).
Begonia tuberhybrida Voss. UNITED STATES: Nance (1939).
Beta vulgaris L. ENGLAND: Greeves and Muskett (1936). PALESTINE: Reichert (1939). UNITED STATES: Edson and Wood (1937); Kadow (1937); Nance (1939); Wood and Nance (1938).
B. vulgaris var. *macrorhiza*. BELGIUM: Roland (1938, 1939). CZECHOSLOVAKIA: Neuwirth (1934). DENMARK: Henning (1922). ENGLAND: Stirrup (1939). GERMANY: Greis (1940a, 1940b). NETHERLANDS: Brandenburg (1931a, 1931b, 1933, 1935); Van Poeteren (1933). UNITED STATES: Buchholtz (1935); Hutchison and Freeborn (1939); Leach (1941); Leach and Houston (1938); Lill (1930); Maxson (1938); Nance (1939, 1940); Wood and Nance (1938).
Brassica oleracea L. var. *botrytis* L. PALESTINE: Reichert (1939).
B. oleracea var. *capitata* L. UNITED STATES: Edson and Wood (1937); Martin (1933).
B. oleracea var. *italica* Plenck. PALESTINE: Reichert (1939).
B. pekinensis Rupr. PHILIPPINE ISLANDS: Fajardo (1934).
B. rapa L. UNITED STATES: Edson and Wood (1937).
Callistephus chinensis Nees. CZECHOSLOVAKIA: Smolak (1925).
Capsicum annuum L. CEYLON: Anonymous (1936a). PALESTINE: Reichert (1939). PUERTO RICO: Cook (1924). UNITED STATES: Edson and Wood (1937); Martin (1933); Nance (1939, 1940).
C. frutescens L. CHINA: Yu (1940).
Carica papaya L. CEYLON: Anonymous (1936a). HAWAII: Parris (1939b, 1941a). PALESTINE: Reichert (1939). TRINIDAD: Baker (1933).
Castanea sativa L. ITALY: Petri (1933).
Catalpa speciosa Warder. UNITED STATES: Wright (1937).
Chrysanthemum cocineum Willd. UNITED STATES: Sandsten (1934).
Cicer arietinum L. ARGENTINA: Fawcett (1941).
Citrullus vulgaris Schrad. UNITED STATES: Clinton (1934); Nance (1939, 1940); Newell (1941); Younkin (1938); Younkin and Melhus (1939); Walker (1939); Wood and Nance (1938).
Citrus sp. ARGENTINA: Blanchard (1930). UNITED STATES: Weindling (1932).
C. aurantifolia Swingle. BRAZIL: Muller (1933).
C. limonia Osbeck. BRAZIL: Muller (1933).
C. maxima Merr. TRINIDAD: Baker (1938).
Cocos nucifera L. JAMAICA: Ashby (1921). UNITED STATES: Weber (1923).
C. plumosa Hook. UNITED STATES: Weber (1923).
Colocasia esculenta Schott. HAWAII: Carpenter (1920); Parris (1936, 1938a, 1939a, 1940, 1941b).
Crassula portulacea Lam. UNITED STATES: Pirone (1937).
Cucumis melo L. var. *inodoratus* Naud. UNITED STATES: Nance (1940).
C. sativus L. ENGLAND: Salmon and Ware (1931). PALESTINE: Reichert (1939). UNITED STATES: Haenseler (1934); Haenseler and Allen (1934); Haenseler and Moyer (1937); Martin (1933); Nance (1940); Ramsey (1936, 1937); Wood and Nance (1938).

Cucurbita sp. UNITED STATES: Kadow (1937).
C. maxima Duchesne. PALESTINE: Reichert (1939).
C. pepo L. var. *condensa* Bailey. UNITED STATES: Edson and Wood (1936, 1937).
Curcuma longa L. CEYLON: Anonymous (1936a).
Cynodon dactylon Pers. UNITED STATES: Nance (1940).
Dalea sp. UNITED STATES: Buchholtz (1935).
Dendrobium sp. MAURITIUS: Wiehe (1941).
Euphorbia pulcherrima Willd. UNITED STATES: Brown (1940).
Fern prothallia UNITED STATES: Dodge (1940).
Festuca duriuscula L. ALSACE: Anonymous (1936b).
F. rubra L. ALSACE: Anonymous (1936b).
Foeniculum vulgare L. ITALY: Borzini (1937, 1938).
Fragaria chiloensis Duchesne. AUSTRALIA: Adam and Pescott (1932). CANADA: Berkeley (1936); Hildebrand (1934); Hildebrand and Koch (1936); Truscott (1934). ENGLAND: Alcock (1929); Berkeley and Lauder-Thompson (1934); Garret *et al.* (1939); Hickman (1940). FRANCE: Labrousse (1933). SCOTLAND: O'Brien and M'Naughton (1928); Wardlaw (1926). UNITED STATES: Bain and Demaree (1938); Brooks, Watson and Mowry (1929); Dowell (1931); Nance (1939); Plakidas (1930).
F. grandiflora Ehrh. PALESTINE: Reichert (1939).
F. vesca L. NETHERLANDS: Bouwens (1937).
Garcinia mangostana L. BURMA: Su (1933).
Gladiolus sp. UNITED STATES: Edson and Wood (1936, 1937).
Gossypium sp. ANGLO-EGYPTIAN SUDAN: Andrews and Clouston (1938); Clouston and Andrews (1940); Massey (1935, 1936). INDIA: Dastur (1931).
G. hirsutum L. UNITED STATES: Edson and Wood (1936); Lehman (1939); Miller (1938); Nance (1939); Weindling, Miller and Ullstrup (1941).
Hevea brasiliensis Muell. MALAYA: Beeley (1929, 1938); Sharples (1926, 1930, 1933); Thompson (1928); SUMATRA: De Jong (1941).
Hordeum vulgare L. CANADA: Robertson (1931). JAPAN: Asuyama (1935). TUNIS: Chabrolin (1927).
Ilex paraguensis St. Hill. ARGENTINA: Blanchard (1928).
Ipomoea batatas Lam. UGANDA: Hansford (1938). UNITED STATES: Brown (1940); Buchanan (1940); Harter (1924, 1925); Manns and Adams (1925); Wood and Nance (1938).
Juglans regia L. PALESTINE: Reichert (1939).
Lactuca sativa L. BERMUDA: Ogilvie (1926); Whetzel (1922). CANADA: Smieton and Brown (1940). PALESTINE: Reichert (1939). UNITED STATES: Clinton (1934); Nance (1940); Newhall (1939); Richards (1937); Wood and Nance (1938).
Lathyrus odoratus L. UNITED STATES: Martin (1933).
Lepidium sativum L. ENGLAND: Salmon and Ware (1938).
Lespedeza sp. UNITED STATES: Buchholtz (1935).
Leucaena glauca Benth. SUMATRA: Palm (1925); Palm and Jochems (1924).
Linum usitatissimum L. NETHERLANDS: Diddens (1932). UNITED STATES: Buchholtz (1935); Flor (1940); Nance (1939, 1940); Tervet (1937).
Lupinus sp. NORWAY: Solberg (1926).
Lycopersicon esculentum Mill. AUSTRALIA: Samuel (1930). CHINA: Yu (1940). ENGLAND: Salmon and Ware (1931). HAWAII: Parris (1938b). JAMAICA: Hansford (1924). PALESTINE: Reichert (1939); Altstatt (1942); Beach and Shuk (1941). UNITED STATES: Harrison, Young and Altstatt (1939); Martin (1933); Nance (1939, 1940); Skinner (1939).
Mangifera indica L. PHILIPPINE ISLANDS: Camus (1935).
Medicago sativa L. UNITED STATES: Buchholtz (1934, 1935); Edson and Wood (1936, 1937); Grandfield, Lefebvre and Metzger (1935).
Melilotus alba L. UNITED STATES: Buchholtz (1935); Heald (1931); Humphrey and Wood (1935).
M. indica L. UNITED STATES: Buchholtz (1935).
Musa textilis Nee. PHILIPPINE ISLANDS: Hernandez (1924); Roldan (1933).
Nicotiana tabacum L. ALSACE: Anonymous (1936b). CANADA: Berkeley (1937); Hildebrand and Koch (1936); Koch (1935, 1937). CEYLON: Anonymous (1936a); Park (1937); Park and Fernando (1937); Paul and Fernando (1938). JAVA: Leefmans (1934). PUERTO RICO: Cook (1925). RUSSIA: Grooshevoy and Levykh (1936). SUMATRA: Jochems (1926,

1927a, b, 1933a, b); Meurs (1931); Sidenius (1922); Van der Goot (1928, 1934, 1935, 1937); Van Hall (1924, 1926). UGANDA: Hansford (1938). UNION SOUTH AFRICA: Doidge and Bottomley (1931); Moore (1926); Moore and Smith (1936). UNITED STATES: Anderson, Swanback and Street (1938); Cooper (1929); Edson and Wood (1936); Johnson (1937, 1939); Nance (1939, 1940); Valleau and Johnson (1939); Wood and Nance (1938).

Oryza sativa L. JAVA: Leefmans (1929, 1930). UNITED STATES: Cralley and Tullis (1937); Edson and Wood (1937); Gray (1934); Ryker (1937).

Panicum barbinode Trin. HAWAII: Martin (1937).

Pelargonium sp. CEYLON: Anonymous (1936a); Haigh (1929). ITALY: Petri (1935). UNITED STATES: Gill (1936); Holdridge and McCubbin (1928).

P. zonale L'Her. UNITED STATES: White (1938).

Persea americana Mill. PHILIPPINE ISLANDS: Roldan (1933).

Phaseolus lunatus L. UNITED STATES: Martin (1933).

P. vulgaris L. CHINA: Yu (1940). NORWAY: Solberg (1926). PALESTINE: Reichert (1939). PHILIPPINE ISLANDS: Fajardo (1934). SIERRA LEONE: Deighton (1936). UNITED STATES: Edson and Wood (1936); Wood and Nance (1938).

Phoenix dactylifera L. PALESTINE: Reichert (1939).

Physalis peruviana L. PALESTINE: Reichert (1939).

Phytolacca octandra L. SUMATRA: Palm (1925); Palm and Jochems (1924).

Pinus sp. UNITED STATES: Edson and Wood (1936); Nance (1939, 1940).

P. banksiana Lamb. UNITED STATES: Hansen *et al.* (1923); Stakman (1923).

P. insignis Dougl. INDIA: C (1927).

P. insularis Endl. PHILIPPINE ISLANDS: Roldan (1933).

P. longifolia Roxb. INDIA: C (1927).

P. massoniana Lamb. PHILIPPINE ISLANDS: Roldan (1933).

P. pinea L. ITALY: Petri (1940).

P. ponderosa Dougl. UNITED STATES: Jackson (1933).

P. resinosa Ait. UNITED STATES: Hansen *et al.* (1923); Stakman (1923); Wiant (1929).

P. strobus L. UNITED STATES: Hansen *et al.* (1923); Stakman (1923).

Pisum arvense L. NETHERLANDS: Brandenburg (1931a, 1931b).

P. sativum L. CANADA: Anonymous (1924a). ENGLAND: Baylis (1941); Croxall and Ogilvie (1940). NETHERLANDS: Brandenburg (1935). NEW ZEALAND: Hyde (1939). NORWAY: Solberg (1926). UNITED STATES: Drechsler (1925); Edson and Wood (1936, 1937); Haenseler (1924); Horsfall and Kertesz (1933); Jones (1925); Jones and Linford (1925); Kadow (1937); Martin (1933); Nance (1940); Sharvelle and Shema (1941); Wood and Nance (1938).

Poa compressa L. ALSACE: Anonymous (1936b).

P. pratensis L. ALSACE: Anonymous (1936b).

Pogostemon cablin Benth. UGANDA: Hansford (1938).

Prunus amygdalus L. PALESTINE: Reichert (1939).

Pseudotsuga taxifolia Britt. UNITED STATES: Jackson (1933).

Pyrus communis L. CZECHOSLOVAKIA: Baudys (1930).

Raphanus sativus L. UNITED STATES: Edson and Wood (1936, 1937).

Ricinus communis L. HAWAII: Weiss (1942).

Robinia pseudo-acacia L. UNITED STATES: Weiss (1942); Wright (1937).

Rubus idaeus L. CANADA: Berkeley (1936).

Saccharum officinarum L. AUSTRALIA: Bell (1929). EGYPT: Rosenfeld (1939). HAITI: Ciferri (1928). HAWAII: Agee (1926); Barnum (1927); Barnum and Zwaluwenburg (1927); Carpenter (1919, 1921, 1928c, 1934); Cooke (1933); Lee (1928); Lee, Barnum, Weller and Carpenter (1927); Lyon (1923); Martin (1937). INDIA: Ramakrishna (1941); Subramaniam (1936b). MAURITIUS: Hill (1931); Shepherd (1925, 1931, 1933); Wiehe (1940a, b). PHILIPPINE ISLANDS: Agati (1931); Ocfemia (1931); Roldan (1930). PUERTO RICO: Bourne (1924); Cook (1930); Earle (1920); Matz (1920, 1921). SUMATRA: Kuyper (1930); Treub (1885). UNION SOUTH AFRICA: McMartin (1937). UNITED STATES: Dowell (1931); Edgerton and Moreland (1921); Edgerton, Taggart and Tims

(1924); Edgerton and Tims (1925, 1927); Edgerton, Tims and Mills (1929); Flor (1930a, 1930b); LeBeau (1939); Lee, Weller and Barnum (1926); Nance (1939, 1940); Stevenson and Rands (1938); Tims (1932a); Tims and Mills (1927).

Secale cereale L. CANADA: Robertson (1931).

Senecio cruentus DC. UNITED STATES: Drechsler (1935).

Sinningia speciosa Benth. and Hook. GERMANY: Landgraf (1932).

Soil. UNITED STATES: Harvey (1927, 1929); Jensen (1912); Raper (1928).

Solanum melongena L. PALESTINE: Reichert (1939). UNITED STATES: Martin (1933).

S. tuberosum L. PALESTINE: Reichert (1939). UNITED STATES: Ramsey (1937); Ramsey and Wiant (1938); Shapovalov and Link (1924).

Sorghum vulgare Pers. UNITED STATES: Edson and Wood (1937); Hoffmaster (1942); Hutchison and Freeborn (1939); Nance (1940).

S. vulgare var. *sudanense* (Piper) Hitchc. HAWAII: Martin (1937). UNITED STATES: Lefebvre and Johnson (1941).

Soya max L. UNITED STATES: Kent (1942).

Spinacea oleracea L. UNITED STATES: Cook and Callenbach (1935); Edson and Wood (1936, 1937); Kadow (1937); Martin (1933); Wood and Nance (1938).

Stevia sp. UNITED STATES: Brown (1940).

Thea sinensis L. INDO-CHINA: Pfältzer (1940). MAURITIUS: Shepherd (1933).

Trifolium sp. NORWAY: Solberg (1926).

T. hybridum L. UNITED STATES: Buchholtz (1935).

T. pratense L. UNITED STATES: Buchholtz (1935).

T. repens L. UNITED STATES: Buchholtz (1935).

Triticum aestivum L. CANADA: Robertson (1931); Simmonds (1939a, b); Simmonds, Russell and Sallans (1935); Vanterpool (1935a, c); Vanterpool and Ledingham (1930). ITALY: Biraghi (1936); Paolis (1931); Petri (1930, 1936). JAPAN: Asuyama (1935). UNITED STATES: Brown (1940); Edgerton, Tims and Mills (1929); Maneval (1937); Nance (1940); Wood and Nance (1938).

Tulipa gesneriana L. ENGLAND: Moore (1940a); Moore and Buddin (1937).

Ulmus americana L. UNITED STATES: Wright (1937).

U. pumila L. UNITED STATES: Wright (1937).

Vicia faba L. NETHERLANDS: Brandenburg (1931a, 1935).

Vigna sinensis Endl. UNITED STATES: Wood and Nance (1938).

Viola tricolor L. ENGLAND: Chesters and Hickman (1939). NETHERLANDS: Van Eek (1938).

Washingtonia robusta Parish. UNITED STATES: Weber (1923).

Wood pulp. ITALY: Goidanich (1938).

Zea mays L. INDIA: Padwick (1940); PHILIPPINE ISLANDS: Agati (1931); Roldan (1930); UNITED STATES: Cooper (1927, 1929); Edson and Wood (1936). LeBeau (1939); Nance (1939, 1940); Robbins (1927); Valleau, Karraker and Johnson (1926); Wood and Nance (1938).

Zingiber officinale Roscoe. CEYLON: Anonymous (1936a). HAWAII: Parris (1939b, 1940). INDIA: Thomas (1939, 1941).

Pythium spp. have been reported from the United States on unspecified hosts such as conifers (Wilde, 1937), flower and vegetable seedlings (Linn, 1937) and herbaceous and woody plants (Osmun, 1934).

Some reports fail to distinguish between damage due to *Pythium* sp. and *Rhizoctonia* sp., listing hosts for both fungi. It is difficult to determine in some cases whether *Pythium* spp. participated at all in the reduction and loss of stand. These lists are often extensive, including ornamentals as well as fruit and vegetable crops. The reader is referred to Adams (1935), Anderson, Kadow and Hopperstead (1937), Doran (1928, 1938), Dunlap (1936a, 1936b) and Kadow and Anderson (1937).

There are a few reports which cite hosts as attacked by either *Pythium* or *Phytophthora* and in some instance by a "Pythium-like" organism. Hosts

listed for such fungus reports are: *Dioscorea batatas* Decne., Jamaica (Hansford, 1923), *Eugenia aromatica* Baill., Madagascar (Heim and Bouriquet, 1937), *Grammatophyllum speciosum* Blume, Seychelles (Squibbs, 1938), *Hibiscus sabdariffa* L., Ceylon (Gadd, 1936) and *Musa* sp., Italian Somaliland (Petri, 1932).

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A Cytotaxonomic Study of the Genus Mnium

By

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A CYTOTAXONOMIC STUDY OF THE GENUS MNIUM*

Robert James Lowry

INTRODUCTION

The purpose of this investigation was to delimit the genus *Mnium* and to determine interspecific relationships of the North American species through an investigation of chromosome numbers and chromosome morphology.

That the present generic limits of the genus *Mnium* are not clearly understood by taxonomists is well illustrated by the uncertain position of *Mnium* (*Leucolepis*) *Menziesii, Mnium* (*Cinclidium*) *hymenophyllum, Mnium* (*Cinclidium*) *hymenophylloides,* and *Mnium* (*Trachycystis*) *flagellare.* Andrews (1940) includes *Mnium Menziesii* in the genus although acknowledging its independent position. Lindberg (1868) however, considered it of sufficient distinctiveness for generic rank and proposed the name *Leucolepis* for it. *"Mnium punctatum"* consists of a series of many puzzling forms and Loeske (1910) suggested that these forms be united with *Cinclidium.* Andrews agrees that its relationship is evidently closer to *Cinclidium* than to other *Mnium* species but he is unwilling to support a taxonomic union.

Several "species-pairs" whose members seem to be very closely related occur in *Mnium. Mnium affine* and *Mnium medium,* although readily separated by sex condition and cell size, are strikingly similar. *Mnium insigne* differs from *Mnium affine* only quantitatively and in the restricted geographic distribution of the former. Concerning these last two species, Andrews says that *"Mnium insigne* is obviously a derivative of the widely distributed and greatly varying *Mnium affine." Mnium marginatum* and *Mnium orthorhynchum,* separated by sex condition and cell size, agree in most other characters. *Mnium punctatum* presents a taxonomic problem. Several species and varieties have been recognized by many authors whereas others, and most recently Andrews, put all North American forms in one species.

With the above problems in mind and with the conviction that cytological data would be of aid in their solution and also because of its large number of easily available species, the genus *Mnium* was selected for this study.

As more data accumulate they demonstrate with increasing clarity that such cytological phenomena as polyploidy, hybridization, and gene mutation, coupled with isolation, are important factors contributing to the formation of new plant species and must be considered in any attempt to establish relationships within plant groups (Dobzhansky 1941). A review of the literature must therefore pay particular attention to those works which have contributed knowledge related to the above phenomena as they occur and affect the mosses.

* Paper from the Department of Botany of the University of Michigan, No. 853.

Pringsheim (1876), Stahl (1876), Brizi (1892), and Correns (1899) demonstrated that the gametophytic generation of mosses could be produced directly from the sporophytic generation by the development of protonemata from the somatic tissues of the seta and capsule. Pringsheim and Stahl, working independently, obtained regeneration of the setae of *Hypnum, Amblystegium, Bryum,* and *Ceratodon*. Brizi found that protonema may arise from the young capsule wall in *Funaria hygrometrica*. Correns was able to confirm the results of Pringsheim and Stahl with *Hypnum, Amblystegium, Bryum,* and *Ceratodon,* but obtained negative results with a number of other mosses.

The work of the Marchals (Élie & Émile Marchal 1906, 1907, 1909, 1911) clearly demonstrated that moss plants produced by regeneration of sporophytic tissues were actually diploid. The Marchals were primarily interested in the effect of apospory upon sex and found that doubling the chromosome number of a strictly dioicous moss, for example *Bryum caespiticium,* produced diploid gametophytes which were potentially bisexual and, in a fair number of instances, produced both antheridia and archegonia in the same perichaetium. They also reported a direct proportionality between the number of chromosomes and the volume of the nucleus and cell. This work definitely established the fact that polyploidy could occur in the mosses, at least experimentally, and demonstrated some of its effects upon the physiology and morphology of the plants, sex condition, nucleus and cell size. The Marchals suggested the possibility of sporophytic regeneration occurring in nature with the consequent formation of new polyploid forms. They mentioned in this connection a sterile, monoicous form of *Bryum atropurpureum* found by them in nature.

During the period from 1912 to 1923, cytological studies on the mosses were largely confined to gametogenesis and the developing androcyte of the antheridium was the favorite subject for observation. The only interest of these studies to the cytotaxonomist is that chromosome numbers were occasionally reported. Typical examples of studies of this kind appear among the works of Wilson (1908–1915), Allen (1912, 1917) and Woodburn (1915). Wilson contributed observations on nuclear division, spore formation, spermatogenesis, and sex condition. His results agree in essential features with those currently accepted for nuclear division and spore formation. However, his report of a body being "budded off" from the nucleolus in the spore mother cell during the metabolic stage preceding meiosis and persisting until synapsis has not been substantiated by subsequent observations of other workers. Wilson also attributed the origin of the blepharoplast, limosphere, and accessory body of the sperm to material derived from the nucleolus.

In 1915, Wilson reported the discovery of a bisexual gametophyte of *Mnium hornum* (a species which is considered to be dioicous) with six chromosomes and since this is the normal gametophytic number he concluded, contrary to the Marchals, that sex determination is not associated with meiosis, "but is brought about by metabolic processes which operate in the organism over a considerable part of its life history." Blakeslee's (1908) comment upon the

Marchals' work is interesting in this connection. He pointed out that a bisexual race of *Phycomyces niteus*, produced in essentially the same manner as the bisexual mosses of the Marchals, lost the bisexual character after more than a dozen vegetative generations and he felt that the Marchals were unjustified in concluding that their bisexual mosses could be maintained indefinitely by vegetative propagation.

Woodburn (1915) studied spermatogenesis in *Mnium affine* var. *ciliare* and found no evidence of polar bodies or plates. He did describe the appearance of the blepharoplast and "a vesicle enclosed within the coiled body of the sperm and containing cytoplasm and probably nuclear material" which disappeared as the sperm approached maturity.

In 1912, Allen assigned the nucleolar phenomena described by Wilson to inadequate fixation and in 1917 published a detailed account of spermatogenesis in *Polytrichum juniperinum*. He described irregular bodies, derived from plastid material, which formed broad plates at the poles of the spindle in the dividing spermatogenous tissue. These polar plates became gradually less extensive until at the last division previous to the formation of the sperms, a single body (centrosome) was present at the sharply focused poles of the spindle (Allen 1912). The limosphere was described as dividing unequally producing a small mass of material which became associated with the apical portion of the sperm nucleus and the remainder of the limosphere became associated with the posterior portion and gradually disappeared as the sperm assumed its mature form (Allen 1917).

In 1923, workers again turned their attention to aposporically produced polyploids. Schweizer (1923) produced aposporic 2N gametophytes of *Splachnum sphaericum* as well as triploid and tetraploid sporophytes by fertilizations involving N and 2N gametes and tetraploid gametophytes by regeneration of tetraploid sporophytes. He obtained results similar to those of the Marchals regarding apospory and its effect upon sexuality. The Marchals (1909), found that their aposporic diploids were for the most part sterile because of nonfunctional gametes. Diploid forms of *Splachnum sphaericum* obtained by Schweizer did produce sporophytes, however. Diploid gametophytes of *Bryum caespiticium* also occasionally produce sporophytes (Wettstein 1924a).

Bornhagen (1930) working with *Splachnum ampullaceum* and *Splachnum sphaericum* obtained results similar to those of Schweizer just reviewed and produced 2N and 4N gametophytes of the former species.

Arens (1940) determined the sex condition of members of the *Splachnaceae*, by means of single spore cultures. *Voitia nivalis, Tayloria serrata, Tayloria tenuis, Tayloria splachnoides, Froelichiana lingulata, Tetraplodon angustatus, Tetraplodon bryoides, Tetraplodon urceolatus, Splachnum ampullaceum*, and *Splachnum australe* were found to be "haplomonoicous." *Splachnum peduculatum* (*sphaericum*), *Splachnum melanocaulon, Splachnum luteum, Splachnum rubrum*, and *Splachnum vasculosum* were found to be "haplodioicous."

In 1923, Wettstein began his famous studies on the morphology, physiology and development of mosses, with special reference to genetics. He obtained the first experimental evidence demonstrating that hybridization could occur in this plant group. Although supposedly natural hybrids had previously been described in taxonomic papers, Wettstein was the first to make actual crosses and analyze the progeny thereof. Working with members of the *Funariaceae,* he made the following successful crosses: *Funaria hygrometrica* × *Physcomitrium eurystomum* and its reciprocal; *Funaria hygrometrica* × *Physcomitrium pyriforme* and its reciprocal; *Funaria hygrometrica* × *Entosthodon fascicularis* and its reciprocal; *Funaria hygrometrica* × *Funaria mediterranea* and its reciprocal; *Physcomitrium eurystomum* × *Physcomitrium pyriforme* and its reciprocal; *Physcomitrella patens* × *Funaria hygrometrica; Physcomitrella patens* × *Physcomitrium pyriforme; Physcomitrella patens* × *Physcomitrium eurystomum* and its reciprocal. Hybrids between experimentally produced polyploid races were also obtained (Wettstein 1923, 1924a,b,c, 1928a).

Sporophytic characters are inherited in the same manner and exhibit the same types of hereditary phenomena as found in other 2N organisms. As would be expected, Wettstein found that gametophytic characters segregate in a 1 : 1 ratio. Dominance is not found to exist for gametophytic characters if the true haploid chromosome complement is present. In polyploid gametophytes, factors are at least duplicated and a condition suitable for the expression of dominance therefore exists. Dominance may be partial or complete, as in other organisms. From the predominance of maternal characters in gametophyte progeny resulting from interspecific and intergeneric crosses, Wettstein (1924b, 1926, 1928a, b) concluded that the cytoplasm, in addition to the chromosomes, is important genetically.

Wettstein carefully analyzed the effects of polyploidy upon morphological characters and found that doubling the chromosome number from haploid to diploid concomitantly roughly doubles the volume of the leaf cells. Doubling from 2N to 4N, however, increased cell volume in the ratio of 1 : 5.2. A comparison of N and 2N protonema cells demonstrated a ratio of volume of 1 : 1.96. Although chloroplast counts in N, 2N, and 4N leaf cells were found to be 14.4, 26, and 63.37 respectively, the size of the chloroplasts seemed unchanged. The total number of leaf cells in N, 2N, and 4N races was found to be approximately the same. Size (length and width) of antheridia, archegonia, leaves, and capsules showed an increase as the chromosome number was increased.

Wettstein (1924c) appears to have been the first to induce polyploidy in mosses by chemical treatment. By injecting young capsules of *Funaria hygrometrica* with .01 % chloral hydrate and also .1 % potassium nitrate, he obtained a few 2N spores. He also caused doubling of the chromosome number by treating protonema with a dilute chloral hydrate solution, ether, and chloroform vapor. Low temperature and centrifuging were also found to be effective in inhibiting cell division since they resulted in the production of diploid cells.

Heitz (1945) obtained polyploid gametophytes of *Aulacomnium androgynum* by treating propagula and protonema with colchicine.

Doubling the chromosome number of a dioicous moss through apospory results in a potentially bisexual race. The ratio of synoicous plants to plants which produce only antheridia or archegonia in recently-produced 2N races is variable. The usual condition seems to be a preponderance of plants which produce only antheridia (Marchal & Marchal 1907). Most 2N races of originally dioicous mosses are highly sterile, although occasionally sporophytes are produced (Wettstein 1924c; Schweizer 1923). The origin of a completely fertile 2N race of *Bryum caespiticium* is very interesting with regard to its loss of sterility. When first produced the race was highly sterile and produced only antheridia the first year. During the following years, archegonia and finally sporophytes were produced in normal numbers. The spores produced 2N gametophytes. After eleven years the race was completely fertile and was named *Bryum Correnssii*. Correlated with increasing fertility was a corresponding decrease in cell size so that by the time the race had achieved complete fertility its cell size had decreased to that typical of the original haploid (Wettstein 1937a, 1940; Wettstein & Straub 1942).

The reports of "sex chromosomes" in a number of dioicous species support the theory that sex determination in mosses is a phenomenon associated with the chromosomes. Many mosses possess one (or rarely two) chromosome from somewhat to much smaller than the others, which has been called an m-chromosome. A chromosome which is much larger than any of the others, termed an M-chromosome, occurs in some species. Either one or both size variations may be present in a particular species. These chromosomes may be heteropycnotic and the sex chromosome is often of the "m" or "M" type. Sex chromosomes have been positively identified in the following species: *Ceratodon purpureus* (Heitz 1932; Shimotomai & Kimura 1934, 1936; Jachimsky 1935), *Mnium punctatum* (Jachimsky 1935), *Pogonatum grandifolium* (Kurita 1937), *Pogonatum inflexum* (Shimotomai & Koyama 1932a,b), *Pogonatum spinulosum* (Kurita 1937) and *Polytrichum formosum* (*P. attenuatum*) (Shimotomai & Kimura 1934, 1936).

In his investigation of heterochromatin, Heitz (1928) studied 70 moss species representing 26 families and 47 genera. He found one or more heterochromosomes in most of the species investigated. His paper is relevant to this work, since a chromosome number or an estimated number of the chromosomes is given for each species studied. These numbers will be found in the list at the end of this section.

In a paper written in 1942, Heitz discusses the relation between polyploidy and monoicism in mosses. Twenty-six species of *Mnium* were considered, of which 17 were dioicous and 9 monoicous. He arranged the species in the following table (quoted in its entirety) which indicates possible relationships between dioicous haploid (or probably haploid) species and monoicous diploid (or probably diploid) species.

	Getrenntgeschlechtlich		*Gemischtgeschlechtlich*	
Sekt. II.	M. hornum	N = 6		
	M. spinosum	N = 6	M. spinulosum (selten)	
	M. stellare	N = 7 (6 + 1)		
	M. Blyttii			
	M. orthorhynchum	N = 6		
	M. marginatum fo. dioica		M. marginatum	
			M. adniviense	
	M. lycopodides			
Sekt. III.	M. cuspidatum		M. cuspidatum	N = 12
	subspec. trichomanes			
	M. affine	N = 6	M. medium	
	M. Seligeri	N = 6		
	(M. insigne		M. insigne var. intermedium)	
	M. rostratum fo. coriaceum		M. rostratum	N = 12
	M. undulatum	N = 6	M. Drummondii	
	M. Maximoviczii	N = 7 (6 + 1)		
Sekt. IV.	M. punctatum	N = 7	M. pseudopunctatum	N = 13
	(6 + x), (6 + y)		(selten!) (12 + 1)	
	M. hymenophylloides	N = 7 (6 + 1)		
Sekt. V.	M. cinclidioides			

The above table is particularly interesting in regard to *Mnium punctatum* and *Mnium pseudopunctatum*. If, as Heitz says, *Mnium pseudopunctatum* arose by sporophytic regeneration of *Mnium punctatum* the expected chromosome number would be 14 (12 + x + y). He postulates hybridization between *Mnium punctatum* and some 6 chromosome race or related species, to account for the 13 chromosomes of *Mnium pseudopunctatum*.

Further papers of interest regarding the effects of polyploidy upon mosses, are those of Schmidt (1931) and Wettstein (1930, 1932). A spore from a tetraploid sporophyte of *Physcomitrium pyriforme* produced a plant having 18 chromosomes or half the normal haploid number. This hemiploid was self-fertile and regeneration of a sporophyte gave a 36-chromosome plant differing in various characters from typical *Physcomitrium pyriforme* (N = 36). The mating of the hemiploid with another hemiploid, presumed to have arisen in a similar fashion, followed by regeneration of the resulting sporophyte, produced plants indistinguishable from *Physcomitrium pyriforme*. The conclusion reached was that *Physcomitrium pyriforme* has two different sets of 18 chromosomes, either of which can produce a constant race.

Springer (1935) reported abnormal gametophytes of *Phascum cuspidatum* from 2N protonemata which gave rise to apogamous sporophytes from the stems and leaves. Spores produced by these sporophytes gave rise to normal and abnormal gametophytes. Springer concluded that meiosis had taken place in the apogamous sporophytes.

Barthelmess (1938) reported the production of over 100 mutations in *Physcomitrium pyriforme* by irradiation of spores with alpha and X rays. Many of the mutations were assumed to be heteroploid in nature because of the high degree of sterility present.

La Rue (1929) obtained protonemata and leafy plants by regenerating the young sporophytes of the following American species: *Amblystegium serpens, Aulacomnium heterostichum, Bryum caespiticium, Catharinea undulata, Cera-*

todon purpureus, Ditrichum pallidum, Fissidens cristatus, Funaria hygrometrica, Mnium affine, Mnium cuspidatum, Mnium rostratum, Physcomitrium turbinatum, Polytrichum commune, and *Polytrichum ohioense*. This work was done with the hope that data for American species similar to that of the Marchals and Wettstein for European species could be obtained. Unfortunately, the diploid gametophytes did not produce sex organs. The *Mnium* species reported to have been regenerated are of particular interest. La Rue found that *Mnium affine* was remarkably slow to regenerate and required 89 days before the beginning of protonematal growth. *Mnium cuspidatum* and *Mnium rostratum* were also reported as "very slow" to regenerate.

It is surprising that no cytotaxonomic work has been done on mosses, on the basis of the many experimental data which have accumulated. With regard to the following list of previously reported chromosome numbers in *Musci,* largely compiled from Tischler's lists (1927, 1931, 1936, 1938), but with additions by the author, it should be pointed out that numbers given for *Mnium* were determined, with one exception, from European or Asiatic representatives of the species.

It is interesting to note that all cases reported in the list of polyploidy within a species were experimentally produced. The naturally occurring polyploids all represent distinct species. This can be explained partially by the fact that many of the gametophytic characters used by taxonomists in delimiting moss species are those which are affected by polyploidy.

NAME OF MOSS	CHROMOSOME NUMBERS	SOURCE
SPHAGNACAE		
Sphagnum squarrosum	20	Melin 1915
POLYTRICHACEAE		
Polytrichum commune	6	Vandendries 1912; Woodburn 1915
Polytrichum commune	7	Heitz 1928; Jachimsky 1935
Polytrichum commune	7 and 14	Kurita 1937
Polytrichum juniperinum	6	Drs. van Leeuwen-Reijnvaan 1907, 1908; Arens 1907; Allen 1912; Vandendries 1912
Polytrichum juniperinum	(6–)7	Heitz 1928
Polytrichum juniperinum	7	Kurita 1937
Polytrichum piliferum	6	Drs. van Leeuwen-Reijnvaan 1907, 1908; Vandendries 1912
Polytrichum piliferum	7	Heitz 1928
Polytrichum formosum	6	Drs. van Leeuwen-Reijnvaan 1907, 1908; Walker 1913
Polytrichum gracile	12–14	Heitz 1928
Polytrichum alpinum	7	Kurita 1937
Polytrichum attenuatum	7	Shimotomai and Kimura 1934, 1936
Polytrichum attenuatum var.	14	Kurita 1937
Polytrichum sp.	14	Kurita 1937
Pogonatum urnigerum	6–7	Heitz 1928
Pogonatum urnigerum	7	Kurita 1937
Pogonatum grandifolium	7	Kurita 1937
Pogonatum nanum	7	Jachimsky 1935
Pogonatum spinulosum	7	Kurita 1937

NAME OF MOSS	CHROMOSOME NUMBERS	SOURCE
POLYTRICHACEAE (*cont.*)		
Pogonatum contortum	7	Shimotomai and Koyama 1932a, 1932b
Pogonatum inflexum	7	Shimotomai and Koyama 1932a, 1932b
Pogonatum rhopalophorum	8	Ikeno 1904
Catharinaea angustata	8	Ikeno 1904
Catharinaea angustata	7	Kurita 1937
Catharinaea undulata	16–17	Wilson 1911
Catharinaea undulata	14–16	Heitz 1926
Catharinaea undulata	(20–)21(–22)	Heitz 1928
Catharinaea undulata	21	Kurita 1937
Catharinaea undulata	14	Lowry, unpublished
Catharinaea angustata	7	Lowry, unpublished
BUXBAUMIACEAE		
Buxbaumia aphylla	7–8	Heitz 1928
FISSIDENTACEAE		
Fissidens adiantoides	(19)–21	Heitz 1928
DICRANACEAE		
Dicranum japonicum	11	Shimotomai and Koyama 1932a, 1932b
Dieranum scoparium	10–12	Heitz 1928
Dicranum undulatum	10–12	Heitz 1928
Rhabdoweisia fugax	ca. 12	Heitz 1928
DITRICHACEAE		
Ceratodon purpureus	11–12	Heitz 1928
Ceratodon purpureus	13	Shimotomai and Kimura 1934, 1936
Ceratodon purpureus	13	Jachimsky 1935
GRIMMIACEAE		
Grimmia apocarpa	>20	Heitz 1928
POTTIACEAE		
Barbula fallax	(9–)10(–11)	Heitz 1928
SPLACHNACEAE		
Splachnum sphaerieum	8	Schweizer 1923
Splachnum sphaerieum var. *bivalens*	16	Schweizer 1923
Splachnum ampullaceum	8	Bornhagen 1930
Splachnum ampullaceum var. *bivalens*	16	Bornhagen 1930
Splachnum ampullaceum var. *quadrivalens*	32	Bornhagen 1930
FUNARIACEAE		
Funaria hygrometriea	14	Wettstein 1923, 1924a,b, 1930
Funaria hygrometrica var. *bivalens*	28	Wettstein 1924b
Funaria hygrometriea var. *quadrivalens*	ca. 56	Wettstein 1924b
Funaria flavieans	ca. 10	Beardsley 1931
Funaria mediterranea	26	Griesinger 1937
Physeomitrella patens	ca. 16	Wettstein 1924a
Physeomitrium pyriforme (hemiploid var.)	18	Wettstein 1930; Schmidt 1931
Physcomitrium pyriforme typ.	36	Wettstein 1930; Schmidt 1931
Physeomitrium pyriforme var. *tetravalens*	72	Wettstein 1930; Schmidt 1931
TIMMIACEAE		
Timmia cueullata	12	Scheuber 1932
Timmia eueullata	16	Lowry, unpublished
AULACOMNIACEAE		
Aulacomnium palustre	(9–)10	Heitz 1928
Aulacomnium androgynum	10–11	Heitz 1945
BARTRAMIACEAE		
Philonotis fontana	7–8	Heitz 1928
Bartramia pomiformis	7–8	Heitz 1928
Bartramia pomiformis	8	Kurita 1937
BRYACEAE		
Bryum capillare	10	Él. et Ém. Marchal 1911; Heitz 1928

NAME OF MOSS	CHROMOSOME NUMBERS	SOURCE
BRYACEAE (*cont.*)		
Bryum capillare var. *bivalens*	20	Él. et Ém. Marchal 1911
Bryum caespiticium	10	Él. et Ém. Marchal 1911; Wettstein 1924b
Bryum cacspiticium	10 and 20	Griesinger (cit. Wettstein 1937a)
Bryum Corrensii	20 and 40	Griesinger (cit. Wettstein 1937a) and Griesinger 1937
Bryum argenteum	10	Ém. Marchal 1920
Bryum argenteum	10	Jachimsky 1935
Bryum pseudotriquetrum	9–10	Heitz 1928
Webera nutans	14	Heitz 1928
Rhodobryum giganteum	11	Shimotomai and Koyama 1932a, 1932b
MNIACEAE		
Mnium hornum	6	Wilson 1908, 1909, 1910, 1911, 1915; Él. et Ém. Marchal 1911; Heitz 1928; Jachimsky 1935
Mnium hornum var. *bivalens*	12	Él. et Ém. Marchal 1911
Mnium affine	8	Motte 1928
Mnium affine var. *ciliare*	6	Woodburn 1915
Mnium sp.	8	Drs. van Leeuwen-Reijnvaan 1908
Mnium Maximoviczii	7	Shimotomai and Koyama 1932a, 1932b
Mnium undulatum	6	Heitz 1942
Mnium spinosum	6	Heitz 1942
Mnium Seligeri	6	Heitz 1942
Mnium orthorhynchum	6	Heitz 1942
Mnium cuspidatum	12	Heitz 1942
Mnium rostratum	12	Heitz 1942
Mnium stellare	7	Heitz 1942
Mnium hymenophylloides	7	Heitz 1942
Mnium punctatum	7	Heitz 1928; Jachimsky 1935; Heitz 1942
Mnium pseudopunctatum	13	Heitz 1942
RHIZOGONIACEAE		
Rhizogonium spiniforme	6	Kurita 1937
Rhizogonium Dozyanum	7	Kurita 1937
LESKEACEAE		
Thuidium japonicum	10	Shimotomai and Koyama 1932a, 1932b
HYPNACEAE		
Hypnum imponens	6–7	Heitz 1928
Ptilium crista-castrensis	10	Kurita 1937
HYLOCOMIACEAE		
Hylocomium squarrosum	6–8	Heitz 1928
CLIMACEACEAE		
Climacium japonicum	11	Shimotomai and Koyama 1932a, 1932b
HOOKERACEAE		
Hookeria lucens	ca. 12	Heitz 1928
HYPOPTERIGIACEAE		
Hypopterigium japonicum	18	Shimotomai and Koyama 1932a, 1932b
AMBLYSTEGIACEAE		
Chrysohypnum stellatum	6–8	Heitz 1928
Calliergon cuspidatum	(9–)10	Heitz 1928
Calliergon Richardsonii	20	Heitz 1928
Amblystegium serpens	12	Él. et Ém. Marchal 1911; Wettstein 1924b
Amblystegium serpens var. *bivalens*	24	Él. et Ém. Marchal 1911; Ém. Marchal 1912; Wettstein 1924b

NAME OF MOSS	CHROMOSOME NUMBERS	SOURCE
AMBLYSTEGIACEAE (*cont.*)		
Amblystegium serpens var. *quadrivalens*	48	Él. et Ém. Marchal 1911
Amblystegium irriguum	12	Ém. Marchal 1912
Amblystegium riparium	24	Ém. Marchal 1912
BRACHYTHECIACEAE		
Scleropodium purum	9–10	Heitz 1928
Eurhynchium Schleicheri	8(–9)	Heitz 1928
Eurhynchium rusciforme	6–8	Heitz 1928
Brachythecium velutinum	10	Él. et Ém. Marchal 1911
FONTINALACEAE		
Fontinalis antipyretica	8	Heitz 1926
Fontinalis antipyretica	ca. 8	Heitz 1928

I wish to express my sincere appreciation to the following institutions and individuals for their valuable assistance and advice: to the University of Michigan for laboratory, library and other facilities, to the Horace H. Rackham School of Graduate Studies for financial aid in the form of a special fellowship; to Professor Ethel I. Sanborn of Corvallis, Oregon, Dr. Ruth D. Svihla of Seattle, Washington, Dr. Lewis E. Anderson of Durham, North Carolina, Dr. Frances E. Wynne of Chicago, Illinois, Dr. Ruth O. Schornherst of Tallahassee, Florida, and others, for the collection of specimens; to Dr. William C. Steere of the University of Michigan for verification of identifications and, as chairman of my committee, for much helpful advice and encouragement, and to the other members of my committee who have so kindly given their interest and valuable time to aid me in the completion of this work.

MATERIAL

The genus *Mnium* was selected for study for several important technical reasons. First, the number of representatives in North America is not so large as to make a complete survey of chromosome numbers and morphology impractical and is yet large enough so that the results should be of significance in considerations involving the entire genus. Secondly, over one-half of the 21 species known to occur in North America are found in Michigan, thus assuring an abundant supply of living material in natural habitats, within easy access. Thirdly, the species are easily maintained in the laboratory since they grow well if supplied with the proper light, temperature, and humidity.

METHODS

Including its embryonic leaves, the apical meristematic tissue of the gametophyte was used exclusively for the chromosome counts and observations of chromosome morphology, since cell divisions take place there over long periods of time. Because the haploid chromosome complement is present, counts are easier than in sporophytic material; furthermore the chromosomes are not so condensed as they are during meiosis and therefore reveal a more accurate picture of their morphology.

Plants collected in the spring during the active growth period were brought directly to the laboratory and placed in closed glass dishes, in order to assure them an atmosphere of high humidity. Light was supplied from a north window of the laboratory. Useful material was obtained until the production of sex organs, at which time mitosis in the apical meristematic region practically ceases. The production of sterile, stoloniform shoots in some species considerably prolongs the time in which cytological material can be obtained.

Plants collected in late summer and fall, with the exception of a few weedy species, for example, *Mnium cuspidatum, Mnium affine,* and *Mnium medium,* would not resume growth when placed in the customary cultural conditions. It was found necessary to "over-winter" these species in seed flats out-of-doors, after which active growth took place when the plants were brought into the laboratory early the following spring, about a month before growth was resumed by the same species under natural conditions.

Paraffin sections stained with iron-hematoxylin were first used (fig. 129), but it was soon found that the meristematic cells of the gametophyte and embryonic leaves contained large numbers of developing chloroplasts which stained deeply and made the observation of chromosomes extremely difficult. Gametophytes were fixed in a weak chrom-acetic solution (1 g. chromic acid and 2 ml. acetic acid to 100 ml. of water) for 24 hours, washed one hour in water and dehydrated by the glycerine method. Clearing was accomplished by means of a closely graded series of absolute alcohol-xylene mixtures. Infiltration with paraffin was also made very gradually by adding small amounts over a long period of time. The material was found to be extremely sensitive to changes in density of the various fluids, making precise technique necessary in order to avoid excessive shrinkage. Staining with crystal violet followed by an iodine mordant was tried, but it was found impossible to make the chromosomes retain sufficient stain for careful study, although iron-hematoxylin was found to produce good results. Because of their ease and rapidity of preparation, as well as their excellent quality, it was decided to use smear preparations exclusively.

For all counts and morphological studies of the chromosomes, the following smear technique was evolved and used, with slight modifications fitting it to the peculiarities of particular species. Gametophyte tips were cut off about 0.5 cm. from the apex and fixed in a mixture of 30 % glacial acetic acid plus 70 % absolute alcohol for at least one hour. It was found that material could be left in the fixing fluid for as long as 24 hours without affecting the subsequent staining properties. The apical meristem and embryonic leaves were isolated in a drop of stain (acetic orcein) on a slide and a cover glass was added. After the slide had been heated for approximately one minute, just below the boiling point of the stain, the cells were separated by tapping lightly on the cover glass with a bone needle handle. Any lateral movement of the cover glass at this point rolls the cells and breaks them, making the preparation useless. The cells were flattened, and excess stain removed, by placing the slide, cover glass down, on a pad of blotting paper and pressing on the back directly above the center of the

cover glass. The correct amount of pressure can be determined only by experience. Too little pressure does not spread the mitotic figures sufficiently to allow positive counts of the chromosomes and too great pressure breaks the cells and damages the chromosomes. The cover glass was sealed with vaseline and after an hour the chromosomes were sufficiently stained for study.

Slides made by the above technique remain in good condition for about one week, after that the cytoplasm gradually becomes colored by the stain, lowering the contrast of the preparation and eventually making it worthless.

Mounts may be made permanent by carefully removing the cover glass in 2 parts glacial acetic acid and 1 part absolute alcohol, dehydrating and clearing both cover glass and slide through the following solutions (Steere 1931): (1) 1 part glacial acetic acid + 2 parts absolute alcohol; (2) 1 part glacial acetic acid + 9 parts absolute alcohol; (3) absolute alcohol; (4) equal parts absolute alcohol and xylene. The slide and cover glass are reunited in thin xylene-balsam from the last solution. Although the moss material sticks to the slide and cover glass fairly well, caution is necessary during dehydration to prevent loss of material. Care is also necessary to prevent absorption of water vapor during the last two steps in dehydration and clearing before the addition of balsam.

Acetic orcein (0.5 % orcein + 0.1 % potassium acetate in 45 % acetic acid) was used in preference to aceto-carmin since it produced a more brilliant stain with moss chromosomes. The addition of the potassium acetate was found to increase noticeably the brilliance and stability of the stain.

The cells of some species of mosses were found to be excessively delicate, so that they break badly in the process of smearing. For these species, the following treatment improved the fixation and allowed good smears to be obtained: after being fixed for the usual one hour period in acetic-alcohol, the material was treated for 5–10 minutes in a solution of 30 ml. of glacial acetic acid, 65 ml. of absolute alcohol, and 5 ml. of formalin. Smears were made after washing for five minutes in acetic alcohol. Too long a treatment with the formalin solution should be avoided because it causes the cytoplasm to stain deeply.

When the cells would not separate readily from one another, it was found that a 5–10-minute immersion, after fixing, in a solution consisting of equal parts of concentrated hydrochloric acid and 95 % alcohol, followed by a 15-minute immersion in acetic alcohol, made a noticeable improvement (Warmke 1935). This method does not produce as good results with moss tissue as it does with root-tip meristems of the flowering plants, perhaps because of differences in the chemical nature of the middle lamella in the two plant groups.

In this study, temporary smears were preferred to permanent slides, since division figures at best are not numerous and the danger of losing them during dehydration is great. The smears were studied and drawings made as soon as possible after their completion.

Smears of the meiotic division in the spore-mother cells were prepared by the following technique. It was found through experiment that meiosis occurs

at about the time the capsule has assumed its mature form and size, but is still bright green. The capsule wall was cut open and the columella with the adhering spore-mother cells was dissected out and fixed for 15 minutes in an acetic-alcohol solution. After fixation the mass of tissue was placed in a drop of acetic orcein on a slide, covered, and the spore-mother cells separated from the tissue of the columella and from each other by tapping on the cover glass with a needle handle. The cover glass was then sealed with vaseline.

A phase-plate filter, having its maximum transmission in the green region of the spectrum, was used between the light source and microscope condenser for all observations because it improved the contrast and visibility of the chromosomes.

All drawings were made with a camera lucida at a magnification of 2000 diameters.

The leaf-cell size of the species-pairs was obtained by using Amann's (1921) technique, which consists of determining the average number of cells per square mm. (cell index). The procedure was as follows: a metal disc, with a 1.5 mm. square aperture in its center, was placed in the ocular of the microscope. The exact area of the field included in the aperture was determined for the various objectives, by means of a stage micrometer slide. The number of cells included within the aperture was counted and by multiplying the result by an appropriate factor the number of cells per square mm. was obtained. All measurements were made in an area halfway between the leaf base and apex and halfway between the leaf border and costa.

RESULTS

A short description, including only important diagnostic characters, is presented for each species studied. The descriptions are condensed versions of those given by Andrews in the *Moss flora of North America* (1940) and have been compared with actual specimens. After the description the following data are listed: geographical distribution, specimen number and collection location for the material studied, chromosome number, chromosome measurements, and notes on the location of spindle attachment regions when such data are available. The cell index is also given for some species.

A tabular summary listing chromosome numbers and average chromosome lengths is included at the end of this section.

MNIUM Hedw. Plants dioicous or synoicous; stems normally erect; leaves of most species with a border of elongated cells, one or more cells in thickness, frequently with single or double teeth; costa strong, but not always reaching the leaf-apex; cells tending to be hexagonal, their walls sometimes thickened or pitted.

Sporophytes single or several from the same perichaetium. Capsules horizontal to pendulous, oblong to oval; neck short; stomata usually confined to neck, cryptopore; operculum convex to rostrate; teeth of outer peristome 16, of approximately same length as inner peristome, strong, not united at base, tending to be bordered, papillose; dorsal longitudinal line zig-zag, dorsal plates low; ventral lamellae numerous; inner peristome yellow to reddish brown, with

high basal membrane which is in some species irregularly perforated; segments tapering to a mostly slender, cuspidate apex, broadly fenestrate to gaping; cilia mostly in 3's, nodulose. Type species, *Mnium hornum* (Andrews 1940).

MNIUM MENZIESII (Hook.) C. Müll. Plants dioicous, 4–8 cm. high, dendroid, with numerous small branches from upper part, the branches slender, spreading, often decurved, rarely reaching 2 cm. in length; stem leaves in lower part of stem distant, erect, closely appressed to stem, small and scale-like, whitish-hyaline at least in upper part of leaf, slenderly lanceolate, long-acuminate, decurrent, dentate throughout with slender, single-celled teeth which stand out at right angles or nearly so to edge of leaf; costa broader at base, slender above, not reaching apex, not toothed; leaf cells in basal part of leaf chlorophyllose, more or less rectangular, rather small, up to 35 × 15 μ, thick-walled, in upper, hyaline part of leaf the cells much longer, up to 100 μ, thin-walled and empty of chlorophyll, the teeth in this part being only the projecting distal ends of elongated border cells, without, however, a distinctly differentiated border being formed. On upper part of stem the leaves less distant, broader, with normal, irregularly rounded, hexagonal, chlorophyllose cells, except in acuminate point; teeth shorter and less prominent; costa stronger, slightly toothed dorsally. The branches issue from the stem at an angle between 45° and 90°, slightly above the axils of the upper stem-leaves, normally one to each leaf-axil; the branches are simple or themselves slightly branched, their leaves are closer, erect-spreading, more ovate-lanceolate, with short acute apex, not hyaline, sharply toothed, especially in upper part; costa also sharply toothed dorsally, decurrent; cells chlorophyllose throughout.

Seta erect, strong, somewhat flexuose, reddish, to 5 cm. high; capsules single or 2, rarely 3, from the same perichaetium, more or less pendulous, up to 8 mm. in length, oval-cylindric, yellowish-green to brown; operculum hemispheric, mammillose at apex; stomata in neck of capsule, cryptopore; outer and inner peristome of approximately equal length, both dark yellow; spores round, greenish-yellow, about 30 μ, papillose, ripening in spring.

Alaska to northern California, inland to Idaho.

The following specimen was studied:

No. 6. Collected in Seattle, Washington, by Dr. Ruth D. Svihla. N = 5.

The gametophytic chromosome number is 5 (figs. 1–10). The chromosomes are 0.75 μ wide and their average lengths are as follows: 7.4 μ, 6.6 μ, 5.6 μ, 5.2 μ, 4.6 μ.

MNIUM STELLARE Hedw. Plants dioicous, 2–3 cm. high; fertile stems simple, erect; sterile stems frequently curved; leaves elliptic-ovate, obtuse to short-pointed, decurrent, not bordered, but outer 1–2 rows of cells may be somewhat elongated or slightly darker-pigmented, upper leaves denticulate on their apical border with broad unicellular teeth which project normally not in the plane of the leaf-blade, but at angles to it on either side; costa ceasing abruptly at some distance from the apex; cells of leaf 20–30 μ in diameter, walls with thickened corners.

Explanation of figures 1–28

FIGS. 1–10. *Mnium Menziesii*. FIGS. 1–9. Chromosomes in metaphase. FIG. 10. Chromosomes in late metaphase showing longitudinal division. FIGS. 11–21. *Mnium stellare*. FIGS. 11–19. Chromosomes in metaphase. FIG. 20. Chromosomes in early anaphase showing spindle-attachment regions. FIG. 21. Chromosomes in late metaphase showing spindle-attachment regions. FIGS. 22–24. *Mnium hornum*. Chromosomes in metaphase. FIGS. 25–28. *Mnium orthorhynchum*. Chromosomes in metaphase. All figures × 1332.


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Sporophytes* single; seta about 2 cm. high; capsule pendulous, oblong with short neck, somewhat bent and asymmetric, brownish green, up to 3 mm. long; operculum convex, not pointed; stomata in neck; outer peristome-teeth greenish-yellow; inner peristome dark yellow.

Spores* 20 μ, greenish yellow, ripening in May or June.

New Brunswick and Ontario south to Virginia, west to Minnesota; also in Europe and Asia.

The following specimens were examined:

No. 1. University of Michigan Biological Station, Cheboygan, Michigan. N = 7.

No. 22. Nichol's Arboretum, Ann Arbor, Michigan. N = 7.

No. 23. Cady's Corners, Washtenaw County, Michigan. N = 7.

The specimens agree closely with the description and are typical of the species.

The haploid chromosome number of *Mnium stellare* as determined from the specimens listed above, is 7.

The average chromosome lengths are as follows: 12.1 μ, 10.6 μ, 10 μ, 9.9 μ, 7 μ, 6.3 μ, 1.8 μ. The spindle-fiber-attachment regions are located as follows: In the long group three chromosomes have approximately median attachment regions, the fourth has a submedian attachment; of the two medium-length chromosomes one is attached terminally and the other subterminally; the short chromosome has a terminal attachment region.

The over-all size of the chromosomes is large compared with those of the other *Mnium* species.

MNIUM HORNUM L. Plants dioicous, to 7 or 8 cm. high; stem usually simple, slender, more or less erect, but often somewhat flexuose; leaves, narrowly elliptic-ovate, somewhat decurrent, sharply acute to short-acuminate, with a swollen border of darker, narrow cells, more than one cell in thickness, with numerous, double, short but sharp teeth; costa not reaching apex, somewhat toothed dorsally; cells of leaf averaging 20–25 μ, tending to be isodiametric except in basal part where they become somewhat elongated with more thickened corners.

Sporophytes single; seta 2–3 cm. high; capsules horizontal to pendulous, oblong, symmetric, about 4 mm. long, greenish-brown, abruptly narrowed into a short, darker-colored neck; stomata in neck; outer peristome-teeth greenish-yellow; inner peristome orange-yellow.

Spores greenish-yellow, 25–30 μ, roughened, ripening in summer.

Labrador to Georgia, west to Ohio and Tennessee: collected once in Missouri.

Plants of the following collection were studied.

No. 24. Thunderhole Creek, tributary of John's River, near Upton, Caldwell County, North Carolina. Collected by H. L. Blomquist. N = 6.

The plants of the collection do not differ from the description and well represent this distinct and stable species.

The chromosomes are about 0.5 μ in width and their average lengths in microns are as follows: 5.7, 5.5, 5.2, 5, 4.5, 4.

* Not seen by the author

Explanation of figures 29–56

FIGS. 29–36. *Mnium orthorhynchum.* FIGS. 29–35. Chromosomes in metaphase. FIG. 36. Chromosomes in late metaphase showing spindle-attachment regions. FIGS. 37–48. *Mnium marginatum.* Chromosomes in metaphase. FIGS. 49–56. *Mnium spinulosum.* Chromosomes in metaphase. All figures × 1332.


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MNIUM ORTHORHYNCHUM Brid. Plants dioicous, 1–2 cm. high; fertile stems simple, erect; sterile stems flexuose, not much longer than others; leaves, oblong-ovate, acute to short-apiculate, decurrent, bordered throughout with thickened border of elongated cells in more than one layer, toothed throughout with double teeth; costa percurrent, joining border to form apiculus, toothed dorsally in upper part, especially in the apical and perichaetial leaves; cells of leaf small, rarely exceeding 20 μ, walls with corners slightly if at all thickened.

Sporophytes single; seta about 2 cm. long; capsules approximately horizontal, oblong-cylindric, straight, urn 3 to 4 mm. long, reddish-brown when ripe, contracted abruptly into a short narrow neck; operculum short-rostrate, about 1.5 mm. long; stomata in neck; outer peristome-teeth light yellow below, darker toward apex; inner peristome golden yellow.

Spores greenish-yellow, 30–40 μ, slightly roughened, ripening in summer.

Widely distributed through the three northern continents in North America from Alaska and Yukon south to New Mexico, in the eastern states to North Carolina.

The following specimens were studied:

No. 4. Archegonial plants, Nichol's Arboretum, Ann Arbor, Michigan. N = 6.

Gametophytic characters agree well with the description. The teeth on the back of the costa, although definitely present, were not so strongly developed as in other collections seen by the author.

The haploid chromosome number of *Mnium orthorhynchum,* as represented by the above specimen, is 6 (figs. 25–36).

The average lengths of the six chromosomes are as follows: 7.2 μ, 6.7 μ, 6.3 μ, 5.8 μ, 5.5 μ, 5 μ. The average width of the chromosomes is 0.75 μ.

The spindle-attachment regions all seem to be median or submedian except one which is either subterminal or terminal (fig. 30).

The leaf cells average 2578 per square mm.

MNIUM MARGINATUM (Dicks.) Beauv. Plants synoicous, to 3 cm. high; fertile stems simple erect; sterile stems flexuose, often twice as long as others; leaves oblong-ovate, short-acuminate, decurrent, bordered throughout with thickened border of narrow cells in more than one layer, toothed throughout with short, double teeth; costa percurrent and joining border to form apiculus; cells of leaf up to 35 μ, walls with thickened corners.

Sporophytes single; seta to 2 cm. high; capsule approximately horizontal, brownish-yellow, oblong-cylindric, sometimes slightly curved, abruptly narrowed into slender neck, urn to 5 mm. long; operculum rostrate, to 2 mm. long; stomata in neck; outer peristome-teeth rusty brown; inner peristome brown.

Spores yellow, about 25 μ or slightly more, finely roughened, maturing in May.

Widely distributed in the three northern continents; in our eastern states

Explanation of figures 57–84

FIGS. 57–60. *Mnium spinulosum.* FIGS. 57–59. Chromosomes in metaphase. FIG. 60. Chromosomes in anaphase showing the approximate locations of the spindle-attachment regions. FIGS. 61–66. *Mnium Drummondii.* FIGS. 61–65. Chromosomes in metaphase. FIG. 66. Chromosomes in anaphase showing the approximate locations of the spindle-attachment regions. FIGS. 67–78. *Mnium cuspidatum.* FIGS. 67–73. Chromosomes in metaphase. FIGS. 74, 75. Chromosomes in anaphase. FIG. 76. Chromosomes in late metaphase showing spindle-attachment regions. FIGS. 77, 78. Bivalent chromosomes of the first meiotic metaphase. FIGS. 79–84. *Mnium cuspidatum* (N = 6). Chromosomes in metaphase. All figures × 1332.

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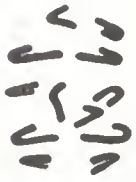

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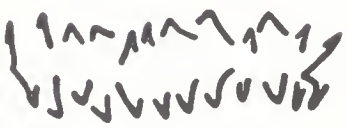

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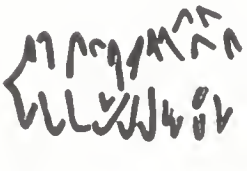

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south to Tennessee, the central states to Missouri, in the west from the Yukon south to Arizona and New Mexico.

The following specimens were studied:

No. 3. School Girl's Glen, Nichol's Arboretum, Ann Arbor, Michigan. Collected by L. Holdridge. N = 12.

The twelve chromosomes are divided into six pairs, the members of each pair being of the same length. The average lengths of the six pairs are as follows: 7.3 μ, 6.4 μ, 6.0 μ, 5.6 μ, 5.2 μ, 4.7 μ. The average width of the chromosomes is 0.75 μ (figs. 37–48).

The leaf cells average 2349 per square mm.

Mnium spinulosum Br. and Sch. Plants synoicous, about 1 cm. high; stems erect, simple; leaves broadly obovate from a narrow base, short cuspidate, decurrent, border throughout with a very strong, thick border which is round in section, its inner cells stereid, toothed in upper part of leaf with long, sharp, double teeth, costa slightly excurrent; leaf cells 18–25 μ, walls without thickened corners.

Sporophytes single in eastern United States, 2 or 3 in western United States and Europe; seta 2.3 cm. high; capsule pendulous, urn oblong-cylindric, straight, about 3 cm. long, very light straw-color, dark brown to purplish at mouth, abruptly narrowed into slender neck which passes gradually into thickened and hooked summit of seta; operculum conic-rostrate; stomata in neck; outer peristome-teeth dark purplish-brown; inner peristome-teeth brown.

Spores brownish-yellow, 15–20 μ, minutely roughened, ripening in May to June.

In Europe, from Pyrenees through the Alps to the eastward, uncommon. Not rare in North America, Nova Scotia to Maryland in the east, westward to Pacific coast from Alaska to Oregon.

Specimens studied:

No. 8. University of Michigan Biological Station, Cheboygan, Michigan. N = 8.

The average lengths of the eight chromosomes are as follows: 6.8 μ, 6.6 μ, 5.8 μ, 5.4 μ, 4.8 μ, 4.2 μ, 2.6 μ, 2.1 μ. The average width of the chromosomes is 0.75 μ (figs. 49–60).

There appears to be at least one long chromosome with a terminal attachment region. The attachment regions of the two small chromosomes appear to be terminal. The remaining members of the complement have median or submedian attachments (fig. 60).

Mnium Drummondii Br. & Sch. Plants normally synoicous, but large antheridial heads with broad perigonial leaves and more or less clavate paraphyses may also occur, even in the same tuft with the synoicous plant, to 2 cm. high; fertile stems simple erect, sterile stems somewhat stoloniform, leaves broadly obovate-spatulate, short-acuminate, decurrent, bordered by 2–4 rows

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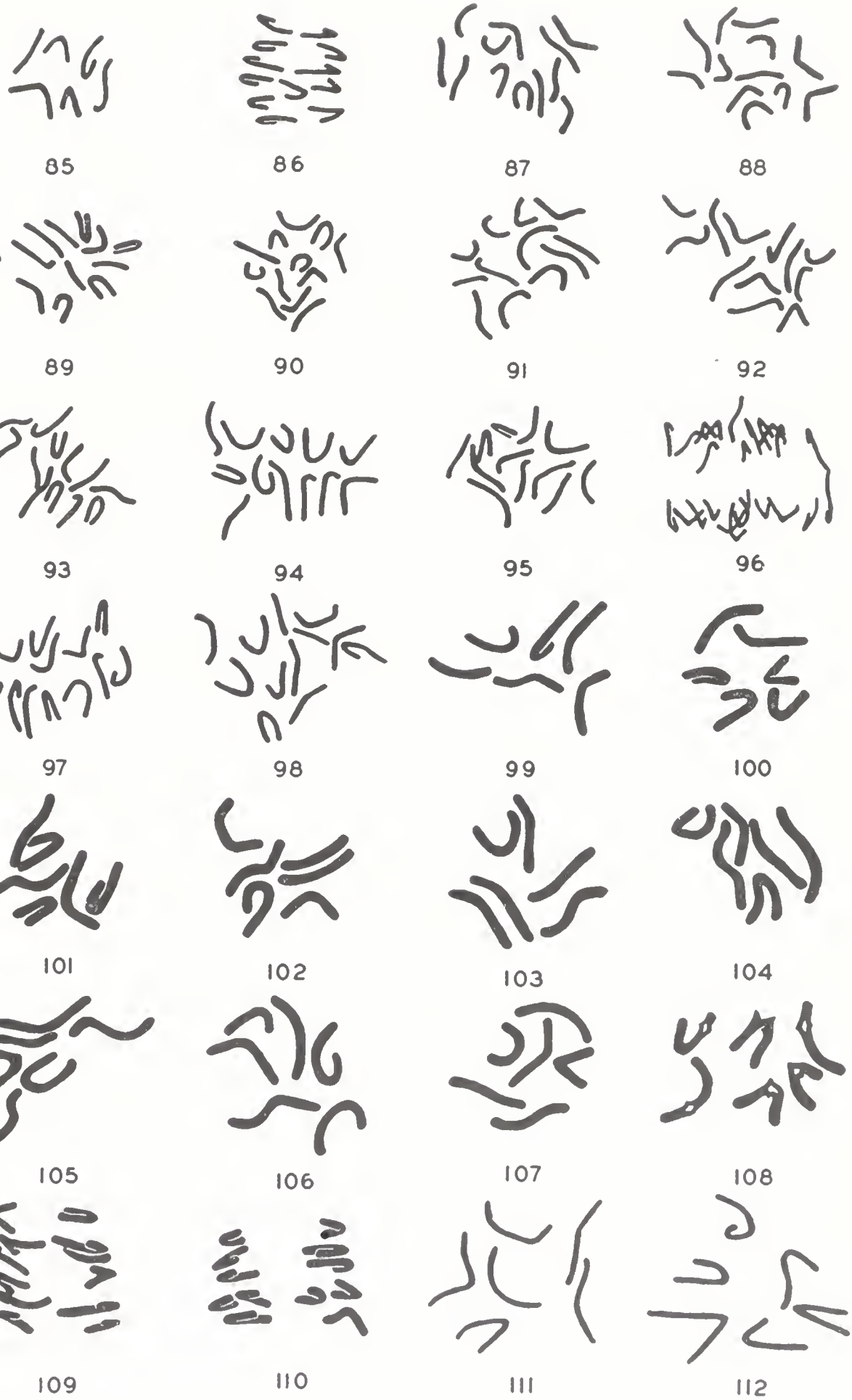
Explanation of figures 85–112

Figs. 85–86. *Mnium cuspidatum* (N = 6). Fig. 85. Chromosomes in metaphase. Fig. 86. Chromosomes in anaphase showing the approximate locations of the spindle-attachment regions. Figs. 87–98. *Mnium medium*. Figs. 87–95, 97, 98. Chromosomes in metaphase. Fig. 96. Chromosomes in anaphase showing the approximate locations of the spindle-attachment regions. Figs. 99–110. *Mnium insigne*. Figs. 99–107. Chromosomes in metaphase. Fig. 108. Chromosomes in late metaphase showing spindle-attachment regions. Figs. 109, 110. Chromosomes in anaphase. Figs. 111, 112. *Mnium cinclidioides*. Chromosomes in metaphase. All figures × 1332.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of elongated cells in single thickness, toothed in upper half of leaf only with single, sharp teeth mostly of one cell each; costa percurrent; cells of leaf distinctly hexagonal, to 40 μ, walls not pitted, corners not thickened.

Sporophytes 1–4 or even 5 from the same perichaetium; capsule pendulous, oblong, urn 3 mm. or less, light yellow, neck short and inconspicuous; operculum convex with a sharp point; stomata in neck; outer peristome-teeth light greenish-yellow; inner peristome brownish-yellow.

Spores yellow, papillose, ca. 22 μ, ripening in May or June.

From Maine to Washington in a narrow belt across the continent, somewhat northward into Canada. In the east southward to Maryland and Pennsylvania; in northeastern Europe and reported from Siberia.

Specimens studied:

No. 15. University of Michigan Biological Station, Cheboygan, Michigan. N = 6.

The average length of the chromosomes is as follows: 5.9 μ, 5.4 μ, 5.2 μ, 5.1 μ, 4.9 μ, 4.5 μ. The average chromosome width is 0.5 μ.

The spindle attachment regions are all median or submedian (fig. 66).

MNIUM CUSPIDATUM Hedw. Plants synoicous, approximately 2 cm. high; fertile stems simple, erect; sterile stems generally much longer than fertile stems, stoloniform, leaves complanate; leaves obovate from a narrow base, acute to short-acuminate, decurrent, bordered by 2–4 rows of narrow cells in single thickness, toothed in upper half only with single, sharp teeth, composed of single cells; costa percurrent; cells up to 25 μ in longest diameter, walls pitted, with thickened corners.

Sporophytes single; to 3 cm. high; seta curved below capsule neck; capsule pendulous, oblong to oval, urn to 3.5 mm. long, yellow to brownish-yellow when mature, neck very short; operculum high-convex without noticeable point; stomata in neck; outer peristome-teeth greenish-yellow; inner peristome brown, basal membrane very conspicuously lacunose.

Spores yellow, 20–25 μ, slightly papillose, ripening in April and May.

Common throughout the United States and northward into Canada, also in Europe and Asia.

Specimens studied:

No. 19. Nichol's Arboretum, Ann Arbor, Michigan. N = 12.

No. 14. Waterloo, Michigan. Collector E. A. Phillips. N = 12.

No. 13. *Mnium cuspidatum,* Florida. Collector Dr. Ruth O. Schornherst. N = 6.

The average length of the six chromosome pairs of *Mnium cuspidatum* is as follows: 6.4 μ, 5.6 μ, 5.2 μ, 4.8 μ, 4.4 μ, 4.1 μ. The chromosomes are 0.5 μ wide. The spindle attachment regions are all median or submedian (figs. 74, 76).

Mnium cuspidatum regularly shows 12 bivalents at metaphase I (figs. 77–78).

No. 13 differs from typical *Mnium cuspidatum* chiefly in its smaller size.

Explanation of figures 113–140

FIGS. 113–120. *Mnium cinclidioides.* FIGS. 113–118. Chromosomes in metaphase. FIGS. 119, 120. Chromosomes in anaphase showing the approximate locations of the spindle-attachment regions. FIGS. 121–129. *Mnium affine.* FIGS. 121–125, 127. Chromosomes in metaphase. FIG. 126. Chromosomes in late metaphase showing spindle-attachment regions. FIG. 128. Chromosomes in anaphase. FIGS. 130–132. *Mnium affine* var. *ciliare.* FIGS. 130, 131. Chromosomes in metaphase. FIG. 132. Chromosomes in late metaphase showing spindle-attachment regions. FIGS. 133–140. *Mnium punctatum.* Chromosomes in metaphase. All figures × 1332.


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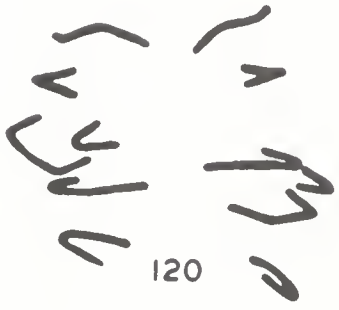
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The leaves are less strongly decurrent and the leaf cell size is smaller, 2881 cells per square mm. in contrast to 1885. The chromosome number is 6 (figs. 79–86). The average chromosome lengths are as follows: 6.6 μ, 6.3 μ, 6.1 μ, 5.3 μ, 4.9 μ, 4.6 μ. The spindle-attachment regions are all median or submedian (fig. 86).

MNIUM MEDIUM Br. and Sch. Plants synoicous, approximately 3 cm. high; fertile stems simple, erect; sterile stems not much longer than others, deflexed; leaves broad-oval to slightly obovate, short-cuspidate, decurrent, bordered by 2–3 rows of narrow cells in single thickness, toothed throughout with single, sharp teeth of one cell each; costa percurrent, combining with border to form cuspidate apex; cells of leaf reaching 80 μ near costa, walls prominently pitted with distinct corner-thickenings.

Sporophytes 1–3 from the same perichaetium; seta to 5 cm. long; capsule pendulous, oblong; urn 3–4 mm. long, yellow to light brown, with short and inconspicuous neck; operculum convex, apiculate; stomata in neck; outer peristome-teeth yellow; inner peristome orange, basal membrane not perforated.

Spores dark yellow, 20–25 μ, papillose, ripening in April and May.

New England to Washington, south to Maryland and California, northward through Canada to Alaska, Yukon and Greenland; also in Europe and Asia.

The following specimens were studied:

No. 7. Cascade Glen, Ann Arbor, Michigan. Collector, W. C. Steere. N = 12.

No. 12. Stinchfield Woods, Washtenaw County, Michigan. N = 12.

No. 25. University of Michigan Biological Station, Cheboygan, Michigan. N = 12.

The gametophytic chromosome number is 12 (figs. 87–98). The 12-chromosome complement consists of 6 pairs, the members of each pair being of equal length. The average lengths of the 6 chromosome pairs are as follows: 6.4 μ, 5.7 μ, 5.3 μ, 5.0 μ, 4.6 μ, 4.1 μ. The chromosomes are 0.5 μ+ wide. The spindle-attachment regions are median or submedian (fig. 96).

There are 575 leaf cells per square mm.

MNIUM AFFINE Bland. Plants dioicous, approximately 3 cm. high; fertile stems simple, erect; sterile stems much longer than others, stoloniform often rooting at tip, leaves complanate; leaves oval to obovate, short-cuspidate, often weakly decurrent, bordered by 2–4 rows of narrow cells in single thickness, usually toothed throughout with sharp teeth of 1–3 cells each, the teeth in some forms much reduced to almost lacking; costa percurrent, ending in cuspidate apex; cells of leaf up to 50 μ in longest diameter, walls pitted, corners not strongly thickened.

Sporophytes mostly single, rarely 2–3 from the same perichaetium; seta about 3 cm. long; capsule pendulous, oblong, about 5 mm. long, brownish-yellow, neck short and inconspicuous; operculum short-convex or broadly conic, apiculate; stomata in neck; outer peristome-teeth greenish-yellow; inner peristome orange, basal membrane not perforated.

Spores yellow, 25 μ, papillose, ripening in spring.

Common throughout the United States and northward to Greenland and Alaska, also in Europe and Asia.

The following specimens were studied:

No. 16. University of Michigan Biological Station, Cheboygan, Michigan. N = 6.

No. 9. University of Michigan Biological Station, Cheboygan, Michigan. N = 6.

No. 10. Little Portage Lake, Washtenaw County, Michigan. N = 6.

No. 18. Nichol's Arboretum, Ann Arbor, Michigan. N = 6.

The gametophytic chromosome number is 6 (figs. 121–132). The average length of the chromosomes for typical *Mnium affine*, as represented by Nos. 16, 10, and 18, are as follows: 6.5 μ, 6 μ, 5.6 μ, 5.2 μ, 4.8 μ, 4.3 μ. The chromosomes are 0.5+ μ wide. Spindle-attachment regions are median or submedian (figs. 126, 128).

No. 9 represents the variety *ciliare*, characterized by the long, multicellular teeth of the leaf margin and by its more robust growth habit. The chromosomes are larger than those of the species (figs. 130–132). The average chromosome lengths are as follows: 8.6 μ, 7.6 μ, 7.4 μ, 7.1 μ, 6.7 μ, 6.3 μ. The spindle-attachment regions are median to submedian (fig. 132).

There are 615 leaf cells per square mm.

MNIUM INSIGNE Mitt. Plants dioicous, very robust, approximately 6 cm. in height; fertile stems simple, erect; sterile stems deflexed; leaves narrowly elliptic, acuminate, prominently long-decurrent, bordered by mostly 3 rows of narrow cells in single thickness, toothed throughout, teeth of lower part of leaf short and blunt, those of apical part sharp, mostly of a single cell each; costa percurrent or nearly so; cells of leaf up to 50 μ in diameter, walls pitted, corners strongly thickened.

Sporophytes 1–9 from the same perichaetium, most frequently 3–6; seta approximately 3 cm. long; capsule pendulous, oblong, about 5 mm. long, yellowish-brown when ripe, neck short; operculum broadly conic, apiculate to almost short-rostrate; stomata in neck; outer peristome-teeth greenish-yellow; inner peristome orange, basal membrane not perforated.

Spores dark yellow, papillose, about 25 μ, ripening in April or May.

Alaska to northern California, inland to northwestern Montana.

The following specimen was studied:

No. 5. Seattle, Washington. Collector, Dr. Ruth D. Svihla. N = 6.

The gametophytic chromosome number is 6 (figs. 99–110). The chromosomes are large, 1 μ wide, and their average lengths as follows: 10 μ, 9.4 μ, 8.8 μ, 8.5 μ, 7.9 μ, 7.3 μ. The spindle-attachment regions are median to submedian (figs. 108–110).

MNIUM CINCLIDIOIDES Huben. Plants dioicous, up to 5 cm. or more in height; fertile stems simple, erect or slightly flexuose; sterile stems erect, flexuose; leaves complanate, obovate, rounded or with a short, blunt point, without a distinct border, though the cells of border region are gradually narrowed in several rows and may show a few short, blunt teeth; costa not reaching apex; cells of leaf so elongated in oblique direction from costa outward as to appear rhomboidal, up to 125 × 30 μ near costa, walls pitted, corners not thickened.

Sporophytes usually single; seta to 6 cm. or more in height; capsule pendulous, oval, with short neck, passing into thickened and much crooked top of seta, urn about 3 mm. long, brown when ripe; operculum convex, apiculate; stomata in neck; outer peristome-teeth brown; inner peristome yellowish-brown.

Spores 35 μ, brownish-yellow, minutely roughened, ripening in May–June.

Greenland to Alaska, southward in peat-bogs to Connecticut, New Jersey, Pennsylvania, Michigan, Minnesota, Montana, British Columbia; also in Europe and Northern Asia.

The following specimen was studied:

No. 11. University of Michigan Biological Station, Cheboygan, Michigan. N = 6.

The gametophytic chromosome complement of 6 is characterized by one chromosome noticeably longer than the others (figs. 111–120). The average chromosome lengths are as follows: 10.3 μ, 7.6 μ, 7.2 μ, 6.5 μ, 5.8 μ, 5.2 μ. The chromosomes are about 0.5 μ in width. The spindle-attachment regions are median to submedian with one subterminal (figs. 119–120).

MNIUM PUNCTATUM Hedw. Plants generally dioicous, varying to synoicous, to 5 cm. or occasionally more in height; leaves large, not or but slightly decurrent, oval to more commonly obovate-spatulate, broadly rounded to slightly emarginate at apex, with a distinct border, not toothed, the border sometimes reddish, 1 or more layers of narrow cells, 1 or more cells wide; costa fairly strong, ceasing below apex or percurrent, sometimes uniting with border to form a short, blunt point at apex, cells of leaf irregularly hexagonal to nearly rhomboidal, frequently elongated and arranged in rows in oblique direction from costa to border, to 150 × 50 μ, sometimes half as large or less, walls thin without pits or thicker and pitted, corners not or slightly thickened.

Capsules generally single, horizontal to pendulous, ovoid to oblong-cylindric, light yellowish to brownish, up to 5 mm. long when deoperculate, neck short; operculum conic-rostrate; stomata in neck of capsule, cryptopore; outer peristome-teeth brown to yellow; inner peristome golden yellow, basal membrane high, not perforated; spores 35–40 μ, brownish-yellow, roughened, ripening in winter or spring.

Greenland to Alaska, southward to Georgia, Ohio, Michigan, Wisconsin, Minnesota, Colorado, California; also in Europe and Asia.

The following specimens were studied:

No. 2. University of Michigan Biological Station, Cheboygan, Michigan. N = 7.

No. 17. Lake City, Michigan. N = 14.

No. 20. Corvallis, Oregon. Collected by Professor Ethel I. Sanborne. N = 6.

No. 2 is the typical dioicous *Mnium punctatum*. The gametophytic chromosome number is 7 (figs. 133–144). The average chromosome width is 0.3 μ and the average chromosome lengths are as follows: 4.7 μ, 4.3 μ, 3.9 μ, 3.6 μ, 3.2 μ, 2.7 μ, 0.9 μ.

There are 465 leaf cells per square mm.

No. 17 is a synoicous form referable to *Mnium pseudopunctatum*. The gametophytic chromosomes consist of 7 pairs. The average chromosome width is 0.3 μ and the average length of the 7 pairs is as follows: 4.8 μ, 4.5 μ, 4 μ, 3.7 μ, 3.2 μ, 2.7 μ, 0.75 μ (figs. 145–149). The spindle-attachment regions seem to be mainly median, submedian, or subterminal. Two of the long chromosomes appear to have terminal attachment regions (fig. 149).

There are 390 leaf cells per square mm.

No. 20 is referable to Kindberg's *Mnium glabrescens*. The gametophytic

Explanation of figures 141–167

FIGS. 141–144. *Mnium punctatum*. Chromosomes in metaphase. FIGS. 145–149. *Mnium pseudopunctatum*. FIGS. 145–148. Chromosomes in metaphase. FIG. 149. Chromosomes in anaphase showing the approximate locations of the spindle-attachment regions. FIGS. 150–158. *Mnium glabrescens*. FIGS. 150–157. Chromosomes in metaphase. FIG. 158. Anaphasic chromosomes. FIGS. 159–167. *Cinclidium stygium*. FIGS. 159–165. Chromosomes in metaphase. FIGS. 166, 167. Chromosomes in anaphase showing the approximate locations of the spindle-attachment regions. All figures × 1332.

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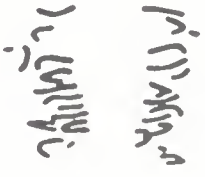

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chromosome number is 6 (figs. 150–158). The chromosomes are 0.3 μ wide and their average lengths are as follows: 4.7 μ, 4.2 μ, 3.7 μ, 3.6 μ, 3.4 μ, 2.4 μ. There are no clear indications of terminal spindle-attachment regions.

There are 480 leaf cells per square mm.

CINCLIDIUM Sw. Not differing from *Mnium* in its gametophytic characters. All species have entire bordered leaves. The capsule differs in its peristome; the exostome having shorter blunt teeth, the endostome not divided as normally into segments and cilia, but consisting of a single dome-like structure with small round openings at apex and somewhat irregular openings at the side, corresponding in number and position with the outer peristome-teeth. Spores rather large. Type species *Cinclidium stygium* Sw. (Andrews 1940).

CINCLIDIUM STYGIUM Sw. Plants synoicous, 3 cm. high or sometimes higher; stems stout, erect, simple or occasionally branching subapically; leaves of good size, from a very narrow base broadly oval to obovate, sharply apiculate, with a reddish border of 3 to 4 rows of very narrow thick-walled cells; costa strong, reddish, generally extending into apex; cells of leaf, except those immediately adjacent to costa or border, elongated in oblique or transverse direction from costa to border, irregularly elongated-hexagonal, to 50 × 30 μ, rather thick-walled without thickened corners, walls pitted.

Seta slender, erect, flexuose, 3 or 4 cm. long or sometimes longer, reddish-yellow, thickened and strongly crooked where it passes into neck of capsule; single, pendulous, up to 4 mm. long, oval-pyriform with prominent, broad neck about one-third length of entire capsule, light yellowish-brown when mature; operculum hemispheric; stomata in neck of capsule, cryptopore; outer peristome-teeth light greenish-yellow, short, broad and blunt or even irregularly emarginate at apex; inner peristome darker, yellowish-brown in color, minutely roughened, columns supporting the dome (which would correspond to segments in normal peristome) slender, carinate without openings at keel; spores varying in size even in same capsule, up to 50 μ, or more, the smaller ones of half this diameter or less, greenish-yellow, roughened, ripening in summer.

Of northern distribution, found in 3 localities in Michigan, occasional northward to Yukon and Alaska, Labrador and Greenland; also in Europe.

The following specimen was studied:

No. 21. University of Michigan Biological Station, Cheboygan, Michigan. N = 14.

The gametophytic complement of 14 chromosomes (figs. 159–167) consists of 7 pairs. The average lengths of the 7 chromosome-pairs are as follows: 4.4 μ, 3.9 μ, 3.6 μ, 3.3 μ, 2.9 μ, 2.5 μ, 0.9 μ. The chromosomes are about 0.3 μ wide. Two of the longer chromosomes have terminal spindle-attachment regions, the attachment regions of the very small chromosomes could not be determined. Other members of the complement have median or submedian attachment regions (figs. 166, 167).

DISCUSSION

The Mniums studied in this work can be separated into groups based on similarities of chromosome number and overall chromosome size, which with few exceptions parallel previous groupings based on morphological similarities alone. The basic chromosome number for the genus is 6. Chromosome size is not generally considered to be significant in considerations involving rather

distantly related organisms; however its importance in a small group of obviously closely related species cannot be ignored.

TABLE 1

SPECIES	CHROMOSOME NUMBER	AVERAGE CHROMOSOME LENGTH IN MICRONS							
Mnium Menziesii	N = 5	7.4	6.6	5.6	5.2	4.6			
Mnium stellare	N = 7	12.1	10.6	10.0	9.9	7.0	6.3	1.8	
Mnium hornum	N = 6	5.7	5.5	5.2	5.0	4.5	4.0		
Mnium orthorhynchum	N = 6	7.2	6.7	6.3	5.8	5.5	5.0		
*Mnium marginatum**	N = 12	7.3	6.4	6.0	5.6	5.2	4.7		
Mnium spinulosum	N = 8	6.8	6.6	5.8	5.4	4.8	4.2	2.6	2.1
Mnium Drummondii	N = 6	5.9	5.4	5.2	5.2	4.9	4.5		
*Mnium cuspidatum**	N = 12	6.4	5.6	5.2	4.8	4.4	4.1		
Mnium cuspidatum	N = 6	6.6	6.3	6.1	5.3	4.9	4.6		
*Mnium medium**	N = 12	6.4	5.7	5.3	5.0	4.6	4.1		
Mnium affine	N = 6	6.5	6.0	5.6	5.2	4.8	4.3		
Mnium affine var. *ciliare*	N = 6	8.6	7.6	7.4	7.1	6.7	6.3		
Mnium insigne	N = 6	10.0	9.4	8.8	8.5	7.9	7.3		
Mnium cinclidioides	N = 6	10.3	7.6	7.2	6.5	5.8	5.2		
Mnium punctatum	N = 7	4.7	4.3	3.9	3.6	3.2	2.7	0.9	
*Mnium pseudopunctatum**	N = 14	4.8	4.5	4.0	3.7	3.2	2.7	0.75	
Mnium glabrescens	N = 6	4.7	4.2	3.7	3.6	3.4	2.4		
*Cinclidium stygium**	N = 14	4.4	3.9	3.6	3.3	2.9	2.5	0.9	

The following grouping was constructed using cytological data as the sole criterion.

Group I.
- Subgroup I.
  - *Mnium hornum.* N = 6.
- Subgroup II.
  - *Mnium orthorhynchum.* N = 6.
  - *Mnium marginatum.* N = 12.
- Subgroup III.
  - *Mnium spinulosum.* N = 8.
- Subgroup IV.
  - *Mnium stellare.* N = 7.

Group II.
- Subgroup I.
  - *Mnium cuspidatum.* N = 6.
  - *Mnium cuspidatum.* N = 12.
  - *Mnium Drummondii.* N = 6.
- Subgroup II.
  - *Mnium affine.* N = 6.
  - *Mnium medium.* N = 12.
  - *Mnium affine* var. *ciliare.* N = 6.
  - *Mnium insigne.* N = 6.

Group III.
- *Mnium punctatum.* N = 7.
- *Mnium pseudopunctatum.* N = 14.
- *Mnium glabrescens.* N = 6.

Group IV.
- *Mnium cinclidioides.* N = 6.

Kabiersch (1936) handled the above species in a similar manner in his critical division of the genus into sections and subsections.

* The average chromosome length for the diploids is given for chromosome-pairs.

The usual and convenient separation of the genus into three groups depending upon the marginal teeth of the leaves, i.e. single teeth, double teeth, and no teeth, is not so artificial as it would seem at first glance. The above species fall into these three groups as follows:

Double-teeth:
- *Mnium hornum.*
- *Mnium orthorhynchum.*
- *Mnium marginatum.*
- *Mnium spinulosum.*
- *Mnium stellare* . . .?

Single-teeth:
- *Mnium cuspidatum* (N = 6).
- *Mnium cuspidatum.*
- *Mnium Drummondii.*
- *Mnium affine.*
- *Mnium affine* var. *ciliare.*
- *Mnium insigne.*
- *Mnium medium.*
- *Mnium cinclidioides* . . .?

No-teeth:
- *Mnium punctatum.*
- *Mnium pseudopunctatum.*
- *Mnium glabrescens.*

The teeth of *Mnium stellare* are not always obviously of the double-toothed group. When well developed, however, they stand out at an angle to the plane of the leaf-blade and when close together give the appearance of double teeth.

The position of *Mnium cinclidioides* is questioned, since cytologically it is not similar to other members of the single-toothed group.

Disregarding the above two exceptions, the grouping is the same as that based on cytological evidence.

The cytological evidence separating the double-toothed from the single-toothed species is not too convincing. The basic chromosome number of 6 is the same in both groups. There is no consistent difference in chromosome size, with the exceptions of *Mnium cuspidatum, Mnium cuspidatum* (N = 6), and *Mnium Drummondii.* The chromosomes of these three are of the same size and noticeably smaller than other members of the two groups. The separation of the two groups cytologically is based upon the presence of subterminal or terminal spindle attachment regions in the double-toothed species and their absence in the single-toothed species.

The non-toothed group, as represented by the three species: *Mnium punctatum, Mnium pseudopunctatum,* and *Mnium glabrescens,* is more clearly natural. The chromosome size is the same for all three species and is much smaller than that of any other *Mnium* studied.

Some cytological evidence regarding the limits of the genus was obtained. *Mnium Menziesii* (see description) is a very distinct species with no apparent close relatives in the genus. Lindberg (1868) removed it from *Mnium* and proposed the name *Leucolepis acanthoneura* for it. Kabiersch (1936) agreed with Lindberg's conception, retaining the plant under the latter name. Andrews, in the *Moss flora of North America,* admits the distinct position of the plant but retains it in *Mnium.* The chromosome number is 5, in contrast to the basic number of 6 for the genus *Mnium* (figs. 1–10). The loss of a small chromosome fragment from a monoploid complement is known to have occurred in higher plants without causing the death of the cells involved but in most cases is accompanied by reduced vigor. However, the loss of a complete chromosome

from a monoploid complement, without lethal effects, is probably impossible. A reduction in chromosome number might possibly be effected by the translocation of all the material of one chromosome to other members of the complement with the exception of the spindle-attachment region which would then have to be eliminated (Sharp 1934). The above possible derivations of a 5-chromosome form from a basic number of 6 chromosomes are at best improbable, and especially when one considers the fact that to establish the new chromosome number in a dioicous plant such as *Mnium Menziesii* the event would have to occur simultaneously in plants of both sexes and the altered gametes would have to be brought together to produce a sporophyte which would then supposedly produce only spores having 5 chromosomes.

In view of the above difficulties in deriving the chromosome complement of *Mnium Menziesii* from some 6-chromosome form, it is more logical to suppose that the plant is not a *Mnium,* that it represents a different chromosome series, and should, following Lindberg, be maintained in the genus *Leucolepis.*

The similarity of *Cinclidium* (see description) to the non-toothed Mniums, *Mnium punctatum, Mnium pseudopunctatum,* and *Mnium glabrescens,* has been recognized as possibly indicating a close relationship. Loeske (1910), in fact, suggested the union of the above Mniums with *Cinclidium.* Morphologically, *Cinclidium* can be distinguished easily from *Mnium* only by peristome characters. The tissue of the inner peristome does not split to form teeth, as in *Mnium,* but remains united in a perforated, dome-like structure. The outer peristome-teeth, although similar to those in *Mnium,* are shorter. The gametophytic plants of *Cinclidium stygium,* the species studied in this paper, are essentially indistinguishable from those of *Mnium pseudopunctatum.* The chromosome number and chromosome morphology (figs. 159–167) suggest a very close relationship with *Mnium pseudopunctatum* (figs. 145–149). Both plants have 14 chromosomes, six relatively long pairs and a dimorphic pair of very short chromosomes. A comparison of chromosome lengths (table 1) between the two species, reveals a surprisingly close agreement and the chromosomes are also of the same width. Correlated with the apparently diploid condition of both species is the synoicousness of both. The chromosomes of *Mnium punctatum* ($N = 7$; figs. 133–144, table 1) agree very closely in length and width with the chromosome pairs found in *Cinclidium stygium.* The chromosomes of *Mnium glabrescens* also agree closely with those of *Cinclidium stygium* with the exception of the small seventh chromosome which is absent. Without doubt, *Mnium punctatum, Mnium pseudopunctatum,* and *Mnium glabrescens* are more closely related to *Cinclidium* than to other groups in the genus *Mnium.*

The suggestion that *Mnium cinclidioides* also shows the above affinities is not well supported cytologically. The chromosome lengths are quite different (figs. 111–120, table 1).

Whether the non-toothed Mniums should be united with *Cinclidium* is a

question to which the answer had best be delayed until more cytological data have been accumulated regarding other *Cinclidium* species, particularly those which are dioicous.

In many respects, *Rhizogonium spiniforme* reminds one very strongly of a double-toothed *Mnium*. The chromosome number is 6 (Kurita 1937, Lowry unpub.). However, the combination of 6 chromosomes and its synoicous inflorescence, a combination only found in *Mnium Drummondii,* coupled with its characteristic tropical distribution and peculiar growth habit, approaching the pleurocarpous type, makes a close relationship seem improbable. Unfortunately, data are lacking which would enable one to make a detailed comparison of chromosome morphology with *Mnium* species.

Five species belonging to the double-toothed Mniums were studied. *Mnium hornum, Mnium orthorhynchum, Mnium marginatum,* and *Mnium spinulosum* have well developed double teeth on the leaf margins, the teeth of *Mnium stellare* are not truly double but, as pointed out previously, are inserted at an angle to the plane of the leaf blade and when close together give the impression of double teeth.

The inclusion of *Mnium stellare* in the double-tooth group is done with considerable uncertainty, although its close morphological similarity to *Mnium Blyttii,* a plant which produces well developed double teeth, indicates that it does belong here. The chromosome number of *Mnium stellare* is 7, 6 long chromosomes with an "m" (figs. 11–21).

Heitz (1942) has found 7 chromosomes (6 + m) in *Mnium hymenophyllum,* a plant often found mixed with or confused with *Mnium Blyttii* (Andrews 1940), which suggests the possibility that a similar chromosome complement will be found for *Mnium Blyttii.* If such proves to be the case the position of *Mnium stellare* in the double-toothed group will be considerably strengthened.

Mnium stellare was placed in the non-toothed group by Limpricht (1893) and if one considers only the chromosome number, 6 with an "m," it apparently does belong there. A comparison of chromosome size between *Mnium stellare* (figs. 11–21) and *Mnium punctatum* (figs. 133–144) makes a possible relationship seem remote. The chromosomes of *Mnium stellare* are much larger.

The presence of the small seventh chromosome in *Mnium stellare* calls for some explanation of its origin, since the basic chromosome number for *Mnium* is clearly six. Fully aware that any explanation is necessarily hypothetical, the following possible origin is presented. If one chromosome with a subterminal attachment region, in an original complement of 6, broke through its attachment region, the result would be a relatively long chromosome and a very short chromosome. One would expect the two fragments to behave normally, since both would possess spindle attachment regions which would be located terminally. Such a chromosome fragmentation has been observed in *Zea* and the resulting fragments were found to behave as complete chromosomes (McClintock 1932, 1938). Since *Mnium stellare* is dioicous the fragmentation would have

to occur in plants of both sexes if the altered chromosome complement were to become established. The most logical point in the life history for the event to occur and involve both the chromosomes of both sexes simultaneously would be during the first meiotic division of the spore-mother cells. The almost complete sterility of the plant suggests an altered chromosome complement.

Mnium hornum, the type species of the genus, does not appear, cytologically, to be especially closely related to the other double-toothed species studied. The 6 chromosomes of *Mnium hornum* average 1 micron shorter than those of *Mnium orthorhynchum.* The plant is dioicous.

Mnium orthorhynchum and *Mnium marginatum* constitute a natural species-pair. The following gametophytic characters represent the only clearly defined differences between them. *Mnium orthorhynchum* is dioicous, there are on the average 2578 leaf-cells per square mm., and teeth are present on the back of the costa. *Mnium marginatum* is synoicous, there are on the average 2349 leaf-cells per square mm., and there are no teeth on the back of the costa. *Mnium orthorhynchum* ripens its spores in summer while those of *Mnium marginatum* ripen in May. The two plants have a nearly identical geographical distribution and are often found growing together, even mixed in the same clump. The chromosome numbers are as follows: *Mnium orthorhynchum,* N = 6, *Mnium marginatum,* N = 12 (figs. 25–36, 37–48). The twelve chromosomes of *Mnium marginatum* clearly represent 6 pairs (figs. 38, 44, 46). A comparison of the average chromosome lengths of *Mnium orthorhynchum* with the six chromosome-pairs of *Mnium marginatum* reveals no significant differences (table 1). *Mnium marginatum* is undoubtedly an autodiploid derived from *Mnium orthorhynchum.* The chromosome doubling must have been the result of apospory, regeneration of sporophytic tissue or the production of 2N spores, since doubling occurring in the gametophytic generation would not bring about the synoicous condition found in *Mnium marginatum.* It appears likely that the diploid does not represent an isolated instance of chromosome doubling, since the two species are often found mixed in a single collection.

Mnium spinulosum is aneuploid, N = 8; 6 + 2, with respect to the other species of the double-toothed group (figs. 49–60). The morphology of the species is distinctive and variable forms are not found (see description). An analysis of chromosome lengths reveals 6 chromosomes ranging in length from 6.8 μ to 4.2 μ, which is approximately the lengths found in the other species of the group. The two extra chromosomes are considerably shorter, 2.6 μ and 2.1 μ. Chromosomes of this size were not found in any of the other species studied. In formulating an hypothesis that would account for the origin of *Mnium spinulosum* it is necessary to postulate conditions which would bring about the synoicous character of the plant in addition to increasing the chromosome number from 6 to 8. The two extra chromosomes may have been derived, as was suggested for *Mnium stellare,* by fragmentation. The presence of terminal attachment regions (fig. 60), with the fact that chromosomes of a similar length to the two short chromosomes of *Mnium spinulosum* are not found in other

members of the genus, supports the fragmentation theory. Another interesting point is demonstrated by adding the two short chromosomes to the two next shortest chromosomes creating a hypothetical set of 6 and comparing with the complement of *Mnium orthorhynchum.*

Hypothetical complement	*7.4*	6.8	6.6	*6.3*	5.8	5.4
Mnium orthorhynchum	7.2	6.7	6.3	5.8	5.5	5

It is not suggested that *Mnium spinulosum* was derived from *Mnium orthorhynchum.* The above comparison merely points out that if *Mnium spinulosum* arose as the result of chromosome fragmentation, the original 6 chromosomes were possibly similar in length to those of the other members of the double-toothed group. The above mode of origin does not satisfy conditions necessary for the creation of the synoicousness of the plant. In the double-toothed group, 6 chromosomes and dioicousness are correlated, thus making it necessary to assume the synoicous character of *Mnium spinulosum* to be the result of genetic change. The synoicousness of *Mnium spinulosum* is more easily explained if the aneuploid were the result of irregular meiotic divisions following hybridization between a dioicous, 6-chromosome form and a synoicous, 12-chromosome form.

Five species and one variety representing the single-toothed group were studied. The basic gametophytic chromosome number is 6. There is considerable variation in chromosome size within the group; *Mnium cuspidatum, Mnium cuspidatum* (N = 6), and *Mnium Drummondii* form one size group with chromosomes somewhat shorter and thinner than the other species. A progressive increase in chromosome size is found in *Mnium affine, Mnium affine* var. *ciliare,* and *Mnium insigne.*

Mnium cuspidatum (N = 12; figs. 67–78) is an autodiploid. Here again the synoicous inflorescence is correlated with a 2N chromosome complement. The chromosomes consist of 6 pairs, the members of each pair being of equal length. If *Mnium cuspidatum* is an autodiploid, one would expect to find a haploid, dioicous moss similar to *Mnium cuspidatum.* Such a plant is represented by collection No. 13 which is referred to in this work as *Mnium cuspidatum* (N = 6). The 6-chromosome form is somewhat smaller than the species and the leaf-cell size is smaller, having 2881 cells per square mm. as compared to 1885 cells per square mm. The form was sterile and the sex condition could not be determined; presumably it is dioicous. The chromosome number is 6 (figs. 79–86). The average lengths of the chromosomes agree reasonably well with those of the 6 chromosome-pairs of *Mnium cuspidatum.* The two forms are obviously very closely related since *Mnium cuspidatum* is the aposporous autodiploid of the form.

Mnium Drummondii is a distinct and stable species easily distinguished from all other Mniums. In morphological characters it approaches *Mnium cuspidatum* more closely than any of the other members of the genus. It differs from the above species in leaf-cell size, 800 cells per square mm. as compared to

1885 cells per square mm. of *Mnium cuspidatum,* the regular hexagonal shape of the cells, the tendency to produce several sporophytes from the same inflorescence, and its restricted geographical distribution (see description). The plants are normally synoicous but occasional male plants are found of the characteristic *Mnium* type, having broad, terminal rosette of perigonial leaves, and clavate paraphyses. The chromosome number is 6 (figs. 61–66), which is surprising since all other members of the genus having 6 chromosomes are dioicous. One is thus forced to consider the possibility that *Mnium Drummondii* may be diploid, having evolved from a 3-chromosome dioicous form. The work of the Marchals, Wettstein and others (see introduction) has shown that recently-produced diploids of dioicous species develop many plants which are apparently unisexual and only a small proportion of synoicous plants. Perhaps the occasional production of a typically male inflorescence by *Mnium Drummondii* is an expression of instability resulting from a not-too-remote chromosome doubling. Allowing for errors in measurements, the 6 chromosomes of *Mnium Drummondii* may be grouped into 3 pairs (table 1). In speculations of this type, one must not overlook the possibility of changes in the germ plasm without accompanying visible alterations in the chromosomes, which would be capable of altering the mechanism governing sex expression.

Mnium affine (see description) and *Mnium medium* (see description) form a natural, closely-related species-pair. There are only two morphological characters which will infallibly separate them, namely leaf-cell size and sex condition. The leaf cells of *Mnium medium* are larger, 575 cells per square mm., than those of *Mnium affine* which has 615 cells per square mm. *Mnium medium* is synoicous and *Mnium affine* is dioicous. *Mnium affine* has 6 chromosomes (figs. 121–132) and *Mnium medium* is diploid with 12 (figs. 87–98). The 6 chromosome-pairs of *Mnium medium* agree closely in length with the 6 chromosomes of *Mnium affine* (table 1). Here, as in the other species-pairs of the genus, *Mnium medium* is apparently an autodiploid derived from *Mnium affine* by some type of apospory.

Mnium affine is variable in the character of the leaf-margin teeth. There are forms in which teeth are much reduced to nearly absent and at the other extreme, forms having long, sharp teeth consisting of several cells. The latter form is represented by specimen No. 9 and is here referred to as *Mnium affine* var. *ciliare* (Grev.) C. Müll. The chromosome number is 6 (figs. 130–132). The chromosomes are larger than those of the species and remind one of the chromosomes of *Mnium insigne* (figs. 99–110). Andrews (1940) says, "*Mnium insigne* is obviously a derivative of the widely distributed and greatly varying *Mnium affine.*" A detailed comparison, in so far as data permit, of the chromosomes, suggests such a relationship. Both species have 6 large chromosomes, those of *Mnium insigne* are next to the largest found in the genus. Subtracting the average lengths of the chromosomes of *Mnium affine* var. *ciliare* from those of *Mnium insigne* yields the following results: 1.4 μ, 1.8 μ, 1.4 μ, 1.4 μ, 1.2 μ, 1 μ. There are no great differences in relative lengths, although the chromo-

somes of *Mnium insigne* are longer. A comparison of spindle attachment regions of the two chromosome sets reveals no great differences. All are submedian or median (figs. 132, 108).

Mnium cinclidioides (see description), although superficially resembling *Cinclidium,* does not show cytological affinities in the direction of this genus or toward the non-toothed Mniums. It is best treated as a separate section of the genus, without close relatives, as was done by Kabiersch. The gametophytic chromosome number is 6. The chromosomes are long and quite thin, which gives the complement a characteristic appearance not found elsewhere in *Mnium* (figs. 111–120). One chromosome is noticeably longer, 2.7 μ, than the others (table 1) and can be designated as an "M" chromosome. The shortest chromosome of *Mnium cinclidioides* is longer than the longest chromosome found in either the non-toothed Mniums or in *Cinclidium.*

The non-toothed Mniums, represented by Andrews' description as *Mnium punctatum,* are characterized cytologically by the small size of their chromosomes. The largest chromosome is only 4.8 μ by 0.3 μ. In Andrews' treatment in the *Moss flora of North America* all forms are included in *Mnium punctatum,* with the statement, "Variable in many of its features. Of the different characters that have been employed to separate *Mnium pseudopunctatum* (*M. subglobosum*) none, even its synoicous inflorescence, appears to correlate with any others and it seems impossible to distinguish under this name even a clearly marked variety." Of the Pacific Coast form, Andrews states, "*M. glabrescens* Kindb. has often been regarded as a valid species of the Pacific coastal region and may give somewhat that impression, but presents no characters that definitely separate it. Its leaf cells average smaller than usual, but vary greatly even in the same leaf." Three collections of *Mnium punctatum* were studied cytologically: Collection No. 2 from Michigan, a dioicous, rather small form corresponding closely to the description of *Mnium punctatum;* No. 17, also from Michigan, a synoicous form referable to *Mnium pseudopunctatum;* and No. 20 from Oregon, a dioicous form representing *Mnium glabrescens.* One finds a basis for separating definitely the above species in their chromosome numbers which are, respectively, 7, 14, and 6 (figs. 133–144, 145–149, 150–158). The chromosome complement of *Mnium punctatum* is made up of 6 long chromosomes plus one very short "m" chromosome (table 1). The "m" chromosome has been identified in European material by Jachimsky (1935), as a sex-chromosome.

The synoicous *Mnium pseudopunctatum* is apparently an autodiploid derivative of *Mnium punctatum* (figs. 145–149). Of the 7 chromosome-pairs, one of them consists of two "m" chromosomes, presumably the x and y sex-chromosomes of *Mnium punctatum.* A detailed comparison of average chromosome lengths reveals a maximum difference of only 0.2 μ between the two species (table 1). In addition to 14 chromosomes and synoicousness, *Mnium pseudopunctatum* presents other diploid characteristics differentiating it from *Mnium punctatum.* The leaf cells are larger, a character which is difficult to

measure, because of the variable size of the cells in different parts of the leaf; however, by carefully selecting the same relative position in each leaf examined, the following reasonably constant results were obtained: *Mnium punctatum,* 465 cells per square mm.; *Mnium pseudopunctatum,* 390 cells per square mm. The rhizoids which cover the lower part of the stem show a different mode of branching in the two species, dichotomously with short internodes in *Mnium pseudopunctatum,* deliquescent with relatively long internodes in *Mnium punctatum.* This character does not seem to be the result of different environmental factors, since the characteristic branching was maintained by the two plants when grown side by side in the same damp chamber.

Heitz (1942) found only 13 (12 + m) chromosomes in the European *Mnium pseudopunctatum* and suggested hybridization between *Mnium punctatum* and some unknown 6-chromosome form as its origin. *Mnium glabrescens* (figs. 150–158) is such a form with 6 chromosomes. The plant is quite similar to *Mnium punctatum* except for the more narrowly oval leaves, slightly smaller leaf cells, 480 per square mm., and its very restricted geographical distribution. The 6 chromosomes are of nearly the same length as the 6 long chromosomes of *Mnium punctatum* (table 1). The above morphological differences, correlated with the chromosome number of 6, are here considered sufficient justification for considering *Mnium glabrescens* a distinct species. The basic chromosome number for *Mnium punctatum* and *Mnium pseudopunctatum* is undoubtedly 6, the "m" chromosome having probably arisen as the result of fragmentation. In figure 149, there are indications of terminal spindle-attachment regions in two of the longer chromosomes, which is the result expected if fragmentation had occurred through this structure.

In general, the cytological data obtained in this work support the concepts of the morphological taxonomist, and in the matter of polyploids imposes a somewhat narrower interpretation upon the separation of species. It has been customary to regard experimentally produced, semi-sterile, diploid forms as varieties, and rightly so, for one of the obvious requirements of a good species is that it must be able to maintain itself in nature. *Bryum Corrensii* was not given specific rank until the plant had established complete fertility. Most naturally occurring polyploids have been given specific rank, although Andrews does not recognize *Mnium pseudopunctatum.* In this connection it is interesting to note the status of the haploid form of *Mnium cuspidatum.* To be consistent with the treatment of *Mnium medium* and *Mnium marginatum,* the form should be given specific status.

Autopolyploidy, although not ordinarily considered as important in the evolutionary process leading to species production as allopolyploidy, does produce changes of a specific nature in the dioicous mosses, for example synoicousness and increased cell size. The "gigas" characters may be lost eventually, as in *Bryum Corrensii,* and there is not always a perfect correlation between cell size and chromosome number. A maximum cell size may be reached in which further increase in chromosome number does not result in further increase in

cell size (Wettstein 1924c, 1928b). In spite of the above exceptions, however, the synoicous character of the inflorescence effectively isolates the autopolyploid moss from its haploid progenitor by making self-fertilization practically certain.

The cytological evidence obtained in my work is necessarily an indirect indication of relationships and the need for experimental corroboration is obvious if such conclusions as have been made are to be removed from the status of hypotheses. Aposporic diploids of the haploid members of the species-pairs should be produced artificially and compared with the natural diploids. The matter of sex determination needs further investigation. Single-spore cultures of each species should be made to determine the true sex condition positively. Many of the Mniums are described as dioicous merely because the antheridia and archegonia are produced on different leafy plants with no assurance that the plants originated from different spores. The European *Mnium hornum* was examined by the Marchals and found to be really dioicous. However, the variable, synoicous to heteroicous nature of *Mnium Drummondii* throws suspicion upon other 6-chromosome, supposedly dioicous species. The synoicousness of the hyperploid *Mnium spinulosum* also emphasizes the need for a more thorough knowledge of the subject.

The presence of the "m" chromosome in *Mnium stellare* and *Mnium punctatum* suggests the possibility of producing chromosome fragmentation through the use of X-rays or other physical means. It would be particularly interesting to irradiate *Mnium glabrescens* ($N = 6$), in order to validate the supposition that one of the chromosomes has a tendency to break through its spindle attachment region, forming a 7-chromosome complement like that of *Mnium punctatum*.

SUMMARY

1. Cytological researches on mosses are reviewed, with special emphasis on chromosome numbers and polyploidy, and a list of published reports of chromosome numbers in mosses is given.

2. The genus *Mnium* was selected because of the availability in Michigan of more than half of the 21 North American species.

3. Special techniques to investigate and count chromosomes of mosses were devised, consisting especially of a smear technique for the apex of the gametophyte plant.

4. Fixation was made with a 1:2 solution of acetic acid and absolute alcohol; staining was accomplished by means of acetic orcein.

5. Fifteen species of *Mnium* are described on the basis of their external morphology, which is correlated with their chromosome morphology.

6. The chromosome number and morphology of 15 North American species of *Mnium* and of *Cinclidium stygium* were determined.

7. The basic chromosome number for the genus *Mnium* is 6.

8. It is suggested that *Mnium Menziesii* be excluded from the genus *Mnium*

and retained in the genus *Leucolepis* Lindb. on the basis of its 5 chromosomes, coupled with morphological differences.

9. Three aneuploid species were found, *Mnium stellare*, N = 7, *Mnium spinulosum*, N = 8, and *Mnium punctatum*, N = 7. It is postulated that the extra chromosomes arose as the result of fragmentation.

10. The average chromosome lengths for 15 species, 1 variety and 1 form of *Mnium* and for *Cinclidium stygium* were determined.

11. It was concluded that the cytological data indicate a relationship between *Cinclidium* and the non-toothed Mniums, represented by *Mnium punctatum* and *Mnium pseudopunctatum*, that is closer than between the latter species and other Mniums.

12. Four species-pairs are recognized:

Mnium orthorhynchum	N = 6	*Mnium marginatum*	N = 12
Mnium cuspidatum	N = 6	*Mnium cuspidatum*	N = 12
Mnium affine	N = 6	*Mnium medium*	N = 12
Mnium punctatum	N = 7	*Mnium pseudopunctatum*	N = 14

It was concluded that the diploid members of the above species-pairs are aposporic autodiploids of the haploid members.

13. *Mnium Drummondii* was found to possess 6 chromosomes and a synoicous inflorescence, a condition not found in other 6-chromosome Mniums. The occasional production of a male inflorescence by this species is interpreted as possibly indicating an unstable condition as the result of a recent, aposporic chromosome doubling of some unknown dioicous, 3-chromosome form. It was pointed out that the 6 chromosomes of *Mnium Drummondii* may be grouped into 3 pairs, the members of each pair being of similar length.

14. Similar chromosome size and spindle-attachment regions of *Mnium affine* var. *ciliare* and *Mnium insigne* are interpreted as supporting evidence to the previously postulated close relationship between these two species.

15. *Mnium cinclidioides* is placed in an independent position on the basis of its unique external morphology and chromosome morphology.

16. The 6-chromosome complement of *Mnium glabrescens*, in contrast to the 7-chromosome complement of *Mnium punctatum*, is considered as sufficient additional evidence for retaining the plant as a good and independent species.

17. It is suggested that the haploid form of *Mnium cuspidatum*, to be consistent, should be given specific rank, since it maintains itself in nature.

18. The synoicous inflorescence was found to be correlated with the diploid gametophyte in all species studied with the exceptions of *Mnium Drummondii*, apparently haploid (N = 6) and *Mnium spinulosum*, hyperhaploid (N = 8).

19. The cytological data support the concepts already proposed by morphological taxonomists, and a classification based on cytological interrelationships is proposed.

20. Autopolyploidy is considered to be more important than allopolyploidy in species production in *Mnium*.

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Reproduction in Petunia

By

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REPRODUCTION IN PETUNIA

A. B. Stout

Chapter 1. The Nomenclature, Character, and Specificities of the Three Species That Were Studied

Nomenclature and Early Introduction of Petunia axillaris. The first and very brief description of this species was by Lamarck in 1793 under the name *Nicotiana axillaris* and this was based on herbarium specimens collected by Commerson near Montevideo in Uruguay, South America. In 1797, Poiret repeated the description given by Lamarck and added to it, but also without illustration. In 1803, Jussieu examined herbarium specimens collected by Commerson near the mouth of the La Plata River and established the genus *Petunia* in which he included two very distinct species which he named *P. nyctaginiflora* and *P. parviflora*. In addition to a fairly complete description of "*P. nyctaginiflora*" there are excellent drawings which show a flower corolla about 1¾ inches in expanse, a flower tube about 1½ inches long and somewhat expanded near the throat, and stamens of three lengths with the two longest slightly shorter than the pistil.

For about one hundred years the names *Nicotiana nyctaginiflora* and *N. axillaris* were used by various authors (Lehman 1818; Otto 1824; Kuntze 1898) while others used the name *Petunia nyctaginiflora* (Persoon 1805; Sims 1825; Sweet 1825; Loudon 1840; Bailey 1911). But in a catalog of plants growing within one hundred miles of New York City and published in 1888, Britton, Sterns, and Poggenburg listed "*Petunia axillaris*" as an introduced species. Thus for the first time the specific epithet applied by Lamarck was used with the generic name *Petunia* established by Jussieu, and the specific name *Petunia nyctaginiflora* Juss. was reduced to a synonym. In his monograph of the genus *Petunia*, Fries in 1911 adopted the name *P. axillaris* and reduced to a synonym the name *P. nyctaginiflora* and also the name *P. propinqua*, which had been applied by Miers in 1846 and afterward used by various writers until 1881 (see Fries 1911). This terminology has very generally been accepted in later botanical and horticultural literature.

According to Loudon (1840) seeds of this species were sent in 1823 to Europe where plants were grown in cultivation. At any rate, good colored illustrations prepared from living plants appeared in 1824 (Otto) and 1825 (Sims; Sweet). These, as well as Jussieu's drawings, definitely indicate that all of the plants referred to were of the type to which the name *Petunia axillaris* is to be applied. Also it is certain that the plants discussed and illustrated in various figures in the present paper under the name *Petunia axillaris* are all of this same type and species.

Recent Introduction and Recognition of Petunia parodii. In 1931 Steere applied the name *Petunia parodii* to plants grown from seeds obtained

from Professor P. Parodi in Argentina. Steere recognized that these were different from plants of *P. axillaris*. Most noticeable of the differences in the character of the flowers are (a) a long slender corolla tube of nearly uniform width, (b) smaller anthers and stigma, and (c) the more slender filaments of the stamens of which four are equal in length. It does not appear that this particular type of flower and plant had previously been recognized, especially by Miers (1846) and Fries (1911). Miers described, under the name of *Petunia propinqua*, plants which he noted were somewhat different in stature and in leaves from plants of his *P. nyctaginiflora*, and he states that they were "probably a variety."

It seems probable, if not certain, that the type of *Petunia parodii* was not included among the large white-flowered petunias that were introduced into Europe and America for culture previous to 1924. In that year Professor Margaret Ferguson grew at Wellesley College plants of this type from seeds obtained from Professor Parodi under the name "*Petunia nyctaginiflora*." In reports by Professor Ferguson (1928) and her associates (Brooks, Walsh & Ferguson 1930) plants of this stock were called *P. nyctaginiflora*, but later (Ferguson & Ottley 1932) they were given the name *P. axillaris*. But the excellent drawings that were published (Ferguson & Ottley 1932, *pl. 33*) definitely show the long slender corolla-tube and the four equal stamens that are characteristic and distinctive of *P. parodii* as described by Steere. Also it is stated (1932, page 39) that the flowers have a "long cylindrical tube which widens scarcely at all before reaching the throat."

Mather (1944), at the John Innes Horticultural Institution in England, obtained seed of this type of *Petunia* from Professor Ferguson. He states that the plants which were grown from this seed and studied by him fully conform to the descriptions given by Ferguson and Ottley. Mather continued to apply the name *P. axillaris* in reports of research with this type. It is evident that the reports which these authors have made under the name *P. axillaris* refer instead to *P. parodii*.

It may be noted here that the reports by Ferguson and associates and also those by Mather are definite that the plants of this type which they studied were "self-fertile." The writer has found this to be the condition for all plants which he has studied of *P. parodii* while all plants of the true *P. axillaris* grown at the N. Y. Botanical Garden, except one mutant, have been self-incompatible and also involved in intra-genotypic cross-incompatibilities.

The Nomenclature, Character, and Status of Petunia integrifolia. *Nomenclature.* Plants which had small, "violet"-colored flowers and which were otherwise very different from *Petunia axillaris*, then well known in England, were described and illustrated by Hooker in 1831 under the name *Salpiglossis integrifolia*. The plants of this first description were grown at the Glasgow Botanic Garden of seeds obtained from a Mr. John Tweedie, a resident in Buenos Aires.

Plants of this same type were also described and illustrated in a colored plate by Don in 1833 under the name *Nierembergia phoenicea*. Later in the

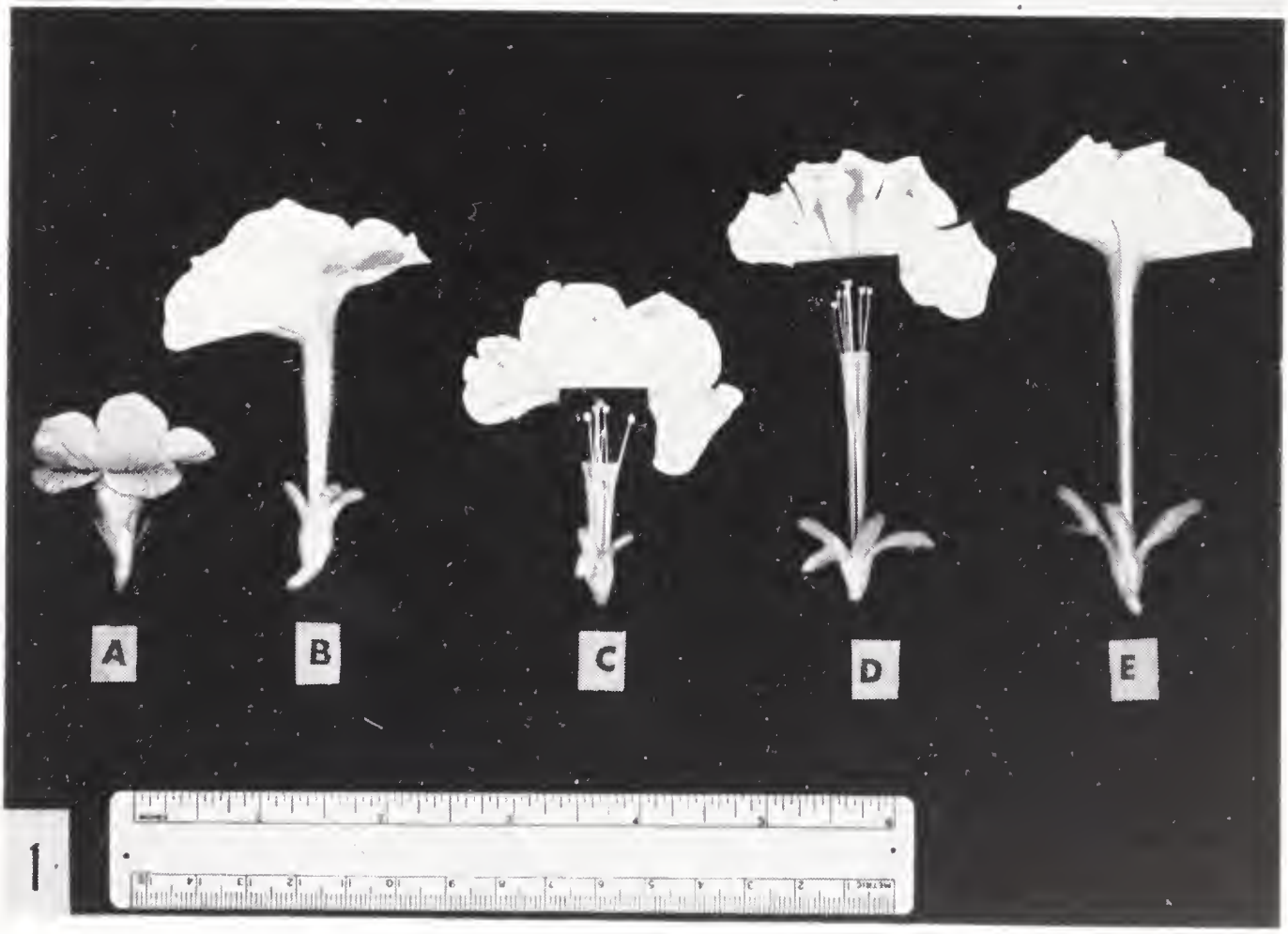


FIG. 1. Flowers of (A) *Petunia integrifolia*; (B and C) *P. axillaris*; and (D and E) of *P. parodii*.

FIG. 2. Young plants of *Petunia parodii*, showing different habits of branching.

same year Lindley published a description with a colored plate of a plant of this same type under the name *Petunia violacea*. In his synonymy, Lindley made an error in citing the publication of the name "*Petunia violacea*" in the description by Don, for there is no mention of this name in this description.

In the synonymy given by Fries, references are also given for the use of

the combination *Petunia violacea*. But in 1915 Schinz and Thellung noted that the correct epithet for this species, according to the present International Rules of Nomenclature, must be *Petunia integrifolia*. The correct citations for this name and for the most important synonyms in the order of the publications are the following:

Petunia integrifolia (Hook.) Schinz & Thellung, Nat. Ges. Zurich, **60**: 361. 1915.

Salpiglossis integrifolia Hooker, Bot. Mag. **58**: *pl. 3113*. 1831. [an Lodd. Bot. Cab. *pl. 1978*. 1833?]

Nierembergia phoenicia, G. Don. in Sweet Brit. Flow. Gard. II **2**: *pl. 193*. 1833–4.

Petunia violacea Lindley, Bot. Reg. **19**: *pl. 1626*, 1833; and Fries, Kgh. Svensk. Vet. Akad. Handl. **46** [5]: 31, 1911.

Nicotiana integrifolia, O. Kuntze, Rev. Gen. Pl. **3** [2]: 223. 1898.

In the synonymy given by Fries, references are also given for the use of the names *Stimoryne purpurea, Nierembergia punicea,* and *Petunia dichotoma*.

Specificity and Variability of P. integrifolia. Fries (1911) recognized that there are several species of a "Violacea group" that are collectively so distinct from *P. axillaris*, which he grouped with *P. pygmaea* in the subgenus *Pseudonicotiana*, that they constitute a different natural subgenus (*Eupetunia*). He described as new two species, *P. inflata* and *P. occidentalis,* which, in part at least, had previously been included with "*P. violacea.*" In fact, Chodat and Hassler (1904) described five forms or varieties of "*P. violacea.*" Fries himself recognized one variety ("*P. violacea depauperata*") which has more prostrate habit of growth and a shorter pedicel than has the type species. Thus it is recognized that there are variations among the wild plants that have been included in the name *P. integrifolia* or its synonyms. At least two of the taxonomic species recognized by Fries resemble the type of his "*P. violacea*" so closely that they had previously been included under this name.

Garden Hybrids. Plants of the "*Salpiglossis integrifolia*" and the "*P. violacea*" described by Hooker and by Lindley in 1831 and 1833 were introduced into garden culture in England. Plants of *P. axillaris* (Lam.) B.S.P. had already been in cultivation since 1823. Hybrids of these two types were soon obtained for culture in gardens. Colored plates of these appeared in 1835 (Don), 1936 (Paxton; Don), and 1837 (Hooker). These plates showed variations in the coloring of flowers of hybrids including shades of purple, pale pink, pink, and almost white. In 1835, the well known hybridist William Herbert (1837) grew seedlings of the hybrids of *Petunia axillaris* (his *P. nyctaginiflora*) × *P. integrifolia* (his *P. violacea*) but he did not describe the flower coloring and he did not observe segregations in the progeny even of the F_2 generation. A note in the *Gardeners' Chronicle* of

1843 (E.J.) mentions that the seedlings of the hybrid *Petunia* "Scarlet" had flowers in colors ranging from rosy-purple to white. The next year (Masters 1844) plants of a dwarf with blue flowers mottled with white were offered under the name "Punctata" at a price of 7 shillings 6 pence each. Also plants of 24 named clones were offered at prices ranging from one shilling to one shilling 6 pence each. Thus these hybrids were grown from seeds and also propagated as clonal varieties and soon there were selections of segregations in a remarkable range of new combinations and modifications, especially in the habits of growth and in the color and size of the flowers.

In the century that has elapsed since these early hybridizations there have been (a) mutations, especially in doubleness, and (b) polyploidy, both of which were factors in the origin of giant-flowered forms. There has been much selective breeding. Today there are in cultivation numerous seed-reproduced garden varieties derived from the hybrids of *P. axillaris* × *P. integrifolia* which present a remarkable range in habits of growth, in size and form of flowers, and in flower coloring.

Pure strains of *Petunia integrifolia* and of *P. axillaris* were apparently not maintained in Europe. This is not surprising, for both of the parent species were far surpassed in beauty and in garden value by many of the hybrids. But various investigators continue even to the present time to apply the name *P. violacea* to hybrids that somewhat resemble the "*P. violacea*" in flower coloring. Ferguson and Ottley (1932) note that the name *P. violacea* has been applied by horticulturists to small-flowered colored petunias of hybrid origin and also that "recent students of the genus have followed their lead and called the garden hybrids on which they have based their studies either *P. violacea* Lindl. or a variety of *Petunia violacea*." To a lesser degree the same situation exists in the application of the name *P. axillaris*, or of the synonyms, to white-flowered segregates in garden culture.

The Kew Clone of Petunia integrifolia. There has been a recent re-introduction of what appears to be the true *Petunia integrifolia*. In 1916 seeds of wild plants of this species were sent by Hon. C. E. R. Rowland, vice-consul at Montevideo, Uruguay to the Royal Botanic Garden at Kew, England (Ferguson and Ottley 1932). In 1930 at Kew the writer saw ramets of one clone evidently derived from one plant of this stock that had been propagated since 1916. The gardener in charge stated that the plants of this clone had flowered profusely at Kew but had never produced capsules and seeds. Professor Margaret Ferguson was also at Kew during 1930 and she obtained cuttings which were propagated at Wellesley College in Massachusetts. Excellent pen drawings of the foliage, flowers, and flower parts of plants of the Kew clone, and also of *P. parodii* (called *P. axillaris*) are presented in plate 33 of a report by Ferguson and Ottley (1932).

Comparative Specificity in the Features of Sexuality in these Species. *Structure and Character of Flowers.* The flowers of each of these three species are hermaphrodite and homomorphic. There is no appreciable

dichogamy except that secretion appears on the stigma before pollen is shed. The plants of each species are notably homogeneous in the structure of their flowers. For the populations of *P. axillaris* and of *P. parodii* and for the ramets of the Kew clone of *P. integrifolia* there are no intra-specific differentiations either in flower structure or in the relative developments of pistil and stamens that restrict self- and cross-pollination and that favor or compel inter-genotypic pollination within the population of each species.

But there are definitely different specificities in the character of the flowers of the three species (see figure 1). The flowers of *P. integrifolia* are much smaller and the pistils and the stamens are much shorter and much smaller than those of *P. axillaris* and *P. parodii*. The stamens are more unequal in length and the two longest stand immediately over and above the stigma in a position that promotes and insures autonomous self-pollination. The anthers and pollen are blue-violet in color. The throat of the tube is much broader. The entire flower is more zygomorphic.

Both *P. axillaris* and *P. parodii* have flowers that are very different from *P. integrifolia*. The flowers are much larger in respect to the corolla, the pistils, and the stamens; the longest stamens usually stand slightly below the stigma. The anthers and pollen are pale yellow. The flowers are less zygomorphic. *P. axillaris* and *P. parodii* are closely related—but definitely distinct. The flowers of *P. parodii* have one short stamen and four long stamens of equal length, a feature apparently not known in any other species of *Petunia*. These features of specificity are to be considered in evaluating the relations of hybridizations and the features of fertility and sterility in hybrid progenies.

Fries (1911) places "*Petunia violacea*" (= *P. integrifolia*) in the subgenus *Eupetunia*, which is a large group of 21 species, all of which have shades of violet-red in the flowers. *P. axillaris* and *P. pygmaea*, which differ greatly in habit of growth and size of flowers, constitute the subgenus *Pseudonicotiana*, to which *P. parodii*, which Fries did not then know, is to be added as closely related to *P. axillaris*.

In their habits of growth, plants of *P. axillaris* and *P. parodii* are noticeably similar and distinctly different from *P. integrifolia*. Both are more robust, the branches are coarser and more upstanding, and the pedicels remain upright or nearly so after the capsules mature. The more slender branches of plants of *P. integrifolia* are often somewhat decumbent; the pedicels become deflexed soon after the petals fall, but this feature is not present in the type known as *P. inflata*.

Size of Pollen. According to Ferguson and Coolidge (1832) the pollen of *P. integrifolia* (called *P. violacea*) is larger than the pollen of *P. parodii* (called *P. axillaris*).

Seeds. The examination and evaluation of the seeds of the three species (*P. parodii*, *P. integrifolia*, and the diploid *P. axillaris*) give the following information. The normal mature seeds of *P. integrifolia* are globose or nearly spherical. The shape of the seeds of *P. axillaris* and *P. parodii* is nearly al-

ways somewhat wedge-shaped with one or more flattened sides due to close contact with each other on the placenta (figure 41, A and C).

The surface of the seeds of all three species is reticulated, owing to ridges in the testa. The ridges join and surround sunken areas or facets that are polygons of as many as seven sides. About the point of attachment of seeds these areas are rather irregular in shape and usually elongated, but elsewhere they are more symmetrical.

Under microscopic examination the ridges on seeds of *P. integrifolia* are almost even in height and have smooth sides, while those of *P. axillaris* and *P. parodii* are serrated, with the crest of the ridge in a zigzag line along which a slender line of darker and more intense brownish red is conspicuous. In size the seeds of *P. axillaris* ranged in greatest diameter from 0.80 mm. to 0.50 mm.; those of *P. parodii,* from 0.71 mm. to 0.50 mm.; those of *U. integrifolia,* from 0.94 mm. to 0.52 mm. But since the seeds of *P. integrifolia* are more spherical their volume as well as their greatest diameter is greater, as is well shown in figure 41. The normal seeds of all three species contain considerable endosperm in which a well-formed embryo is embedded. Lindley (1833) states that the embryos in seeds of *P. axillaris* (called *P. nyctaginiflora*) are curved or twisted while those of *P. integrifolia* (called *P. violacea*) are straight. We find both curved and straight embryos in the seeds of each of these three species.

It may be reported here that the seeds of *P. parviflora* have reticulations like those of *P. integrifolia* but the size in greatest diameter ranged from 0.53 mm. to 0.42 mm.

Chromosome Numbers. Ferguson (1928) reported that the somatic or *2n* number of chromosomes in what she then called "*P. nyctaginiflora,*" but which was without doubt *P. parodii,* was *14* (*7+7*). In 1932, Ferguson and Coolidge reported that "the true breeding species *Petunia violacea* Lindl." had a somatic number of *14* chromosomes. Kostoff et al. (1935) reported a somatic number of *14* in plants which were called *P. axillaris,* but since these plants were grown from seeds obtained from Dr. Margaret Ferguson they were probably *P. parodii.* Kostoff et al. report that seedlings grown from seeds of the Kew clone of *P. integrifolia* also had a somatic number of *14.* It may be noted that these particular seeds were some of those obtained by the writer by premature pollination of ramets of the Kew clone and *not* from plants raised from seed as stated by Kostoff. In the original description of *P. parodii,* Steere (1931) states that the plants which he described had *14* chromosomes.

In the studies here reported the chromosome number has been determined for individuals of *P. axillaris, P. parodii,* for ramets of the Kew clone and for various of its seedling progeny. In all these the somatic number was *14* (Sullivan 1947).

In chromosome number the three species under consideration are alike with a somatic number of *14* (*7+7*). It may be noted that plants of the

species *P. parviflora* are reported to have *18* ($n = 9$) for the somatic number of chromosomes (Ferguson & Coolidge 1932).

Comparison of Intraspecific Reproduction in the Three Species. *Potential Fertility.* The flowers of each species are bisexual and homomorphic. The plants have numerous flowers all of which have high, if not complete, potential fertility for reproduction within the species. There are marked specificities in the parts of the flowers as to size and position of stamens, size and color of anthers and pollen, and in the more hidden physiological properties of pistils, ovules, and pollen tubes. These specificities are involved in interspecific hybridizations which will be reported and discussed later.

Two readily observed differential features of potential fertility are (a) the size of the capsules and (b) the number of the seeds which they contain. The capsules of *P. integrifolia* are smaller and the number of seeds per capsule is much lower than in either of the other two species, and these differences are to be considered especially in evaluating the potential fertility of hybrids.

Intraspecific Incompatibilities. Data will be presented which show that all the plants of *P. axillaris* of diploid ($2n = 14$) constitution which were studied at the N. Y. Botanical Garden, except aberrant individuals, had self- and cross-incompatibilities which enforce bigenotypic reproduction, but that all members of *P. parodii* had no intraspecific incompatibilities. Thus there was a very distinctive difference between the cultures of these two more closely related species in this important feature of intraspecific reproduction.

The cultures of the two very different species, *P. axillaris* and *P. integrifolia*, possessed hereditary self- and cross-incompatibilities. Hence they were similar in this limitation of intraspecific reproduction. But the cultures of the two species possessed entirely different hereditary factors (which will be called *S* factors) for the incompatibilities.

Interspecific Relations. It will be reported in this publication that the specificities of these three species also involve physiological properties that are expressed in unilateral or one-way hybridizations. Data on the genetics of these relative specificities and on their role in the fertilities and sterilities of hybrid progenies will be presented and evaluated.

Petunias of Wild Species Grown by the W. Atlee Burpee Company. *Cultures of Presumably P. inflata Derivation.* In the annual catalog for 1936 of the W. Atlee Burpee Company there were listed seeds of *P. inflata* (2483), which was described as having small flowers of purplish carmine coloring, and seeds of *P. inflata* Sapphire (2484) which had small flowers of sapphire blue coloring. In the catalog for 1937 there was added the seed (2468) of a *Petunia* called " Phoenicea " which had bright rosy purple flowers about two inches across. All these were discontinued in the catalog of 1943 but certain seedling cultures were continued in the experimental plots. In 1950, the writer evaluated four such cultures. All had small flowers of a size and a shape that corresponded closely to the flowers of the Kew clone of *P. integri-*

folia (figures 1 and 14). But all the stamens were shorter than the pistil, the pollen was not blue, the pedicels did not become deflexed, and the stems were much thicker and more erect. In the field notes these were called "primrose flowering."

It may be stated here that in none of the more than 800 cultures of *Petunia* that were grown at Floradale Farms in 1950 did a plant have deflexed pedicels. This feature of *P. integrifolia* (figure 16) was absent in the entire group of horticultural varieties as well as in the progenies of breeding of which many had strong anthocyanin coloring. This feature of deflexed pedicels is recessive in F_1 progenies of *P. axillaris* × *P. integrifolia* and it is reported to be absent in *P. inflata* (Fries 1911). Possibly this character is the chief feature of differentiation between *P. inflata* and *P. integrifolia*.

In 1941, the W. Atlee Burpee Company grew two cultures, that were evidently *P. inflata*, of seeds collected from wild plants in Argentina and distributed by the University of California Botanical Garden (U.C.B.G. Nos. 40–507 and 515). In 1942 a further progeny of one of these was grown and an excellent photograph was obtained. This, together with the field notes, indicates that the leaves, stems, flowers, calyx lobes, and capsules of these plants were like those of the Kew clone of *P. integrifolia* but that the pedicels did not become deflexed. During 1951, 19 plants were grown of seeds labeled *Petunia inflata* that came from the Instituto de Fitotécnica, Quinta Agronómica, at Tucumán, Argentina. These plants had no specific character that distinguished them from the Kew clone of *P. integrifolia* except that the pedicels did not become deflexed. Tests of 15 of these plants revealed that eleven were completely self-incompatible and three were feebly self-fertile (pseudofertility). Ten were crossed in one or more relations, all of which gave fine capsules and numerous seeds.

Cultures of White-Flowered Petunias. In 1941 and 1942 the W. Atlee Burpee Company grew white-flowered plants of eight different collections of seeds from wild plants in Argentina that were distributed by the University of California Botanical Garden. In 1942, excellent photographs were obtained of the flowers, branches, and leaves of representative members of seven of these lines. These together with the notes of descriptions indicate that most members of six lines may be identified as *P. axillaris*, with, however, minor variations in size of flowers and the character of the coloring in the throat of the flowers. Two other lines were definitely *P. parodii* in respect to the long slender tube of the flowers; but there are no data on the lengths of the stamens.

One line of the type of *P. axillaris* was continued in 1946 and in 1950. Only four plants were grown in 1950. These had a habit of growth and leaves, stems, flowers, and capsules that were almost identical in character with the cultures of *P. axillaris* that were grown at The New York Botanical Garden. But there was one difference in that each plant was self-fertile and all were reciprocally cross-fertile. There were no self- and cross-incompatibilities in

these plants unless they were hidden. The field notes reveal that in the second generation of this line grown in 1942 there were two divergent members that had long flower tubes.

Another line was grown in 1946 and 1950. All six members grown in 1950 had long slender flower tubes, relatively slender branches, and long calyx lobes but the anthers were of three lengths as is typical for *P. axillaris*. All members were self- and cross-fertile. The records of the University of California Botanical Garden reveal that the original collection of seed (U.C.B.G. 40–516) from which this line arose was received via Dr. Horacio Descole, Instituto Miguel Lillo, Tucumán, Argentina.

Data from the Herbarium and the Botanical Garden of the University of California. Several of the specimen sheets in this herbarium, of collections in Argentina, appear to conform closely to the true *P. axillaris* grown at the N. Y. Botanical Garden. Four of these (23198, 23201, 23428, and 23687) were collected in the Province of Buenos Aires and the notes indicate that the type of *Petunia axillaris* is common in this Province.

Four other specimens had flowers with tubes that were conspicuously long and slender (two or more inches in length) and otherwise similar to plants of *P. parodii* grown at The N. Y. Botanical Garden, but no data were recorded on the lengths of the stamens. Three of these specimens were collected in the Providence of Jujuy. These four specimens have been identified by Dr. Ivan M. Johnston as *Petunia axillaris* var. *leptantha* with no reference to the true *P. parodii* which had already been named and described by Steere. The writer has thus far failed to locate any publication of this varietal name.

It may be noted that one specimen (548687),which had been collected in the Province of Mendoza and identified as *P. axillaris*, had flowers that were pale lilac streaked with purple and that the corolla tube was slender and about two inches in length. Another specimen (22441) from the Province of Jujuy which had small purple flowers but a habit "very like *P. axillaris*" was identified as *Petunia occidentalis*.

At the Botanical Garden of the University of California the writer observed (1) a considerable number of plants that closely resembled the Kew clone of *P. integrifolia* except that the pedicels did not become reflexed; (2) numerous plants that may be classed as *P. axillaris;* (3) some plants that were like *P. axillaris* except that the flowers were noticeably tinged with rose-purple; and (4) a number of plants whose flowers were like those of *P. parodii* except that the stamens were of three lengths.

Concluding Remarks. From the information noted above it appears that the true *Petunia axillaris* has wide distribution in certain areas of Argentina. But there are data on self-fertility for only four plants of one culture of these particular plants. There are also wild plants that conform closely to *P. parodii*. But the six members of one derived culture (Burpee 50 No. 600) and all plants of this type that were growing in the U. C. Botanical Garden in 1950 had stamens of three lengths and hence were not typical of *P. parodii*. It is

possible and probable that there has been natural hybridization in Argentina between the two types, *P. axillaris* and *P. parodii*, and that this may have given rise to genotypes and races that have long slender flower tubes and three lengths of stamens.

The experimental studies reported in the following chapters of this monograph involve pure-breeding lines of the two distinctly different types designated as *P. axillaris* and *P. parodii*. There may be some questions regarding the abundance and distribution of these two types of *Petunia* in South America and also as to the extent to which the members of *P. axillaris* have intraspecific self- and cross-incompatibilities.

Chapter 2. Intraspecific Reproduction in *Petunia parodii*

Source of Material. One collection of seeds of *Petunia* was supplied to the writer in 1937, two in 1939, and one in 1942 by Professor Lorenzo Parodi. The seeds received in 1937 were collected from plants that grew in the Jardín Botanico of the University of Buenos Aires. Fourteen plants grown from the seeds obtained in 1937 were self-fertile and definitely *Petunia parodii* and one other was self-incompatible and typical of *P. axillaris*. Of the two collections received in 1939, one (series 140 in figure 3), from the wild in the province of Buenos Aires, gave 17 plants of *P. parodii* of which 10 were studied. The other collection (series 139), from the Jardín Botanico in the city of Buenos Aires, gave 29 plants of *P. axillaris*, 11 of *P. parodii*, and two that appeared to be hybrids of these two species. The collection of 1942 was obtained in the "campaña" (level country) near Buenos Aires and all of the 17 plants which were grown were *P. parodii*, but in comparison to the plants from the seeds of 1939 they had somewhat smaller flowers and the foliage was paler green. The plants grown from these seeds will be called the *parent generation*.

Counts of chromosomes during early stages of meiosis were made for several of the plants of *Petunia parodii* and in all cases the somatic number was *14* ($2n = 7 + 7$).

Data for the Intraspecific Reproduction of Petunia parodii. *The Parent Generation: Series 139 and 140.* Figure 3 presents the results of experimental tests by controlled pollinations of 8 plants (of the total of 11) grown from seed collected in the Jardín Botanico and of 10 plants (series 140) grown from seed obtained in the province of Buenos Aires. Each plant was fully self-fertile to self-pollinations. All combinations of cross-relations that were tested by controlled pollinations were fully cross-fertile, and these tests included nearly all the possible cross-relations between all the members of each of the two groups.

Data for a Pedigreed First Generation. Selfed progenies were grown of 3 plants of series 139 and of one plant of series 140, and for these the data are as follows:

Of plant 140–10, the 14 individuals of a selfed progeny were all self-fertile and the 212 cross-relations which were tested were fully fertile. Of plant 139–8, the 37 plants of a progeny were all fertile and all the 119 cross-relations which were tested were fertile. Twenty-seven members of a selfed progeny of plant 139–28 were self-fertile and the 51 cross-relations tested were fertile. Of a progeny of plant 139–42 twenty-five plants were self-fertile and the 62 cross-relations that were tested were fertile.

		3	8	15	20	26	28	33	42	5	6	8	9	10	11	12	14	15	16	P.i.	P.a.
139	3	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	H
	8	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	H
	15	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	H
	20	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	H
	26	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	H
	28	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	H
	33	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	H
	42	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	H
140	5	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	
	6	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	
	8	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F		
	9	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	
	10	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F		
	11	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	
	12	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	
	14	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	H	
	15	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F		
	16	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F		
P. integrifolia		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O		
P. axillaris		O	O	O	O	O	O	O	O												

FIG. 3. Chart indicating reactions of the parent generation of *Petunia parodii*. Symbols: H refers to unilateral hybridization; O to unilateral sterility; F to intraspecific reproduction.

The Second Generation. In two progenies of a second generation of controlled selfing there were 51 plants all of which were self-fertile.

The Third Generation. In 1947, eleven plants were grown from selfed seed of a plant of the second generation for special use as testers. Each was self-fertile. Another series (No. 346) was grown for a special study of the heredity of habits of growth and of these the 25 plants that were tested were self-fertile.

The Progenies of the 1942 Collection. The 17 members of the parent generation were all self-fertile and the 10 members of a selfed first generation were also self-fertile.

Summary. The parent progenies of *P. parodii* comprised 14, 11, and 17 plants all of which were self-fertile. There was a total of 103 plants of the first generation of selfed seedlings, 51 of the second generation, and 36 of the third generation which were tested and found self-fertile. More than 500 cross-relations were tested, mostly of intra-series relations but also of relations of seedlings with a parent and of the relations of seedlings of different parents. Every one of these cross-relations was fertile. Most frequently the capsules had from 500 to 1,000 seeds each. The highest number counted was 1,220.

Character of these Progenies of P. parodii. Without exception all plants of these cultures of *P. parodii* were distinct from those of *P. axillaris* in both flowers and foliage. There were slight variations in the size of open flowers and in the length of the corolla tube but remarkable uniformity in its slender form, in the equal length of four of the stamens, and in the more slender filaments and smaller anthers, all of which were sharply differential features (see figure 1) in comparison with flowers of *P. axillaris*. Also the lobes of the calyx were more elongated and leafy in *P. parodii*.

Seedling plants of *P. parodii* developed more rapidly than did plants of *P. axillaris*. They reached flowering maturity, completed their flowering and died much sooner, especially as grown in the greenhouse for the experimental studies. A larger proportion of them died from attacks of a root-rot fungus. Propagation by cuttings was more difficult. The plants had more slender branches and the leaves were smaller.

Individual differences among the seedlings were noticeable in habits of growth (see figure 2) and comparable differences were also observed in seedlings of both *P. axillaris* and *P. integrifolia*. In one extreme type of growth the main stem of a seedling made good and often vigorous axial growth while the formation or the growth of lateral branches was inhibited. At the other extreme the elongation of the main stem was inhibited but there was vigorous growth of laterals from the axils of leaves of the rosette. There were also cases of both axial and lateral growth, and also variations in the number and vigor of laterals. These differences were noted among the members of the parent generations and also among the members of most series of the first and the second generations which were all of selfed seeds. One series of the third generation was derived from the selfed seeds of a plant that had both good axial growth and several well-developed laterals. Every one of the 75 members of this progeny had strong axial growth and well-developed branches along the axis below the first flower. This result indicates that there is probably a genetic basis for the different habits of growth observed in the cultures of all three of the species of *Petunia* that were studied.

There were no noticeable reductions in vigor of growth or of fertility in any of the selfed progenies of *P. parodii* that could be attributed to the degenerative effects of self-reproduction and inbreeding.

Evaluation of the Intraspecific Reproduction in Petunia parodii. *Po-*

tential Fertility. The potential fertility of plants of *P. parodii* is to be evaluated by the number of flowers on a plant, the character of the pollen and ovules, the number of seeds per capsule, and the ability with which seeds are produced in any relation. The potential fertility for all flowers of all plants of *P. parodii* was high, as it was also in *P. axillaris.*

Complete Intraspecific Reproduction. In the plants of *P. parodii* which were studied the actual fertility in terms of seed production was close to the maximum potential fertility. There was frequently effective autonomous self-pollination at the time when the stigma was ready to receive pollen, and there were no self- or cross-incompatibilities to limit or restrict the favorable action of any pollen that was brought from other plants of this species. Relatively few flowers failed to produce capsules and seeds even when enclosed in the wire cages employed in the experimental work and left to autonomous pollination. Of the controlled hand-pollinations of both self- and intraspecific cross-relations very few failures of individual flowers occurred. It appears that the chief cause of failure in these tests was that open flowers were sometimes "flooded" with water when plants were watered.

The evidence seems fully adequate and conclusive that there were no self- or cross-incompatibilities among the plants of *P. parodii* obtained in these studies. In full agreement are the reports by Ferguson and Ottley (1932) and by Mather (1944) for the plants of their studies which they called *P. axillaris* but which were evidently of the species *P. parodii.*

Genetical Constitution. On the basis of the results reported above it appears that complete and unrestricted intraspecific reproduction is possible in the species *Petunia parodii.* There was a remarkable homozygosity for all characters and qualities of both the male and the female structures and elements of sexual differentiation. There was no selective functioning of either the male or the female structures, including (a) stamens and pollen and (b) pistils and ovules, which effected any limitation of reproduction. Thus the genetic constitution in respect to the factors of intraspecific reproduction and fertility of such a diploid ($2n = 14$) population of *P. parodii* can be represented by the formula PP. Since special accessory hereditary "*S*" factors which effect self- and cross-incompatibility were absent in these plants the genetic constitution for reproduction can be represented, for comparison with such a species as *P. axillaris*, as PP *S 0.0.*

Chapter 3. Intraspecific Reproduction in *Petunia axillaris*

Data for the Parent Generation: Series 139. *Material and Methods.* Of the plants of this series grown from seeds collected in the Botanical Garden in Buenos Aires, 29 were definitely diploid *Petunia axillaris* (others were *P. parodii*).

The reactions to self-pollination in these plants were tested by controlled normal self-pollination. Branches with flowers were enclosed in glassine

paper bags or an entire plant was enclosed in a fine-mesh, wire cage constructed as a cylinder that was closed at one end (figure 34). At the time of normal anthesis the anthers of the flowers of *P. axillaris* are not dehisced but usually there is some secretion on the stigma. Soon the secretion increases in quantity and then the anthers of the flower dehisce. But the anthers stand somewhat below the stigma in a position that does not promote autonomous selfing. Hence all the self-pollinations of these tests were made by hand at a time when secretion was visible on the stigma and while pollen was abundant and in good condition.

The cross-relations between individual plants were tested by controlled pollinations. The flowers were emasculated in the bud shortly before anthesis and later when there was abundant secretion on the stigma the fresh pollen for the desired test was obtained directly from a flower and used in a fully controlled pollination. It was found that cross-relations, both fertile and incompatible, can be determined accurately for plants of *2n P. axillaris* without the emasculation of the flowers of the self-incompatible plant that is used as a female parent. In the tests of later progenies of *2n P. axillaris* the plants were enclosed in a cage, there was a normal anthesis of the flowers, and the stamens were removed at the time when the controlled cross-pollinations were made. Any stray self-pollinations did not influence the reactions of self-incompatibility, cross-incompatibility or cross-fertility. But in all cases there was emasculation and complete control in obtaining seeds that were used in planting for pedigreed progenies.

Self-Incompatibility: figure 4. Every one of the 29 plants of this series which were judged to be *Petunia axillaris* was self-incompatible. Preliminary tests by fully controlled cross-pollinations were made and the maximum number of individual plants of any one intra-incompatible group, or genotype, that were kept for more complete tests, was limited to three. This explains why there are no more than three plants in any of the genotypes reported in figure 4.

Cross-Incompatibility and Cross-Fertility. In the relations between these 22 self-incompatible plants there were both cross-incompatibility and cross-fertility. There were 11 mating groups or genotypes. For the members of each group there was cross-incompatibility and for members of any two groups there was cross-fertility. In each of five of these genotypes there was only one plant, owing, it is believed, to the rather limited number of plants studied.

The data obtained demonstrate two important features of intraspecific incompatibility:

a. Each self-incompatible plant was potentially able to function both as a seed parent and as a pollen parent in seed reproduction. Hence the conditions of self- and cross-unfruitfulness were relative and selective. They did not involve abortion of spores or gametes or the inability of any one or more of the essential elements of reproduction to function.

b. There was not only self-incompatibility but also cross-incompatibility. This condition was not recognized by Darwin (1876) or, at least definitely, by others of the horticulturists, botanists, and plant breeders until the reports by de Vries (1906) and especially by Correns (1912, 1913) appeared. Correns recognized that both self- and cross-incompatibilities within a species are determined by the same hereditary factors, and that self-incompatibility does not exist as a purely individual condition that is determined by an " in-

139		19	36	40	12	21	31	7	13	38	9	11	17	16	24	41	10	25	27	30	32	34	37	P. p.	P. int.
	19	S	s	s	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	0	H
S 1.2	36	s	S	s	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	0	H
	40	s	s	S	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	0	H
	12	F	F	F	S	s	s	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	0	H
S 1.3	21	F	F	F	s	S	s	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	0	H
	31	F	F	F	s	s	S	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	0	H
	7	F	F	F	F	F	F	S	s	s	F	F	F	F	F	F	F	F	F	F	F	F	F	0	H
	13	F	F	F	F	F	F	s	S	s	F	F	F	F	F	F	F	F	F	F	F	F	F	0	H
	38	F	F	F	F	F	F	s	s	S	F	F	F	F	F	F	F	F	F	F	F	F	F		H
	9	F	F	F	F	F	F	F	F	F	S	s	s	F	F	F	F	F	F	F	F	F	F	0	H
	11	F	F	F	F	F	F	F	F	F	s	S	s	F	F	F	F	F	F	F	F	F	F	0	H
	17	F	F	F	F	F	F	F	F	F	s	s	S	F	F	F	F	F	F	F	F	F	F	0	H
	16	F	F	F	F	F	F	F	F	F	F	F	F	S	s	s	F	F	F	F	F	F	F	0	H
	24	F	F	F	F	F	F	F	F	F	F	F	F	s	S	s	F	F	F	F	F	F	F	0	H
	41	F	F	F	F	F	F	F	F	F	F	F	F	s	s	S	F	F	F	F	F	F	F		H
	10	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	s	F	F	F	F	F	0	H
	25	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	s	S	F	F	F	F	F	0	H
S 4.5	27	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	F	F	F	F	0	H
S 4.6	30	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	F	F	F	0	H
	32	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	F	F		H
	34	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	F	0	H
	37	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	0	H
P. parodii.		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H		
P. integri.		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

FIG. 4. Chart indicating reactions of 22 seedlings of the parent generation of *Petunia axillaris*. The symbol S indicates self- and intragenotypic incompatibility.

dividual stuff " as had previously been believed (see especially Jost 1907). The first recognition of the diplont-haplont reaction of intraspecific self- and cross-incompatibilities was by Prell in 1921 (see discussions by Stout 1938, 1945b).

Potential Fertility and Intraspecific Reproduction. Each plant that exhibited self-incompatibility and cross-incompatibility was able to function in seed reproduction in certain relations. Thus a potential or basic fertility operated through the fertile cross-relations to provide continued reproduction of the species. It is certainly inadequate to state, as is frequently seen in

publications, that a "species is self-sterile." In such a species the members of the successive generations which comprise it have what may be called a fundamental basic or potential fertility of intraspecific reproduction. As in *P. parodii*, the plants of *P. axillaris* are remarkably uniform and homozygous for all basic features of sexual differentiation and there is the possibility of complete intraspecific reproduction except for the limitations due to the physiological action of *S* factors.

The potential fertility of the individual members of the species *P. axillaris* is very uniformly high when it is judged by the size of the capsules and the number of seeds obtained by fertile intraspecific cross-relations. Counts of seeds in typical capsules were obtained of "fertile" relations from various diploid genotypes and generations. The number ranged from 405 to as high as 813. A total of 6,051 of these seeds were placed in water and only 108 did not sink. Dissection and microscopic examination showed that the seeds which sank contained endosperm and embryo and that those which floated were more or less empty. The normal seeds of diploid plants of *P. axillaris* are somewhat spheroid and those that were measured ranged from 0.52 mm. to 0.75 mm. in greater diameter. The well-formed capsules typical of diploid plants are as much as 13.5 mm. in length and 6.0 mm. in width. There are, or course, differences in the size of capsules and in the number of seeds per capsule according to vigor and age of a plant, position of capsules on the branches of the inflorescence, and adequacy of pollinations. The number of seeds obtained in capsules of *P. axillaris* was somewhat less than in capsules of *P. parodii*.

Pollen-tube Behavior in Self-Incompatibility. This was determined for normal self-pollinations of flowers of the plant 139–12, which was a member of the genotype designated as (*S 1.3*). The original diploid plant was propagated as a clone and a suitable number of flowers was available for study over a period of one week. Controlled normal close-pollinations were made so that the time when pollen was applied was known. The pistils were collected at various intervals, split lengthwise, spread and somewhat flattened by pressure, and stained with acetocarmine. The most advanced tubes were about half-way down the style at the end of 34 hours after pollination and there was almost no further growth in length. Six days after pollination, when the style dropped at the touch, the longest tube observed had grown to a length of 29 mm. in a style that was 43 mm. in length. In these studies the pollen tubes ended at various levels in the upper portion of the styles but there was no definite zonation of the ends at two different levels which would definitely indicate a differential growth of classes (as *S 1* and *S 3*) of pollen-tubes after normal self-pollination. It will be shown later in the experimental studies that the *S 1* class of pollen functions in premature self-pollinations of the plant 139–12. But in the normal self-pollinations of this representative plant of *S 1.3* the incompatibility reactions involved a limited growth of all pollen-tubes in the tissues of the style.

The Experimental Evaluation of Intraspecific Incompatibilities. The information obtained in the tests reported in figure 4 and in similar pre-

liminary studies of other plants, does not indicate the precise type of incompatibility or the genetic constitutions involved in the control of the incompatible reactions.

For the adequate identification of the type of genetic determination and expression of intraspecific incompatibilities in any species or group of plants or animals, it is necessary to obtain and analyze what may be called pedigreed collateral progenies. This term may be applied to two or more of the progenies that can be derived in successive generations from any two cross-fertile genotypes of a population of self-incompatible plants. The number of the cross-fertile genotypic combinations, including reciprocals, for the eleven genotypes of the original population (see figure 4) is 110. To obtain and study all of these was impossible, hence the effort was mainly directed to a study of the collateral progenies of a minimum sustaining population derived from two self-incompatible but cross-fertile individuals. Also these two original parents were propagated vegetatively as clones for use as testers of the progenies.

Data for Reciprocal Collateral Progenies of a Pedigreed First Generation. *Series 161: Progeny of Genotype I × II; figure 5.* The seed parent of this series was the plant 139–40 and the pollen parent was 139–12. Each of the 58 members of this progeny was self-incompatible. Twenty were tested in most of the possible cross-relations and all were sufficiently tested to determine the genotypic constitutions. In order to reduce the size of figure 5, data are presented for only ten plants.

There were only two mating groups or genotypes. The members of one group were reciprocally cross-incompatible with their pollen parent (139–12) and hence were of the same genotype (II). The members of the other group were reciprocally cross-fertile with both parents and were hence of a different and new genotype.

Thus the progeny of I × II was composed of II and III. The genotype of the seed parent did not appear in the progeny. That this was the case could only be demonstrated and determined by the reactions with the parents which had been kept in propagation.

The Reciprocal Progeny: Series 155; II × I. The seed parent of this progeny was 139–12 and the pollen parent was 139–40. Each of the 28 plants which were grown and tested was self-incompatible. Figure 6 presents the pattern of the reactions and these data are representative of the behavior of all 28 plants. There were two mating genotypes in respect to intra-incompatibility and inter-fertility. One group was reciprocally incompatible with the pollen parent and hence its members were of the same genotype (1). The other was reciprocally cross-fertile with both parents.

Explanation of figures 5 and 6

Fig. 5. This chart indicates the reactions of five representative members of each of the two mating groups in the progeny of 139–40 (*S. 1.2*) × 139–12 (*S. 1.3*). Fig. 6. A chart indicating the reactions of representative members of the two genotypes in the progeny of 139–12 (II, *S 1.3*) × 139–40 (I, *S 1.2*).

161		24	25	27	28	34	7	22	31	33	36	139-40	" -12
S1.3 (II)	24	S	S	S	S	S	F	F	F	F	F	F	S
	25	S	S	S	S	S		F	F	F	F	F	S
	27	S	S	S	S	S		F	F	F	F	F	S
	28	S	S	S	S	S		F	F	F	F	F	S
	34	S	S	S	S	S		F	F	F	F	F	S
S2.3 (III)	7	F					S	S		S		F	F
	22	F	F	F	F	F	S	S	S	S	S	F	F
	31	F	F	F	F	F	S	S	S	S	S	F	F
	33	F	F	F	F	F	S	S	S	S	S	F	F
	36	F	F	F	F	F	S	S	S	S	S	F	F
139-40, S1.2		F	F						F	F	F		
" 12, S1.3		S	S						F	F			

5

155		5	7	9	15	21	2	8	12	18	23	Testers. 139-12	" -40	(III) 161
I (S1.2)	5	S	S	S	S	S	F	F		F	F	F	S	
	7	S	S	S	S	S	F	F		F	F	F	S	
	9	S	S	S	S	S		F		F	F	F	S	F
	15	S	S	S	S	S		F	F		F	F	S	
	21	S	S	S	S	S				F	F	F	S	
III (S2.3)	2	F				F	S	S		S	S	F	F	
	8	F				F	S	S		S	S	F	F	
	12	F			F	F	S	S	S	S	S	F	F	S
	18		F	F	F	F	S	S	S	S	S	F	F	
	23	F				F	S	S			S	F	F	
Testers 139-12 (II)		← F →					← F →							
" -40 (I)		← S →					← F →							
161 (III)		← F →					← S →							

6

The two groups, the one of 161 and the one of 155, which were reciprocally cross-fertile with both parents were tested in as many as ten of each of their reciprocal relations. There was complete incompatibility. Hence both groups were of the same genotype and the formula for series 155 is II × I = I and III.

Evaluation of the Reactions and the Genetic Constitutions. In each of the two reciprocal progenies only two mating groups appeared; one was the same as that of the pollen parent; the other was a *new genotype;* the genotype of the seed parent did not appear in either progeny. The new genotypes of the two series were the same in constitution. All these conditions conform to the assumptions first postulated by Prell (1921) for the relations between two self-incompatible heterozygous plants that possess one oppositional *S* factor in common. In the application to the two plants of *Petunia axillaris* which were the parents of series 161 and 155 the assignment of incompatibility factors may be *S 1.2* for the plant 139–40 and *S 1.3* for the plant 139–12. In the relation of *S 1.2* × *S 1.3* the segregation of *S* factors in the pollen parent gave two classes of pollen, one of *S 1* and one of *S 3*. The haploid pollen tubes which carried the common factor *S 1* were inhibited in the pistil whose diploid cells possessed *S 1*. But the pollen tubes which carried *S 3* functioned and there was full production of seeds. The cross-fertile reaction was selective for pollen of the *S 3* class and there was *hidden incompatibility* for all pollen of the *S 1* class and this resulted in the *elimination* of the genotype of the seed parent from the progeny. The same conditions operated in the reactions that were involved in the reciprocal progeny. All the tests indicated (a) that the two parents, and the two genotypes which they represented, differed in only one *S* factor, (b) that the genotype of the seed parent was eliminated in the immediate progeny, and (c) that the same new genotype appeared in each of the two reciprocal collateral progenies of the first generation. These conditions and reactions may be represented as follows: (I) *S 1.2* × (II) *S 1.3* = (II) *S 1.3* and (III) *S 2.3;* and (II) *S 1.3* × (I) *S 1.2* = (I) *S 1.2* and (III) *S 2.3*.

If the assignments of genetic constitutions given above are correct each of the three genotypes obtained in the first generation of the reciprocal progenies 161 and 155 was heterozygous and collectively they included only the three *S* factors which were present in the two parents. Also the population of the next generation which could be derived from all possible cross-relations between the three genotypes would include six different collateral progenies of which four would be additional to the two reciprocal progenies of the previous generation.

Data for Three Other Collateral Progenies. *Data and Evaluations.* Seeds of each of the fertile relations between the three genotypes were obtained in abundance from the test pollinations that were made for members of series 161 and 155. Also the two parents and several individuals of each of the four groups in these progenies were propagated for use as testers.

Three progenies were grown for the analysis of the offspring of *S 2.3* and the parentage and numbers of these were as follows:

Series 250, *S 1.3* × *S 2.3*, 19 plants;
" 251, *S 2.3* × *S 1.3*, 23 plants;
" 253, *S 1.2* × *S 2.3*, 22 plants.

Except for the pollen parent of series 251, which was 139–12, all the parents were segregates of the series 155 and 161.

Each of the 64 plants in these progenies was self-incompatible. Every plant was adequately tested for the determination of its genotypic constitution. Each progeny was composed of only two mating groups, one group was cross-incompatible only with the pollen parent, and the other was cross-fertile with both parents. Thus the patterns of the intra-series relations of all of these three progenies and of series 155 and 161 were exactly alike. But the reactions with the parents and with testers known to be *S 1.2*, *S 1.3*, and *S 2.3* proved that the formulae for these three series were as follows:

Series 250, *S 1.3* × *S 2.3* = *S 1.2* and *S 2.3*;
Series 251, *S 2.3* × *S 1.3* = *S 1.2* and *S 1.3*; and
Series 253, *S 1.2* × *S 2.3* = *S 1.3* and *S 2.3*.

When the plants of series 253 were in bloom, homozygous plants of *S 1.1* were available for use as testers. Also some members of each of the mating groups in all series which were grown were propagated and later these were tested in relations with *S 1.1* and *S 3.3*. Without exception all reactions confirmed the analyses given above and were as indicated in figure 11.

Progenies of Premature Selfing. *Premature Pollinations.* A diploid plant of any of the genotypes *S 1.2*, *S 1.3*, or *S 2.3* was completely self-incompatible when normal self- or close-pollinations were made either autonomously or by hand. But some capsules and viable seeds were obtained when pistils of plants of all three of these genotypes were close-pollinated before anthesis at a time when the pistils were not mature and before there was visible secretion on the stigma. Many of these pollinations failed to induce any development of capsules and the capsules that were obtained were smaller than those obtained on the same plant in fertile cross-relations. Also the number of seeds obtained per capsule was definitely below that of the potential fertility. The highest number obtained of any premature pollination of any plant of *2n P. axillaris* was 256, but often the number was less than 100.

Data for the Progenies of Selfing. Four of these progenies were grown and tested. One, of 17 members, was a progeny of a plant of *S 1.2*; two, of 22 and 18 members, were progenies of two different plants of *S 1.3*; and one, of 15 plants, was a progeny of a plant of *S 2.3*.

The patterns of the intra-relations in all these four progenies were alike but different from the pattern of any of the collateral progenies arising from

the crossing of *S 1.2*, *S 1.3* and *S 2.3*. There were only two intra-incompatible groups in each progeny but in their reciprocal cross-relations there was one-way fertility and one-way incompatibility (figures 7 and 8).

Each one of the 72 members of these four progenies was self-incompatible. The members of each progeny were very fully tested for intra-progeny relations, and also many members of each group were tested in critical determinations with known genotypes.

268		S 3.3					S 2.3					Testers		
		5	7	8	9	17	2	3	4	10	11	S 2.3	S 1.3	S 1.2
S 3.3	5	S	S	S	S	S	F	F	F	F	F	F	F	F
	7	S	S	S	S	S	F	F	F	F	F	F	F	F
	8	S	S	S	S	S	F	F	F	F	F	F	F	F
	9	S	S	S	S	S	F	F	F	F	F	F	F	F
	17	S	S	S	S	S	F	F	F	F	F	F	F	F
S 2.3	2	S	S	S	S	S	S	S	S	S	S	S	F	F
	3	S	S	S	S	S	S	S	S	S	S	S	F	F
	4	S	S	S	S	S	S	S	S	S	S	S	F	F
	10	S	S	S	S	S	S	S	S	S	S	S	F	F
	11	S	S	S	S	S	S	S	S	S	S	S	F	F

Testers											
S 2.3	S	S	S	S	S	S	S	S			
S 1.3	S	S	S	S		F	F	F	F	F	
S 1.2	F	F	F	F	F	F	F	F	F	F	

FIG. 7. This chart indicates the reactions of plants obtained by the premature selfing of a plant that was *S 2.3*. The genotypes *S 3.3* and *S 2.3* were obtained but there were no members that were *S 2.2*.

Figure 7 presents data for ten of the fifteen plants of a selfed progeny of *S 2.3*. The tests revealed that one genotype was *S 2.3* and the other was *S 3.3*. The critical tests for the latter were that, as pollen testers, the members of this group were cross-incompatible with *S 1.3* but cross-fertile with *S 1.2*. Hence they were *S 3.3* and not *S 2.2*. Thus the formula for this progeny is *S 2.3* = *S 2.3* and *S 3.3*. There were no plants of *S 2.2* in the progeny of 15 plants that were grown.

The tests of the progeny of 17 plants grown from *S 1.2* selfed were fully conclusive that there were only the genotypes *S 1.2* and *S 1.1*.

295		$S_{1.1}$ 3	4	14	17	18	19	20	21	26	27	$S_{1.3}$ 5	9	10	13	15	23	25	16
$S_{1.1}$	3	S	S	S	S	S	S	S	S	S		F	F	F	F	F	F	F	F
	4	S	S	S	S	S	S	S	S	S	S	F	F	F	F	F	F	F	F
	14		S	S	S	S						F			F				
	17	S	S	S	S	S	S	S	S	S	S	F	F	F	F	F	F	F	F
	18	S	S	S	S	S	S	S	S	S	S	F	F	F	F	F	F	F	F
	19	S	S		S	S	S	S	S	S	S	F	F	F	F	F	F	F	F
	20					S	S	S	S				F	F	F	F			F
	21	S	S	S	S	S	S	S	S	S	S						F	F	F
	26		S		S	S			S	S		F	F	F	F	F	F	F	
	27	S	S		S	S	S	S	S	S	S	F	F	F	F	F	F	F	F
$S_{1.3}$	5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	13	S	S	S	S			S		S	S	S	S	S	S	S	S	S	S
	15	S	S	S	S	S		S	S	S	S	S	S	S	S	S	S	S	S
	23	S	S	S			S	S				S	S				S	S	
	25	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	16	F					F					F						F	F

Testers	$S_{1.1}$	$S_{3.3}$	$S_{1.2}$	$S_{1.3}$	$S_{2.3}$
	S	F	F	F	F
	S	F	F	F	F
	S	F	F	F	F
	S	F	F	F	F
	S	F	F	F	F
	S	F	F	F	F
	S	F	F	F	F
	S	F	F	F	F
	S	F	F	F	F
	S	F	F	F	F
	S	S	F	F	F
	S	S	F	F	F
	S	S	F	F	F
	S	S	F	F	F
	S	S	F	F	F
	S	S	F	F	F
	S	S	F	F	F
	F	S	F	F	F

Testers																			
	$S_{1.1}$	S	S	S	S	S	S	S	S	S	S	F	F	F	F	F	F	F	F
	$S_{3.3}$	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	$S_{1.2}$	S	S	S	S	S	S	S	S	S	S	F	F	F	F	F	F	F	F
	$S_{1.3}$	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	$S_{2.3}$	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F

FIG. 8. The progeny of the premature selfing of a plant of *S 1.3* was composed of only the genotypes *S 1.1* and *S 1.3* and one member whose self-fertility was due to a somatic mutation.

There were two progenies of *S 1.3* selfed. One was from 139–12 which was of the parent generation grown from seed obtained from Argentina. This progeny of 22 members was composed of *S 1.1* and *S 1.3* genotypes only. Another series (295; figure 8) grown from the selfed seed of a plant 249-4 (*S 1.3*) was also composed of *S 1.1* and *S 1.3*, but there was one aberrant self-fertile member whose status will be considered later.

Evaluations. The fertility of all premature close-pollinations of *S 1.2*, *S 1.3*, and *S 2.3* was much less than the potential fertility in regard to number of seeds produced.

The analysis of the progenies obtained show that there was a selective fertility in that only one class of pollen functioned and a selective incom-

patibility or competition for the other class of pollen which eliminated one of the possible homozygotes.

In the progenies of *S 1.2* and *S 2.3* no plant of *S 2.2* was obtained. This indicated that the *S 2* factor in pollen tubes had incompatibility reactions with the tissues of young pistils at a time when *S 1* and *S 3* did not have such reaction. Thus *S 2* was different in the strength or degree of the incompatibility reaction. There was also a differential and relative quality of the *S 3* factor according to whether it was associated with *S 1* or *S 2*. The *S 3* pollen tubes did not function in the premature self-pollinations in *S 1.3* pistils but did function in *S 2.3* pistils.

There was, it should be noted, no differential reaction in normal self-pollinations of any member of the genotypes *S 1.2*, *S 1.3*, or *S 2.3*. Numerous individuals of each genotype were grown and many self-pollinations were made. But not one instance of self-fertility was observed except that of the plant 295-16 (figure 8).

Secondary Collateral Progenies of S 1.1 × S 1.2 and S 1.3. *Series 249 and 252; figure 9.* The progeny 249 was derived from *S 1.1 × S 1.3*; series 252 was from *S 1.1 × S 1.2*. In distinction from the *primary collateral progenies* that are obtained of heterozygous plants, the progenies that arise from one or more parents that are homozygous for an *S* factor may be called *secondary collaterals*. The evaluations of these two series provide further data on the constitution of the plants considered to be *S 1.1*, on the behavior of the *S 1* factor in pistils when it is duplicated in diploid pistils and is not associated with a different allele, and on the reactions of the *S 3* factor in such pistils.

The parentage of series 249 was 164–7 (*S 1.1*) × 139–12 (*S 1.3*). Thus a seedling of premature selfing was crossed with its own parent. The capsule contained 571 seeds almost all of which were normal in size and shape. Sixty seeds which were dissected had endosperm and embryo. There was excellent germination and the seedlings were remarkably uniform in vigor. Twenty-five were selected at random for potting and twenty were grown to flowering age and tested. Each plant was self-incompatible and the intra-series relations were adequate to show that the twenty plants comprised one intra-incompatible genotype. Ten plants were propagated and tested in all possible cross-relations and also in reciprocal relations with known plants of *S 1.1*, *S 1.2*, *S 1.3*, and *S 2.3*. The results proved that all of the twenty members of this progeny were *S 1.3*. Thus the progeny of *S 1.1 × S 1.3 = S 1.3* only. There were no members that were *S 1.1*, hence there was hidden incompatibility for the *S 1* pollen tubes and only *S 3* pollen tubes escaped incompatibility reaction.

Of series 252, the twenty-three members that were tested were self- and cross-incompatible and the tests with the known genotypes mentioned above showed conclusively that all were *S 1.2* in constitution. Thus the progeny of *S 1.1 × S 1.2 = S 1.2* only and there had been hidden incompatibility for *S 1* pollen tubes while *S 2* pollen tubes had functioned in fertility.

Evaluations. The *S 1* factor was inactive in the premature self-pollinations of plants of *S 1.2* and *S 1.3* and this had given some plants of *S 1.1*. But in the normal self-pollinations of these plants there was effective self-incompatibility, and also complete incompatibility with the *S 1* pollen of *S 1.2* and *S 1.3* genotypes. In the normal pollinations of *S 1.1* × *S 1.2* and *S 1.3* it was only the *S 2* and the *S 3* pollen tubes which functioned. In the premature selfing of *S 1.2* the *S 2* pollen tubes did not function and in the premature selfing of *S 1.3* the *S 3* pollen tubes did not function.

			S1.3			S1.2			S1.1	= 1.2	= 1.3	= 2.3
			2	4	5	1	3	5				
249	S1.3 20 plants	2	S	S	S	F	F	F	S	F	S	F
		4	S	S	S	F	F	F	S	F	S	F
		5	S	S	S	F	F	F	S	F	S	F
252	S1.2 23 plants	1	F	F	F	S	S	S	S	S	F	F
		3	F	F	F	S	S	S	S	S	F	F
		5	F	F	F	S	S	S	S	S	F	F
Testers		S1.1	F	F	F	← F →						
		= 1.2	F	F	F	← S →						
		= 1.3	S	S	S	← F →						
		= 2.3	F	F	F	← F →						

FIG. 9. The 20 members of series 249 were of the parentage *S 1.1* × *S 1.3* and all were *S 1.3*. The 23 members of series 252 were of the parentage *S 1.1* × *S 1.2* and all were *S 1.2*.

Data for a Progeny of S 4.6 × S 4.5. *The Series 297: 139–30 × 139–27.* Five members of the parent generation of *P. axillaris* were cross-fertile with all others of this generation which were tested (see figure 4). It was assumed that each of these five plants was a member of an intra-incompatible genotype that differed from any other genotype by at least one *S* factor. To test this assumption a progeny of eleven plants was grown from seeds of 139–30 × 139–27. The small size of this progeny was due to limitations in greenhouse space.

Each of the eleven plants was self-incompatible. There were only two mating genotypes and the patern of reactions was like that of figure 6. All the plants were reciprocally cross-fertile with plants of *S 1.1, S 3.3, S 1.2, S 1.3,* and *S 2.3*. Hence none of them possessed the factors *S 1* or *S 3*. It was considered that the genotypes of the two parents could be assigned as *S 4.6* × *S 4.5* in which case the genotypes of the progeny were *S 4.5* and *S 5.6*. Members of these two genotypes were propagated and used as testers when it was desired that there be no common *S* factor of *P. axillaris*. Various of the evaluations which are reported later could not have been made without the use of these or similar genotypes as testers.

The Case of Mutation to Self-fertility. *Data for the Self-Fertile Plant.* Number 16 of series 295 (figure 8) was the only individual of all the progenies of *2n P. axillaris* which was self-fertile. Its chromosome number was *14* (*7+7*) and it was typical of *P. axillaris* in all features of appearance and potential fertility; but it was self-fertile. It was one of a progeny obtained by the premature close-pollination of a plant of *S 1.3*. As is shown in figure 8 all others of the progeny were either *S 1.1* or *S 1.3*. Hence it was expected that the self-fertility involved a loss or a mutation of either *S 1* or *S 3*.

In the reactions of the pollen of this self-fertile plant there was cross-fertility with pistils of *S 1.1, S 3.3, S 1.2,* and *S 2.3,* and there was cross-incompatibility with pistils of *S 1.3*. Each of these reactions was the normal incompatibility reaction of the two classes of pollen of an *S 1.3* genotype in which one pollen class has a factor not present in the pistil.

As a seed parent the self-fertile plant was fertile with pollen of *S 1.1, S 1.2, S 1.3,* and *S 2.3* but cross-incompatible with pollen of *S 3.3*. There was incompatibility reaction in the pistil with *S 3* pollen tubes but none with *S 1* tubes.

Thus the reactions of the various tests indicate that the mutation which allowed self-fertility was confined to the pistil, that the plant had *S 1* and *S 3* pollen which exhibited normal incompatibility reactions, and that either a loss of, or change in, the *S 1* factor in pistils allowed *S 1* pollen tubes to function. Further critical evidence on this condition was obtained by an analysis of a selfed progeny.

Data for a Selfed Progeny of 296–16: Series 347 in figure 10. The capsules and seeds obtained by selfing 295–16 were fully normal and like those obtained from fertile relations between *2n* plants of *P. axillaris*. One hundred seeds were taken at random from a selfed capsule and planted on June 14, 1947. Almost every seed germinated; thirty-two were taken at random for potting. Twenty of these were repotted three times and they were then in six-inch pots. All were vigorous and fully normal in growth and in the character of their flowers. The first of their flowers opened on September 30. Flowering decreased during the latter part of October and only a few flowers were formed after November 15. The 12 plants which were held longer in the smaller pots did not flower. The flowers which developed were fully normal in the character and reactions of the pollen and pistils.

Each of the 20 plants was self-incompatible, and from two to five flowers per plant were selfed. The reactions with testers (see figure 10) definitely proved that six members of this progeny were *S 1.1* and that fourteen were *S 1.3*. There was not a single reaction which did not conform fully to this analysis. The progeny of the self-fertile plant was the same in composition as the progeny which was obtained of a normal *S 1.3* by premature close-

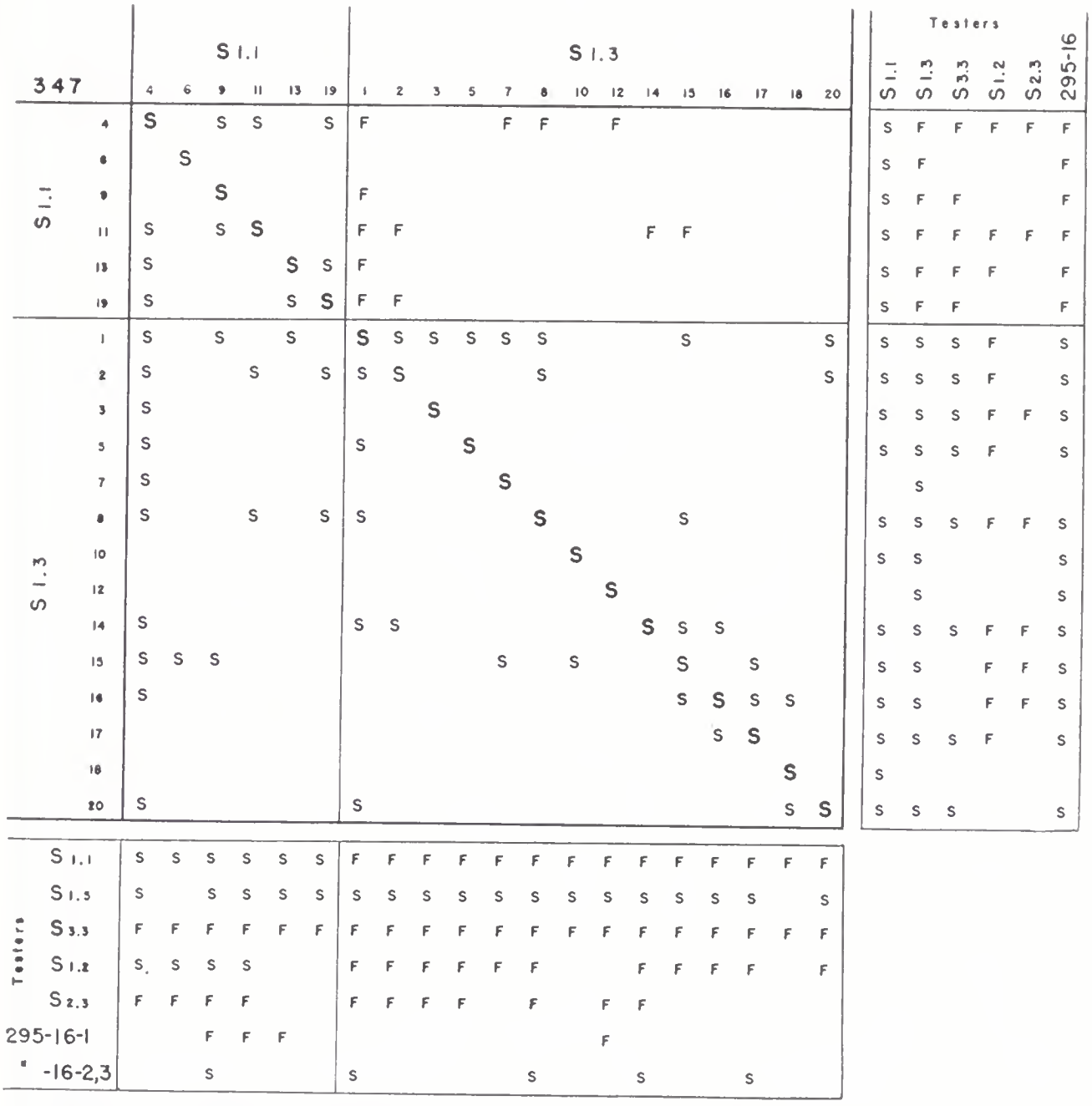


FIG. 10. The selfed progeny of the self-fertile plant No. 295–16 was composed of *S 1.1* and *1.3* members that were self-incompatible.

pollination. No feature of the self-fertility of the plant 295–16 was transmitted to any of the progeny. Its egg cells which were fertilized carried normal *S 1* or *S 3*; but the pollen-tubes which functioned in the pistils were normal *S 1*. The mutation which allowed the *S 1* pollen tubes to function was confined to somatic cells of the pistils.

Somatic Segregation in the Plant 295–16. The original seedling of this plant had a dominant main axis and two laterals from the base. The first

flower, which was terminal on the main axis, opened on June 3, 1946. It was selfed and produced a fine capsule. As soon as this was observed three other flowers were selfed, and they also gave good capsules. By this time each of the two laterals as well as the main axis had a bostryx in good bloom. In the axil of the second leaf below each inflorescence there was, as is frequent in petunias, a vegetative lateral and these three laterals were removed for propagation. About 20 flowers on the plant were used in controlled pollinations for the reactions reported in figure 8. Late in the season of bloom six more flowers were selfed of which four produced capsules and two did not, but the exact locations of the latter were not noted. The original plant did not have flowers during the following winter and became so poor in its vegetative condition that it was discarded. But the three ramets from cuttings lived over winter in excellent condition and bloomed well during the summer of 1947. Then it was learned that all flowers on two of these ramets were self-incompatible and that the other ramet continued to be self-fertile in all of its flowers. The two ramets which were self-incompatible were found by adequate tests to be normal *S 1.3* in the reactions of both pollen and pistils. The self-fertile ramet gave reactions as did the original seedling plant; the pollen gave the normal reactions of both *S 1* and *S 3*, but the pistils reacted as though they possessed *S 3* only.

The self-fertile ramet (295–16–1) had only four flowers after plants of series 347 began to flower. Three of these were tested with pollen of *S 1.1* segregates (347 Nos. 9, 11 and 13) and one tested with a segregate of *S 1.3* (347–12) and there was fertility in every case. Also four flowers of the self-incompatible ramet 295–6–3 were tested with pollen of *S 1.3* segregates (347–1, 8, 14, and 17) and the reactions were incompatible, as was the case when tests with other *S 1.3* plants were made.

Evaluations. The evidence is conclusive that the mutation to self-fertility in the plant 295–16 was not transmitted by any of the germ cells, but was present in certain somatic cells of the pistil to a degree that allowed pollen grains of normal *S 1* to function, not only in selfing but in any cross-relation. The somatic segregation of self-incompatible branches which was fully normal for *S 1.3* also indicates that the original seedling was itself a chimera which probably had an outer histogen of cells in which there was a loss or a mutation of the *S 1* factor only, and that the tissues which contributed to spores and germ cells were normal for both *S 1* and *S 3*.

It is to be noted that this case of mutation to self-fertility was not like that reported by Crane (1947) in *Oenothera organensis* which was transmitted to a selfed progeny of 34 plants. Also the tests which were made by Crane showed that the mutant allele was inactive in haploid pollen on pistils which carried this factor; his *S 3.6* × *S 3.6*$_x$ was fertile, but in the reciprocal as *S 3.6*$_x$ × *S 3.6*, there was incompatibility. Crane considered that his data indicate "that the *S* gene is a double structure consisting (1) of a highly specific part which determines the characteristic incompatibility reaction of

a particular *S* allele, and (2) of a less specific part which has the function of an activator or "primer" and that, in this case, the mutant allele had lost its activator and could not act in haploid pollen but could be active in diploid tissue when accompanied by an allele that possessed the same activator.

The mutation to self-fertility in the plant of *Petunia axillaris* was not transmitted to any of the seed-grown progeny and hence there was no opportunity for further tests of its genetical status.

Natural Populations and the Integration of Collateral Progenies. *A Minimum Number of Factors and Genotypes.* The three heterozygous genotypes, (I) *S 1.2*, (II) *S 1.3*, and (III) *S 2.3* were combinations of the

Integration of progenies		Parentage	Progeny					
			1.2	1.3	2.3	1.1	2.2	3.3
	161	I (S1.2) × II (S1.3)	E	II	III	E		
	285	" " × III (S2.3)	E	II	III		E	
	155	II (S1.3) × I (S1.2)	I	E	III	E		
	250	" " × III (S2.3)	I	E	III			E
	251	III (S2.3) × II (S1.3)	I	II	E			E
	Not grown	" " × I (S1.2)	I	II	E		E	
		Eliminations	2	2	2	2	2	2
		Frequencies	4	4	4			

FIG. 11. This chart indicates the integration of the genotypes of the primary collateral progenies in the reproduction of a sustaining population that consists of three genotypes that are combinations of three *S* factors of oppositional status.

factors *S 1*, *S 2*, and *S 3*, and collectively they comprised the minimum number of genotypes in a succession of generations in a natural population. A progeny of any cross-fertile relation will contain the genotype of the pollen parent and the third genotype. The two reciprocal progenies of any cross-fertile relation will contain all three genotypes. Thus in the natural reproduction of successive generations from seed there will be at least three genotypes in any population.

The Integration of Primary Collateral Progenies: figure 11. Any two of the three genotypes of such a population that are isolated in nature or in experimental studies can give a next generation of reciprocal progenies, as I × II = II and III; II × I = I and III, that is composed of all three genotypes in unbalanced frequency of 1 : 1 : 2. But in the chance cross-pollinations of

the three genotypes of this generation there will be six fertile relations and the collective and integrated progenies will tend to give a balanced frequency for the three genotypes in the next generation. The hidden incompatibilities eliminate the genotype of the seed parent from each progeny and also the homozygous genotypes are eliminated and for these there is also balance (E in figure 11).

Composite Populations. The feature of balanced genotypes may be extended to an entire population when four *S* factors are involved. The addition of one more factor, as *S 4*, to a minimum sustaining population of *P. axillaris*, will automatically provide three more genotypes or mating groups; (IV) *S 1.4*, (V) *S 2.4*, and (VI) *S 3.4*. Then, thirty fertile inter-genotypic relations will be possible. Of these, six will involve no common factor, the reactions will have no hidden incompatibility, and each progeny will consist of four genotypes, none of which is the same as either of the two parents. For example, I (*S 1.2*) × VI (*S 3.4*) = (II) *S 1.3*; (III) *S 2.3*; (IV) *S 1.4*; and (V) *S 2.4*. This condition was postulated by Prell (1921) and demonstrated by East and Mangelsdorf (1925), by Sirks (1927), and by Lehmann (1927). The integration of all the possible progenies will give a balanced frequency for all four genotypes. But from such a composite population four minimum or sustaining units could be isolated as follows:

S 1, *S 2*, and *S 3* in genotypes I, II, and III;
S 1, *S 2*, and *S 4* in genotypes I, IV, and V;
S 1, *S 3*, and *S 4* in genotypes II, IV, and VI;
S 2, *S 3*, and *S 4* in genotypes III, V, and VI.

Any one of these units could be isolated as a sustaining population both in nature and in pedigreed breeding.

The Status of the Wild Population of P. axillaris. In the rather small sample or segment of the wild species of *P. axillaris* that was obtained in the parent generation (figure 4) there were at least eleven genotypes and twelve *S* factors. Ten different cross-fertile relations were possible for each of the twelve genotypes and the number of composite collateral progenies that were possible is 110.

It would require very complete pollinations by insects to effect all these relations so that every possible progeny would be represented in the next generation with a balance of the genotypes. It would seem that the natural distribution of the species would involve the geographic segregation of different composites and even of minimum primary groups.

Concluding Evaluations. *The Genetic Basis of Incompatibilities in Petunia axillaris.* The incompatibilities in the cultures which were studied of this species were determined by a single allelic series of hereditary factors. An individual plant was heterozygous for these "*S*" factors, and each of its gametes carried only one of these factors.

The Nature of the Incompatibility Reactions. The incompatibility was

a reaction between diploid tissues of a pistil and the haploid pollen-tubes. There was independent and efficient self-antagonism involving a gene present in the nuclei of a pollen-tube and the same gene in the cells of a pistil. Each individual plant was normally heterozygous for the *S* factors and the somatic tissues of its pistils had potentially two different incompatibility reactions. The pollen of a plant comprised two different haploid genotypes, and each had its one potential incompatibility reaction. In selfing and in intragenotypic relations both reactions occurred and there was complete incompatibility and no reproduction. In a cross-relation between two plants which had one factor in common there was incompatibility reaction only for the pollen tubes which carried the common factor and this was hidden by the functioning of the other class of pollen. When a cross-relation involved no common factor, as *S 1.2* × *S 4.5*, there was *no action by any S factor* and there was complete and unrestricted intraspecific reproduction.

Both the diplont-haplont reaction in flowering plants and the independent self-antagonism of independent and "oppositional" genetic factors were postulated by Prell (1921) and well demonstrated soon thereafter in experimental studies especially by East and Mangelsdorf (1925, 1926), Lehman (1927), and Sirks (1926, 1927).

The studies of the past twenty years have revealed other classes of incompatibility and also greater complexity in the diplont-haplont class. In the fungi the incompatibilities are often mating reactions between individuals that are haploid, bisexual and gametophytic. In the diplont-diplont incompatibilities, seen in certain animals, there is selective conjugation between individuals that are bisexual and diploid. In all these types there is effective operation of *S* factors and bigenotypic reproduction. The simplest reaction is that of the self-antagonism of independent or oppositional factors, but in the more complex reactions there are two or more allelic series of factors with complementary reactions (Stout 1938, 1945b).

The diplont-haplont reaction, well exemplified in the cultures of *P. axillaris,* operates in the mating reaction between two individuals of different alternating generations. The pistillate plant is sporophytic and the pollen tube is gametophytic. This reaction is an essential one in the series of developments and reactions which effect sexual reproduction in flowering plants. The operation of incompatibility reactions enforces selective fertilizations which effect bigenotypic reproduction that is, nevertheless, intraspecific.

It is to be noted that in all the main classes of incompatibilities mentioned above the reactions are not gametic. They occur previously to the interactions between gametes. Other types of sterility operate in the reactions of gametes and in the death of embryos and some of these may be selective and similar to incompatibilities in results.

The Status of Intraspecific Reproduction in P. axillaris. The successive seed-grown generations of a species such as *P. axillaris* are from seeds of

fertile cross-relations in which *S* factors are inactive. Any one of the self-incompatible plants has a high degree of potential fertility and can function in reproduction in inter-genotypic relations.

Also the members of the species are remarkably alike in the character of their flowers, which are hermaphrodite and homomorphic. Except for the *S* factors there is homozygosity for the members and homogeneity for the population in respect to the genetic factors which determine differentiations in the structure and function of pistils and stamens. Except for the action of *S* factors there is no restriction to full and complete intraspecific reproduction made possible by potential fertility. Until one or more of the characters of differentiation become heterozygous in determination, as for example in species having dimorphic or dichogamous flowers, the entire complex operates as a collective unit. In *P. axillaris* this diploid mechanism which determines intraspecific reproduction may be represented as AA just as the corresponding mechanism of *P. parodii* may be represented as PP.

The Secondary Status of Incompatibilities. It has previously been recognized (Stout 1931, 1938, 1945b) that the genetic and physiological mechanism of incompatibilities is accessory to, and superimposed upon, the more fundamental and basic mechanism of intraspecific reproduction. This is especially obvious in the "personate" (Correns 1916) type of the diplont-haplont class, exemplified in the cultures of *P. axillaris,* for the following reasons:

1. The *S* factors comprise an allelic pair in every diploid plant. No gene of any feature of intraspecific fertility is, or can be, allelic to these factors.

2. No special organs or cells are exclusively involved in the reactions of incompatibility. These reactions operate by inhibiting an action that is already established as an essential feature of intraspecific reproduction.

3. Of closely related species one, as *P. axillaris,* may have incompatibilities and the other, as *P. parodii,* may have no incompatibilities. This emphasizes the fact that no aspect of incompatibilities is an obligate concomitant of reproduction.

4. It is obvious that the mechanism of intraspecific fertility operates when the *S* factors are inactive. The antagonism reaction in a pollen tube is epistatic to its potential and inherent reactions of intraspecific fertility.

5. The reactions of incompatibility may be removed or reduced by removing the secretion on stigmas and by premature pollination. There are also such differences in expression as (a) end-season fertility, (b) cyclic incompatibility, (c) border-line action of certain *S* factors, and (d) complementary reactions with other factors including those that favor fertilization. These features of reduction and inactivation of *S* factors indicate the accessory status of the incompatibility mechanism.

6. For purposes of abbreviated representation and comparisons the two mechanisms operating in *P. axillaris* may be represented as AA + *S x.y.* In much of the preceding presentations of data, only the genotypes of the in-

compatibility mechanism were represented, as *S 1.2*, *S 1.3*, and *S 2.3*. But it should be kept in mind that the fertilities of reproduction are effected by the fundamental or basic mechanism represented by AA which operates when the incompatibility mechanism is inactive.

Chapter 4. Intraspecific Reproduction in *Petunia integrifolia*

The Kew Clone of Petunia integrifolia. *Status of the Kew Clone.* The studies here reported for *Petunia integrifolia* involve (1) ramets of the one clone, here designated the Kew clone, which was derived from the Royal Botanic Gardens at Kew, England, and obtained from Professor Margaret Ferguson. (2) ramets of what is here designated a Kew subclone, and (3) seedlings derived (a) by premature self-pollination of ramets of the Kew clone, (b) by normal selfing of ramets of the self-fertile Kew subclone, and (c) by the fertile bigenotypic cross-fertilizations of homozygous seedlings × heterozygous and homozygous seedlings.

Ferguson and Ottley (1932) very fully described the foliage and flowers of the Kew clone of which they obtained cuttings from the Royal Botanical Gardens at Kew, and they published an excellent plate of drawings which show the features of the flower structure. The chromosome number for this clone, as reported by Ferguson and Coolidge (1932) is $2n = 14$. This number was found in each of several examinations made at The New York Botanical Garden of ramets of the Kew clone and of various seedlings, and the pairing during meiosis was regular and complete for the seven pairs of homologs. Kostoff and associates (1935) report that plants grown from seeds supplied by the writer, and obtained by premature selfing of the Kew clone, had a somatic number of *14* chromosomes. This report also states that the plants grown at New York were from "seeds originally obtained from Wellesley College (M. Ferguson)" but this is not correct, for only cuttings of the Kew clone were received by the writer from Professor Ferguson.

Self-Incompatibility of the Kew Clone. (1) Reports by Ferguson and Ottley (1932) for this clone include the following statements and data: "Many attempts were made to secure seeds by artificially selfing the flowers of *Petunia violacea*. As a result of numerous selfings but three capsules with viable seeds were formed." In one capsule 88 seeds were obtained and seedlings were grown which at the age of flowering were reported as "not distinguishable from the young cuttings of our original stock." But in their studies several capsules were observed on ramets of the Kew clone which were grown both in the open and in a greenhouse under conditions that suggested autonomous self-fertilization. But it was noted that "for several months during the winter and spring, when the plants of *P. violacea* were surrounded on all sides by other species and cultivated strains of *Petunia* not a single capsule set on them as a result of either natural crossing or of selfing."

(2) Mather (1944) reports that the ramets which he grew of the clone obtained at Kew and the 43 seedlings which he obtained from premature pollinations were all self-incompatible. His report on "*P. violacea*" which evidently refers only to the ramets of the Kew clone of *P. integrifolia* and its seedlings is as follows: "Here self-pollination is rarely followed by the production of seeds. A small quantity of seeds has been obtained in this way from a few flowers, but these cases may be attributed to a low degree of pseudo-compatibility, the plants being, in the broad sense, incompatible. Bud pollinations did not appear to lead to any greater pseudo-compatibility than the pollination of open flowers."

(3) The data obtained at The New York Botanical Garden may be summarized as follows: Very complete autonomous self-pollination naturally occurs in the flowers of the Kew clone. The two longest stamens stand with their anthers directly and slightly above the stigma in a position that provides for normal pollination. The stigmas show secretion somewhat before the shedding of pollen. Few flowers can escape abundant self-pollination even without the aid of insects.

During 1934–1943 inclusive, not one capsule was obtained of numerous normal self- and close-pollinations of flowers of the ramets of the Kew clone. Also not one capsule developed to any open pollination. But during 1943 capsules which contained seeds were observed on some of the ramets, and from cuttings of these a *subclone* was obtained, all of whose flowers were self-fertile (figure 16) while certain other pedigreed cuttings of the self-incompatible Kew clone continued to be self-incompatible.

The Potential Fertility of Pollen. The pollen of the Kew clone was very uniform in appearance and highly viable. As shown in figure 49 D the tricolpate or trilete pollen grains of the Kew clone were very uniform and relatively few were aborted, undersized or empty. In cultures on a medium of 1 gram agar and 15 grams of sugar in 100 cc. water a high percentage of all grains germinated and produced pollen tubes (figure 49 E).

Studies of Pollen-Tube Behavior. Pollen-tube growth was studied during 1940 both (a) for the self-incompatibility of normal pollinations and (b) for the fertility of premature pollinations. The normal pollinations were made when previously emasculated flowers were fully open and there was abundant secretion on the stigma. Premature pollinations were made approximately 20 to 24 hours before anthesis when there was no secretion on stigmas. It was not possible to make all these pollinations on the same day or at the same hour of the day. In the premature pollinations it was somewhat difficult to select buds of exactly the same age. The pistils were collected at intervals

Explanation of figures 12–14

FIG. 12. Seedlings of *Petunia integrifolia* Kew clone, showing normal foliage and different habits of growth. FIG. 13. Seedlings of the Kew clone with abnormal foliage. FIG. 14. (A) Normal flower and (B) normal stamens and pistil of Kew clone. Others are abnormal flowers of seedlings that also had abnormal foliage.

12

13

14

after pollination and were placed in 70 per cent alcohol to which 12 per cent formalin had been added. They were held in this solution until the dissections were made. The material was easier to dissect and was stained more readily when only a short time intervened between fixations and dissection. The styles were first dissected on a clean glass slide and then stained with acid fuchsin as described by Chandler (1931) or lacmoid as reported by Nebel (1931). Pollen tubes could readily be recognized in the stylar tissue. They were measured and the length of the longest tubes was plotted in relation to the length of the style in which they grew.

Of the pollen-tube growth after normal controlled hand-pollination the following behavior was noted. At the end of 6 hours the longest tubes had grown about ⅙ of the length of the style. After 24 hours the longest tubes were about ½ of the distance to the ovary, and at 36 hours a few tubes were about ¾ of the distance to the ovary. In one pistil, collected 48 hours after pollination, 32 tubes were observed in the upper part of the ovary chamber but none was seen at or in a micropyle of any ovule. The hundreds of tubes that entered a single style were distributed at various levels but the majority were in the apical half of the style. There was no definite bimodal distribution. A considerable amount of coiling and twisting of the tubes at their tips, and what appeared to be aimless wandering of some of the tubes through the style were all observed in preparations of pistils after normal self-pollinations were made.

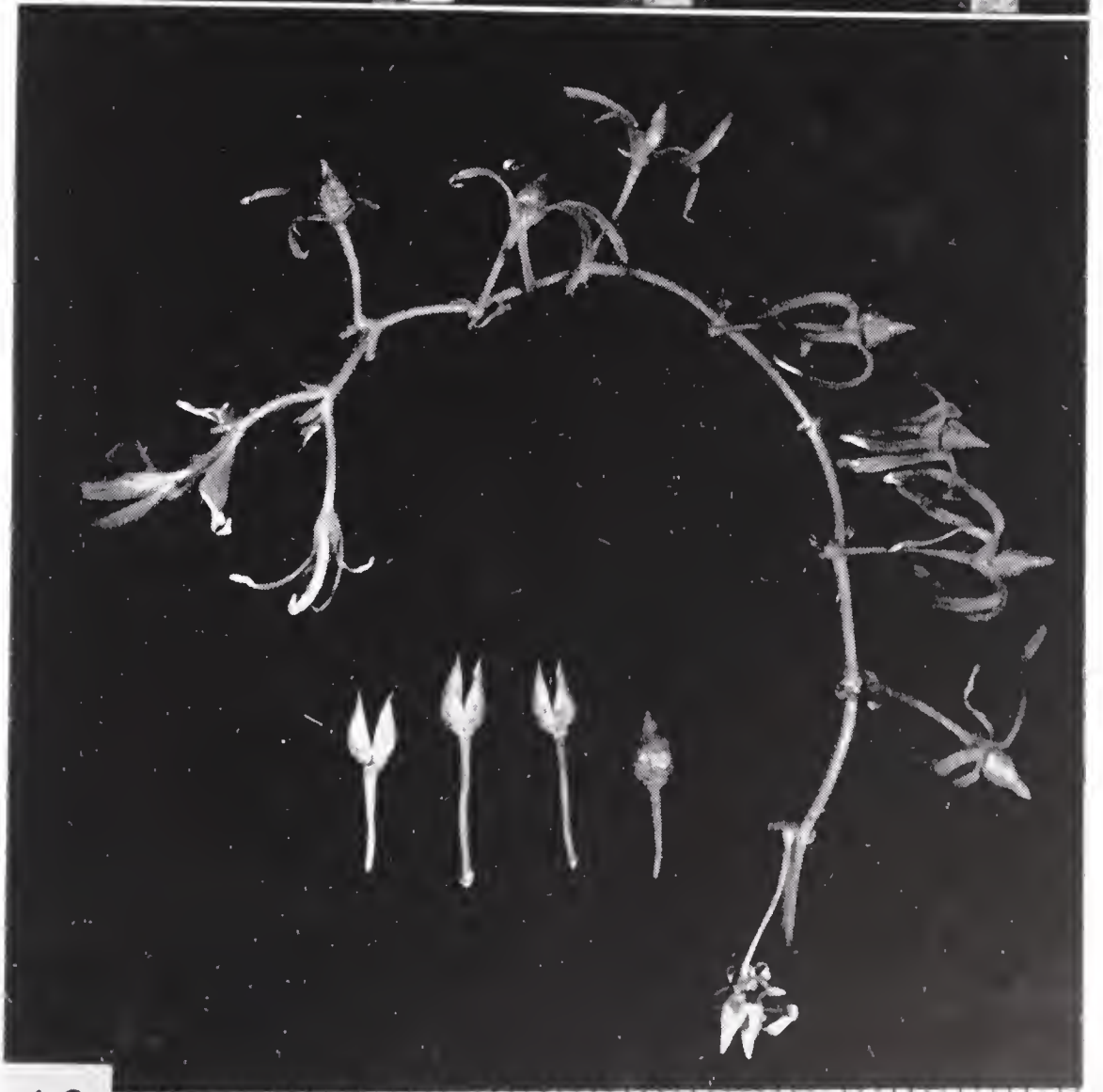
Pollen-tube behavior in premature close-pollination was as follows: When flowers of the Kew clone of *Petunia integrifolia* were emasculated and the pistils were prematurely close-pollinated, a few tubes were observed in the ovary cavity only 30 hours after pollination. At the end of 36 hours many tubes were in the cavity and some were entering the micropyles of the ovules in the upper portion of the ovary cavity. In each preparation numerous ungerminated grains were observed on the stigma and there were ends of tubes at various distances throughout the style. Thus the more advanced pollen tubes of premature pollinations reached the ovary in 30 hours, which was when the pistils had developed to full size and anthesis was beginning, and there was the production of some seeds. After normal pollination the most advanced tubes were 47 hours in reaching the ovary and seeds were not obtained.

The Capsules and Seeds of Premature Pollination. The ramets of the Kew clone yielded some capsules and viable seeds to premature close-pollination. During 1934 such seeds were obtained, some of which were sent

Explanation of figures 15 and 16

FIG. 15. A and D, two seedlings with abnormal foliage and flowers and many flowers that were reduced to small green buds. At B and C, seedlings with normal foliage and flowers. FIG. 16. Showing capsules of the self-fertile Kew subclone, obtained by autonomous self-pollination. Each flower was terminal for a section of the false axis of the bostryx which appears to be a continuous stem.

15



16

to Dr. D. Kostoff in Bulgaria. During each of the years since 1934 seeds have also been obtained. As an example of the results the following data may be given. Of 24 premature pollinations made over a period of three weeks only four failed completely. The number of well-formed seeds in the ten best capsules were 111, 75, 55, 51, 41, 41, 39, 31, 30, and 17. In the other 14 capsules the seeds per capsule ranged from 2 to 11. During the same period and on the same branches not one of the 23 flowers which were normally selfed produced a capsule. It seems certain that there was a reduced or partial seed production in the relations of premature selfing and that the seeds which were thus obtained usually did not represent the total number of functional ovules in the mature ovaries.

Progenies of Premature Selfing of the Kew Clone. *Series 119.* A total of 100 seeds obtained by prematurely selfing were planted in soil in a seed pan. Only 19 seedlings appeared above ground. Five were weak in growth and had *abnormal* leaves; four of these died early and the one that lived to flowering age also had abnormal flowers and was discarded.

The 14 seedlings that had normal flowers and leaves were vigorous in growth. The flowers of nine were colored like the flowers of the Kew clone but five had flowers of rose-pink coloring. The 14 seedlings were tested by normal selfing and all were fully self-incompatible. All possible cross-relations were tested by emasculations and controlled pollinations. The 11 members of one group were intra-cross-incompatible and also cross-incompatible reciprocally with the Kew clone. On the assumption that there was the simpler personate type of incompatibility, as in *P. axillaris,* the genotype of the Kew clone and of these 11 seedlings was assigned as *S a.b.* The three plants of the other intra-incompatible group were fertile as seed parents with the Kew clone and with the eleven sisters of the genotype *S a.b;* but they were cross-incompatible with them as pollen parents. Thus the genotype for these was either *S a.a* or *S b.b.* The plants of series 119 became badly infected with mosaic and hence were all discarded.

Series 165. The 74 seeds in a capsule of the premature pollination of a flower of the Kew clone were planted in soil in October, 1943. All these seeds were almost spherical, unshrunken, and very uniform in size and color. But only 16 seedlings appeared above ground. Two of these died while very small; another that had abnormal leaves died later. Four plants which had abnormal foliage were carefully tended and these grew to flowering age as did the nine that had normal foliage.

The foliage and flowers of the abnormal plants were most conspicuously different from the normal. The leaves were more slender and often contorted with margins inrolled and tips upturned (figure 13). The veining was reduced to a midrib and a single vein parallel and rather close to each margin of the blade. The flowers were also abnormal. The corolla (figure 14) was often split into segments with some or all segments poorly developed or absent. Often some or even all of the stamens and also the pistils of such

165			S a.b 1	7	2	3	4	8	11	12	13	S a.a 6	9	10	S b.b 5	Kew C.	Kew S.	P. p.	P. ax.
S a.b	1	Normal: Pink	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		
	7	" "	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		
	2	" : Kew	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		
	3	" "	S	S	S	S	S	S	S	S	S	S	S		S	S	S		
	4	" "	S	S	S	S	S	S	S	S	S	S	S		S	S	S	O	O
	8	" "	S	S	S	S	S	S	S	S	S	S	S		S	S	S		
	11	" "	S	S	S	S	S	S	S		S	S	S		S	S			
	12	" "	S	S	S	S	S	S	S	S	S	S			S	S			
	13	" "	S	S	S	S	S	S	S	S	S	S	S		S	S			
S a.a	6	Abnormal: Kew	F	F	F				F			S	S	S	F	F	F		
	9	" "	F	F	F	F	F	F	F	F	F	S	S		F	F	F	O	O
	10	" "	F	F		F		F				S	S	S	F				
S b.b	5	" : Pink		F	F	F		F	F		F	F	F		S	F	F	O	O
Kew clone, S a.b			S	S	S	S							S		S	S	S	O	O
Kew subclone			F	F	F	F	F	F	F	F	F	F	F		F	F	F	O	O
P. parodii			←				H				→		H		H	H	H	F	H
P. axillaris			←				H				→		H		H	H	H	O	F

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	Progenies of Kew clone																of Kew sub-clone							
Series	119 : 165			211			212			213				244	1st. generation						2 d. g.	
No. plants	24*			16			9			11				23	67						7	
S a. a.	3			2			4			1					11							
S a. b.		20			11				5		7			23			53				7	
S b. b.			1			3						3							3			
Normal		20		2	11	3	2		5		7	2		23	1		47		1		6	
Abnormal	3		1					2		1			1			10		6		2		1
Kew color	3	15					1		4	1	7	2	1	23	1	9	28	5	1	2	4	1
Rose pink		5	1	2	11	3	1	2	1							1	19	1			2	

18

FIG. 17. This figure indicates the character and the reactions of a progeny obtained by the premature selfing of the Kew clone. The unilateral reactions of hybridization are indicated by the symbols H and O. FIG. 18. This figure is a tabulation of all seedling progenies of the Kew clone and the Kew subclone.

flowers were malformed, aborted, and sterile. There was much variation in the extent and degree of the abnormalities among different flowers of a plant and also for different plants. In the extreme cases the flowers af a branch were reduced to a small cluster of entirely sterile green rudiments and in a few plants almost all the flowers were of this condition (figure 15). When pistils were formed they were most often asymmetric in form, twisted, lacking in secretion, and either functionally sterile or low in potential fertility.

The tests (figure 17) revealed that all of the plants were self-incompatible. The nine plants which had normal foliage and flowers were reciprocally cross-incompatible with the Kew clone and hence were judged to be *S a.b.* Two of these had flowers of rose-pink coloring; seven had flowers of coloring like that of the Kew clone, which has been described by Ferguson and Ottley (1932) as "between mallow purple and tyrian pink with a deeper purple along the midveins." The segregated rose-pink coloring was very distinct from, and much paler than, the coloring of the flowers of the Kew clone.

The four abnormal plants that were tested, using the best of their flowers, were cross-incompatible as pollen parents with the Kew clone and with their *S a.b* sisters but as seed parents they were all cross-fertile. These reactions indicated that all four were homozygous for an *S* factor. But in their cross-relations there were two genotypes: three that were reciprocally cross-incompatible were classed as *S a.a;* the other seedling was reciprocally cross-fertile with all three of the homozygous sisters and was obviously *S b.b.* Only one abnormal plant had rose-pink flowers. There were also noticeable differences in habits of growth (figure 12) which were similar to those already noted for *P. parodii* (figure 2) and *P. axillaris.* A few seedlings had only axial growth but most seedlings were well branched (figure 15).

Thus in this one progeny of 13 plants there were segregations (a) of the three genotypes, *S a.a, S a.b,* and *S b.b,* (b) of two classes of flower coloring, (c) of abnormal and normal types of foliage, and (d) of different habits of growth. In this progeny all members that had abnormal foliage and flowers were homozygous for an *S* factor. Seedlings were propagated of each of the three different genotypes for *S* factors including some that had rose-pink flower coloring and some that had abnormal foliage.

Tests by premature close-pollination were made of all nine plants that had normal flowers and from one to seven capsules per plant were obtained. The number of apparently fully developed seeds in eleven of the best capsules ranged from 17 to 114 with an average of 67 +.

The Second Generation. *Series 211: figure 18.* A progeny of 16 plants was grown from the seeds of a premature selfing of a plant of *S a.b* (165–1) that had normal flowers of rose-pink coloring. All 16 had normal foliage and rose-pink flowers. One plant had anthers that were aborted and contained almost no pollen and the grains that were present were shrivelled and evidently not functional, but the pistils produced good capsules with seeds to pollen of *S a.b* and *S b.b* and hence the constitution was *S a.a.* This

was the only case of male sterility that was observed in any seedlings of *P. integrifolia* that had normal flowers. The other 15 plants were self-incompatible. Eleven were *S a.b;* two were *S a.a,* and three were *S b.b* as determined by tests with plants of series 165 and with the Kew clone. Thus there was segregation for the *S* factors, but all of the progeny had rose-pink flowers and normal foliage and flowers.

Series 212: figure 18. A progeny of 10 seedlings was obtained from seeds of a premature selfing of a plant of *S a.b* (165–2) that had normal foliage and flowers of the coloring like that of the Kew clone. One seedling died before true leaves appeared. All nine that flowered were self-incompatible; five were *S a.b;* four were *S a.a.* Seven had normal foliage and flowers; two had abnormal foliage and both were *S a.a.* Five had the Kew clone flower coloring; four had rose-pink coloring, and of these two had abnormal foliage. Thus in this progeny there were independent segregations for character of foliage and for flower coloring.

Series 213. The eleven members of this progeny were from premature selfing of a plant of *S a.b* (165–3) which had normal foliage and flowers of the Kew clone coloring. All had flowers with the coloring of the Kew clone. Seven were *S a.b;* one was *S a.a,* and three were *S b.b.* Nine had normal foliage, and of the two which had abnormal foliage one was *S a.a* and one was *S b.b.* Thus the parent plant was homozygous for the flower coloring of the Kew clone.

Series 244. This progeny was obtained from the fertile cross 165–6 (*S a.a,* abnormal, Kew clone coloring) × 165–5 (*S b.b,* abnormal, rose-pink). Thirty-six seeds were planted and thirty seedlings appeared above the soil. Seven were weak and four of these died before true leaves were formed, and the other three had only axial growth and died early, but their foliage was normal. Twenty-three plants were grown to flowering age. All had normal foliage and flowers, and the flowers had Kew clone coloring. All were self-incompatible to normal pollinations. Numerous tests of the cross-relations were made, all of which indicated that all members of this progeny were *S a.b* In reciprocal tests with ramets of the Kew clone and with seedlings known to be *S a.b* there was complete incompatibility indicating that none was homozygous. Eleven plants were tested with the homozygous parents as seed parents, and in evey case there was fertility indicating that the relation was homozygous × heterozygous.

Each of the parents of this series had abnormal foliage and was homozygous for one of the *S* factors. One was *S a.a* and one was *S b.b.* All of the progeny had normal foliage and flowers, and were *S a.b.* These facts suggest that there were at least two factors for the abnormal condition, that these factors were allelic to a factor for the normal, and that the abnormal character appeared only when a plant was homozygous for one of these factors, as: $Ab_1\ Ab_1$, and $Ab_2\ Ab_2$. The data also suggest that there was a linkage of these factors with the *S* factors. Plants that were homozygous for *S* factors

were most likely to have abnormal foliage. On this basis the *S a.b* plants which had abnormal foliage and the *S a.a* and *S b.b* plants which had normal foliage resulted from cross-overs.

It may be noted that the factors which determined abnormal flowers and leaves were distinct from *S* factors and that they were probably semi-lethal or even lethal in action.

The data on flower coloring indicate that the rose-pink coloring was due to a recessive factor and that the Kew clone was heterozygous for allelic factors for color, and that some of the seedlings of the Kew clone were homozygous for one or the other of these factors.

The Kew Subclone. *Origin and Character.* At least ten ramets of the Kew clone were kept from year to year and these were propagated under one number. During the spring of 1944 it was observed, for the first time, that capsules were developing on certain of the ramets of the Kew clone to autonomous self-pollination. It was noted that some of the ramets did not have capsules while others had them rather frequently, especially on certain branches. Each ramet was then given an individual number and pedigreed cuttings were made (a) of ramets and of branches which had capsules and (b) of those which had no capsules.

It soon became evident that a *new subclone had been obtained* which produced capsules abundantly to an autonomous selfing (figure 16) and that other ramet propagations of the original Kew clone continued to be self-incompatible to normal self- and close-pollinations.

The Kew subclone was identical with the Kew clone in every particular except that capsules were formed by almost every flower. There appeared to be no change in the degree of protogeny seen in the flowers of the Kew clone. Secretion occurred on the stigmas early in anthesis before the anthers dehisced, hence there appeared to be no change or delay in the formation of the secretion that could effect premature pollinations. Capsules were produced when plants were enclosed in wire-screen cages and left to self-pollination. No capsules were obtained when the flowers were emasculated as soon as they opened. Capsule and seed production began when the first flowers were formed in March and continued until the end of flowering in late autumn. There was no difference in this behavior according to age of a ramet or to the time of the year.

The Reactions of Cross-Relations. The pollen of the Kew subclone was incompatible in all normal pollinations of flowers of the Kew clone and of all seedlings of *S a.b* constitutions, but was cross-fertile on pistils of seedlings of either *S a.a* or *S b.b* constitution. As seed parents the ramets of the Kew subclone were fertile with pollen of the Kew clone and with any seedlings of either *S a.b, S a.a,* or *S b.b* constitution.

Thus the pollen tubes of the self-fertile Kew subclone continued to possess the incompatibility reactions of normal factors *S a* and *S b* in all relations except *on and in its own pistils.* Also the pistils of the Kew subclone allowed fertility not only to pollen of the same flowers but also to any pollen of the

Kew clone or of the seedlings of *S a.b*, *S a.a*, and *S b.b* constitution as shown by the reactions with members of series 165 (figure 17). Hence the pistils alone had changed in respect to their reaction with *S a* and *S b* pollen-tubes. Progenies were grown and studied to determine if this condition was transmitted to the offspring.

The Potential Fertility of the Kew Subclone. Forty capsules of self-pollinations were evaluated. Eight of these were of autonomous self-pollinations and thirty-two were of controlled hand-pollinations in which an abundance of pollen was applied from freshly dehisced anthers. Of forty-four such pollinations only four failed to develop capsules. The seeds in the eight that were selfed autonomously ranged from 30 to 76; those in the hand-pollinated capsules ranged from 3 to 67 per capsule. The average number of seeds for all capsules was 38 +. In all cases nearly every one of the seeds was well-formed and they were very uniform in appearance and nearly spherical. There was no class of shrivelled seeds or of partly developed seeds.

Capsules and seeds obtained of cross-relations were also studied. The numerous tests made during 1944, 1945, and 1946 indicated conclusively that the self-fertile ramets of the Kew subclone also produced capsules and seeds to controlled pollinations with pollen of the Kew clone and of any seedling of either *S a.b*, *S a.a*, or *S b.b* constitution. Seventeeen such capsules were evaluated. The number of seeds per capsule ranged from 6 to 97 with an average of 23. With the exception of three seeds that were collapsed, all of these seeds were well-formed and normal in appearance. In the capsule that had 97 seeds there were about 25 ovule scales.

A First Generation of Selfed Progeny. A total of eighty-two seedlings were obtained of seeds of normal self-pollination of the Kew subclone. Fifteen died when young and of these ten had abnormal foliage. The sixty-seven that flowered were self-incompatible and none produced any capsules to autonomous selfing as did all ramets of the Kew subclone. All were sufficiently tested to determine their genotypic constitutions as summarized in figure 18. Fiftly-three were *S a.b*, eleven were *S a.a*, and three were *S b.b*. There were segregations for normal (49) and abnormal foliage (18), and also for Kew coloring (46) and rose-pink coloring (21).

A Second Generation Progeny of S a.a × S b.b. The seed parent had abnormal foliage and flowers of rose color; the pollen parent had normal foliage and flowers of Kew coloring. Only seven seedlings were grown; all were self-incompatible and *S a.b* in constitution. Six had normal and one had abnormal foliage. Five had flowers of Kew coloring; two had rose-pink flowers.

Evaluation of the Kew Subclone. Each seedling plant of the progeny was self-incompatible. Thus the condition in the pistils which allowed self-fertility of the Kew subclone and also cross-fertility with the pollen of the Kew clone or of any *S a.b*, *S a.a*, or *S b.b* seedling was not transmitted to any of the progeny that was grown.

The evidence indicates that the condition responsible for the self-fertility

of the Kew subclone was confined to the pistils for the egg cells in the ovules carried the *S* factors which operated effectively in the progeny in both the haploid pollen and in the diploid tissues of their pistils. Thus the somatic mutation which effected the self-fertility of the Kew subclone was like that in the one self-fertile plant of diploid *P. axillaris* except that there was loss or inhibition of the incompatibility reaction with both classes of pollen (*S a* and *S b*) instead of with only one class (*S 1* and not *S 3*).

The segregations (a) of the three different genotypes for *S* factors, (b) of the normal and abnormal types of foliage and flowers, and (c) of the two classes of flower coloring were quite as in the progeny of the Kew clone. This was additional evidence that the self-fertility of the Kew subclone involved true fertilizations.

Data Obtained by Mather for a "Selfed" Progeny of the Kew Clone. Mather (1944) has reported on a progeny of 43 plants obtained by selfing ramets of the Kew clone of *P. integrifolia* (called "*P. violacea*"). All were self-incompatible and the reactions with parental clone indicated that 25 were of the same heterozygous class as the Kew clone (*S 1.2* in Mather's terminology), 15 were of one homozygous group (Mather's *S 2.2*), and one was of another homozygous group (*S 1.1*). Mather made no mention of segregates which had abnormal flowers and foliage or that had rose-pink flowers. He noted that the deficiency of one class of homozygotes may involve either selective death of zygotes or a difference in the strength of the *S* factors.

Summary and Evaluations. *Type of Incompatibility.* The reactions of the seedling progenies indicate that the Kew clone of *Petunia integrifolia* had only two of an allelic series of oppositional factors, here designated as *S a* and *S b*, which effected the incompatibility reactions. The self-inhibiting actions of these two factors in the diplont-haplont reactions were complete and effective in all relations of normal pollinations except in the pistils of the Kew subclone.

No seedling of *S a.b* set seed to normal self-pollination. The relations of *S a.b* × *S a.b*, *S a.a*, and *S b.b* were incompatible. The relations of *S a.a* and *S b.b* × *S a.b* were fertile but involved hidden incompatibility for the pollen tubes which carried the common factor. In the relations between the two different homozygous genotypes there was inactivation of the *S* factors and the expression of the inherent intraspecific fertility that is the basis of all reproduction of the species.

The Self-Fertility of the Kew Subclone. This involved a somatic change in the reactions of the diploid pistils which allowed the pollen of both *S a* and *S b* to function not only in selfing but in cross-relations with the pollen of any plant of *S a.b*, *S a.a*, or *S b.b*. But this condition was not transmitted to any of the seedlings that were obtained. Also the pollen of the Kew subclone continued to have incompatibility reactions on the pistils of the Kew clone or of any *S a.b* seedling.

The progenies obtained from the selfed seed of the Kew subclone were self-incompatible and they gave the same segregations that were obtained in the progenies of the premature selfing of the Kew clone.

The character of the somatic mutation to self-fertility was like that of the one self-fertile plant of *Petunia axillaris* except that in the Kew subclone both of its classes of pollen tubes functioned while in the plant of *P. axillaris* only one pollen class functioned.

Premature Fertilization. In the premature selfing of the Kew clone and of seedlings of *S a.b* there was a functioning of both *S a* and *S b* pollen tubes.

Semi-Lethal Factors. The abnormal type of foliage and flowers was determined by two genetic factors, Ab_1 and Ab_2, which effected the abnormal character when *either* was homozygous. Plants with abnormal foliage were often weak in growth and frequently they died when they were young seedlings. Thus it appears that the excess of *S a.b* normal plants over abnormal homozygous plants in the survivals of the progenies did not necessarily involve selective action of pollen-tubes. The abnormal character of the flowers involved much abortion and sterilization of stamens and pistils and greatly reduced potential fertility.

Potential Fertility. The fundamental or basic fertility in the intraspecific reproduction of the species *P. integrifolia* may be judged by the production of capsules and seeds by normal plants in such fertile relations as homozygous × heterozygous and the reciprocals of *S a.a* with *S b.b*. In such relations good capsules and seeds were obtained. The capsules were always much smaller than those of either *P. parodii* or *P. axillaris*. For the 25 typical capsules that were evaluated the number of well-formed seeds ranged from 60 to 192. The average per capsule for *S a.a* × *S a.b* was 111; for *S b.b* × *S a.b* it was 107; for *S a.a* × *S b.b* it was 131; and for *S b.b* × *S a.a* it was 116. The average for all capsules evaluated was 124. Thus the basic potential fertility of these plants of *P. integrifolia*, in terms of number of seeds per capsule, was much less than that of the other two species that were studied. The seeds of *P. integrifolia* were somewhat larger and more spherical and evidently the seeds were more widely spaced on the surface of the placenta.

The Status of the Population That Was Obtained. The total population of the progenies derived from the Kew clone and the Kew subclone comprised only the genotypes *S a.a*, *S a.b*, and *S b.b*. These did not comprise a natural sustaining population. The progeny of any normal fertile cross-relation was composed of *S a.b* plants which were self- and cross-incompatible. The addition of one more factor, as *S c*, would provide three heterozygous genotypes (*S a.b*, *S a.c*, and *S b.c*) which could establish successive generations of seed-grown progenies. There are probably numerous *S* factors in the constitution of the wild populations of this species in its natural distribution in South America.

Taxonomic Status of the Kew Clone. The Kew clone appears to be typical of the species *Petunia integrifolia* in all its characters, habits of growth,

and specific features of foliage, flowers, capsules, and seeds. It is, however, heterozygous for flower coloring. The rose-pink coloring was obtained in *S a.b, S a.a,* and *S b.b* genotypes, and by selection this type may be had in lines that breed true. Possibly this type may exist in the wild population of this species.

The abnormal type of foliage and flowers is also a condition that is represented in the genetic constitution of the Kew clone.

The writer has attempted, with no success, to obtain seeds of *P. integrifolia* from wild plants in South America. Hence these studies of this species were limited to the Kew clone, to the Kew subclone, and to the seedling progenies derived from them. Whether these are, or are not, representative and typical of the wild population of this species cannot be stated.

Chapter 5. The Interspecific Relations of *Petunia axillaris, P. parodii* and *P. integrifolia*

The Hybridization Reactions between Diploid P. axillaris and Diploid P. parodii. *Tests with 2n P. axillaris as the Female Member.* During 1941 nineteen plants of *2n P. axillaris*, including some of all of the genotypes (figure 4) of the parent generation grown from seeds received from Professor Parodi, were tested as female members in relations which involved six different plants of *P. parodii.* In every relation ovaries did not start to develop. The same result was obtained in numerous tests during the next four years using plants of the successive generations of *P. parodii* and plants of various genotypes obtained of *P. axillaris.*

During 1946 and 1947 numerous tests were made of emasculated flowers of caged plants of some of all the later series of *2n P. axillaris* including members of the genotypes *S 1.1, S 3.3, S 1.2, S 1.3, S 2.3.* Twenty-five different plants of *P. parodii* were involved as pollen members. There was complete failure for every relation. The ovaries did not even start to enlarge.

Data for P. parodii as the Female Member: figures 3 and 4. All the tests with the parent generations grown from seeds obtained from Argentina indicated that plants of *P. parodii* would produce capsules and seeds to pollen of *P. axillaris.* In 1944, special tests by controlled pollinations were made using 12 plants of *P. parodii* as female members and plants of *P. axillaris* as pollen members. The latter included genotypes *S 1.1, S 1.2, S 1.3,* and *S 2.3.* It was necessary to emasculate about 24 hours before anthesis because the flowers of *P. parodii* were not only self-fertile but they usually shed pollen before the corolla opens. Every combination gave capsules and numerous viable seeds and failures were incidental.

In 1946, ten different seedlings of later generations of *P. parodii* were tested as seed parents in hybridization pollinations with pollen of twenty-three different *2n* plants of *P. axillaris* comprising genotypes of *S 1.1, S 3.3, S 1.2, S 1.3,* and *S 2.3.* In every relation fine capsules with viable seeds were obtained.

Eighteen of the capsules were evaluated; four of *S 1.1* pollen parentage, six of *S 1.2*, six of *S 1.3* and two of *S 2.3*. The number of apparently normal and fully formed seeds per capsule ranged from 291 to 1506, with an average of 931. In size and shape the capsules were typical of those obtained by the intraspecific reproduction of *P. parodii*. In size, form, color and appearance these seeds were typically *P. parodii*. Of the total of 16,770 seeds that were examined only 8 seeds were shrivelled and somewhat collapsed. There were few scale ovules of both the *a* and *b* classes; the *b* class was most frequent; the number of such scales in capsules that had highest number of seeds was as low as 10, but they were more numerous when the number of seeds was relatively low. It is to be noted that there were no intermediates in seed-size between the class of *b* scale ovules and the large well-formed seeds.

There was excellent germination of seeds in each of the four plantings that were made to obtain F_1 progenies. The potential fertility of plants of *P. parodii* was relatively greater than that of *P. axillaris* in respect to the number of seeds per capsule. The relative fertility of the unilateral hybridization, in which *P. parodii* was the female, was almost if not equal to that of the intraspecific fertility. If the latter is rated at 100 the hybridization fertility was at least 90. In the relation PP ♀ × A ♂ there was a high degree of fertility but in the reciprocal relation of AA ♀ × P ♂ there was complete sterility (figure 19).

The Interspecific Relations of Petunia axillaris and P. integrifolia. *Early Data.* In his reports of hybridization between these two species of *Petunia,* Herbert (1837) stated that seeds were obtained when plants of *P. axillaris* (his *P. nyctaginiflora*) were the seed parents. Later, in 1849, Gärtner reported that the reciprocal relations of hybridization between what he considered to be these same two species exhibited one-way hybridization, but he also reported progeny of each of the two reciprocal relations. The first generation hybrids which Naudin (1865) reported under the name "*P. violaceo-nyctaginiflora*" had "*P. violacea*" for the pollen parent. In considering the reports of so-called hybrids of these species by Correns (1912), Lotsy (1914), Tjebbes (1932), and others, it is certain that some, if not all, of their stocks were of garden cultivation and more or less heterogeneous for hybridity. Especially may this be the case when Tjebbes reported that ten out of eighteen hybridization pollinations of what he considered to be *P. integrifolia* × *P. axillaris* were successful in producing capsules and seeds. Of the various references in the literature, of which the citations above are only a few, possibly the data presented by Herbert are most reliable for plants that were definitely of the species *P. axillaris* and *P. integrifolia*. It is also certain that some of the most important of the recent experimental and cytological studies have applied the name *Petunia axillaris* to white-flowered segregates among cultivated hybrids and that others have used the name *P. axillaris* for the type that is *P. parodii*.

Data Obtained at The New York Botanical Garden. The data on the reactions of *P. integrifolia Kew clone* × *2n P. axillaris* may be summarized as

follows: The twenty-two plants of *P. axillaris* which were grown from seed received from Argentina were all fully tested during 1941 with ramets of the Kew clone in both of the reciprocal relations. When the Kew clone was the seed parent capsules did not even start to develop. In these tests the pollen members included eleven different genotypes in the constitution of the *S* factors (figure 4). Numerous tests were made during each year since 1941 and these included plants of *P. axillaris* of the genotypes *S 1.1, S 1.2, S 1.3, S 2.3,* and *S 3.3*. In every case there was no development of capsules when the Kew clone was the female. Studies of pollen-tube growth were made for the normal pollinations of Kew clone × *S 1.3*. Considerable pollen germinated on the stigma and numerous pollen-tubes extended into the styles but no tubes were found, even after 72 hours, at a point more than ¾ of the distance to the ovary.

The relation of *P. axillaris* × *P. integrifolia Kew clone* gave capsules which contained some viable seeds. Each one of the twenty-two plants grown from wild seed, which included 11 different genotypes, produced such capsules. The same results were also obtained with plants of *S 1.1, S 1.2, S 1.3, S 2.3,* and *S 3.3*. Capsules were not, however, obtained to every pollination, but when several pollinations were made on any one plant some of them resulted in capsules.

Pollen-tube growth was studied for the relation AA: *S 1.3* × Kew clone. The growth of the tubes was relatively slow but at the end of the third day after normal pollinations there were some tubes within the chamber of the ovary. It is to be noted that this one-way hybridization involved special activity on the part of the pollen-tubes of *P. integrifolia*. They grew the entire length of the styles of *P. axillaris,* which were approximately four times the length of the pistils of *P. integrifolia,* and accomplished fertilizations. In the reciprocal relation the pollen-tubes of *P. axillaris* had a relatively short distance to travel yet they not only did not effect fertilizations but they failed to stimulate the development of capsules.

The Degree of the Fertility of the One-Way Hybridization. Nine representative capsules of *2n P. axillaris* × *P. integrifolia* Kew clone were evaluated. The numbers of well-developed seeds per capsule were 88, 112, 144, 151, 226, 310, 315, 343, and 436. The seeds were typical of the normal seeds of *2n P. axillaris* produced by normal cross-fertile intra-specific relations. It was obvious and definite that in respect to the number of seeds obtained the fertility of this one-way hybridization was always much less than that of the potential fertility of the ovules of *P. axillaris* as this was expressed in intra-specific reproduction. Along with the seeds, and especially when the number of seeds was less than 300, there were what may be called scale ovules. These developed from ovules that had enlarged somewhat but which were very much smaller than any seeds and also entirely empty at the time the normal sister seeds were developing. There were two classes of these scale ovules (figure

41). Those which will be referred to as class "*a*" scales were almost transparent and those of the "*b*" class were somewhat larger, brownish in color, and the surface was reticulated but the ridges were low in comparison with those of well-formed seeds. In the ripe capsules of AA ♀ ♀ × Kew clone ♂ ♂ the greater number of these scale ovules represented ovules which were able to function in intraspecific fertility. Hence the fertility of the unilateral hybridization had a relatively low value both in the number of pollinations that failed and in the reductions in the number of seed in a capsule.

The Interspecific Relations of P. parodii and P. integrifolia. *The Data Reported by Mather.* It is certain that the plants which Mather (1944) called *P. axillaris* were typical of *P. parodii* and hence his references will here be applied to this species. He states that hybrids between his plants and the Kew clone of *P. integrifolia* (his *P. violacea*) were obtained only when the latter was the pollen parent. He reports, however, that in 1939 the use of such pollen failed on 101 flowers of *P. parodii* in the greenhouse but that of "about twenty similar crosses made under a muslin cage in the open three gave good capsules of hybrid seed." Later in 1940 three capsules were obtained of 35 flowers pollinated in a greenhouse. It was concluded that the frequency of successful pollinations in this hybridizing relation "depends on external conditions," but that of the tests which were made many flowers failed and few were successful. No report was made concerning the number of seeds per capsule in the successful pollinations.

The Results Obtained at The New York Botanical Garden. Eighteen seedlings of the parent generation (figure 4) of *P. parodii* were tested as pollen parents with the Kew clone. At least several seedlings of each of the generations of *P. parodii* that were grown since then were also tested as pollen parents on ramets of the Kew clone and the Kew subclone and on seedlings of *S a.b*, *S a.a*, and *S b.b* constitution. No capsule of any size was obtained. The specificity barrier to hybridization in this direction was complete.

For the relation of *P. parodii* × *P. integrifolia*, fourteen of the parent generation of *P. parodii* were tested in fully controlled pollinations with pollen of the Kew clone and at least some of each later generation were tested reciprocally with the Kew clone, the Kew subclone, and seedlings that were *S a.b*, *S a.a*, and *S b.b*. These tests were all made of plants grown in a greenhouse. The tests were made at various dates throughout the entire periods of bloom and for young seedlings as well as for propagations one or more years old. Capsules with some seeds were obtained in each relation. It was necessary to emasculate the highly self-fertile flowers of *P. parodii* when they were in the bud, and this may have somewhat reduced the normal development of capsules. There were some complete failures, but when several pollinations were made on the pistils of any plant there were always some capsules. The highest number of seeds observed in any capsule was 173, but most capsules had less than 50 seeds. As a rule the seeds were well-formed and many were

viable. There were numerous scale ovules of both *a* and *b* classes. The fertility of this one-way hybridization was much less than the normal potential fertility.

The Relative Values of Interspecific Reproduction (*figure 19*). The comparative values of the potential fertility of the three species, based on maximum seed production, may be fairly well represented as 100 for *P. parodii,* 60 for *P. axillaris* and 20 for *P. integrifolia* (see figure 19).

The resultants of the unilateral hybridizations admit of the following comparisons. The highest degree of interspecific fertility was for PP ♀ ♀ × A ♂ ♂. This relation involved closest resemblance, least zygomorphism, largest flowers and ovaries in both parental species, and greatest potential fertility. There was slight decrease in the number of seeds obtained in comparison

	P ♂	A ♂	I ♂
P. parodii ♀	100	90	10
P. axillaris ♀	0	60	20
P. integrifolia ♀	0	0	20

FIG. 19. Diagram that indicates the relative values of intraspecific reproduction (100, 60, and 20) in the three species of *Petunia* and the comparable values of the unilateral hybridizations.

with the potenital fertility but this may have been purely incidental to experimental methods, such as premature exposure of pistils after emasculation. If the potential fertility of *P. parodii* is rated at 100 the actual fertility of the PP ♀ ♀ × A ♂ ♂ relations was at least 90.

The lowest degree of the interspecific fertilities was that of PP ♀ ♀ × A ♂ ♂. There were many failures of the pollinations which is an indication that the period when the physiological condition of the pistil is most favorable for the growth of the foreign pollen tubes is much more limited than that which effects intraspecific reproduction. But also the best capsules obtained were of smaller size and the number of normal seeds was always much less than that obtained in selfing the adjacent flowers. The relative value of this hybridization was no more than 10. This relation involved the greatest extremes in the size and zygomorphic characters of the flowers and in potential fertility.

The ovaries of *P. parodii* could produce as many as 1000 seeds but an average of no more than one in ten of the ovules functioned in the hybridization.

The fertility of AA ♀♀ × I ♂♂ was definitely greater than that of PP ♀♀ × I ♂♂. There were fewer failures of the pollinations. The proportion of the ovules that produced seeds was about one of three. Thus the relative or reduced fertility was evaluated at 20. In this relation, compared with PP ♀♀ × I ♂♂, there were less contrasts in size of flowers, potential fertility, and degree of zygomorphism.

The Appraisal of the Unilateral Relations and Reactions. *Basic Considerations.* The three important features of the hybridization resultants are: (1) that there are unilateral reactions, one of sterility and one of fertility, for the reciprocal relations between any two of the three species, (2) that the fertility of the three hybridizations differ in relative values, and (3) that *P. parodii* ♀♀ is fertile with both of the other species but *P. integrifolia* ♀♀ is sterile with pollen of the other two.

Each of the three species has its own mechanism of intraspecific reproduction that is highly efficient. The genetic basis of the ♀♀ and the ♂♂ complexes is in part generic and common to all species of the genus; in part multi-specific or common to three or more species; in part bi-specific or common to only two species; and in part strictly specific. Hybridization brings these genic components into new associations in hybrids and the coordinations of their interactions operate in effecting fertility and sterility. Also two of the three species possess the superimposed mechanism of intraspecific incompatibilities, the genic factors of which can be identified. The particular genic components involved in a unilateral sterility are also transferred to the hybrids. The genic components which determine the interspecific sterility are unknown and it is the particular aim to apply the experimental techniques which will identify them in hybrids and provide an adequate analysis of their relations to the components which effect intraspecific incompatibilities and the more basic fertility.

The Locus of the Unilateral Reactions. The mechanism of reproduction in flowering plants that are hermaphrodite and homomorphic, as are these species of *Petunia*, involves the functions of the so-called ♀♀ and ♂♂ organs and elements that arise by somatic differentiation. The ♀♀ complex includes pistils, ovaries, ovules, macrospores, embryo-sacs, and egg cells. The ♂♂ complex includes stamens, pollen (microspores), pollen-tubes, and sperms.

Many of the properties and characters of the pistils and stamens are adaptations which influence pollination and are in no sense physiological reactions between pistils and stamens. The first of the direct physiological interactions between a ♀♀ and a ♂♂ element is effected by the secretions of the pistil on the germination of pollen and on the growth of pollen-tubes.

In the complete failure of the unilateral sterilities of AA × P, H × P, and H × A the ovaries do not even start to enlarge. They shivel without even a

trace of parthenocarpic influence. It appears that the reaction occurs previously to a union of gametes. The particular unilateral sterilities in the relations under consideration are essentially a diplont-haplont reaction between pistils and pollen-tubes. Its locus is hence the same as that of the intraspecific incompatibilities. The reactions in question are to be distinguished from interactions between gametes, death of embryos, or abortions of seeds as these occur in other relations. Particularly are these initial physiological reactions to be evaluated in the hybrid offspring which may exhibit abortions of stamens and pistils and abortions of spores, all of which effect absolute sterilities previously to pollination and pollen-tube growth.

An obligatory prerequisite to the action of unilateral sterility in the Petunias is that the flowers are bisexual and that the ♀ ♀ and ♂ ♂ complexes are functional in certain other relations.

There are, therefore, no organs or structural units which are exclusively involved in the various fertilities, the intraspecific sterilities, and the unilateral interspecific sterilities under consideration. All operate in diplont-haplont reactons between the pistils and one species and the pollen-tubes of another species. There is obviously less relative specificity in the ♀ ♀ and ♂ ♂ relation that is fertile than in the reciprocal that is sterile.

The Genic Components. In 1944, Mather proposed that there is a simple genic control of the interspecific fertility and sterility in the hybridization of *P. parodii* (his *P. axillaris*) and *P. integrifolia* (his *P. violacea*) which can be demonstrated in the behavior of the hybrids. He presented evidence that a single fertility gene of *P. parodii* has reactions that control both the unilateral reactions of hybridization with *P. integrifolia*. This gene is considered to be allelic to the true *S* factors of *P. integrifolia*. Mather called this gene "*S a*" but for present designation it will be called *S p*. The interpretation by Mather is that pollen tubes that have this gene do not grow down a style that has either one or both of the *S a* and *S b* factors, as *P. integrifolia S a.b* ♀ ♀ × *P. parodii S p.p* ♂ ♂. In the reciprocal relation, in the pistil these factors never inhibit the pollen tubes that possess *S a* or *S b*, as *P. parodii S p.p* ♀ ♀ × *P. integrifolia S a.b* ♂ ♂. But also in the pistils of hybrids of the *S p* factor inactivates *S a* or *S b* to the extent that the incompatibility of syngenic relations does not recur. Thus the "switch action" of the *S p* gene is such that the self-fertility of the F_1 member that is PI: *S p.a* is due to the functioning of *S a* pollen and not to *S p* pollen.

At the time of Mather's publication in 1944 the writer's studies were under way and it was evident that the conception of a switch gene did not apply to the behavior of the hybrids of *P. parodii* × *P. axillaris* or of *P. axillaris* × *P. integrifolia*. In order to make the evaluating tests more definite, homozygous genotypes of *S 1.1* and *S 3.3* were obtained, propagated as clones, and extensively used in testing. Reports of these studies will be made and a more complete examination of Mather's studies will be made in the concluding remarks concerning unilateral reproduction in chapter 15 of this monograph.

Chapter 6. Hybrid Progenies of *P. parodii* × *P. axillaris*

The F_1 Generation. *General Data.* Four series comprising one hundred and five of these hybrids were grown of pollen parents that were *S 1.2*, *S 1.3*, and *S 1.1*. The numbers of seeds in the four capsules were 959, 858, 780, and 490. With few exceptions the seeds appeared to be normal. Seeds selected at random were planted in the numbers of 240, 278, 230, and 160 and the respective numbers of seedlings obtained were estimated at 200, 250, 200, and 150. Seedlings selected at random were grown to flowering age and tested in the numbers of 29, 26, 25, and 25.

Character of the F_1; figures 20 and 21. In only two of the contrasted characters of the flowers was there definite dominance of expression. (1) The stamens were of three lengths as in *P. axillaris* but the anthers dehisced shortly before anthesis as in *P. parodii*. There were intermediate expressions of the length of stamens and pistils, of the size of anthers, of the length and width of the corolla tube, and of the length of pedicels and calyx lobes. (2) The F_1 hybrids were more branched and the stems and leaves were less coarse than in *P. axillaris* but the plants lived longer than did plants of *P. parodii* and were more robust.

Self- and Cross-Fertility. Every one of the one hundred and five members of the F_1 was self-fertile and the tests were adequate to demonstrate that any one was cross-fertile with any other of the entire F_1 population. These resultants showed that the basic mechanisms which operated in the intraspecific fertilities of the two species were coordinated in effecting fertility when combined in the hybrids. This condition simplified the techniques of testing for the action of intraspecific *S* factors and and for the behavior of the components involved in interspecific sterility.

Potential Fertility and Seed Production. The anthers of all the F_1 hybrids dehisced normally and pollen was abundant. Several tests for germination of pollen were made. In every case the pollen was remarkably uniform in size and shape, no grains with four pores were found, and very few grains were shrivelled. On a medium of 1 per cent agar and 5 per cent sugar there was excellent germination of at least 90 per cent of the pollen grains. Thus the microspores as well as the ovules were highly functional and potential fertility was high.

Every one of the 105 F_1 seedlings produced capsules with high numbers of viable seeds to controlled self-pollinations. Extensive tests by cross-pollinations showed that any cross-relation, either intra-series or inter-series, was also fertile. The numbers of apparently normal seeds that were counted in typical selfed capsules of seedlings of all genotypes in the F_1 progenies ranged from 600 to 919. When the number was 800 or more there were few scale ovules, which indicated that almost all of the ovules had functioned in seed formation. Thus the F_1 hybrids had a potential fertility in respect to seed production that was almost, if not, equal to that of plants of *P. parodii*. It was

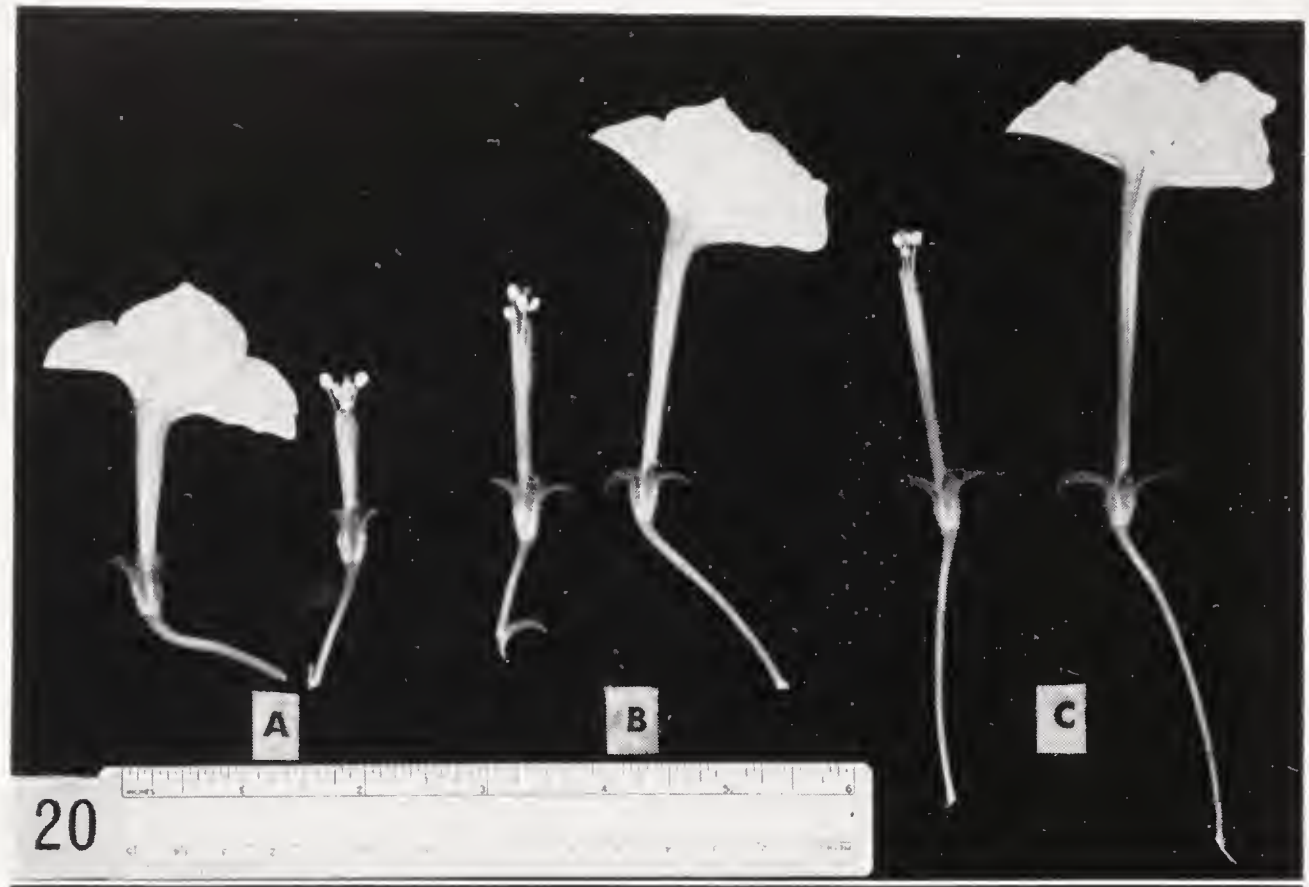


FIG. 20. Typical flower and view of stamens and pistil: A, *2n P. axillaris*; B, F_1 hybrid; and C, *P. parodii*. The hybrid has stamens of three lengths, as in *P. axillaris*. FIG. 21. Typical plants: A, *2n P. axillaris*: E, *2n P. parodii*; B, F_1 hybrid; C, an F_2 plant strongly like *P. parodii* but more robust. D, F_2 very much like *P. axillaris*.

somewhat higher than that usually seen in plants of *P. axillaris*. Very few ovules failed to develop into viable seeds.

Reactions of the F_1 with P. axillaris ♂ ♂. Every F_1 hybrid was fertile as a ♀ ♀ with pollen of *P. axillaris S 1.2, S 1.3,* and *S 2.3*. But the reactions with testers that were homozygous for *S 1.1* and *S 3.3* revealed that there was hidden syngenic incompatibility and the entire F_1 population was composed of plants that had one *S* factor that was still active in syngenic relations.

The progeny of PP ♀ ♀ × AA: *S 1.3* was composed of two groups. The members of one group were incompatible with AA: *S 1.1* ♂ ♂ but fertile with *S 3.3*. They were PA: *S 0.1*. The members of the other group were incompatible with AA: *S 3.3* ♂ ♂ but fertile with *S 1.1* ♂ ♂, and hence they were PA: *S 0.3*. Both groups were fertile with *S 1.2, S 1.3,* and *S 2.3*.

F_1 P. parodii x P. axillaris	n, P⇆A ♂			P ♂	A ♂					P. int.
	S 0.1	S 0.2	S 0.3	S 0.0	S 1.2	S 1.3	S 2.3	S 1.1	S 3.3	
PA: S0.1, ♀	F F S	F	F	F	S F F	S F F	F	S	F	H
" S0.2, "	F	F F S	F	F	F F S	F	S F F	F	F	H
" S0.3, "	F	F	F F S	F	F	F F S	F F S	F	S	H

P. parodii ♀		F	F	F
P. axillaris	S 1.2	F F S	F F S	F
	S 1.3	F F S	F	F F S
	S 2.3	F	F F S	F F S
	S 1.1	F F S	F	F
	S 3.3	F	F	F F S
P. int. S a.b		0	0	0

FIG. 22. This chart indicates the reactions of all members in each of the three genotypes obtained in the F_1 progenies of *Petunia parodii* × *P. axillaris*. The genotypes and the hidden incompatibilities of each *S* factor were revealed by the reactions with testers. All hybrids were reciprocally cross-fertile with both parental species except for syngenic action of a *S* factor. All had unilateral sterility and fertility in relations with *P. integrifolia*.

The twenty-nine members of one progeny and the twenty-six of another had a plant of AA: *S 1.2* for their ♂ ♂ parent. Approximately one-half of the members were cross-incompatible with AA: *S 1.1* but cross-fertile with *S 1.2* and hence were PA: *S 0.1*. There were no plants of AA: *S 2.2* available for use as testers but it is considered that those which were cross-fertile with *S 1.1* were PA: *S 0.2*.

The twenty-five hybrids of one progeny had AA: *S 1.1* for the ♂ ♂ parent. Every one was cross-incompatible with *S 1.1* ♂ ♂ but cross-fertile with *S 3.3, S 1.2, S 1.3,* and *S 2.3* as ♂ ♂. All were PA: *S 0.1*.

The resultants of these tests demonstrated that each of the self-fertile hybrids of the F_1 had one *S* factor that retained its incompatibility reaction in relations with *P. axillaris* ♂ ♂. This indicates that there was also hidden incompatibility in the self-fertility of any F_1 and in the cross-fertility of any two which possessed the same *S* factor. The analysis of F_2 and back-cross progenies, reported later, revealed that these reactions occurred.

The entire F_1 population that was obtained included the three genotypes PA: *S 0.1*, *S 0.2*, and *S 0.3* and their reactions were as indicated in figure 22, without a single exception.

The seed production of the F_1 with *P. axillaris* ♂ ♂ was judged to be equal to that of the self-fertility of the same ♀ ♀ plants. There were definitely no reductions in the number of seed below that of the potential fertility. It should be stated that the dehiscence of anthers before anthesis made it necessary to emasculate for controlled pollinations earlier than in *P. axillaris* and the early exposure of pistils rather frequently affected their ability to function fully in any relation. But repeated tests demonstrated that this feature was incidental to the manipulation.

Data on the reactions of the F_1 hybrids as pollen members with ♀ ♀ P. axillaris. The tests were sufficiently extensive and definite to prove that each member of all four of the F_1 hybrid progenies was fertile as a ♂ ♂ with any genotype of *P. axillaris* ♀ ♀. But each hybrid possessed one *S* factor which was active in its syngenic relations with *P. axillaris* and hence there was hidden incompatibility when the ♀ ♀ of a relation had a factor that was present in the hybrid that was the ♂ ♂ member. The analysis of the back-cross progenies that were grown confirmed this evaluation.

The seed production by the ♀ ♀ *P. axillaris* with pollen of the hybrids was judged to be equal to that of intraspecific reproduction. The capsules were large and well-formed and the number of normal seeds in a capsule was as high as 791. Since the relation AA ♀ ♀ × PP ♂ ♂ was sterile the pollen of the PA hybrids may have included some grains that were so fully *P. parodii* in character that they did not function. In other words such pollen may have possessed the genetic component that was involved in the AA ♀ ♀ × P ♂ ♂ unilateral sterility. But there was no immediate effect of such hidden one-way specific sterility on the number of seeds that were produced.

Reactions of the F_1 Hybrids with P. parodii. Every one of the numerous members of the three genotypes of the F_1 hybrids of PA that were tested was fertile both as a ♀ ♀ and as a ♂ ♂ with plants of *P. parodii* (figure 22). The application of the conception of an *S p* factor that is allelic to *S 1*, *S 2*, and *S 3* would be as follows. The hybridization relation of AA: *S 1.3* ♀ ♀ × PP: *S p.p* ♂ ♂ is sterile. The F_1 PA: *S p.3* × PP: *S p.p* is fertile. Hence the *S p* pollen tubes were functioning in the pistils. Also in the selfing of the F_1 it was the *S p* pollen tubes that were functioning while the syngenic *S 1*, *S 2* or *S 3* factors were active. It is certain that the switch gene, described by Mather in his stock of *P. parodii*, in the hybrids of *P. parodii* × *P. integrifolia* was

either not present in the writer's stock of *P. parodii* or that it had different functions in hybridizations with *P. axillaris*.

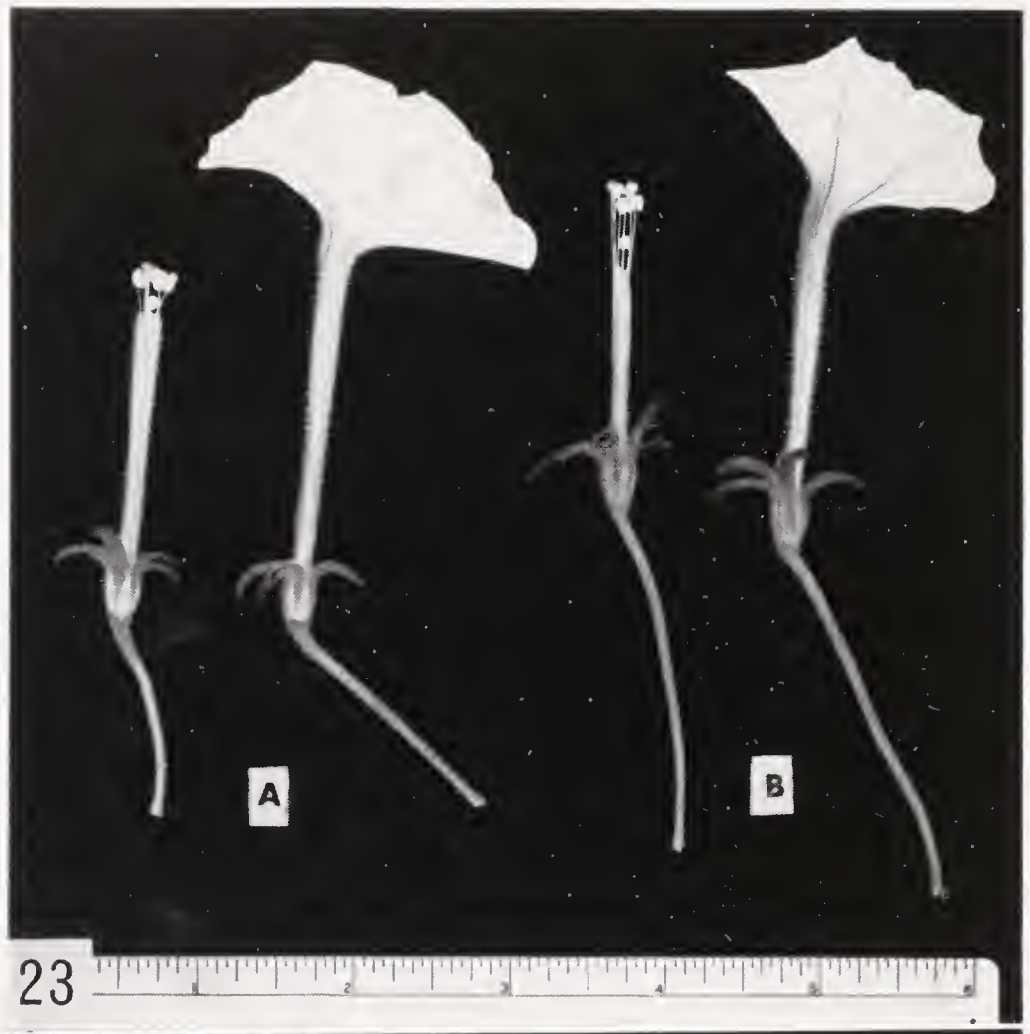
The F_2 Generation; figures 21, 23 and 25. *General Data.* Four progenies of the F_2 were grown of seeds of controlled self-pollinations. A series of twenty-eight members was a selfed progeny of a plant that was PA: *S 0.2*. A series of twenty-nine plants, and another of twenty-five plants, were progenies of PA: *S 0.1*. It was the intention to grow a progeny of PA: *S 0.3* but there was an error in selecting the seeds that were planted and another series of twenty-one plants was also a progeny of PA: *S 0.1*.

Character of the F_2. The members of the F_2 were more diverse than the F_1. Most of them had branches, foliage, and flowers that were intermediate in character, but some, as D in figure 21, were segregates that were strongly like *P. axillaris* in most features. Others, as C in figure 21, were more like *P. parodii*. Twenty-three had stamens of four lengths and eighty had stamens of three lengths which was a segregation of 1 : 3.4+. But there were very few that had stamens as short, or anthers as large, as in *P. axillaris*. In the greater number of plants the flowers were more or less intermediate as shown in figure 23. Yet the F_2 provided much greater diversity than did the F_1, especially in the lengths of the stamens and in the size of the anthers. There was noteworthy correlation between length of corolla-tube and length of pistil and stamens. No plant had the short length of pistil seen in *P. axillaris* and the long stamens of *P. parodii*. In the majority of the plants the anthers dehisced before anthesis.

Potential Fertility. All members of the F_2 had a high degree of potential fertility. The stamens and pistils were fully normal and every plant produced fine capsules with numbers of seeds that were at least equal to those obtained in *P. axillaris*. The best capsules contained few scale ovules.

Data for the Progeny of PA: S 0.2. The twenty-eight members of this series were self-fertile and cross-fertile in the numerous relations that were tested. The sixteen that were tested with *S 1.1* were fertile. There were no genotypes of *S 2.2* that could be used as testers. The members were very fully tested as ♂ ♂ with AA: *S 1.2* and *S 2.3* and found fertile, hence it was evident that no member was *S 2.2*, especially as none was self-incompatible. This progeny was presumably composed of the genotypes PA: *S 0.0* and *S 0.2*.

Progenies of PA: S 0.1. There was a total of seventy-five plants in the three progenies. All were fully self-fertile but one plant. This plant had several intermediate characters and was definitely a hybrid. It was propagated and very fully tested reciprocally with all genotypes of *P. axillaris*. As a ♀ ♀ it was incompatible only with *S 1.1*; as a ♂ ♂ it was cross-incompatible with *S 1.1*, *S 1.2*, and *S 1.3* but fertile with *S 2.3* and *S 3.3*. It was also fertile with sister plants. The self-sterile member was PA: *S 1.1*. Evidently in the selfing of the parent PA: *S 0.1* one pollen-tube that carried *S 1* had functioned and its sperm had fused with an egg that also had *S 1*. This was the only instance



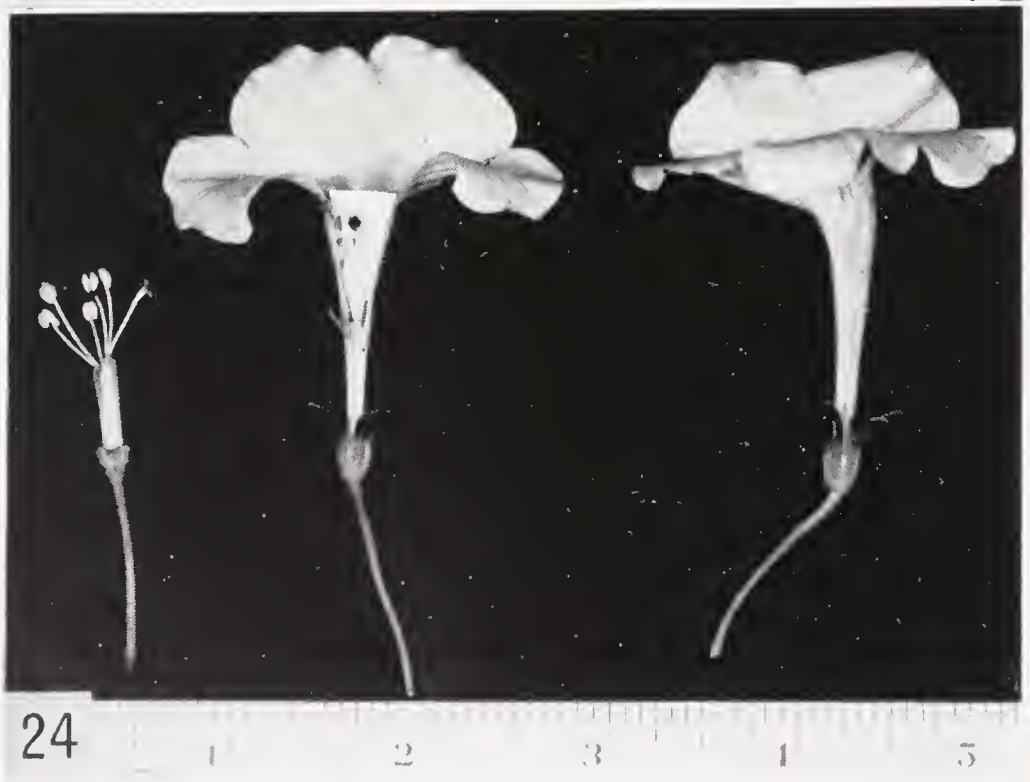


FIG. 23. Flowers of F_2 *P. parodii* × *P. axillaris*: A, flower and stamens near *P. parodii* but pedicels and calyx lobes (and foliage) almost as in *P. axillaris*; B, stamens of three lengths, but anthers, pedicels, and calyx lobes like *P. parodii*. FIG. 24. Typical flowers of F_1 plants of *P. axillaris* × *P. integrifolia*. Typical F_1 plant shown at C in figure 35.

when a pollen-tube which carried an *S* factor functioned in the selfing of any of the F_1 hybrids of PA

The members of each group were very fully tested as ♀ ♀ with pollen of the AA genotypes. The resultants definitely indicated that twenty-one were incompatible only with *S 1.1* ♂ ♂ and were hence PA: *S 0.1* and that fifty-three were fertile with all genotypes and were PA: *S 0.0*. The reactions were without exception as indicated in figure 25. But the two genotypes were definitely unbalanced in relative members in each of the three progenies. The numbers of *S 0.1* and *S 0.0* genotypes were respectively 7 and 22, 9 and 15, and 5 and 16. It is evident that there had been selective fertilization in the

F_2		P ⇌ A ♂			
		S 0.1	S 0.2	S 1.1	S 0.0
P ⇌ A ♀	S 0.1	F F S	F	S	F
	S 0.2	F	F F S	F	F
	S 1.1*	F F S	F	S	F
	S 0.0	F	F	F	F

	A ♂					P. p.	P. int.
	S 1.1	S 1.2	S 1.3	S 2.3	S 3.3	S 0.0	S a.b
S 0.1	S	S F F	S F F	F	F	F	H
S 0.2	F	F F S	F	S F F	F	F	H
S 1.1*	S	S F F	S F F	F	F	F	H
S 0.0	F	F	F	F	F	F	H

P. axillaris					
	S 1.1	F F S	F	S	F
	S 1.2	F F S	F F S	S	F
	S 1.3	F F S	F	S	F
	S 2.3	F	F F S	F	F
	S 3.3	F	F	F	F
P. parodii		F	F	F	F
P. int. S a.b		0	0	0	0

* Only one plant of S 1.1

FIG. 25. Patterns of the reactions of all the F_2 genotypes obtained by selfing F_1 members of *P. parodii* × *P. axillaris*. The members of one genotype had no *S* factors. All members were reciprocally cross-fertile with *P. parodii*, hence no member had the AA constitution that effected the unilateral sterility with *P. parodii* ♂. But all had unilateral reactions with *P. integrifolia*.

selfing of the three *S 0.1* parents. There was, except in one instance, hidden self-incompatibility for the *S 1* pollen tubes and only pollen-tubes which did not possess the *S 1* factor had functioned. But these pollen-tubes functioned 53 times in ovules whose eggs had no *S 1* factor and 21 times with eggs which had the *S 1* factor.

Reactions of the F_2 with P. parodii. The F_2 were especially tested as ♀ ♀ with pollen of *P. parodii* to determine if there were segregations of the genetic component of *P. axillaris* which would give the reaction of unilateral sterility with pollen of *P. parodii*. From three to ten members of each genotypic group of each progeny of the F_2 were thus tested. There was full fertility in every test.

The reciprocal relations were less fully tested but all that were tested were fully fertile. But this relation involved only components that gave fertile reactions; for PP ♀ ♀ × PP ♂ ♂ was one of intraspecific fertility and PP ♀ ♀ × AA ♂ ♂ was one of interspecific fertility, and both favored fertility.

Hence in the reactions of the F_2 hybrids of PA there were no features of hybridization sterility in any of the reactions of reproduction including the relations with *P. parodii.*

The Back-Cross Progenies of P. axillaris × F_1 Hybrids. *General Data and Status.* Two series of plants (341 and 342) were of the parentage AA: *S 1.1* × PA: *S 0.1* and one series (344) was of AA: *S 1.2* × PA: *S 0.3.* Each of the seventy members of these progenies received a haploid constitution of *P. axillaris* which carried one set of the genetic determiners for both the ♀ ♀ and the ♂ ♂ complexes of that species. These included a haploid complement of the ♀ ♀ component that was concerned with unilateral sterility. But each back-cross progeny also received a haploid genetic constitution from its F_1 pollen parent, and these differed according to the segregations in the pollen. It was possible that the extremes of these segregations ranged from a haploid A complement to a haploid P complement. Segregations of a factor or of more than one of the factors of the AA ♀ ♀ complex that was involved in the AA ♀ ♀ × P ♂ ♂ sterility would, in some of the back-cross members, be added to that received from the AA ♀ ♀ parent.

In respect to the known *S* factors, all members of series 341 and 342 received only the factor *S 1* but certain of series 344 could receive two *S* factors and be either *S 1.3* or *S 2.3.*

Appropriate tests were made which identified the *S* factors that were present, and also revealed that the genetic component of AA ♀ ♀ which effected unilateral sterility with P ♂ ♂ had been reassembled.

Data for Series 341 and 342: figure 26. The ♀ ♀ parents were AA: *S 1.1* and the ♂ ♂ parents were PA: *S 0.1.* Fifty-three (35 and 18) seedlings were grown. All were self-fertile and also cross-fertile in all of numerous tests. Every one was cross-incompatible with pollen of AA: *S 1.1* but the reciprocals were fertile. The tests showed that all members of these two series had the factor *S 1* but that pollen of *S 0* (or *S p*) had functioned and there had been hidden incompatibility for all *S 1* pollen-tubes in reactions with the *S 1.1* pistils. The tests indicated that all the members were fertile reciprocally with all genotypes of *P. axillaris* except *S 1.1* ♂ ♂.

Tests with pollen of *P. parodii* were made for forty-two (28 and 14) of these progenies. Twenty (13 and 7) were fertile and twenty-two (15 and 7) were completely sterile. Thus it was demonstrated that the AA ♀ ♀ genic component which effected the hybridization sterility in reactions with P ♂ ♂ had been reassembled in half of the members of these back-cross progenies all of which were *S 0.1.*

In figure 26, in the two columns under "Character" there are given evaluations of the character of the corolla and of the anthers. Plants whose flowers

		Character		Selfed	P. axillaris					P. parodii
		Flowers	Anthers		S 1.1	S 1.2	S 1.3	S 2.3	S 3.3	
341 PA♀ S0.1	1	A-	A-	F	S	F			F	H
	12	Int.	"	F	S	F	F	F	F	H
	5	"	"	F	S		F		F	H
	10	"	P-	F	S	F	F	F	F	H
	16	"	"	F	S	F	F		F	H
P<A♀ S0.1	6	A-	A-	F	S	F	F	F	F	O
	26	"	"	F	S	F	F	F	F	O
	9	Int.	"	F	S	F	F	F	F	O
	18	"	P-	F	S	F	F			O
	22	"	"	F	S	F	F	F	F	O
342 PA S0.1	5	"	A-	F	S	F	F	F	F	H
	10	A-	P-	F	S	F	F	F	F	H
	6	Int.	"	F	S	F	F	F	F	H
P<A♀ S0.1	7	A-	A-	F	S	F	F	F	F	O
	9	"	"	F	S	F	F	F	F	O
	12	Int.		F	S	F	F	F	F	O

FIG. 26. There were 53 seedlings of AA: *S 1.1* × PA: *S 0.1*. All were *S 0.1*. Forty-two were tested with P ♂. Twenty were fertile; but 22 were sterile and in these there had been a recombination of the AA ♀ genic component that had the reaction of unilateral sterility with *P. parodii* ♂. The recovery of unilateral sterility is indicated by the symbol O. Probably the symbol H in this chart should be changed to F.

were near *P. axillaris* in length are listed as A−; those that were near *P. parodii* are rated P−, and others were intermediate. All members had stamens of three lengths, but some were near *P. axillaris* in size and positions (A−) and others were near *P. parodii,* especially in size. It is apparent that there was no close correlation of either of the specificity reactions with any one feature of the flower structure.

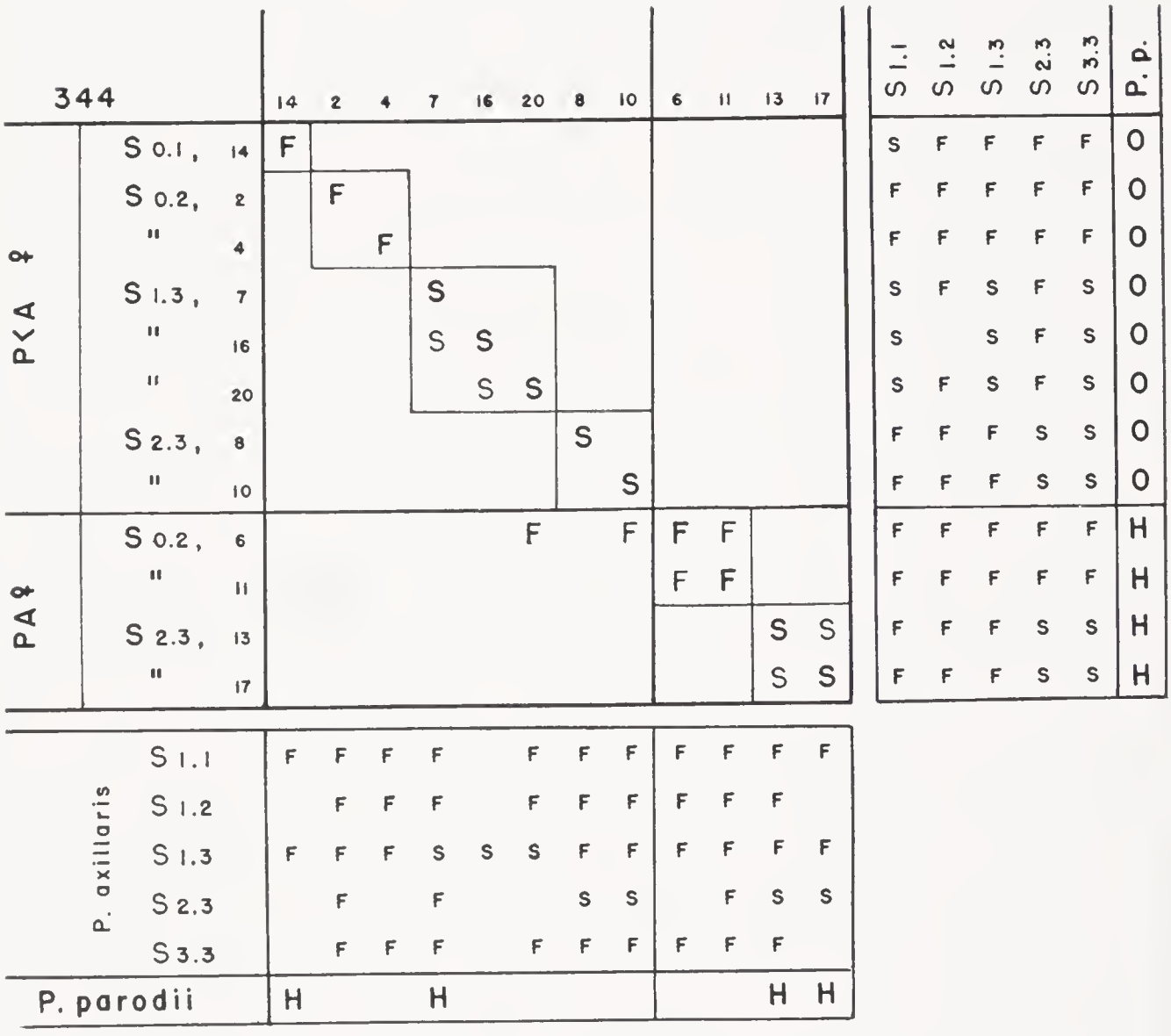


FIG. 27. The back-cross progeny of AA: *S 1.2* × PA: *S 0.3* included four genotypes. Those that possessed one *S* factor were self-fertile but had hidden incompatibility. Those that had two *S* factors were self-incompatible. Of the 12 members that were tested, 8 had unilateral sterility with *P. parodii* ♂ and 4 had fertility.

Data for Series 344; figure 27. The parents of this progeny were AA: *S 1.2* × PA: *S 0.3*. In this relation no incompatibilities were involved and it was expected that both classes of pollen tubes would function and that four genotypes would appear in the progeny.

Twenty plants were grown and tested. Seven were self-fertile and the tests revealed that one of them was *S 0.1* and six were *S 0.2*. Thirteen were self-incompatible and of these three were *S 1.3* and ten were *S 2.3*. Figure 27 in-

dicates the extent to which these were tested. The two members of *S 0.2* and the six of *S 2.3*, which were omitted from figure 27 to conserve space, were equally well-tested and all their reactions were conforming. The segregations of the four genotypes of the incompatibility mechanism and the reactions of all the members demonstrated that the *S 3* factor had retained its identity and character while it was in the self-fertile F_1 hybrid where it had no allelle and had reacted in hidden incompatibility.

The reactions with P ♂ were critical for evaluations of unilateral sterility. At the time when the members of series 344 were in flower few plants of *P. parodii* were in good condition but twelve of the relations with P ♂ and four of the reciprocals were tested (figure 27). Four of these back-cross hybrids were fertile with P ♂ and eight were sterile. The four reciprocals which were tested were fertile.

Twelve of this progeny were tested in reciprocal relations with five genotypes of *P. axillaris* to the extent indicated in figure 27. There was complete fertility, except for the action of incompatibility factors. There were no nonconforming resultants.

Evaluations of the Data on Unilateral Sterility. The AA ♀♀ complex which reacted with P ♂ in a hybridization sterility was reassembled in approximately half of each of the three backcross progenies. The question arises whether this condition was correlated with any one or more of the observable specific characters of *P. axillaris*, as, for example, the relatively shorter pistil. All members of these three series had stamens of three lengths, but so did all of the F_1 and about three-fourths of the F_2 hence it is obvious that the length of stamens and pistil was not correlated with the unilateral reactions of sterility. Some members of the back-cross progenies were more fully like *P. axillaris* than were any of the F_2, but none was as strongly like *P. parodii* as were certain members of the F_2. Special attention was given to the testing of the six plants that were most like *P. axillaris* in the size and shape of the flowers, length of pistil, and size of anthers. As indicated in figure 26 four of these (341 Nos. 6 and 26; 342 Nos. 7 and 9) were sterile with P ♂ but two (341 Nos. 1 and 12) were fertile. There were eighteen others that were also sterile with P ♂ and these had flowers and flower parts more or less intermediate in character. The twenty that were fertile with P ♂ displayed quite the same range in these same characters that was seen in those that were sterile. In the character of their flowers the members of series 344 were almost like the members of 341 and 342.

The segregations in the three series of back-cross progenies which possessed the AA ♀♀ component that effected sterility with P ♂ were not correlated with any one of the observable contrasted features of the flowers. It is to be noted that the two groups of plants that reacted differently with pollen of *P. parodii* were alike in their reactions with *P. axillaris*. The genic components of AA which effected unilateral sterility were not linked with any of the *S* factors and had no direct interactions with any of them.

Reactions of PA Hybrids with P. integrifolia. The F_1, F_2, and back-cross progenies of the PA hybrids were very fully tested in reciprocal relations with *P. integrifolia S a.b.* There was unilateral sterility when *S a.b* was the ♀ ♀ member and hybridization when *S a.b.* was ♂ ♂ with the reduced seed production characteristic of the hybridization when members of the species *P. axillaris* and *P. parodii* were ♀ ♀ members.

SUMMARY AND EVALUATIONS

1. All members of the F_1, F_2, and back-cross progenies of *P. parodii* × *P. axillaris* had a high degree of potential fertility. The basic mechanisms which effected intraspecific reproductions separately in the two parental species were equally efficient when combined in the hybrids.

2. The mechanism of intraspecific incompatibilities derived from *P. axillaris* continued to operate effectively in all hybrids and in all reciprocal relations of these with each other and with *P. axillaris*. Only one instance of a non-conforming reaction was observed. In all cases the incompatibility mechanism was superimposed upon the basic hybrid mechanism which effected fertility and reproduction.

The incompatibility mechanism of *P. axillaris* was entirely inactive in the reactions of the initial hybridization. The pollen tubes of A ♂ found no conditions in the pistils of PP ♀ ♀ which were unfavorable. The seed number obtained per capsule was, apparently, slightly below that of selfing but this did not involve selective action of any *S* factor.

3. All members of the F_1 progenies and all selfed progenies of the F_2, with the exception of one plant, were fully self-fertile and cross-fertile. This was due to the fact that these plants possessed only one *S* factor. The self-fertility was made possible by the presence of only one *S* allelle in the pistils and no *S* factor in half of the pollen. In the F_2 there were plants that had no *S* factor. In certain members of back-cross progenies, self-incompatible plants heterozygous for *S* factors were obtained and in them the basic mechanism of reproduction functioned in selfing and in certain cross-relations quite as in *P. axillaris.*

4. The hybridization relation of AA ♀ ♀ × P ♂ was one of sterility. All F_1 and F_2 hybrids were fertile with pollen of P ♂. Hence the genic component of AA ♀ ♀ which was involved in this sterility was either absent or inoperative. Since no F_1 hybrid was sterile with P ♂ it is concluded that no haploid complement of AA ♀ ♀ had the particular genic complex that was necessary for the reaction with P ♂. Hence it could not be a single factor or any haploid group of complementary factors. But in the back-cross progenies approximately half of the members received that portion of the original AA ♀ ♀ component that effected the sterility with P ♂. The facts suggest that the back-cross recombinations provided an allelic duplication of a single gene (as *X a.a*) and that their combined action effected sterility with *X p*. Since all pollen of *P. parodii* gave this reaction, it may be assumed that this species is homozygous for a single factor (*X p*) which is involved in the reaction.

5. The mechanism of intraspecific incompatibilities in *P. axillaris* and the mechanism of unilateral interspecific sterility that operates in the hybridization relations between *P. axillaris* and *P. parodii* are distinct in genetic control, in status in relation to reproduction, and in their actions in the hybrid progenies.

Chapter 7. The Hybrids of Diploid *Petunia axillaris* × *Petunia integrifolia*

The F_1 Generation. *General Data and Status.* Ninety-nine seedlings were grown to flowering age of the parentage AA: *S 1.3* (139–12) × II: *S a.b* (Kew clone). Both parents had a mechanism of intraspecific incompatibility and the two were alike in that each had a single series of allelic oppositional factors which operated in the simple personate type of behavior. Since the two series were allelic in the hybrids there was no opportunity for a "switch gene" to be also allelic. The hybrid progenies provided material for a study of the behavior of the known *S 1* and *S 3* factors of *P. axillaris* and the known *S a* and *S b* of the Kew clone, not only in the reactions of the initial hybridization but also in the new environments of the hybrid constitutions.

Character of the F_1. There was noteworthy uniformity among the mature plants of the F_1 generation in the character of the foliage, stems and petioles, and in the size and character of the flowers and flower parts. In comparison with the Kew clone the foliage and stems were coarser and more robust, and the flowers and their organs were much larger (figure 24). The spread of the open corolla was nearly equal to that of *P. axillaris* but the throat of its tube was broader. The anthers were larger than those of the Kew clone but smaller than those of *P. axillaris*. The stamens were of three lengths but the anthers of the two longest ones were almost even with the stigma and not well above the stigma as in the Kew clone. After flowering and while capsules were forming the pedicils moved almost to a right angle to the main axis but were not strongly deflexed as in *P. integrifolia*. The capsules were larger than those of *P. integrifolia* but smaller than those of *P. axillaris*.

All the 99 F_1 hybrids had flowers of some shade of purple. The intensities, shades, and tints of coloring of freshly opened flowers included light phlox purple, Hortense violet, amparto-purple, phlox purple, and purple as these are designated in plate XI of the *Color Standards and Nomenclature* by Ridgway. No plant had a flower coloring that closely matched that of the Kew clone or that approached the rose-pink of certain seedlings of the selfed progeny of this clone. As the flowers aged the coloring became more intense in some seedlings but decreased or faded in others.

The Kew clone of *P. integrifolia*, which was the pollen parent of these hybrids, was heterozygous for certain of the factors determining flower color (Mather & Edwards 1944; also chapter 4) and hence some diversity in the F_1 was expected. But the entirely new shades of purple coloring in the F_1

showed the effects of modifying factors that were probably derived from the white-flowered *P. axillaris*.

In habits of growth, the young seedlings displayed the same diversities that were observed in the parental species. Some had axial growth only, and such plants had few flowers. The majority had at least several lateral branches from the rosette and such plants were more branched than was the rule for plants of *P. axillaris*. The hybrids surpassed all seedlings and ramets of the Kew clone in stature, vigor of growth, and coarseness of stems and leaves (figure 35). None had the abnormal type of foliage and flowers which appeared in some of the seedlings of the Kew clone.

The Potential Fertility of the F_1. All members of the F_1 had anthers that were well-formed and normal in dehiscence and there was much loose pollen that was functional in certain cross-relations. Special examinations were made of the pollen of three typical plants of the series (Nos. 21, 16, 15) which were most fully tested and kept under propagation. The percentage of aborted and empty grains in the pollen of No. 15 was 45; the normal grains (55%) varied from 28.5 μ to 36.1 μ in diameter and were very uniformly trilete in shape. In the pollen of No. 16 there were abortions of 60 to 75 per cent and the grains that had contents varied in shape from trilete to quadrilete and in size to 49.4 μ. In the pollen of No. 21 there were abortions of approximately 5 per cent and the ranges in size and shape were like those of No. 16. Other examinations were made which indicated that the abortions of pollen in these F_1 hybrids were seldom less than 50 and often as high as 75 per cent.

Thus there was a high degree of abortion in the microspores of all these F_1 hybrids in comparison with the pollen of the parents and of the hybrids of *P. parodii* and *P. axillaris*. Also there was greater variation in the shape and size of pollen which reached a diameter of 49.4 μ in comparison with the maximum of 38.0 μ for the parents. Several of the F_1 hybrids were found to be diploid and it is believed that all were diploid.

The data for the capsules and seeds may be summarized as follows: Every plant produced capsules and viable seeds in certain relations with sister plants. The best of these capsules were somewhat larger than any obtained of fertile relations between seedlings of the Kew clone (as *S a.a* × *S a.b*) but the capsules were smaller than the typical capsules of *P. axillaris*. The number of seeds in numerous capsules of cross-fertile relations involving many of the F_1 ranged from 83 to 304. Almost without exception these seeds were nearly spherical, plump, and the majority contained an embryo and endosperm. But there were always ovule scales and frequently as many as 250 of them could be counted.

The evidence indicated that approximately half of the pollen grains and ovules (with egg cells) were functional. The abortions were, it seemed, due to irregularities in the chromosomal mechanism of meiosis and sporogenesis. But these abortions did not prevent the production of capsules and viable seeds. The abortions of ovules did reduce the number of seeds below the num-

ber of ovules. Measured in terms of the number of seeds per capsule, the potential fertility was much below that of *P. axillaris*, but in most cases, at least, it was equal to that of *P. integrifolia*.

Self-Incompatibilities: figure 28. Every one of the ninety-nine members of the F_1 were self-incompatible to all controlled normal self-pollinations or close-pollinations and to all autonomous selfing under cage control. There was

			n, A ⇄ I ♂			A ♂						I ♂			P. parodii
F1, 154			21	16	15	S 1.2	S 1.3	S 2.3	S 1.1	S 3.3	S 4.6	S a.b	S a.a	S b.b	
A I ♀	S 3.b	21	S	F	F	F	F	F	F	S	F	F	F	S	0
	S 1.b	16	F	S	F	F	F	F	S	F	F	F	F	S	0
	?	15	F	F	S	S_n	S_n	S_n	S	S	F	F	F	F	0
A A ♀	S 1.2		F	F	F										
	S 1.3		F	F	F										
	S 2.3		F	F	F										
	S 1.1		F	F	F										
	S 3.3		F	F	F										
	S 4.6		F	F	F										
I I ♀	S a.b		0	0	0										
	S a.a		0	0	0										
	S b.b		F_n	F_n	F_n										
P. parodii			H	H	H										

S_n & F_n = nonconforming reactions

FIG. 28. This chart indicates the reactions of a member of each of the three mating groups obtained in an F_1 progeny of AA: *S 1.3* × II: *S a.b*. Two of the genotypes were *S 3.b* and *S 1.b*, but the identity of the third group was uncertain.

no shedding of pollen before anthesis or before there was secretion on the stigma. There were no cases of feeble or partial fertility to selfing. The self-incompatibility indicated that each plant possessed two *S* factors, one from each parent, and that in the hybrid constitutions both *S* factors were active in self-incompatibility.

Cross-Incompatibilities. In the tests of the cross-pollinations numerous irregularities were observed, especially in the cross-fertile relations between mating groups. As soon as several members of an intra-incompatible group

were identified one or more of them were used as testers of other members. In this way two groups of 27 and 26 members respectively were obtained. One large group of 46 members were very irregular in cross-reactions. All five of them that were tested with pollen of the parent plant of *P. axillaris S 1.3* were sterile. Eleven other plants that were tested with the same pollen were cross-fertile. Sixteen plants were fertile with pollen of *P. integrifolia*. This series soon became badly infected with a mosaic disease the virus of which was evidently introduced in tobacco smoke. One plant of each of three mating groups was kept in propagation for several years until homozygous plants of AA: *S 1.1* and *S 3.3* and of II: *S a.a* and *S b.b* were available for testing.

The Analysis of the Genotypes. The results of the tests (figure 28) indicated that the member selected of the group of 27 members was AI: *S 3.b* and that the member of the group of 26 members was *S 1.b*. As ♀ ♀ these two plants were cross-fertile with all genotypes of *P. axillaris* and of *P. integrifolia* except only for normal syngenic incompatibilities. Thus it appeared that the large group of 46 members that gave irregular reactions were *S 1.a* and *S 3.a*. The one member that was kept and tested (154 No. 15) gave nonconforming reactions in that it was cross-fertile with II: *S a.a* ♂ ♂ but cross-sterile with AA: *S 1.2*, *S 1.3*, and *S 2.3* as well as with *S 1.1* and *S 3.3*. It was not possible that plant No. 15 could possess the *S 2* factor.

As pollen parents the three F_1 hybrids were cross-fertile with all genotypes of *P. axillaris*, which indicates that either the *S b* pollen functioned or that *S* factors in pollen were inactivated in such relations as AA: *S 1.1* × AI: *S 1.b*; AA: *S 3.3* × AI: *S 3.b*. The three plants were cross-sterile as male members with II: *S a.b* and II: *S a.a* ♀ ♀ but cross-fertile with II: *S b.b* ♀ ♀, which were nonconforming both for the action of *S* factors or for reactions of unilateral hybridization. At the time of the tests the pressure of other work, and especially with the tetraploids, was such that it was decided to grow only a progeny of the two genotypes *S 1.b* and *S 3.b* that had been identified. It is to be noted that three plants had uniform unilateral sterility and fertility with the third species *P. parodii*.

The Fertility in Terms of Seed Production. Ten capsules were evaluated that were produced by seedlings No. 21 and No. 15 with pollen of AA: *S 1.3* and *S 2.3*. There were from 104 to 190 apparently normal seeds per capsule. Twenty seeds selected at random had an endosperm and an embryo. From 100 to 300 scale ovules were also present in the capsules. The capsules of *P. axillaris* × pollen of the F_1 hybrids were usually as large as the capsules obtained of intraspecific relations and the numbers of seeds in the capsules that were evaluated ranged from 140 to 514. Ten capsules that were obtained with pollen of *P. integrifolia S a.b*, *S a.a.*, and *S b.b* were evaluated. The number of seeds per capsule ranged from 87 to 322 and the twenty-four that were dissected were all normal.

The F_2 Generation. *General Data.* Only one progeny (255) of the F_2

was grown. Its parentage was 154–21 (AI: *S 3.b*) × 154–16 (AI: *S 1.b*). The capsule contained 83 plump seeds but only twenty-nine seedlings appeared above the soil. Twenty-five were potted; twenty-three survived and flowered and were very fully tested. Except for one plant (No. 19) the reactions with all testers were uniform as indicated in figure 29.

Character of Series 255. There was much diversity in the appearance of these plants, in the expressions of the contrasted characters, and in the com-

F_2 255		A⇌I, S 1.b						A⇌I, S 1.3					154-21	154-16	S 1.1	S 3.3	S 1.3	S 1.2	S 2.3	S a.b	S a.a	S b.b	P. p.
		1	9	12	6	2	19	16	4	23	14	21											
S 1.b 16 members	1	S	S	S	S	S	Fn	F	F	F	F		F	S	S	F	F	F	F	F	F	S	O
	9	S	S	S	S	S	Fn	F	F	F	F	F	F	S	S	F	F	F	F	F	F	S	O
	12	S	S	S	S	S	Fn	F	F	F	F	F	F	S	S	F	F	F	F	F	F	S	O
	6	S	S	S	S	S	Fn	F	F	F			F	S	S	F	F	F	F	F	F	S	O
	2	S	S	S	S	S	Fn	F	F	F	F	F	F	S	S	F	F	F	F	F	F	S	O
	19	S	S		S	S	S	F	F		F	F	F	S	S					F	F	S	O
S 1.3 5 members	16	F	F	F	F	F	F	S	S	S	S	S	F	F	S	S	S	F	F	F	F	F	O
	4	F	F	F	F	F	F	S	S	S	S	S	F	F	S	S	S	F	F	F	F	F	O
	23	F	F	F	F	F	F	S	S	S	S	S	F	F	S		S	F					O
	14	F	F	F	F	F	F	S	S	S	S	S	F	F	S	S	S	F	F	F	F	F	O
	21	F	F	F		F	F					S			S	S	S	F		F	F	F	O
154-21 (S 3.b)		F	F	F	F	F	F	F	F	F	F	F											
154-16 (S 1.b)		S	S	S	S	S	Fn	F	F	F	F	F											
S 1.1		F	F	F	F	F	F	F	F		F	F											
S 3.3		F	F	F	F	F	F	F	F		F	F											
S 1.3		F	F	F	F	F	F	S	S		S												
S 1.2		F	F	F	F	F	F	F	F	F	F												
S 2.3		F	F	F	F			F	F		F	F											
S a.b		O	O	O	O	O	Fn	O	O	O	O	O											
S a.a		O	O	O	O	O	Fn	O	O		O												
S b.b		Fn	Fn	Fn	Fn	Fn	Fn	Fn	Fn		Fn												
P. parodii		H	H	H	H	H	H	H	H														

Fn = a nonconforming reaction

FIG. 29. This chart indicates the reactions of an F_2 progeny of *P. axillaris* × *P. integrifolia*. The F_1 parents were *S 3.b* × *S 1.b*. The progeny was composed of *S 1.b* and *S 1.3*, with one nonconforming member.

binations of the different characters. The extremes approached those of the two parental species (figures 30, 31, and 32). Four members closely resembled *P. axillaris* in most characters, seven were decidedly like *P. integrifolia* and eleven were more or less intermediate in most of their characters. The majority of the series had more slender stems and smaller leaves than had the plants of *P. axillaris* even though the flowers were much like those of this species (No. 16 in figure 30 B). Five members had flower coloring almost like that of the Kew clone. Only two had flowers that were almost white and in these the flowers had the size, shape and the color of the pollen that were

almost as in *P. axillaris*. For the others of the series the range of flower coloring included rose-pink and several shades of rose and purple. All had stamens of three lengths. Twelve had the pair of longest stamens slightly above the stigma, as No. 2 in figure 31 A, but not as far above as in *P. integrifolia*. In figure 32 the members of this series are arranged according to their degrees of resemblance to the two parents.

Potential Fertility. Each of the F_2 hybrids had normal flowers with well-formed anthers and pistils. Every plant produced capsules and seeds to certain pollinations. Special studies were made of the pollen of eleven of these hybrids which represented the range of segregations in the character of flowers and stamens. There were definite differences in the pollen. Three plants (Nos. 2, 4, and 14) had pollen in which the abortions were estimated at 28%, 65%, and 65%. Five (1, 11, 12, 13, and 19) had abortions that ranged from 32 to 70% and some of the normal grains were quadrilete. In the pollen of Nos. 2 and 19, which were most like the Kew clone in combination of characters, there were abortions of 75 and 85% and many of the grains that had contents were four-pored and of large size. One plant (No. 16) which was very like *P. axillaris* had at least 60% of the pollen aborted, and of the grains which had contents many were extra small in size and also many of the smaller as well as the larger grains were four-pored, a condition not observed in any other plant of the series.

There were definite individual differences among the members of this F_2 in the size of the capsules and the number of seeds per capsule. Seedling No. 1, which was most like *P. axillaris*, produced capsules of a size near that of capsules *P. axillaris* and the number of seeds was as high as 398, with also about 200 scale ovules. In contrast, seedling No. 2, which was most like the Kew clone, had capsules about half the size of those of No. 1, and the highest number of good seeds in the best capsules was 5. Some of the best capsules produced by plants Nos. 16, 23, 13, 10, and 7 were evaluated. The numbers of good seeds per capsule for different plants ranged from 38 to 464. The largest capsules and the highest numbers of seeds were from plants that were either most like *P. axillaris* or intermediate in the character of the flowers. Undersized and shrivelled seeds were few but there were at least 200 scale ovules of the *B* class on the placenta in most capsules. Presumably these represented ovules that were unable to function in any relation and their abortions corresponded to those of microsporogenesis. But every one of the F_2 had a potential fertility that allowed for reproduction and for tests of the various reactions of fertility and sterility.

Data for the Self- and Cross-Relations: figure 29. Each of the twenty-two members of this progeny was fully self-incompatible. There were two intra-incompatible groups which were reciprocally cross-fertile. One other member was nonconforming in certain of its reactions as a pollen member.

There was complete fertility of all the F_2 members in all reciprocal relations with the genotypes of *P. axillaris* except for syngenic incompatibilities.

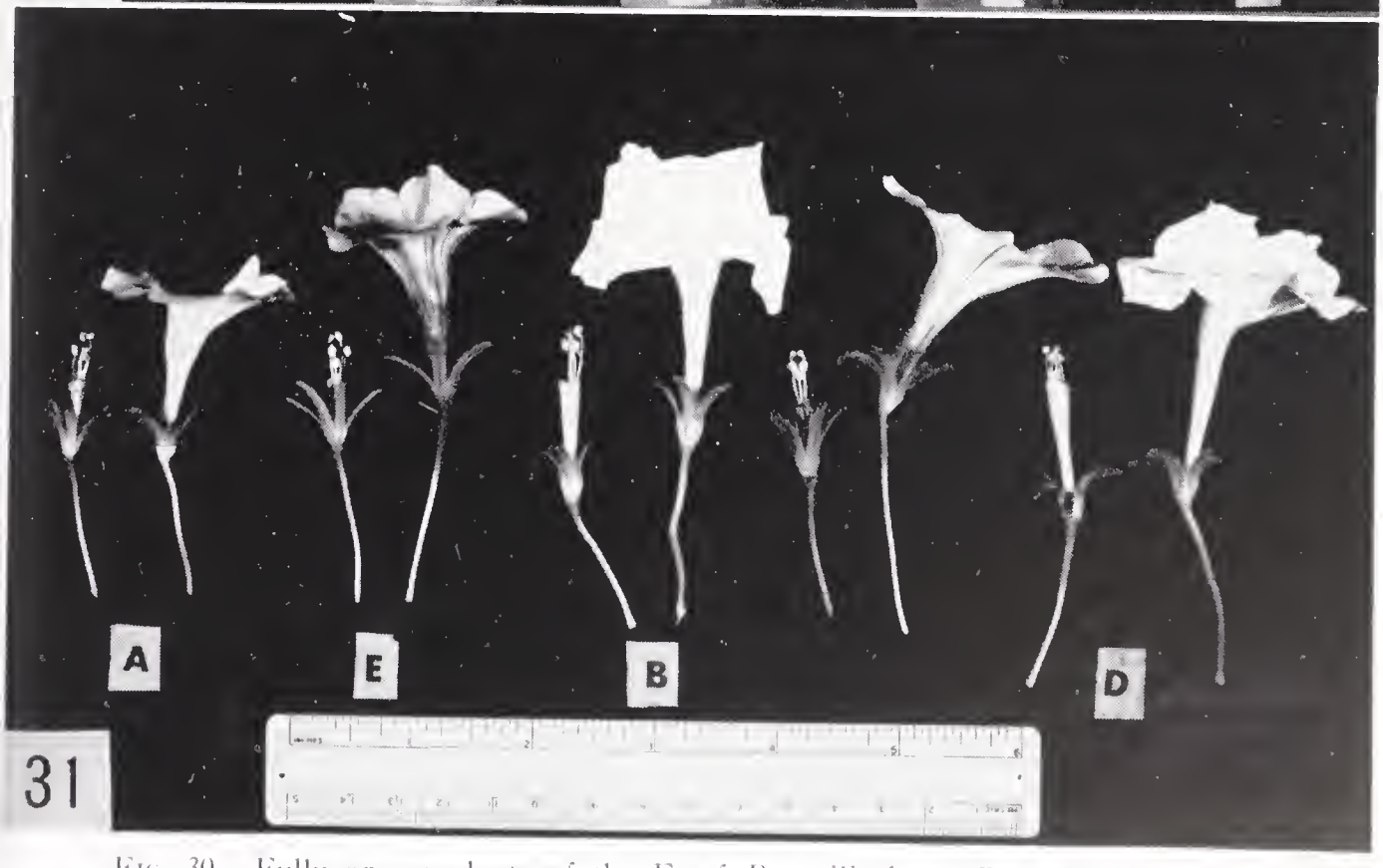


FIG. 30. Fully grown plants of the F_2 of *P. axillaris* × *P. integrifolia*. FIG. 31. Flowers of the same plants, as lettered. Plants A and E were very like *P. integrifolia*; B, C, and D were near to *P. axillaris* but had small anthers.

The one nonconforming member (225-19) was tested only with *S 1.1*. None of the other seedlings had the genic component of *P. integrifolia* that effected unilateral sterility with *P. axillaris*.

As female members the F_2 members were fertile with the three genotypes of *P. integrifolia* ♂ ♂ except for normal syngenic action of the *Sb* factor. But as pollen members there was sterility of all plants of AI: *S 1.b* and *S 1.3* with II: *S a.b* and *S a.a* but fertility with *S b.b* ♀ ♀. The failures could not be due to normal reactions of syngenic *S* factors. They suggest that there were reactions of unilateral sterility in which all pollen had the genic or cytoplasmic component of *P. axillaris* ♂ (*X a*) that reacts with *P. integrifolia* ♀ (*X i.i*) in unilateral sterility. But the reactions of fertility of II: *S b.b* ♀ ♀ with all members of AI: *S 1.b* and *S 1.3* were nonconforming in respect to unilateral sterility and incompatibility. That there were segregations and recombinations of modifying factors and perhaps also mutations in the *S b.b* genotype are indicated. The analysis of these would require the evaluations of a number of the collateral progenies that could be obtained.

255	Most like P. axillaris	⟶ intermediate ⟵	Most like Kew clone
A⇌I S 1.b	1 – – – 18 –	9,12,–8,15–6,5,13,22,– – – 10 –	– 3,7,11,17 – 2 –
" S 1.3	– 16 – 4 – –	– – – – 23 – – – – – – 14 – –	– 21 – – – – –
Aberrant			– – – – 19 – –

FIG. 32. This tabulation indicates the relative character of 22 of the F_2 of series 255 that were most fully evaluated. No. 1 is D in figure 30; No. 16 is B; No. 9 is C; No. 2 is A; and No. 19 is E.

Evaluation of Genotypes. The various critical tests that gave what appear to be normal and conforming resultants indicate that sixteen of the F_2 were A< >I: *S 1.b* and five were A< >I: *S 1.3*. These are the genotypes expected of a parentage that was AI: *S 3.b* × *S 1.b*, provided that the *S b* pollen did not function because of incompatibility. The analysis of the progeny indicates that only the *S 1* pollen had functioned and that the *S b* pollen tubes had syngenic incompatibility in pistils that were *S 3.b*.

One member (No. 19 in figure 29) had various reactions expected of *S 1.b* except that as a ♂ ♂ it was fertile with its sisters that were *S 1.b* and it differed from them in reactions with II: *S a.b* and *S a.a* ♀ ♀.

The unbalanced numbers of sixteen of *S 1.b* and five of *S 1.3* in the F_2 suggest that there was some selective action in pollen-tube growth or in fertilizations that favored the union of *S b* egg cells with *S 1* sperms.

Reactions with P. parodii. With *P. parodii* all members of the F_2 were sterile as ♀ ♀ but fertile as ♂ ♂. This behavior corresponds to that of the F_1 and it was the same as that of the two parental species with *P. parodii* ♂ ♂. The hybrids maintained uniform reactions of unilateral fertility and sterility

with *P. parodii*. Each individual of both the F_1 and the F_2 possessed a genic ♀ ♀ complement which effected sterility with *P. parodii* ♂. Thus the various recombinations and segregations that modified and gave nonconforming reactions of unilateral reactions by AI hybrids had no influence on the reactions with *P. parodii*.

Reproduction in, and among, the AI Hybrids. The syngenic action of *S* factors effected the self- and cross-sterilities that occurred in the reactions between diploid pistils and haploid pollen in both the F_1 and the F_2. The abortions of spores and the reduction in potential fertility of the F_2 were due to chromosomal and genic behavior in processes of meiosis and microsporogenesis and these were independent of the diploid-haploid reactions between pistils and pollen tubes.

Except for plant No. 19 there were no reactions between F_2 members that may indicate a reaction of unilateral sterility. This plant was sterile as a ♀ ♀ with its sisters that were *S 1.b* but fertile with those that were *S 1.3*. It was strongly like *P. integrifolia* in appearances. Possibly it had that component of *P. integrifolia* ♀ ♀ that effected unilateral sterility with pure *P. axillaris*. Unfortunately the only test that was made with genotypes of *P. axillaris* ♂ ♂ was with *S 1.1*.

It is certain that the association of *S b* with *S 1* or *S 3* in the F_1 and F_2 hybrids did not modify the reactions of either in selfing, in intra- or intergroup relations or in relations with either *P. axillaris* or *P. integrifolia* as ♂ ♂. Also there were no segregations in the ♀ ♀ constitution, except possibly for No. 19, that effected unilateral sterility or switch reactions. The cross-fertility of these hybrids as ♀ ♀ with both parents corresponds to the comparable reactions of the PA hybrids but not to those of the PI hybrids studied by Mather which will be discussed later in this paper.

Chapter 8. Autotetraploidy of *Petunia axillaris*

Introduction. In three preliminary publications (Stout & Chandler 1941, 1942: Stout 1945a) it was reported that all of the somatic autotetraploids which were obtained from fifteen different self-incompatible plants of diploid *P. axillaris* were self- and cross-fertile, that all the selfed progenies of these *4n* branches were also tetraploid, self-fertile and cross-fertile, and that in the cross-relations between a *4n* and its *2n* there was a one-way sterility barrier which was an expression of a new specificity in the character of the *4n*.

Further and more complete data with charts will now be presented on the various features of fertility, sterility, and reproduction in intraspecific and interspecific relations which involve the new *4n* genotypes. The relations of the *4n* with the *2n* genotypes are now presented with new data (a) on the influence of *S* factors, (b) on the features of pseudospermy and induced parthenocarpy, and (c) on the syngenic and disgenic reactions in the expressions of the new specificity of the tetraploids. The evaluation of these features indi-

cate that the $2n \times 4n$ relation for these cultures of *P. axillaris* was one of almost complete sterility and that the $4n \times 2n$ reaction for disgenic relations produced some viable seeds.

Methods of Obtaining Tetraploids. Vigorous young seedlings which had several laterals were selected for treatment. Two methods of applying colchicine were employed. Some tetraploid branches were obtained when diploid buds were immersed for four hours in aqueous solutions of colchicine ranging from 0.5 to 0.2 per cent (Blakeslee & Avery 1937; Nebel 1937; Nebel & Ruttle 1938, a, b, c).

Also some tetraploid branches were obtained after buds were thoroughly sprayed with 1.0, 0.4, and 0.02 per cent of colchicine in water in an emulsion of lanolin, stearic acid, and morpholine (Warmke & Blakeslee 1939), in which case there were three applications at intervals of three days. The percentage of the treatments which gave tetraploid branches was low. All of numerous treatments of branches of the Kew clone and of later treatments of *S 1.1* and *S 3.3* genotypes of *P. axillaris* failed to give any tetraploid branches. Three or more tetraploid branches were obtained on sixteen different plants of *2n P. axillaris* and one hexaploid branch was obtained on a triploid hybrid of *4n P. axillaris* × *2n P. integrifolia*.

Character of the Tetraploids of P. axillaris. *The Vegetative Characters.* The *4n* branches had thicker stems and large, coarse leaves. The young leaves that were treated often became somewhat twisted and unevenly expanded. At the time of flowering the *4n* ramets of propagations were more robust than were ramets of *2n* plants. Tetraploid seedlings were much slower in early growth and were later in flowering than were diploid seedlings of the same age. But ultimately the tetraploids grown from seeds or from cuttings surpassed in stature any of the *2n* plants, as shown in figure 35. Tetraploid plants flowered more continuously throughout the winter months than did diploids. Frequently the first internode of the false axis of an inflorescence, which is a bostryx, was fused with the pedicel of the flower to which it was axillary and the point of separation of the two was carried to some distance above the bracteole (figure 34). Occasionally such fusions were repeated in several successive sections of the bostryx.

The Character of the Flowers. In every case the flowers of an original *4n* branch were noticeably larger than the flowers on the *2n* parts of the same plant (figure 33). There was a definite increase in the size of both the anthers and the stigma. These increases in size, together with the thicker stems and coarser leaves, were continued in the cuttings and in the selfed seed-grown progenies of all *4n P. axillaris*. The anthers of all *4n* flowers dehisced normally and pollen was abundant. Numerous examinations were made of the stages of microsporogenesis and some were made of mitosis in the young leaves which showed that the somatic number of chromosomes was *28*, or four times the *n* number of *7*.

Self-Fertility and Cross-Fertility. As soon as the first tetraploid branches

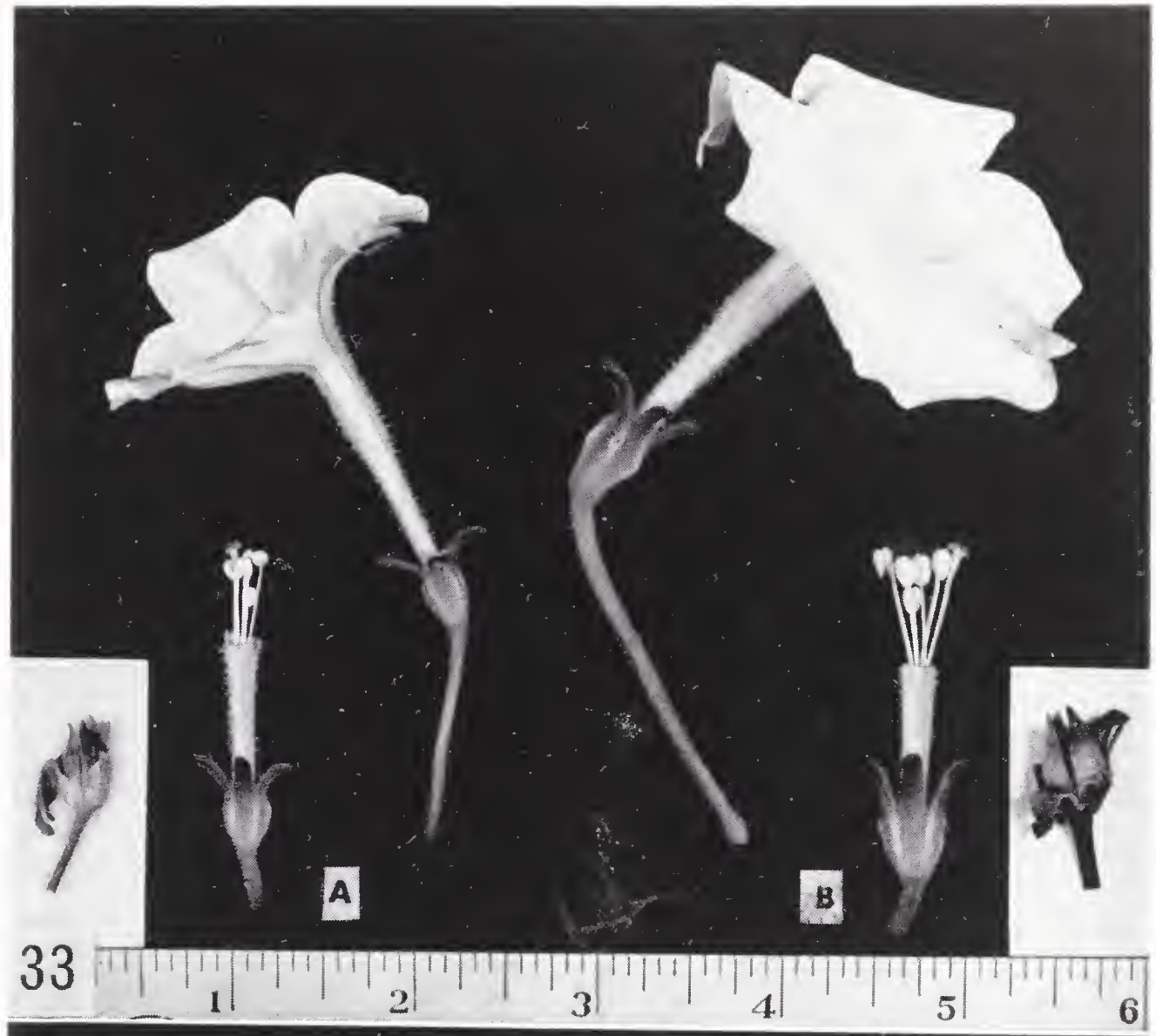


FIG. 33. A, flower and capsule of *2n Petunia axillaris*; and B, flower and capsule of the somatic autotetraploid. FIG. 34. Young tetraploid seedlings showing habits of branching. At right, a wire cage of the type extensively used in controlling pollinations.

had flowers it was found that fine capsules (figure 33 B) which contained numerous viable seeds were obtained of normal self-pollinations. Each of the sixteen original tetraploid branches had flowers that were highly self-fertile while the flowers of *2n* branches on the same plant were fully self-incompatible. It was also determined that there was cross-fertility in all reciprocal relations between any of the different *4n* branches and the ramets grown from them by vegetative propagation. The ovaries of emasculated *4n* flowers on

FIG. 35. Well-grown plants about one year old from cuttings. A, a typical diploid plant of *P. axillaris*; B, an autotetraploid of *P. axillaris*; C, a diploid F_1 hybrid of *P. axillaris* × *P. integrifolia*; D, a ramet of the Kew clone of *P. integrifolia*. FIG. 36. First generation seedlings of the somatic double triploid AAAAII.

plants which were enclosed in cages did not enlarge, which indicated that pollination was necessary for the formation of capsules and seeds. The capsules on the *4n* branches were somewhat larger than those on the *2n*.

The Seedling Progenies of S 1.1.3.3. *Series 142 of the First Generation.* The data for this progeny of seedlings are here given in some detail. Three selfed capsules were obtained from the *4n* branch of the *2n* plant 139–12 which was *S 1.3*. The numbers of seeds judged to be normal in these capsules were 415, 470, and 583. These seeds were planted as soon as the cap-

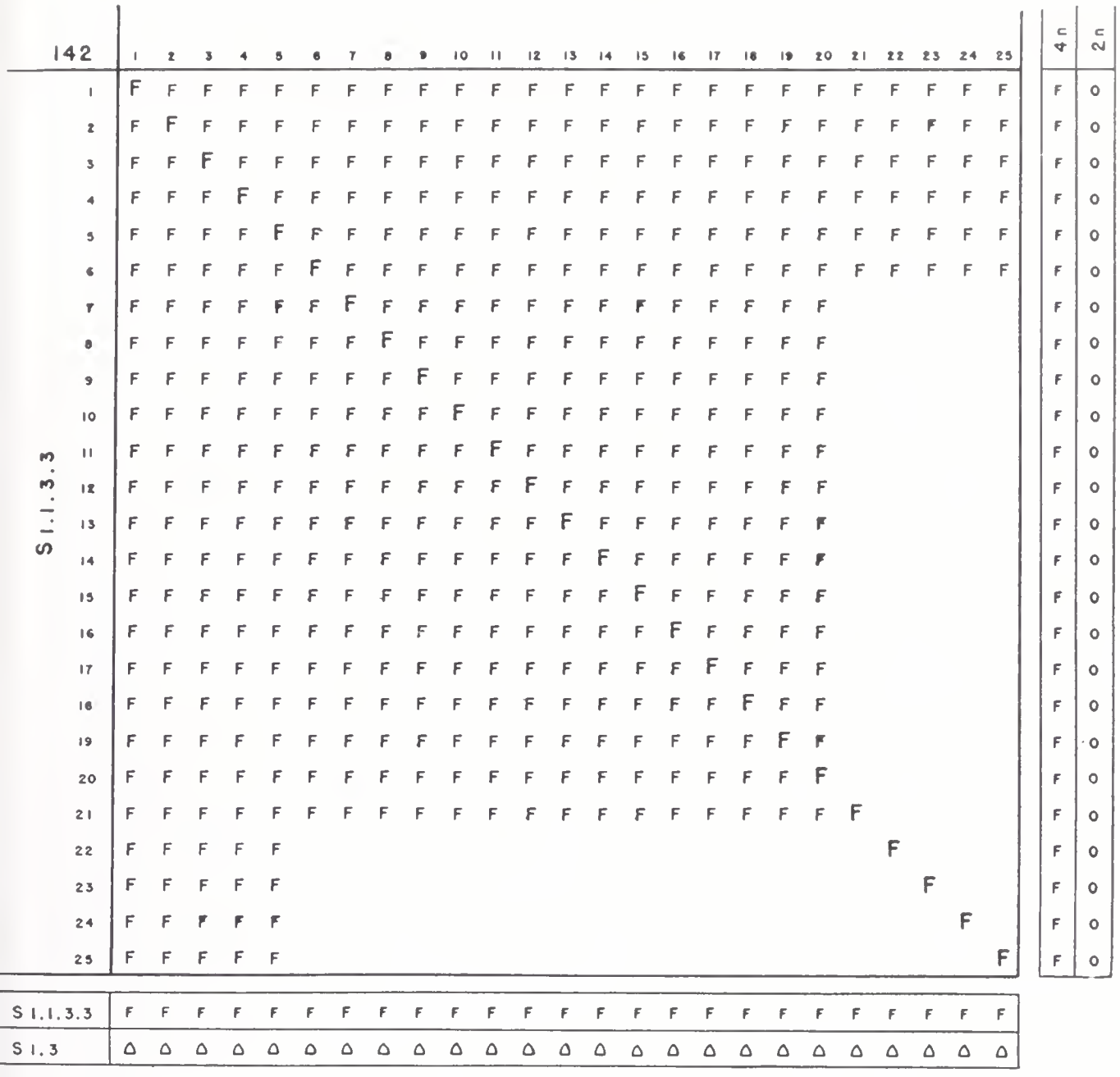


FIG. 37. Chart indicating reactions of a selfed progeny of a somatic autotetraploid of AAAA (*S 1.1.3.3*). The cone-shaped symbol indicates capsules with pseudoseeds. The symbol 0 here indicates complete failure that was due to the incompatibility of a syngenic relation of *4n* × *2n*.

sules were ripe, which was on May 29, 1944. Thus the seeds had no rest period. Germination was irregular and delayed beyond that usually obtained when seeds were planted several months after collection. Seedlings continued to appear until late in the following October. At several intervals throughout the period of the germinations, seedlings were selected for potting and the numbers that were grown to flowering age were 43, 54, and 21.

The first of these seedlings to bloom had flowers in March 1945, ten months after the seeds were planted. But when the ripe seeds of tetraploids were planted during December after several weeks of storage most of the seeds germinated within a period of about four weeks and with good care they began to flower in about six months.

There was no doubt that each of the 118 plants of these three series was a tetraploid. Every one was self-fertile to normal self-pollinations. The term "cross-relation" is used in this report to mean a relation between two plants grown from seed. About 1,500 cross-relations were tested by controlled pollinations of emasculated flowers and every one was fertile. Figure 37 indicates how extensive these tests were. The first 20 plants of series 142 that were numbered were plants that were either well-branched or that were propagated and all of their cross-relations were tested. From three to six of these were tested in reciprocal relations with each of the 23 other members of the series. From three to forty cross-relations were tested for the seventy-five other seedlings of the selfed progeny of the *4n* plant 139–12 (*S 1.1.3.3*). Also many of this progeny were tested in reciprocal relations with their *4n* seed parent and also with the *2n* plant of *S 1.3*.

There was self-fertility for every member of this rather large progeny of tetraploid seedlings and the evidence was conclusive that there was complete fertility for any cross-relation between tetraploids. The *S* factors of the diploid plant 139–12 which effected self and intra-genotypic cross incompatibilities were inactive in the self and cross-relations of all of these *4n* plants. The self-fertility which had first developed in the original *4n* branches was transmitted to all of the seedling progeny.

Data for Series 237. On December 4, 1944, another planting of selfed seed of the *4n* plant 139–12 was made. These seeds had several months of a rest period in storage. Four weeks later there were about 100 seedlings of which 35 were selected at random for potting. These plants were given extra care and on May 24, six months after the seeds were planted, their first flowers opened. Thus these seedlings began to bloom about ten weeks later by calendar dates than did plants of series 142.

Each plant of this progeny was definitely tetraploid in all characters and fully self-fertile. The cross-relations were tested in from three to ten relations for each plant and there was complete fertility for all tests.

Data for Series 329 of the 2d Generation of S 1.1.3.3. In 1947, a series of fourteen plants was grown from selfed seeds of 237 No. 1, and tested. All these plants were tetraploid and self-fertile and there was fertility for each of the numerous relations with the other tetraploids.

The Seedling Progenies of S 1.1.2.2. Four generations were grown from selfed seeds beginning with the seed of the tetraploid branch on the plant 139–40. All members were tetraploid and self-fertile. The cross-relations were not as fully tested as in series 142. Every plant was tested with at least three of its sisters and with some other tetraploids. Many of the reciprocal relations with the *4n* parent were tested. All these relations were fertile. The results of the tests for each progeny were uniform and completely in accord with those presented in figure 37, and hence the data for the cross-relations may be condensed and summarized.

Data for the 1st Generation. A progeny (series 145) of thirty-one mem-

bers was grown: fifteen were tested with the seed parent (139–40, *S 1.1.2.2*); numerous cross relations with sisters and with *4n* plants of *S 1.1.3.3* were tested. All tests were fertile.

Fifty members were grown from the selfed seed of the *4n* plant 139–36. One hundred and seven intra-cross-relations and twenty-seven cross-relations with the seed parent were tested and found fully fertile. Another series of fifty members was grown from the seeds of 139–36 × 139–40 which were both *S 1.1.2.2*. Tests were made for 10 intra-cross-relations and 26 reciprocal relations with both parents. Every relation was fertile.

Data for the 2d Generation. Thirty-six seedlings (series 171) were a progeny of one of the 1st generation of *S 1.1.2.2*. Sixty-seven intra-series cross-relations were tested, several members were tested with the original *4n* clone (139–40) and with three or more of each of all progenies of *4n* seedlings. Each relation was fertile and all plants were self-fertile.

Another series (172) of sixty members of this second generation which had a different plant for the seed parent was grown. All were self-fertile. Numerous of their cross relations were tested and from three to five were tested with each of the genotypes *S 1.1.3.3* and *S 1.1.2.3*. All relations were fertile.

Data for the 3d Generation. Two hundred and ninety-five seeds from one capsule of 171–7 were planted in November. All these seeds were apparently normal and all sank when placed in water. Four weeks later there were at least two hundred seedlings of which twenty-five were selected at random and potted. They grew more slowly than *2n* seedlings of the same date of seed planting. All were self-fertile. The few cross-relations that were tested were fertile.

Another series was from the selfed seeds of a plant of the series 172. Two hundred and nine seeds were planted and about 175 seedlings appeared but there were irregularities in the time of germination. Thirty-six, including some of both the early and the latest to germinate, were potted. Twenty-five were tested. All were tetraploid and self-fertile, and all of the several cross-relations that were tested were fertile.

Data for Plants of the 4th Generation. The members of series 331 (figure 38) were of the fourth generation obtained by successive self-fertilizations of a line descended from the tetraploid branch of *S 1.1.2.2*. Ninety seeds were selected at random from the seeds of a selfed capsule and planted on Jan. 24, 1947. On Feb. 17 there were 75 seedlings and only three appeared later. Twenty-five which were selected at random and the three of late germination were grown to maturity. All were self-fertile and all of various tests with other *4n*'s were fertile. The special tests of the relations of the members of this series with *2n* genotypes are reported later.

The Seedling Progeny of S 2.2.3.3. *Series 327.* This series was a first generation from selfed seeds of the original *4n* branch of the diploid plant 155–19 which was *S 2.3*. Twenty-five plants were potted but four died of root

rot. Figure 34 shows the variations in habits of growth from the extremes of dominant axial growth to dominance of laterals. Each plant was tetraploid and self-fertile. Members of this genotype were tested in reciprocal relations with plants of *S 1.1.2.2* and *S 1.1.3.3* and found fully fertile. Also there were special tests in syngenic and disgenic relations with *2n* genotypes (figure 40) which indicate that all of the plants which were tested were diallelic and possessed both *S2* and *S3* factors.

Progenies of Unknown 4n Genotypes. *Data.* Four progenies were grown from selfed seeds of flowers on the original tetraploid branches of four seedlings of series 139. The parents were not kept in propagation and the *4n* seedlings were discarded after they were tested. The data for these four series are as follows: series 143, parent was 139–25, twelve members; series 144, parent was 139–27, thirty members; series 147, parent was 139–41, twenty-one members; series 218, parent was 139–30, fifteen members. Every plant was tetraploid and self-fertile. From 100 to 278 cross-relations in a series were tested and found fertile. At least three of each series were tested with members of each of the other series, with the *4n* ramets of 139–12 (*S 1.1.3.3*) and 139–40 (*S 1.1.2.2*), and with *4n* seedlings of series 142. There was fertility in every relation.

Data for Triallelic Progenies. *Series 160.* The parentage of this series was 139–40 (*S 1.1.2.2*) × 139–12 (*S 1.1.3.3*) which were ramets of original tetraploid branches. Forty-five seedlings were grown. All were typically *4n* and self-fertile. Twenty-nine were included in 217 tests for cross-relations all of which were cross-fertile; 24 were female parents and 22 were pollen parents in tests with the two parents and all relations were fertile.

Series 222. The parents of this series were 139–12 (*S 1.1.3.3*) × 157–20 of *S 1.1.2.2* parentage. The 24 members were all self-fertile and typical of *4n*. Some cross-relations among the members of the series, with the parents, and with other *4n* were tested and all were fertile.

The Fertility of the Tetraploids. *Self- and Intra-Cross Fertility.* The tests were sufficiently extensive and the results fully definite to prove that each of the seedlings which were grown from seeds of either self- or intra-cross relations of any *4n* plant was not only self-fertile but also cross-fertile with any other *4n*. Even after four generations of selfed progenies all individuals were *4n* and every plant produced viable seeds to self-pollination and to any cross-pollination. If there were any self- or cross-incompatibilities they were hidden. Also if there were any gametes, sperm cells or egg cells that possessed other than *2n* chromosomes these did not function in the fertilizations that gave the selfed or crossed progenies. There was an *inactivation* of the incompatibility mechanism which was derived from the diploid parents and this condition allowed the basic mechanism of reproduction to effect fertility in all the selfing and intra-tetraploid relations. There were no segregations such as were reported by Atwood (1944 a, b) of mating groups in the *4n* of *Trifolium repens*. There were no self-incompatibilities of any tetraploid seed-

ling such as Howard (1942) reported in tetraploids of *Brassica* and *Raphanus* and such as Hecht (1944, 1949) reported for tetraploids of *Oenothera rhombipetala*. Lewis (1947) has reported that none of the numerous seedlings of *4n Oenothera organensis* were fully self-fertile. In the progeny of a prematurely selfed diploid plant of a garden variety of *Petunia*, Kostoff and Kendall (1931) obtained one tetraploid plant that was "self-sterile" but the 16 tetraploid members in a progeny that was obtained when this *4n* plant was crossed with a *2n* plant were self-fertile. The significance of these differences in the behavior of tetraploids derived from self-incompatible plants will be discussed later.

The Evaluation of Capsules and Seeds. The well-developed capsules of all *4n* plants obtained in self- and cross-relations were larger than the capsules of *2n* plants and the walls were somewhat thicker but the surface area of the placenta was at least equal to that of the capsules of *2n* plants.

The number of apparently normal seeds in the typical capsules that were studied ranged from 127 to 583, with an average of 341. The average for capsules which were examined of the F_1 of *S 1.1.2.2* was 322; for the F_1 of *S 1.1.3.3.* it was 257; for the F_2 and F_3 combined the average was 255; and for the triallelic series the average was 291. In most selfed capsules that were examined there were no more than ten shrivelled and empty seeds in a capsule. Otherwise the seeds were plump and most of them had an endosperm and embryo. The majority of these seeds were somewhat larger than the largest seeds of any *2n P. axillaris* (figure 42 L), and they were more spherical, because, it is believed, the seeds were less crowded on the placenta. There was always a high percentage of germination. Frequently when 300 seeds were planted as many as 250 seedlings were obtained, but the germination was usually somewhat slower and more irregular than that of the seeds of *2n* plants of *P. axillaris*. Possibly the matter of a rest period was a factor in the germination of both *2n* and *4n* seeds.

There were always from 100 to 200 scale ovules (figure 41 K) along with the highest numbers of seeds. As a rule these were scattered at random over the placenta and were placed between the good seeds. In their appearance these scales were like those seen in the capsules of hybrids and various polyploids. Such ovule scales were few and apparently only incidental in well-developed capsules of *2n* plants of *P. axillaris*.

The potential fertility of the tetraploids, judged by the number of normal seeds and scale ovules in the capsules, was less than that of *2n* plants. The number of ovules in an ovary was not much different but a greater number of them aborted. Approximately one-fourth of the ovules in tetraploids did not function in seed production. It is considered that this was due to abortions in macrosporogenesis or to abortion of zygotes.

The Evaluations of Microsporogenesis. Microscopic studies of the stages of microsporogenesis were made of smear preparations stained with aceto-carmine. In all *2n P. axillaris* there was very uniform pairing of all of the

seven homologs and four microspores, each with a complement of seven chromosomes, were very regularly formed from a pollen-mother-cell and aborted microspores were few and incidental.

Microsporogenesis was studied in the original tetraploid clones of *S 1.1.2.2* and *S 1.1.3.3* and *S 1.1.2.3* and in several seedlings of each of the generations. The number of cells that were formed from a pollen-mother-cell were often more than four and the highest number observed was eight. A total of 161 pollen-mother-cells in the anthers of a plant of *4n S 1.1.3.3* (139–12) were evaluated during the late stages of spore delineation. There were 94 which produced four microspores that were very uniform in size. In diakenesis the majority of the chromosomes were in pairs; but also groups of four were observed. It was evident that in all of the *4n* plants that were studied a majority of the pollen-mother-cells produced four microspores.

At the late stages of the first meiotic division a separation of *14* and *14* (each with two chromatids) was often observed. In the late stages of the second meiotic division cases were found in which each of the four nuclei derived from a single pollen-mother-cell had *14* chromosomes. But frequently the four young microspores were uneven in size and in the number of chromosomes. Supernumerary microspores were frequent. Of the 161 pollen-mother-cells that were especially studied, 94 produced 4 spores each, 31 produced 5, 32 produced 6, 2 gave 7, and 2 gave 8. In some of the smallest of these, less than 7 chromosomes were counted. There were also some giant cells which contained more than *14* chromosomes.

The Evaluation of Pollen. At the time of the normal dehiscence of the anthers the dry pollen of the tetraploids showed much variation in size and shape (I & J in figure 50). In comparison with pollen of the diploids there were more undersized empty and aborted grains, some of larger sizes and many that were quadrilete in shape.

When the pollen of *4n*'s was placed in glycerine jelly and stained with magenta and menthyl green, or were placed on sugar-agar media for tests of germination, the grains which contained cytoplasm soon swelled to their full or nearly full size. In such preparations the normal grains of *2n* and *4n* plants always stood on one of the two ends where the sutures converged. This placed the equatorial plane parallel to the eye and hence all the pores were in view. It must be that the normal pollen grains have a center of gravity nearer to one end. When pollen grains were almost distended the equatorial cross-section view was three-sided, four-sided, or even five-sided according to the number of germinal pores. Special evaluations of pollen in glycerin-jelly stains were made for seventeen *4n* plants and sugar-agar mounts were made for some twenty-five others.

The percentage of aborted grains in the mounts ranged from 11 to 39. In size the equatorial diameter of grains that had contents ranged from 30.4 μ to 47.5 μ. In one preparation (of 331–8) there were eight pollen grains with five pores each, but otherwise such grains were rather rare. Many grains,

and possibly a majority in some plants, had four pores and when such grains were not fully distended they appeared almost square in outline. About half of the grains which had contents were trilete and usually, but not always, smaller than the quadrilete grains.

The data that were obtained indicated that the degrees of abortions of pollen were fully as great in the later generations of *4n* plants as in the earlier ones. The abortions in members of the fourth generation were as high as 39 per cent and there was also no greater uniformity in the size and shape of the pollen. Numerous tests of the germination of pollen of *4n* plants were made on sugar-agar media of 1 gram agar, 100 ml. water, and 5, 10, and 15 per cent cane sugar. The percentage of grains that were germinated was lower than that of pollen of *2n* plants of *P. axillaris*. Both trilete and quadrilete grains germinated.

The Occurrence of Four-Pored Pollen Grains. Ferguson and Coolidge (1932) report that the pollen of two cultivated tetraploid petunias had some four-pored pollen grains and that the size of the pollen ranged from 49 μ to 58.5 μ while the largest grains of diploid *P. parodii* (called *P. axillaris*) were 49.95 μ in diameter. The occurrence of four-pored pollen of tetraploids that were derived from diploids whose pollen is wholly or usually three-pored has frequently been observed. In one report (Warmke & Davidson 1943) it is stated that diploids had "as little as 1% of four-pored pollen and the tetraploid as high as 90% of such grains" and also the following statements were made: "it has been the opinion of various workers that this increase of pore number results from a different arrangement of the four microspores within the wall of the microspore mother cell. It has been postulated that the three germ pores result from a tetrahedral arrangement of the spores in the tetrad, and that the four-pored condition results from a square or rhomboidal arrangement. . . . Preliminary studies by Dr. Kaiser indicate that there is not sufficient difference in the arrangement of spores within the tetrads of the tetraploid to account for more than a fraction of the four-pored pollen grains observed, and suggest that factors other than tetrad arrangement are of importance in determining germ-pore number."

In the pollen of *2n* plants of *P. axillaris* no quadrilete grains were observed but in the tetraploids there was a majority of such grains. This condition was evidently associated with the increase from *14* to *28* chromosomes in the pollen-mother-cells, but it seems evident that not all the pollen grains which had four pores had *14* chromosomes.

Pollen-Tube Growth in Pistils. A study was made of pollen-tube behavior after normal self-pollination of a ramet of the tetraploid 139–12 (*S 1.1.3.3*) and also of a selfed seedling of series 142. In all cases many tubes were near the base of the style 32 hours after the pollination and at 46 and 48 hours there were numerous tubes in the chamber of the ovary and some were traced into the micropyles of ovaries.

Fertilizations. Every individual that was grown of all the selfed and

intra-bred progenies of the *4n* plants was evidently tetraploid. This indicated that each of the egg-cells and sperms that functioned in such relations had two sets or genomes of chromosomes. That there were some pollen grains, and possibly also egg cells, that had one set of chromosomes is certain. These did not function in the self- and cross-reproduction of the *4n* plants. That some of such gametes may function in certain other relations will be shown later.

The Reactions between 4n and 2n Genotypes of P. axillaris. *The Earlier Reports.* The preliminary reports (Stout & Chandler 1941, 1942; Stout 1945 a) were based on the resultants which involved the flowers of a *4n* branch, or clone derived from it, and the flowers of the *2n* plant or clone from which the particular *4n* was derived. In all such relations the *4n* × *2n* relation was completely sterile in that capsules did not begin to develop but the *2n* × *4n* relation gave fine capsules which contained many seeds of good size. But the later evaluations of such seeds revealed that very few had an embryo and endosperm. They were "pseudoseeds" and the development of the capsule was chiefly a stimulated parthenocarpy. This was a new feature of sterility not observed in the diploid species or in any of the *2n* hybrids that were grown.

Disgenic and Syngenic Relations. In 1944 it was determined that a *4n* × *2n* relation would give capsules and many pseudoseeds when the pollen member possessed at least one *S* factor not present in the *4n* member. When there are no *S* factors common to both the pistil and the pollen a relation may be called *fully disgenic.* When only one factor of a pollen member is also present in a pistil member (*S 1.2* × *S 1.3* or *S 1.1.2.2* × *S 1.3*) the relation is disgenic for one *S* factor and syngenic for the other. The term "fully syngenic" may be applied to a *4n* × *2n* relation in which the pollen member has all the *S* factors present in the seed member. In these relations the reactions were between *4n* pistils and *n* pollen tubes. The same terms may be applied to the *2n* × *4n* relations in which the reactions are between *2n* pistils and segregations in pollen of which many are known to be *2n*.

The original clones of *S 1.1.2.2* and *S 1.1.3.3* were kept in vegetative or clonal propagation and in 1945 a tetraploid clone of *S 2.2.3.3* was also obtained. There were also clones of three or more selfed seedlings of each of the original tetraploids and also some of each of the later generations of seedlings. These were very fully tested in reciprocal relations with both the heterozygous and the homozygous genotypes of *P. axillaris.* All *2n* × *4n* relations gave parthenocarpic capsules, pseudoseeds, and a few normal seeds. But the *4n* × *2n* relations gave differential resultants for which special tests were made of the members of three series which will now be reported.

The 4n × *2n Reactions of Series 331.* This series was of the fourth generation of successive selfing beginning with the original somatic tetraploid of *S 1.1.2.2.* With *S 1.1* and *S 1.2* testers (figure 38) there was complete sterility; with testers of *S 3.3, S 1.3, S 2.3,* and *S 4.5* there were capsules, pseudoseeds and an occasional good seed.

31	Selfed	Syngenic		Disgenic			
		1.1	1.2	3.3	1.3	2.3	4.5
1	F	0	0	△		△	
2	F	0	0	△		△	
3	F	0	0	△	△	△	△
4	F	0	0	△	△	△	△
5	F	0	0	△	△	△	
6	F	0	0	△	△	△	△
7	F	0	0	△	△	△	△
8	F	0	0	△		△	△
9	F	0	0	△		△	△
10	F	0	0	△	△	△	△
12	F	0	0	△	△	△	
19	F	0	0	△	△	△	
24	F	0	0	△	△	△	△
25	F	0	0	△	△	△	△
26	F	0	0	△	△		
27	F	0	0	△			

329	Selfed	Syngenic			Disgeni		
		S 1.1	S 3.3	S 1.3	S 1.2	S 2.3	
1	F	0	0	0		△	△
2	F	0	0	0	△	△	△
3	F	0	0	0	△	△	△
4	F	0	0	0	△		
5	F	0	0	0	△	△	△
6	F	0	0	0	△	△	△
7	F	0	0	0	△	△	△
8	F	0	0	0	△	△	
9	F	0	0	0	△	△	△
10	F	0			△		
11	F	0	0	0	△	△	△
12	F	0	0		△		
13	F	0	0	0	△		
14	F	0	0	0	△	△	

4n, S 1.1.3.3

39

FIG. 38. A selfed progeny of *S 1.1.2.2* produced no capsules to pollen of the *2n* pollen parent, but when the *4n* × *2n* relation was disgenic for one class of pollen there were large capsules which contained many pseudoseeds and few viable seeds. FIG. 39. The reactions of the progeny of selfed *S. 1.1.3.3* indicate that each member had at least one factor of both *S 1* and *S 3* and was diallelic (*S 1.1.3.3*).

The 4n × 2n Reactions of Series 329 This series was a second generation of selfed progenies derived from the original branch of *S 1.1.3.3*. The resultants (figure 39) were, without an exception, complete sterility in every relation with pollen of *S 1.1*, *S 3.3*, and *S 1.3* but with pollen of *S 1.2*, *S 2.3*, and *S 4.5* there were capsules, pseudoseeds and some good seeds.

The 4n × 2n Reactions of Series 327. This was a progeny of the first generation obtained by selfing the original branch of *S 2.2.3.3*. The members were sterile with pollen of *S 3.3* and *S 2.3* but with pollen of *S 1.1*, *S 1.2*, *S 1.3*, and *S 4.5* there were capsules, pseudoseeds and a few good seeds (figure 40).

Evaluations. For the *4n* × *2n* relations, capsules and seeds were obtained only when a relation was disgenic for at least one *S* factor. The capsules were

usually equal in size to those produced by *4n* plants to self-pollination. The number of seeds of full size in the well-formed capsules ranged from 175 to 514, but many of them were more or less collapsed. There were also numerous

4n, $S_{2.2.3.3}$ 327	Selfed	$S_{1.1.2.2}$	$S_{1.1.3.3}$	$S_{2.2.3.3}$	Syngenic $S_{3.3}$	Syngenic $S_{2.3}$	Disgenic $S_{1.1}$	Disgenic $S_{1.2}$	Disgenic $S_{1.3}$	Disgenic $S_{4.5}$
2	F				0	0	Δ	Δ	Δ	Δ
3	F				0	0	Δ	Δ		
4	F	F			0	0	Δ	Δ	Δ	Δ
5	F	F	F	F	0	0	Δ	Δ	Δ	Δ
6	F	F			0	0	Δ	Δ	Δ	Δ
7	F				0	0	Δ	Δ	Δ	Δ
8	F	F			0	0	Δ	Δ	Δ	Δ
9	F	F	F	F	0	0	Δ	Δ	Δ	Δ
10	F	F			0	0	Δ	Δ	Δ	Δ
11	F	F			0	0	Δ	Δ	Δ	Δ
12	F	F		F	0	0	Δ	Δ	Δ	Δ
13	F	F		F	0	0	Δ	Δ	Δ	Δ
14	F	F			0	0	Δ	Δ	Δ	Δ
15	F				0	0	Δ	Δ	Δ	Δ
16	F				0	0	Δ	Δ	Δ	Δ
17	F				0	0	Δ	Δ	Δ	Δ
18	F				0	0	Δ	Δ	Δ	Δ
19	F	F	F	F	0	0	Δ	Δ	Δ	Δ
20	F	F	F	F	0	0	Δ	Δ	Δ	Δ
21	F				0	0	Δ	Δ	Δ	Δ

FIG. 40. The reactions of a progeny of selfed *S 2.2.3.3* indicate self-fertility, cross-fertility with any other autotetraploid, cross-incompatibility with a fully syngenic *2n* ♂, and capsules with mostly pseudoseeds when the *2n* ♂ was syngenic for at least one *S* factor.

ovule scales, and their relative number corresponded closely to those of the selfed capsules. Dissections revealed that the collapsed seeds and most of the plump seeds were empty but that a few seeds were normal and from them a total of 31 seedlings were grown.

Capsules of the *2n* × *4n* relations were obtained in any syngenic or disgenic relation. Figure 41 J shows seeds that were typical of those obtained in the disgenic relation of *S 1.2* × *S 1.1.3.3*. The number of the pseudoseeds and good seeds ranged from 234 to 485. Many of them were collapsed and many of the plump seeds had no embryo. In one test seventy-five plump seeds in a capsule of *S 1.3* × *S 1.1.2.2* were planted: three germinated.

The *4n* × *2n* reactions involved *4n* tissues of the pistil and haploid pollen tubes. The reaction was one of sterility whenever the *4n* pistil contained an *S* factor for which the diploid ♂ ♂ plant was homozygous (as *S 1.1.3.3* × *S 3.3*) or whenever a ♂ ♂ member had both of the *S* factors of a ♀ ♀ member (as *S 1.1.3.3* × *S 1.3*). These resultants indicate that an incompatibility reaction occurred in the syngenic relations of *4n* pistils and *n* pollen tubes. But in all disgenic relations of *4n* × *2n* there were parthenocarpic capsules, pseudoseeds, and a few normal seeds. Possibly there was incompatibility reaction for the tubes that possessed an *S* factor. But there was only limited functioning of those tubes which had no common *S* factor. The *4n* × *n* resultant of feeble or limited fertility expressed a new specificity of the *4n* tissues in comparison with the *2n* tissues of the same genotype.

The reciprocal *2n* × *4n* relation involved *2n* pistils and pollen that was chiefly *2n* but of which some grains were haploid. Hence a selective action was possible. The analysis of the members grown from seeds of the *4n* × *2n* in regard to their chromosomes and reactions in critical relations will provide evidence on the kind of gametes that functioned in their production.

The new specificity of the *4n* is, in part, displayed in the change to self- and unrestricted inter-fertility and in the almost complete failure to "hybridize" with the diploids of *P. axillaris*.

The Interspecific Relations of Tetraploid P. axillaris with Diploid P. parodii. *Data for the Tetraploids as Females.* Fifty-two of these relations were tested and all were fully sterile to the degree that capsules did not start to develop. Some members of all the different tetraploid genotypes were included in the tests and members of three different progenies of *P. parodii* were used as pollen testers.

Data for the Tetraploids as Pollen Members. These tests involved thirty-two seedlings of *P. parodii* and members of the genotypes *S 1.1.2.2*, *S 1.1.3.3*, *S 2.2.3.3*, and *S 1.1.2.3*. In every relation capsules of good size were obtained and the numbers of seeds in the typical capsules that were evaluated ranged from 161 to 836. In capsules which had the higher numbers there were relatively few scale ovules. The seeds were all, or nearly all of full size, but at least one-fourth of them were collapsed and empty. Many of the plump seeds had embryo and endosperm and were viable. Seedlings were grown to flowering age but not studied. Presumably these plants were triploids.

Evaluation. The hybridization of *4n P. axillaris* with *2n P. parodii* was one of sterility when *P. parodii* was the pollen parent but one of fertility in the reciprocal relation. These unilateral reactions corresponded to those of diploid *P. axillaris* with *P. parodii*.

The Interspecific Relations of Tetraploid P. axillaris with P. integrifolia. *Data for P. integrifolia × 4n P. axillaris.* In every test there was complete sterility; the ovaries did not begin to enlarge. The tests included ramets of the Kew clone and Kew subclone, twelve seedlings of *S a.b*, four of *S a.a*, and three of *S b.b*, and about twenty-five seedlings of each of the genotypes *S 1.1.2.2* and *S 1.1.3.3*, and some of *S 2.2.3.3* and *S 1.1.2.3*. The sterility corresponds to the unilateral sterility of *P. integrifolia* × diploid *P. axillaris*.

Data for the Reciprocal Relation. Numerous tests were made of this relation. Pollen was used of all clones and genotypes of *P. integrifolia* with some of all the different tetraploid genotypes. At least half the pollinations entirely failed and there were numerous tiny capsules with no seeds of any size. Only twelve capsules with seeds were obtained. The number of "seeds" in the five best capsules ranged from 50 to 253, but most of them were collapsed and these were of various sizes intermediate between scale ovules and full-sized seeds. The best capsule was about one-third the size of the typical selfed capsules of the *4n*. It had 120 almost globular seeds, 133 shrivelled pseudoseeds, and about 200 scale ovules that could be counted. All the seeds were planted in soil. Six weeks later one seedling appeared and at ten weeks there were eight which were grown to maturity (series 170).

Thus the unilateral hybridization resultants seen in the relations of *2n P. axillaris* with *P. integrifolia* were maintained in the corresponding relations of *4n P. axillaris*.

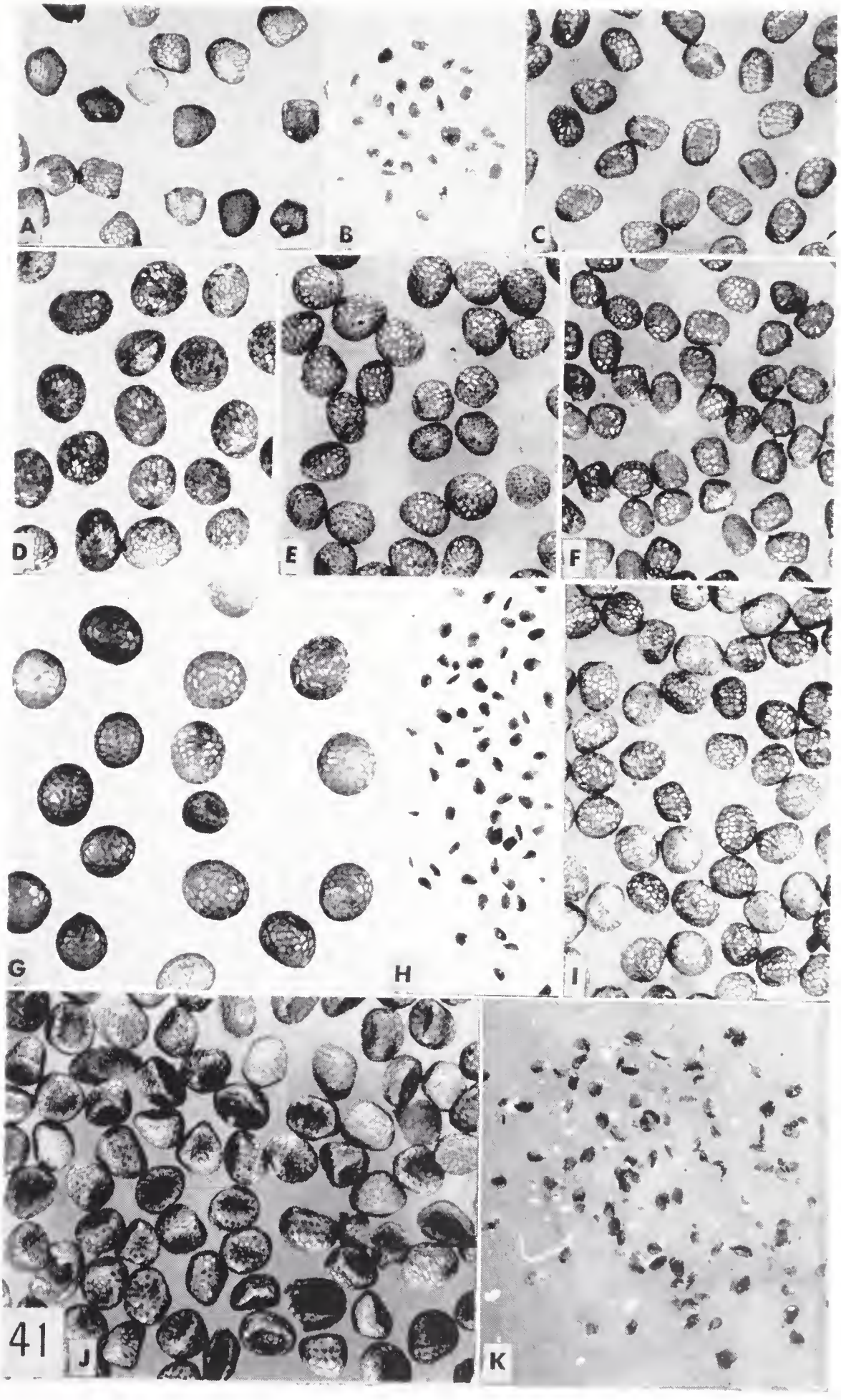
Summary and Conclusions

Self- and Cross-Fertility. The eighteen series of selfed *4n* progenies comprised 720 individuals. Each plant was self-fertile. Several thousand cross-relations between members of a series, members of different generations, and members of different genotypes were tested and every one was fertile.

The three factors *S 1*, *S 2*, and *S 3*, were inactivated in all the self- and cross-reactions of *4n* plants, at least in the functioning of certain of the *2n* pollen tubes.

Explanation of figures 41 and 42

Figs. 41 and 42. Unless otherwise stated the photomicrographs of these figures are approximately 8 ×. A, typical seeds of *2n P. axillaris*; B, scale ovules of the *b* class that are few in cross-fertile relations of *2n P. axillaris*. C, typical seeds of *P. parodii*; D, seeds of *P. integrifolia* of *S a.a* × *S b.b*; E, seeds of an F_2 AI hybrid; F, seeds of an F_1 PA hybrid; G, seeds of a triploid AAI, of premature selfing, and H, ovule scales; I, seeds of AAA × AA, of a fully disgenic relation, but very few will germinate; J, pseudoseeds of *2n (S 1.3)* × *4n (S 1.1.3.3) P. axillaris*, about 12 ×, and K, scale ovules that were present but not numerous; L, seeds of selfed AAAA, that were larger and more spherical than seeds of AA shown by A; and M, scale ovules that were abundant; N, seeds of the original somatic double triploid AAAAII, and O, scale ovules that were abundant; P, seeds of a *6n* seedling; Q, seeds of a pentaploid seedling obtained from *6n* × *4n*; R, seeds of an aneuploid of *sn: 31*; S, seeds of a triploid AAI *(S 1.3.b)* × AAAA *(S 1.1.3.3)*.


A
B
C
D
E
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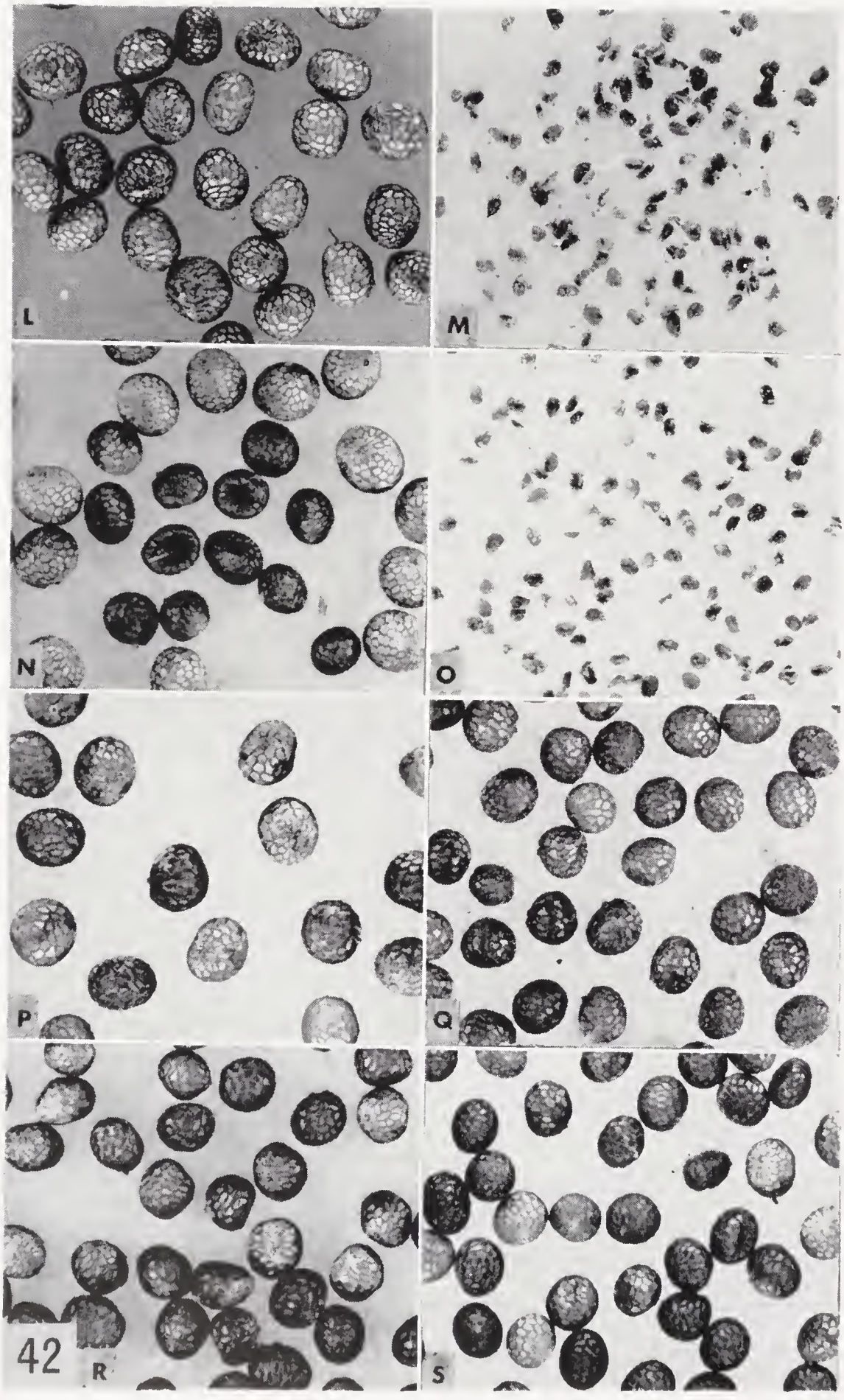
I
41
J
K


L
M
N
O
P
Q
42
R
S

Potential Fertility. In the tetraploids there was abortion of approximately one-fourth of the pollen and ovules (presumably of macrospores) and this did not decrease in the 1st, 2nd, 3rd, or 4th generations that were grown. Yet all the tetraploids had a potential fertility that gave an average of about 400 viable seeds per capsule which was ample for continued reproduction of the tetraploid constitution.

Genotypic Constitution. Every member of every selfed and cross-breed progeny of *4n* parentage was tetraploid. This indicated that all the egg cells and sperm cells that functioned in this reproduction were diploid.

The resultants of the tests with different *2n* genotypes of *P. axillaris* definitely indicate that the diallelic constitution of each original tetraploid obtained by doubling *S 1.2*, *S 1.3*, or *S 2.3* was maintained in all of its selfed progenies, and for *S 1.1.2.2* there were four generations. The tests of *4n* × *2n* reactions in syngenic and disgenic relations were critical and definite for this analysis. Such uniformity in constitution could result from the preferential pairing and disjunction of chromosomes that carried the same *S* factor or from the selective functioning of only heterogenic pollen.

One seedling progeny was grown from *S 1.1.2.2* × *S 1.1.3.3* and one of *S 1.1.3.3* × *S 1.1.2.2*. These plants were presumably *S 1.1.2.3*, but no further generations were obtained.

Specificity. The autotetraploids of *P. axillaris* possessed several fundamental features of specificity. (1) They had distinctive vegetative and floral characters, and they were self- and intra-cross fertile. (2) There was stability in their reproduction. They bred true to their own type. (3) They exhibited a physiological barrier to hybridization with *2n P. axillaris* which was almost complete in that few seeds with an embryo were formed. Thus when such a tetraploid arises, even as a somatic branch, it can maintain an efficient and almost completely independent reproduction even if the progenies grow beside members of the parental diploid species.

The Inactivation of S Factors. The inactivation of *S* factors in the pollen-tubes of the self-fertile tetraploids that are derived from self-incompatible diploids has been conceived to be due to a competition between two different *S* alleles that are present in nuclei of the pollen-tube (Lewis & Modliboroska 1942; Lewis 1947). Also the basis of the incompatibility reaction is assumed to be an antigen-antibody reaction (Lewis 1943a). According to this view tetraploids that were monoallelic (as *S 1.1.1.1*) would be self-incompatible and also monoallelic diploid pollen (as *S 1.1*) of a tetraploid plant (as *S 1.1.2.2*) would be incompatible in selfing. The efforts to obtain monoallelic tetraploids by colchicine treatment of *P. axillaris S 1.1* and *S 3.3* were unsuccessful and hence critical experimental tests of such plants were not possible. But the data indicate that all tetraploids which were obtained of *P. axillaris* were either diallelic or triallelic and fully self-fertile which supports the view that there is inactivation of *S* factors in heterogenic pollen tubes. It is also to be recognized that certain reactions in tetraploids may involve dominance and modifying influences.

Chapter 9. Progenies of Crosses between Tetraploid and Diploid *Petunia axillaris*

Data for the Seeds and the Seed Plantings. *Seeds of Syngenic 2n × 4n.* Several hundred capsules obtained by controlled pollinations of *S 1.2 × S 1.1.2.2*, of *S 1.3 × S 1.1.3.3*, and of *S 2.3 × S 2.2.3.3* were examined. There were always numerous seeds of good size but nearly all were somewhat shrivelled (figure 41 J). Many of these seeds were dissected but no embryos were found. The best-looking seeds selected from about 10,000 of these seeds were planted to the number of 2,879 but no seedling appeared above the soil.

Seeds of disgenic 2n × 4n. Approximately 2,500 seeds were planted of *S 1.1 × S 1.1.2.2* and *S 1.1.3.3*, of *S 1.2 × S 1.1.3.3 and S 1.1.2.3*, and of *S 1.3 × S 1.1.2.2* and *S 1.1.2.3*. Only three seedlings were obtained and these were of *S 1.3 × S 1.1.2.2*. The capsule which provided these seedlings contained, it was estimated, 350 seeds of good size of which only 30 were judged to be fully plump. Three of these seeds germinated but one seedling died early.

The Fully Syngenic Relations of 4n × 2n. These relations were so fully incompatible that capsules did not start to develop. In the early tests (Stout & Chandler 1941) flowers of each *4n* branch were tested with pollen of flowers on the diploid part of the same plant. All such relations failed to give capsules of any size. Later it was found that all of the selfed seedlings of any *4n* plant were also *4n* and completely incompatible to the pollen of the parental *2n* clone and to all pollen of any member of the same genotype. The haploid pollen was incompatible in any syngenic relation with ♀ ♀ tetraploids.

Seeds and Seedlings of Disgenic 4n × 2n. Any disgenic relation of *4n × 2n*, in which the male member possessed one or more *S* factors not present in the female member, gave fine capsules and numerous seeds of full size. But dissections and germination tests revealed that not more than one in fifty of these seeds had an embryo and endosperm. A total of 2,448 of these seeds were planted in soil and twenty-seven seedlings were obtained and grown to maturity.

Data for the Chromosome Numbers in the Seedlings: figure 59. *The 2n × 4n Progeny.* One member had *18* chromosomes and one had *28*. It is probable that the egg cells of the diploid ♀ ♀ parent carried one set of chromosomes and that the plant which had *18* chromosomes received eleven from the *4n* ♂ ♂ parent. There is a possibility that the seedling which had *28* chromosomes grew from a stray seed that was included by error, but possibly an egg-cell was *3n*.

The Progeny of 4n × 2n. Nine of these plants had *14* chromosomes, one had *18*, and seventeen had *21*. Hence it seems rather certain that the *4n* ♀ ♀ had produced some egg cells with *7* and *11* chromosomes, as well as egg cells with *14* chromosomes, which had functioned with haploid male gametes of the diploid parents. Rev. Dr. T. D. Sullivan (1947) determined the chromosome members of 16 of these plants. Miss Selma Kojan and the writer

determined the others by studies of the smear mounts of pollen-mother-cells in stages of meiosis.

Data for the Diploid Members. *The Nine Diploid Members of S 1.1.2.2 × S 1.3* All were self-incompatible and otherwise like diploids of intraspecific breeding. Two died before tests of cross-relations were made but the tests showed that four members were *S 1.3* and three were *S 1.2* (figure 43). It seems certain that the four plants that were *S 1.3* had received *S 3* from a pollen tube and that haploid egg cells of the *4n* ♀ ♀ parent which carried *S 1* had functioned. In each of the fertilizations that resulted in *S 1.2* embryos, a sperm that carried *S 1* must have fused with a haploid egg cell

		S 1.3					S 1.2			S 1.1	S 3.3	S 1.2	S 1.3	S 2.3
		1	2	3	8	9	5	7	8					
187 S 1.1.2.2 x S 1.3	1, S 1.3	S	S	S		S	F	F	F	S	S	F	S	F
	2, "	S	S	S		S	F	F	F	S	S	F	S	F
	3, "	S	S	S		S	F	F	F	S	S	F	S	F
246 " "	8, ?				S									
	9, S 1.3	S	S	S		S	F	F	F	S	S	F	S	F
264 " "	5, S 1.2	F	F	F			S			S	F	S	F	F
	7, "	F	F	F			S	S	S	S	F	S	F	F
	8, "	F	F	F			S	S	S	S	F	S	F	F
Testers	S 1.1	F	F	F		F	F	F	F					
	S 3.3	F	F	F		F	F	F	F					
	S 1.2	F	F	F		F	S	S	S					
	S 1.3	S	S	S		S	F	F	F					
	S 2.3	F	F	F		F	F	F	F					

FIG. 43. The reactions are indicated for the diploid members of the progeny of *S 1.1.2.2 × S 1.3*.

that carried *S 2*. The *S 1.1.2.2 × S 1.1* relation was one of complete sterility but *S 1.1.2.2 × S 1.3* gave capsules and a few normal seeds and evidently at least three pollen tubes that carried *S 1* had functioned along with some that carried *S 3*. But there were no seedlings in the progenies that were obtained that were either *S 1.1* or *S 3.3*.

The selfed progenies of tetraploids consisted of tetraploids only. The appearance of some diploids in these *4n × 2n* progenies demonstrated that the tetraploids produced some haploid macrospores and female gametes that could function in certain relations other than selfing. The occurrence of diploid plants in the progenies of *4n × 2n* relations in petunias has been noted by others.

Data for the Triploid Members (AAA): figure 44. *Character of the Plants.* The seventeen members that were known to be triploids were somewhat more robust than the diploids of *P. axillaris* but less robust than the tetraploids.

Character of the Pollen. The pollen of twelve of these triploids was evaluated. There were abortions of from 25 to 50 per cent of the microspores. The size of the pollen grains that had contents ranged from 28.5 to 45.6 μ while that of tetraploids ranged from 32.3 to 47.5 μ. The majority of the grains were trilete and quadrilete grains were less numerous than in the pollen of tetraploids (figure 49, G, H).

Meiosis. In the several plants that were studied the stages of meiosis showed univalent, bivalent and trivalent grouping and the last named were often in chains, rings, and various irregular bridge configurations. In the first anaphase there were frequent distributions of *8* and *13, 9* and *12,* and *10* and *11* chromosomes (as pairs of chromatids) and there were also many cases of lagging chromosomes with scattered and irregular distributions. In the second division there were chromosome groups of *4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,* and *15* and the numbers of microspores formed from a pollen-mother-cell were 3, 4, 5, 6, and 7.

The Potential Fertility of the AAA Triploids. The potential fertility of these plants was evaluated by the production of seeds in typical capsules obtained in fully disgenic relations with diploid plants of *P. axillaris* that were *S 4.5* and *S 5.6.* The numbers of large-sized and plump or almost plump seeds in eight capsules were 398, 449, 449, 497, 507, 573, 581, and 661. The highest number of shrivelled or collapsed seeds in any of these capsules was 79. Numerous dissections revealed that at least half of the plump seeds had an endosperm and an embryo. There were at least 200 scale ovules intermingled with the seeds on the placenta of a capsule. Thus the ovaries of these triploid plants of *P. axillaris* had an average of about 700 ovules and in fully disgenic relations with diploid *P. axillaris* at least half of them formed seeds of full size but of such seeds about half possessed endosperm and embryo. Thus at least 20 per cent of all ovules produced seeds that had contents.

Self- and Cross-Incompatibilities: figure 44. Each of the seventeen triploid plants was self-incompatible to normal self-pollinations. Thirteen of them were propagated and used in numerous tests. In most of the cross-relations between these triploids no capsules were formed, but in fifteen relations, indicated in figure 44 by the letter " C," there were small poorly developed capsules which usually contained only small scale ovules.

Reactions of Triploids with Diploids. There was complete incompatibility with pollen of *P. axillaris* of the genotypes *S 1.1, S 3.3, S 1.3,* and *S 2.3* except that with pollen of *S 2.3* there were occasionally small capsules that contained only scale ovules or a few shrivelled seeds. But with pollen of *S 4.5* and *S 5.6* there were fine capsules with seeds whose quality is noted above in the statements regarding the potential fertility of the triploids. The

resultants indicate that as female members these triploids had incompatibility reactions with any syngenic haploid pollen but that there was the best production of seed possible for them when haploid pollen was disgenic.

The reactions of the triploids as male members with diploids as female members were very fully tested. Capsules of good size which contained numerous plump seeds that had endosperm and embryo were obtained with ♀ ♀ *S 4.5* and *S 5.6*. But the number of such seeds per capsule was noticeably less than that obtained when the same female members were crossed

			188-1	246-2	" -6	" -7	260-1	" -2	264-3	245-1	" -5	247-1	" -2	" -3	" -8
S 1.1.2.2 × S 1.3	188-1	S 1.2.3	S	S	S	S	C	S	S	C	S	S	C	S	C
	246-2		S	S	S	S	S	S	S	S	S	S	S	S	S
	" -6		S		S	S			C			S		S	S
	" -7		S	S	S	S	S					S			S
	260-1		S	S	S	S	S	S	S	S	S	S	S	S	S
	" -2		S	S	S	S	S	S	S	S	S	S	S	S	S
	264-3		C	C	C	S	C	C	S	S	S	C	C	S	S
S 1.1.3.3 × S 1.2	245-1		S	S	S	S	S	S	S	S	S	S	S	S	S
	" -5		S	S	S	S	S	S	S	S	S	S	S	S	S
	247-1		S	S	S	S	S	S	S	S	S	S	S	S	S
	" -2		S	S		S	S	S	C	S	S	S	S	S	S
	" -3		S	C	S	S	S	C		S	S	S	S	S	C
	" -8		S	S	S	S	S	S		S		S	S	S	S

	S 1.1	S 3.3	S 1.2	S 1.3	S 2.3	S 4.5	S 1.1.2.2	S 1.1.3.3	S 2.2.3.3	S 1.1.2.3
188-1	S	S	C	S	S	F	Δ	Δ	Δ	Δ
246-2	S	S	S	S		F				
" -6	S	S		S						
" -7	S	S	S	S	C	F				
260-1	S	S	S	S	S	F	Δ	Δ	Δ	Δ
" -2	S	S	S	S	S	F	Δ	Δ	Δ	Δ
264-3	S	S		S	S	F	Δ	Δ	Δ	Δ
245-1	S	S	S		S	F	Δ	Δ	Δ	Δ
" -5	S	S	S	S	S	F	Δ	Δ	Δ	Δ
247-1	S	S	S	S	S	F	Δ	Δ	Δ	Δ
" -2	S		S	S	C	F				
" -3	S	S	S	S	S	F	Δ	Δ	Δ	Δ
" -8	S	S	S	S	S	F	Δ	Δ	Δ	Δ

S 1.1	S	S		C	S	S	C	C	S	S	S	S	C	
S 3.3	C		C	C	C	C	C	C	C	C		C	C	
S 1.3			S	S	C	C	S	C	C	S	C	S	C	
S 1.3	S	S	S	S	S	S	S	C	C	S		S	C	
S 2.3	C	C	C	S	S	S	C	C	S	S	S	C	C	
S 4.5	F			F	F	F	F	F		F	F	F	F	
S 1.1.2.2	C	O	C		O	O	O	O	O	O	O	O	O	
S 1.1.3.3	O	O	O	C	O	O	O	O	O	O		O	O	

FIG. 44. The patterns of the reactions of the triploids obtained of *4n* × *2n Petunia axillaris*.

in disgenic relations with other diploids (as *S 1.3*). In all relations with *S 1.1, S 3.3, S 1.2, S 1.3*, and *S 2.3* as ♀ ♀ and these triploids as ♂ ♂ there were either complete failures or poor capsules which contained few collapsed seeds.

The Genotypic Constitution of the AAA Triploids: figure 44. On the basis of the reactions of self- and intra-cross-incompatibility and of the reactions with ♂ ♂ *2n*, all of these AAA triploids were *S 1.2.3*. Six were of *S 1.1.2.2* × *S 1.3* parentage and evidently egg-cells that carried *S 1.2* had been fertilized by sperms that carried *S 3*. Six were from *S 1.1.3.3* × *S 1.2*

and for these presumably *S 1.3* eggs and *S 2* sperms had fused. This analysis indicates that selective and hidden incompatibilities operated in the reactions of *4n* × *2n*. The *S 1* pollen tubes were incompatible in pistils of *S 1.1.2.2* but *S 3* pollen tubes functioned. In the relation *S 1.1.2.2* × *S 1.3* only *S 3* pollen tubes functioned.

It would seem that such relations as *S 1.2* × *S 1.2.3* would produce viable seeds with any *S 3* pollen. But it may be that relatively few such grains were produced and that the number which may have functioned were too few to induce the development of capsules and viable seeds.

It is to be noted that each of the AAA triploids that were obtained had three different *S* factors; none was monoallelic or diallelic.

Reactions with 4n Genotypes. Nine of these *3n* plants were tested with pollen of all of the four genotypes of *4n*. All of these relations were syngenic in that no one of the pollen members possessed an *S* factor not present in the ♀ ♀ member. In every relation capsules of good size were obtained but the highest number of seeds of good size in any of the twenty capsules that were examined was 118 and of these 87 were much shrivelled. Twenty seeds that were plump were dissected; only two had an embryo. Many of the capsules contained only shrivelled pseudoseeds and scale ovules. Since these triploids were *S 1.2.3* there were no disgenic pollen grains either of *n* or *2n* constitution in any of the *3n* × *4n* relations that were tested. The resultants of these *3n* × *4n* relations were very like those of the *2n* × *4n* relations except that there was greater fertility in respect to the production of seeds with embryos.

Of the reciprocal relations (*4n* × *3n*), plants of *S 1.1.2.2* and *S 1.1.3.3* were tested in 24 combinations with the pollen of triploids. All were complete failures except three which gave poorly developed capsules that contained only scale ovules. Since the *3n* were *S 1.2.3*, these *4n* × *3n* relations were disgenic for one *S* factor. Yet the resultants were, with three exceptions, fully sterile and hence different from the comparable relations of *4n* × *2n* which were parthenocarpic and feebly fertile when the relations were disgenic.

The Members That Had 18 Chromosomes. *Seedling 245–4.* This plant had the parentage *S 1.1.3.3* × *S 1.2*. Evidently an egg-cell that had eleven chromosomes was fertilized by a sperm that had seven chromosomes. This plant was self-incompatible. As a female it was cross-incompatible with *S 1.1*, *S 3.3*, *S 1.2*, *S 1.3*, and *S 2.3*. It was cross-incompatible with the four members of the *3n* with which it was tested. Its reactions were the same as those of the *S 1.2.3* triploids and it evidently had the same *S* factors.

Seedling 220–1. This plant had the parentage *S 1.3* × *S 1.1.2.2*. Presumably it received eleven chromosomes from its male parent and seven from the female parent. It was self-incompatible; as a female it was incompatible with males of *S 1.1*, *S 3.3*, *S 1.2*, *S 1.3*, and *S 2.3*. Hence it was probably *S 1.2.3*, in which case the male gamete of eleven chromosomes had carried both *S 1* and *S 2*.

The Member That Had 28 Chromosomes. This plant was a typical tetraploid. It was fully self-fertile, incompatible with pollen of *S 1.1* and *S 3.3*, but parthenocarpic with *S 1.2*. Possibly it grew from a stray seed instead of one that had a parentage of *S 1.3* × *S 1.1.2.2*. It is however possible that a pollen grain that functioned was *3n* or that an egg cell was *2n*.

Reactions of the AAA Triploids with P. parodii and P. integrifolia. As females the triploids of *P. axillaris* were fully sterile with pollen of *P. parodii* but there were some capsules which contained some plump seeds when pollen of the Kew clone was used. In the reciprocal relations some capsules with seeds were obtained with ♀ ♀ *P. parodii* but no capsules formed with ♀ ♀ Kew clone. In these interspecific relations the *3n* of *P. axillaris* behaved as did the *2n* and the *4n* of this species. There were at least some seeds which had embryos in the hybridization fertilities of *3n* × *Kew clone* and of *P. parodii* × *3n*.

Summary and Evaluations

1. Of the 27 members of the progenies of *4n* × *2n P. axillaris* nine had fourteen chromosomes (*2n*), one had eighteen (*2n* + *4*) and seventeen had twenty-one (*3n*). Thus it appears that the egg cells of the *4n* female parents which functioned were haploid (*n* = *7*) in 9 cases, *n* + *4* in one case, and *2n* in seventeen cases. The one member of *2n* × *4n* which had eighteen chromosomes probably received eleven of them from an egg cell of the *4n* parent. The plant that was *4n* was probably included by an error.

2. All of the *2n*, *2n* + *4*, and *3n* members of these progenies were self-incompatible and there were intra-genotypic cross-incompatibilities. The *S* factors which had been inactivated in the self- and cross-relations of the tetraploids had resumed incompatibility reactions in the self- and intra-cross relations of the *2n*, *2n* + *4*, and *3n* members of the progenies.

3. The diploids of the progenies were either *S 1.2* or *S 1.3*; the two that were *2n* + *4* and all that were *3n* were *S 1.2.3*. Thus the haploid pollen tubes which had functioned in the *4n* × *2n* relations of the parents were those which had non-incompatible reactions. The *4n* pistils had incompatibility reactions with haploid pollen; they did not have such reactions with their own diploid pollen which was heterogenic for *S* factors.

4. All the triploids and the one plant of *4n* × *2n* that was *2n* + *4* were triallelic, hence the diploid egg cells that functioned were heterogenic for *S* factors. This is additional evidence that the pairing of chromosomes in the meiosis of the balanced diallelic tetraploids of *P. axillaris* may have been autoallelic.

5. The triploids obtained of *P. axillaris* were potentially fertile to a degree that about one-fourth of their ovules and pollen grains functioned in certain relations. The production of seeds which had embryos was highest when the pollen was fully disgenic (*S 4.5*).

6. The sterility resultants between *3n* and *2n* of *P. axillaris* were effected

chiefly by incompatibility factors. There was fertility in the reciprocal relations with *S 4.5*, which were fully disgenic, and in these relations the degree of fertility was that of the potential fertility. Various of the *2n* × *3n* relations that were disgenic, as *S 1.1* × *S 1.2.3*, should, it would seem, have given viable seeds provided there were viable pollen grains which carried only *S 2* or *S 3*. Possibly such pollen grains were not sufficiently numerous to effect the formation of capsules in such relations.

7. The reactions between *3n* pistils and the *2n* pollen of self-fertile tetraploids are of special significance in considering (a) the nature of the inactivation of *S* factors and (b) the possible action of a new specificity in the triploids. The self-fertility of any *4n* of *P. axillaris* is an expression of an inactivation of *S* factors. The view that this is due to "competition" (Lewis & Modlibowski 1942; Lewis 1943 a, 1947) postulates that there is mutual suppression of both *S* alleles in heterogenic pollen independently of the condition in the pistil. In this case such pollen should be incapable of an incompatibility reaction in any pistil.

8. Each triploid was self-incompatible, hence it either did not have any heterogenic diploid pollen or such pollen was involved in incompatibility reactions or was too limited in number to effect development of capsules. The resultants of the syngenic relations of *3n* × *4n* provide data on this matter. There were definitely fewer viable seeds in the *3n* × *4n* disgenic relations and also a much greater degree of induced parthenocarpy. There was higher seed production in the *3n* × *2n* syngenic relation than in the *3n* × *4n* relation hence it seems that *2n* heterogenic pollen was less effective than non-incompatible *n* pollen in relations with *3n* pistils.

9. It is to be recognized that the *3n* × *4n* resultants, as well as those of *2n* × *4n*, probably involved abortions of zygotes after fertilization. Capsules were obtained which contained many pseudo-seeds. Seed abortion is known to be a barrier to the production of viable seeds in *4n* × *2n* and *2n* × *4n* relations, and especially between an autotetraploid and the diploid from which it was derived (see recent discussion in Brink & Cooper 1947).

10. The triploids of *P. axillaris* displayed less specificity and less incompatibility than did the *2n* in reactions with *4n*. Progenies of *3n* × *4n* were not grown.

Chapter 10. The Triploid Hybrid Progenies of *4n P. axillaris* × *2n P. integrifolia*

The First Generation: Series 170; figure 47. *Parentage and Character.* The seed parent was *S 1.1.3.3* and the pollen parent was the Kew clone (*S a.b*). Eight seedlings were obtained of the 253 seeds in one capsule that were planted; but it was determined that numerous of the seeds of this relation were empty even when they were plump and of good size. These

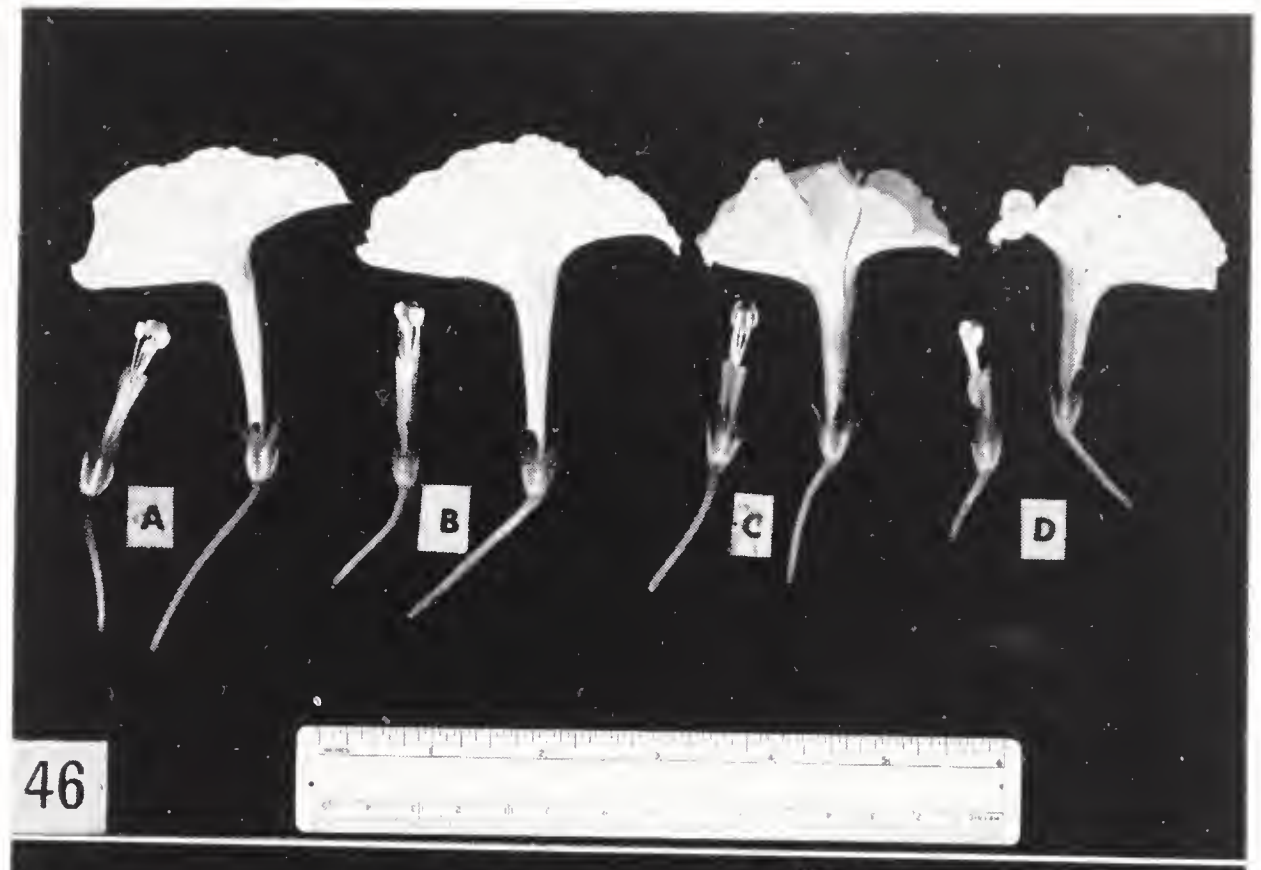


FIG. 45. A, branch, flower, and stamens and pistil of a triploid of AAI: *S 1.3.b.* B, hexaploid or double triploid of AAAAII. All comparable parts are larger in the hexaploid. FIG. 46. Flowers and stamens and pistil: A, triploid No. 247–3; B, pentaploid No. 258–19; C, hexaploid No. 217–14; D, hexaploid No. 280–9.

plants were somewhat more robust than the F_1 of *2n P. axillaris* × Kew clone and they had coarser leaves, stems, and flowers but the width of the open corollas was about the same (figure 45 A). The position of the stamens was near that of the stamens of *P. axillaris* but the anthers and stigma were smaller, the throat of the corolla tube was broader, and the color of the corolla, anthers, and pollen was purplish. There were only slight differences in the shade of the coloring of the different members. One seedling died before its chromosome number was determined; six had *21* chromosomes and one had *20*. Hence six of the egg cells of the *4n* parent which functioned had *14* chromosomes and one had *13*.

Potential Fertility. All members had well-formed anthers that dehisced normally. Pollen was abundant but very irregular in size and about three-fourths of all grains were more or less shrivelled and many of these were of small size (F in figure 49). Most of the microspores that had contents were trilete; a few were quadrilete.

Each of the seven plants produced large capsules in disgenic relations with both *2n P. axillaris* and *P. integrifolia*. Frequently in each of these relations a capsule had between 300 and 400 seeds that were larger than seeds of *2n P. integrifolia* and these were mostly plump and spherical like those shown in G of figure 41. There were some undersized and usually shrunken seeds and ovule scales were always numerous. When the seeds were placed in water, in numbers of 300 to 379 per capsule, only from eleven to twenty-eight seeds sank immediately. Those that floated were empty or had mere traces of contents. One hundred of the seeds that sank in water were dissected; 63 had what appeared to be a normal embryo and endosperm but most of the others had only endosperm.

The six hybrid triploids and the member that was *3n – 1* were potentially able to function in the production of seed. Apparently there were two conditions that reduced the number of viable seeds. There were abortions of microspores and macrospores which effected a high degree of absolute sterility and there were seed abortions after fertilization in the relations with *2n*. Incompatibilities enforced selective reproduction in the expressions of potential fertility.

Self- and Cross-Incompatibilities: figure 47. Each of the seven seedlings was self-incompatible to *normal* self-pollinations at the time when the anthers of a flower were dehiscing. All intra-cross relations of the seven members were incompatible when pollinations were made on fully developed pistils. There was irregularity and some delay in the appearance of secretion on the stigmas, especially when the flowers were emasculated before anthesis and the pistils were prematurely exposed to the air. Some capsules and viable seeds were obtained in some of the close- and intra-cross pollinations that were made at the time when the flowers were opening, and seedlings were obtained from such seeds.

Reactions with Diploid P. axillaris. All the members of this progeny,

except No. 5, were cross-incompatible with pollen of *S 1.1, S 3.3, and S 1.3* but fertile with pollen of *S 1.2, S 2.3, and S 4.5*. These resultants indicate that these seedlings had received both *S 1* and *S 3* from the female gametes of the parent that was *S 1.1.3.3*. Plant No. 5 was also fertile with pollen of *S 1.3.* and *S 3.3* which suggests that it did not possess the factor *S 3*. The plant was, however, cross-incompatible reciprocally with all of its siblings.

The relations of *2n P. axillaris* as females with the *3n* of Series 170 were fully tested and the tests were repeated in 1946. There was sterility with the females of the genotypes of *S 1.1, S 3.3, S 1.2, S 1.3*, and *S 2.3*. In all of these relations the triploid hybrids had at least one *S* factor not present in the female member. In 1947 these relations were tested again with also complete

Series 170			Chr. No.	1	2	3	4	7	6	8	P. axillaris ♀ S 1.1	S 3.3	S 1.3	S 1.2	S 2.3	S 4.5	S 1.2.3	4n	P. integ. ♀ S a.b.	S a.a.	S b.b	6n	P. parodii
3n hybrids A AI	1	S1.3.b	21	S	S	S	S	S	S	S	S	S	S	F	F	F	all O, probably S	Δ	H	H	S	Δ	all O, unilateral
	2	"	"	S	S	S	S	S	S	S	S	S	S	F	F	F		Δ	H	H	S	Δ	
	3	"	"	S	S	S	S	S	S	S	S	S	S	F	F	F		Δ	H	H	S	Δ	
	4	"	"	S	S	S	S	S	S	S	S	S	S	F	F	F		Δ	H	H	S	Δ	
	7	"	"	S	S	S	S	S	S	S	S	S	S	F	F	F		Δ	H	H	S	Δ	
	6	"	20	S	S	S	S	S	S	S	S	S	S	F	F	F		Δ	H	H	S	Δ	
	5	S1.?.b	21	S	S	S	S	S	S	S	S	F_n	F_n	F	F	F		Δ	H	H	S	Δ	

P. axillaris									
	S 1.1; S 3.3; S 1.2; S 1.3; S 2.3	S	S	S	S	S	S	S	
	2n, S 4.5	← F or Δ →							
	3n, S 1.2.3	← O, probably S →							
	S 1.1.2.2; S 1.1.3.3; S 2.2.3.3	0	0	0	0	0	0	0	
P. integrifolia, S a.b; S a.a; S b.b		0	0	0	0	0	0	0	← Unilateral
6n hybrids, S 1.1.3.3.b.b		← O →							

F_n = *nonconforming*
H = *hybridization*
Δ = *few good seeds*

FIG. 47. Pattern of the reactions of the six hybrid triploids (AAI) and the one paratriploid (*3n — 1*) that were seedlings of *4n P. axillaris* × *2n P. integrifolia*.

sterility except that occasionally the relation *S 1.1* × *S 1.3.b* gave a small parthenocarpic capsule. But in 1947, tests were made with the genotype *P. axillaris S 4.5* as females and every relation gave fine capsules and numerous seeds. In the spring of 1948 the tests were repeated for *S 1.2* and *S 1.1* × 170 (*S 1.3.b*), all of which were sterile; and also for *S 4.5* × *S 1.3.b*, all of which gave capsules and some seeds.

Reactions with P. integrifolia. All members of series 170 produced fine capsules and some viable seeds to pollen of *S a.b* and *S a.a* but the ovaries did not begin to enlarge to pollen of *S b.b*. Evidently each seedling had received *S b* from the pollen parent (*S 1.1.3.3* × *S a.b*) and this factor operated with efficient incompatibility with pollen of *S b*.

The reciprocal relations were adequately tested and there was complete sterility as males with *S a.b*, *S a.a*, and *S b.b*. These sterilities cannot be at-

tributed to incompatibilities. The pollen of all these *3n* and *3n – 1* hybrids reacted in these relations as did the pollen of pure diploid *P. axillaris*. Evidently all the pollen of these plants possessed the genic complement of *P. axillaris* ♂ ♂ which effected sterility with any genotype of *P. integrifolia* in the hybridization relation.

Reactions with Triploid P. axillaris, S 1.2.3. Twelve of the triploids of *P. axillaris*, of those listed in figure 45, were tested in reciprocal relations with the members of series 170. Seventy-two relations were tested in which plants of 170 were ♀ ♀ members. Sixty-one tests were complete failures and in eleven tests there were some parthenocarpic capsules which contained only scale ovules and some pseudoseeds. Fifty-six relations were tested in which the *S 1.3.b* plants were male and the *S 1.2.3* plants were female. There was complete failure in all but five relations which were parthenocarpic and pseudospermic.

These *3n* plants had some ♀ and ♂ gametes that were potentially able to function. The evidence indicates that the *3n* pistils of these plants had incompatibility reactions (a) with their own pollen tubes in selfing, (b) in all intra-genotypic crossing, and (c) in reciprocal relations between the two triploid genotypes. The tests with diploids proved that *S 1.2.3* pistils would function with haploid pollen tubes of *S 4.5*, and that pistils of *S 1.3.b* would function with pollen tubes of *S 2* in relations with *S 1.2*.

Reactions with 4n P. axillaris. All the members of series 170 produced capsules of good size to pollen of either *S 1.1.2.2* or *S 1.1.3.3*. Fifteen capsules were evaluated. There were from 42 to 150 seeds of good size per capsule but very few of them had contents. Of 96 seeds in one capsule only three had contents; in another capsule of 127 seeds there were 18 that sank in water; of the 114 seeds in another capsule only eleven had contents. In the examinations it was noted that various amounts of endosperm were present in certain seeds in which there was no trace of an embryo. The number of normal and pseudoseeds per capsule which were obtained of plants of 170 × Kew clone was as high as 379 but no capsule obtained with *4n* ♂ had more than 150 seeds.

Fifteen relations of *4n P. axillaris* × *3n* of series 170 were tested. All were failures including those that were *S 1.1.2.2* × *S 1.3.b*. Thus the *4n* × *3n*, *S 1.3.b* was completely sterile in all relations and at least one test was made with a member of each of the three *4n* genotypes as female with each of the members of 170.

Reactions with Hexaploids. The relation of *3n* (*S 1.3.b*) × *6n* (*S 1.1.3.3.b.b*) was tested in 46 combinations. In all but five of the relations capsules of good size were obtained and there were numerous seeds of good size in some of which there was an embryo and endosperm. The 20 reciprocal relations of *6n* × *3n* (*S 1.3.b*) were complete failures except that three small parthenocarpic capsules were obtained.

Reactions with P. parodii ♂ ♂. As females the seven members of series

170 were sterile with pollen of *P. parodii* which was comparable to the reactions of the two diploid parents of series 170. Thus the members of the triploids of AAI constitution possessed the component which effected unilateral sterility in the AA ♀♀ × PP ♂♂ relation and also that which effected sterility in the II ♀♀ × PP ♂♂.

Summary. 1. The relation of *4n P. axillaris* × *2n P. integrifolia* involved unilateral hybridization and differences in ploidy and the resultant fertility was one of reduced seed production.

2. There were no diploids in the members of series 170. The egg cells of the *4n* parent which functioned carried *14* chromosomes in six instances and *13* in one. But in the progeny of *4n* × *2n P. axillaris* some haploid egg-cells functioned. Possibly a larger population of the F_1 hybrids of *4n* × *2n P. integrifolia* would also have included some diploids.

3. Six members of the AAI progeny were *S 1.3.b*. One member gave non-conforming reactions for the factor *S 3* and may not have possessed this factor. Obviously all of the *2n* ♀ gametes of the *4n* parent which functioned had been heterogenic and *S 1.3*.

4. The incompatibility factors in the AAI triploids effected full self-incompatibility, intra-cross-incompatibility between the *3n* hybrids, and incompatibility in syngenic relations with the ♂♂ of both parents. The component of *P. integrifolia* in the pistils of the triploid hybrids (AAI) had no influence in effecting unilateral sterility, unless it was hidden, with haploid pollen of diploid *P. axillaris*. The action of the AA component was epistatic.

5. As male members all of the F_1 hybrids of *S 1.3.b* and the member that was *S 1.2.b* failed to produce capsules with the ♀♀ of any *P. integrifolia* (*S a.b*, *S a.a*, and *S b.b*) and of any diploid of *P. axillaris* that carried any combination of *S 1*, *S 2*, and *S 3*. All of these failures could not have been due to the usual reactions of *S* factors. For example, the relation of *S 1.1* × *S 1.3.b* should have involved some pollen tubes that carried no *S 1* factors and some that were heterogenic. Neither could all of these failures have involved the unilateral sterility of AA × I, for the relation AA: *S 4.5* × AAI gave capsules and some viable seeds. To evaluate the sterility resultants in these reactions one needs to know the constitution of the pollen tubes and of the ♂ gametes that are functional. Evidence on this matter may be gained in the analysis of (a) the F_2 of premature selfing and (b) the analysis of progenies of *S 4.5* × *S 1.3.b*, which, it is to be regretted, were not grown.

The Second Generation: figure 48. *General Data.* All of the plump seeds of good size in the fifteen capsules that were obtained by the premature cross-pollinations of members of series 170 were planted. The number of seeds per capsule ranged from 10 to 64 and the total was 327. Only 21 seedlings appeared of which seven soon died. The chromosome members were determined in thirteen plants: one had *19*; four had *20*; two had *23*; one had *24*; three had *25*; one had *26*; and one had *28*. Two of the members that had *20* chromosomes were weak in growth and died before they flowered.

Self-Incompatibility. The three members that had *19, 20,* and *20* chromosomes were fully self-incompatible and the tests indicated that they were all *S 1.3.b.* They were sterile with ♂ ♂ *S 1.1* and *S 3.3* but fertile with ♂ ♂ *S 4.5* of *P. axillaris.* They were fertile with ♂ ♂ *S a.b* but incompatible with ♂ ♂ *S b.b* of *P. integrifolia.*

Self-Fertility. Eight members were fully self-fertile to normal self-pollinations and these included all the members that had *23* or more chromo-

		Ch.	Self	S 1.1	S 3.3	S 1.3	S 1.2	S 2.3	S a.b	S b.b
S 1.3.b (?)	230-3	19	S	S	S	S			H	S
	" 1	20	S	S	S	S			H	S
	" 4	20	S	S	S	S			H	S
	318-1	23	F	S	S	C			H	S
	321-3	"	F				Δ	Δ		
	319-3	24	F	S	S	S	Δ	Δ	H	S
	230-2	25	F	S	S	S	Δ	Δ	H	S
	319-1	"	F	S	S	S	Δ	Δ	H	S
	" 5	"	F	S		S	Δ	Δ	H	S
	320-1	26	F	S	S			Δ	H	
	321-1	28	F	S	S	S		Δ	H	S

FIG. 48. This chart indicates the reactions of the seedlings obtained by the premature selfing of triploids that were AAI: *S 1.3.b.* Those that had *23* or more chromosomes were self-fertile.

somes (figure 48). These plants were grown during 1947, chiefly for the determination of their chromosomes and their reactions to selfing, and after these features were known only few tests for cross-relations could be made.

Resultants of Cross-Relations. A total of twenty-seven relations as females with *S 1.1, S 1.3,* and *S 3.3* were sterile and one gave small parthenocarpic capsules. Twelve relations of self-fertile plants with ♂ ♂ of *S 1.2* and *S 2.3* gave capsules and seeds that were nearly all pseudospermic and typical of the *4n* × *2n* relation of *P. axillaris.* Ten members produced capsules with

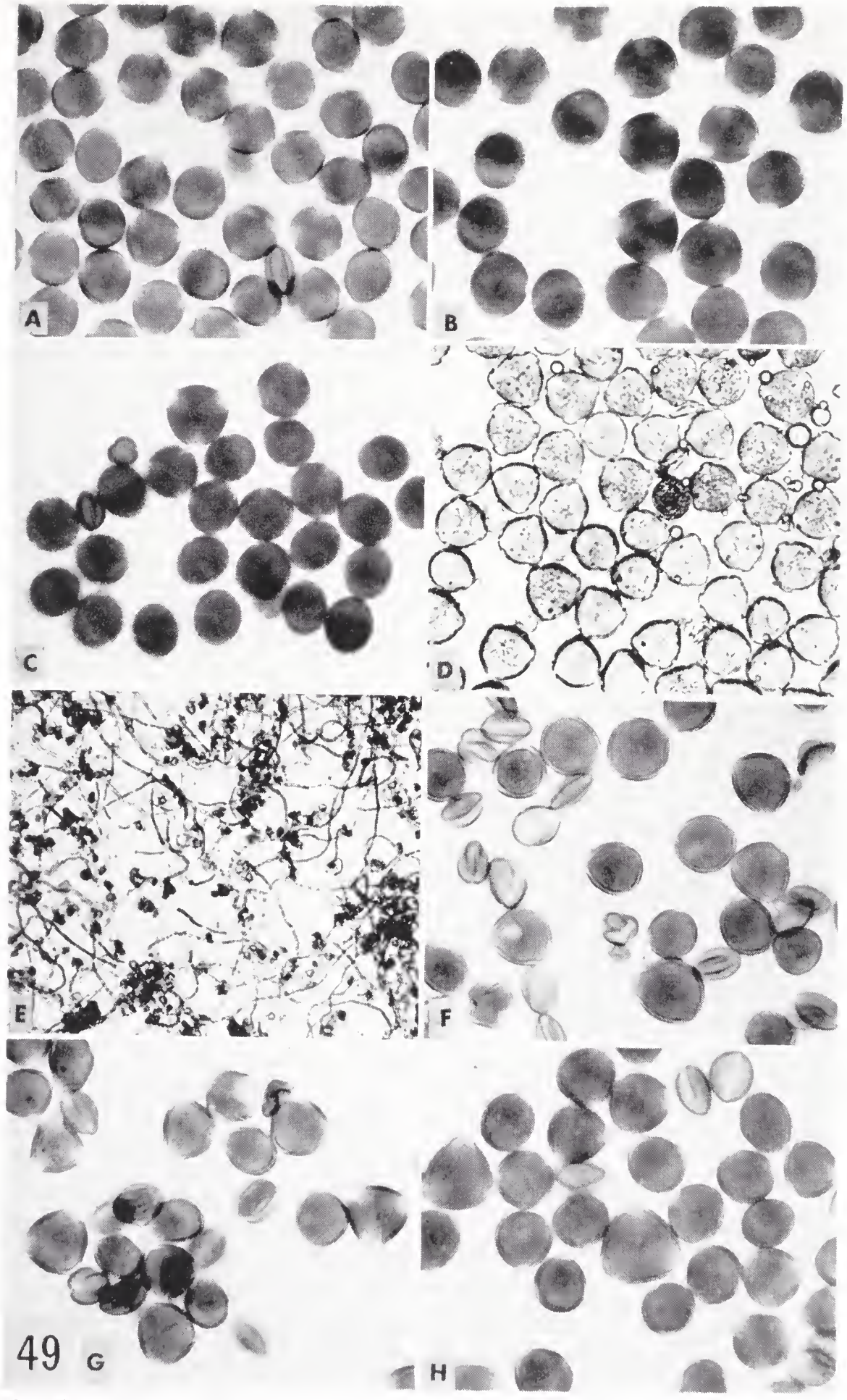


FIG. 49. Photomicrographs of the pollen of petunias. Except for D, E, and J the magnification is about × 240, and the preparations were glycerine-jelly mounts double-stained with methyl green and magenta. This technique was worked out by Mr. Alfred Owczarzak who also made the photographs. A, *2n P. axillaris*; B, *2n P. parodii*; C, *2n P. integrifolia*; D, *2n P. integrifolia* Kew clone, on agar, and E, pollen tubes of Kew clone, × 35; F, hybrid triploid AAI, No. 170–5; G and H, triploids of AAA.

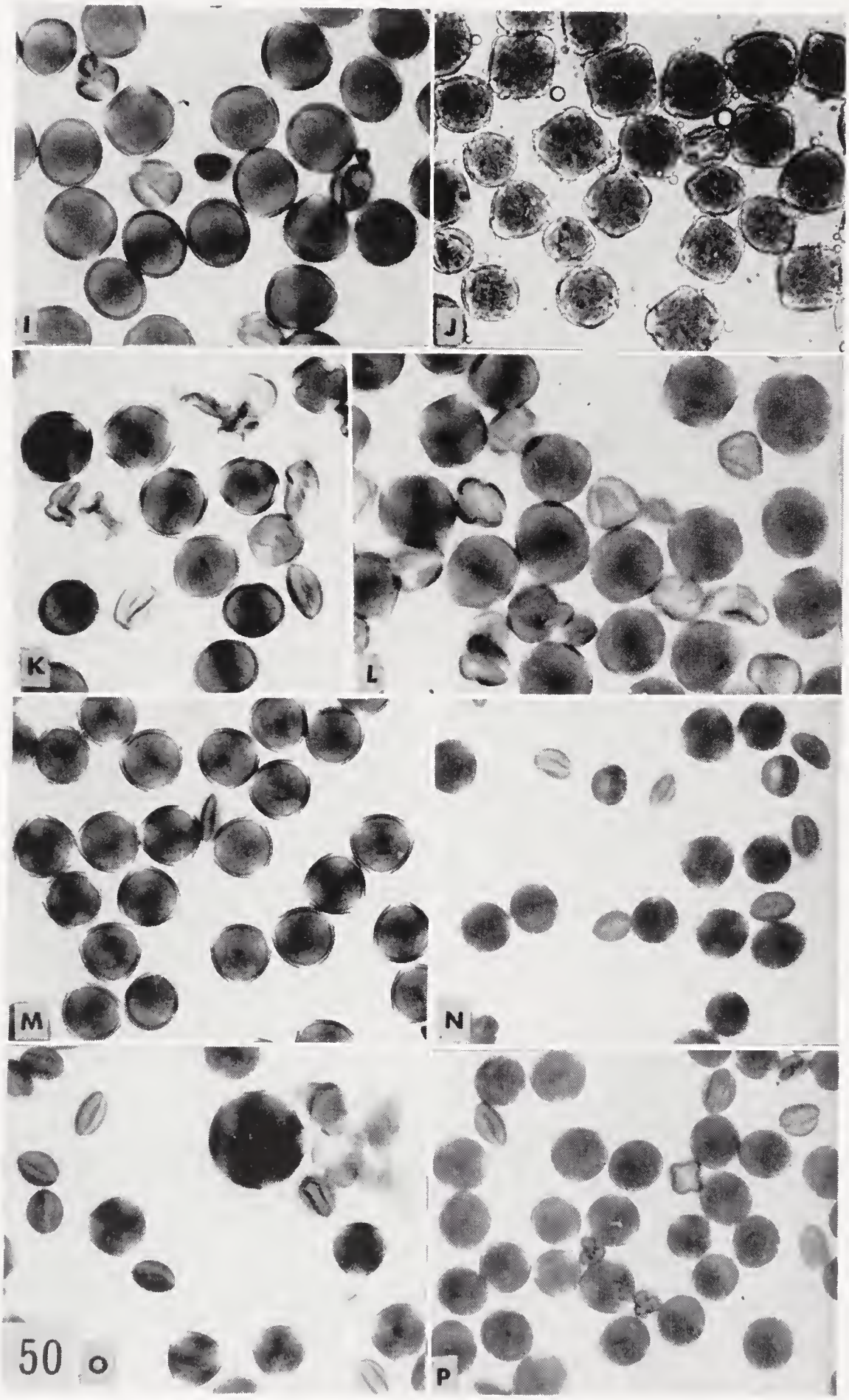


FIG. 50. I, somatic autotetraploid 139-12, and J, same on agar; K, tetraploid seedling of AAAA; L, double triploid seedling of AAAAII; M, F_1 PA hybrid; N, F_1 AI hybrid No. 154-15, and O, 154-21; P, F_2 of AI No. 255-4.

some seeds which had embryo and endosperm to pollen of the Kew clone (*S a.b*) but the six relations that were tested with ♂ ♂ *S b.b* were incompatible.

Evaluations. 1. There were no diploids in the F_2 hence in their origin there were no fusions, or at least survivals, of *n* egg cells with *n* sperms. There were no triploids and hence no fusions of *2n* with *n* gametes had survived. If it be assumed that no microspore or macrospore with less than seven chromosomes was viable, the one plant that had *19* chromosomes could have arisen from a fusion of gametes that had *7* and *12*, *8* and *11*, or *9* and *10* chromosomes. The two that had *20* chromosomes could have arisen from *7* and *13*, *8* and *12*, *9* and *11*, or *10* and *10*. The plants whose chromosomes numbers ranged from *23* to *26* inclusive could have arisen, collectively, from fusions of gametes that had *14* chromosomes with gametes that had numbers of *9*, *10*, *11*, or *12*. The one member that was tetraploid probably arose from gametes that were diploid. It is evident that the gametes that functioned in the production of viable seeds in the premature close-pollinations of the triploids of *S 1.3.b* probably included, collectively, chromosome numbers of *7*, *9*, *10*, *11*, *12*, *13*, and *14*.

2. Each member of this second generation possessed all three of the *S* factors present in the F_1. This indicates that no homogenic gamete had functioned, at least for the production of those plants that had *20* or less chromosomes and were self-incompatible. The members that had *23* or more chromosomes were self-fertile and also triallelic and in these probably some one of the three *S* factors was duplicated due to the fusion of sperms that were heterogenic but had an *S* factor in common. Apparently the presence of four *S* factors was correlated with the inactivation of *S* factors and self-fertility, provided that the constitution was at least diallelic. No monoallelic tetraploid was obtained in any of the cultures. All of the triploids that were obtained were triallelic but self-incompatible.

Chapter 11. Progenies of AAI × the 2n Parental Species

Data for AAI × P. integrifolia. *General Data.* Thirty seedlings (series 235) were grown of 170–3 (*S 1.3.b*) × Kew clone (*S a.b*) and thirty-eight (series 238) were grown of 170–1 (*S 1.3.b*) × Kew clone. Chromosome counts, determined for twenty-eight of them (Sullivan 1947), ranged from *14* to *20* inclusive with distributions (figures 54 and 59) that included some of all of the intervening numbers. Presumably these plants received a genome of seven chromosomes from the male parent. In this case the ♀ gametes that had functioned collectively had chromosome numbers of *7*, *8*, *9*, *10*, *11*, *12*, and *13*. Evidently no egg-cell that had *14* chromosomes had functioned, but the analysis of the progenies of prematute selfing of the triploids of series 170 indicated that some gametes that had 14 chromosomes could function. The *S 1.3.b* × *S a.b* relation gave large capsules which contained as many as

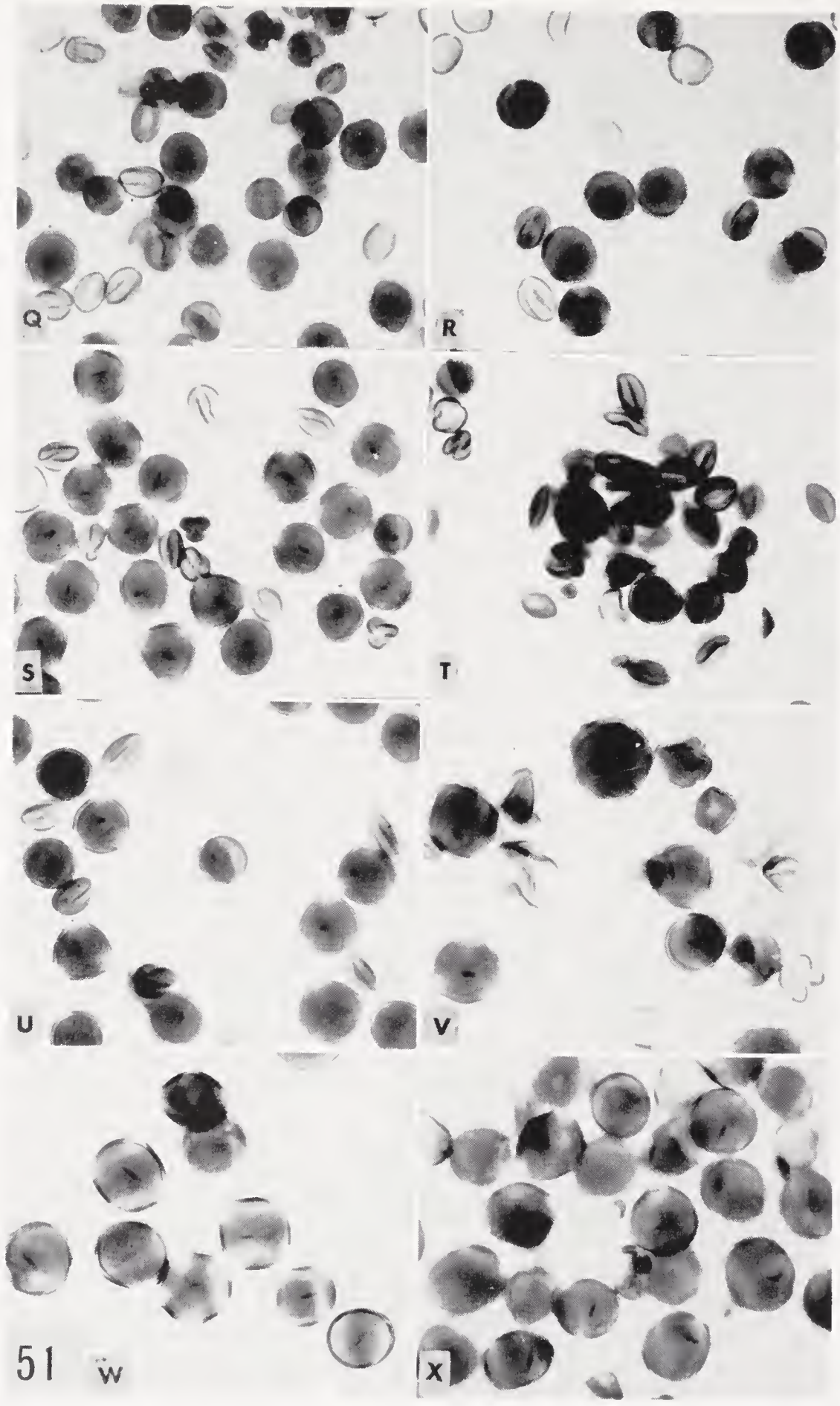


FIG. 51. Q, a *2n* segregate in a progeny of AAI prematurely selfed; R, *sn: 15* seedling (No. 235–1) derived of AAI × *2n P. integrifolia*; S, *sn: 16* seedling No. 235–36; T, *sn: 18* seedling No. 238–7; U, *sn: 20* seedling No. 239–14; V, *sn: 25* seedling No. 319–1; W, a *5n* seedling of *6n* × *4n*; X, an *sn: 40* seedling No. 284–11 of the 2d generation of AAAAII.

300 seeds of good size of which at least 10 per cent germinated in the seed plantings that were made.

The seedlings of the AAI × II parentage received different proportions of the AA and I chromosomes from their triploid female parent which were combined with the genome of seven from the male parent. In general the constitution may be represented as AI and the constituents evaluated according to the character of the plants and their behavior in the reactions of *S* factors.

Character. All members of series 235 were more like plants of *P. integrifolia* than like *P. axillaris* and their chromosome numbers ranged from *15* to *17* inclusive. The members of series 238 had chromosome numbers that ranged from *15* to *20* inclusive and several of them were strongly like *P. axillaris*, as shown in figure 52. Also the two series differed in the combinations of *S* factors (figure 54).

But nearly all of each of these progenies were strongly like *P. integrifolia* in habit of growth and in the size and shape of the flowers and of these, plant 238 No. 7 shown in figure 52 B is representative. In nearly all cases the two longest stamens were above the stigma. Yet all plants displayed some influence of their *P. axillaris* ancestry. The two that were most like *P. axillaris*, as 238–8 and 10 in figure 52, had flowers that were purplish in color and the anthers were smaller than in *P. axillaris*.

Self- and Cross-Incompatibility. Each of the sixty-eight members of these series was self-incompatible. About half of them were propagated and the reactions of all members were sufficiently tested to allow the identification of the five genotypes indicated in figure 54 which gives the data for the 18 plants most fully tested.

Data for AI: S a.b. Of the thirty members of series 235 there were twenty-two that were *S a.b.* The reactions which were obtained for three of these are indicated in figure 54. Each of the other nineteen were tested in from one to twelve intra-group relations, all of which were incompatible. Also each member was involved in tests as a female with one or more of the genotypes of *P. integrifolia* (*S a.b, S a.a, S a.b*) all of which were incompatible.

All the many tests of these plants as females with pollen of *P. axillaris* were fully sterile but each of the 22 reciprocal relations that were tested gave capsules and seeds of which some were found to be normal. These reactions were those of the unilateral hybridization between *P. axillaris* and *P. integrifolia*. These twenty-two members of series 235 had only the *S* factors of the Kew clone. In character they were strongly like *P. integrifolia*. In their reactions with *P. axillaris* they showed that they possessed the genic component of *P. integrifolia* which gave unilateral reactions of sterility and fertility with *P. axillaris*.

Data for AI: S b.b. There were eight plants of this group and all were members of series 235. The chromosome numbers determined for three

plants were *16*, *16*, and *17*. All eight members were self-incompatible and cross-incompatible in all the tests which included at least one relation for each. The members of this group were fertile as ♀ with the pollen of the sister group AI: *S a.b* but as ♂ there was incompatibility. These reactions indicate that the members of the group were homozygous for one of the factors present in the other group (AI: *S a.b*). Since their seed parent was *S 1.3.b* and the pollen parent was *S a.b* this factor was *S b* and this was verified by the reactions with *S a.b*, *S a.a* and *S b.b* (figure 54).

The members of this group had the reactions comparable to those of the unilateral sterility and fertility in the reactions between *P. integrifolia* and *P. axillaris* and in this respect were like the twenty-two sisters of the *S a.b* genotype.

The AI: S 1.b genotype. Six members of series 238 were identified as *S 1.b*. Their chromosome numbers were *15*, *15*, *15*, *15*, *17*, and *18*. These plants were self- and cross-incompatible. Their reactions as females with both *P. axillaris* and *P. integrifolia* were fertile except for the incompatibilities in those relations that involved *S 1* and *S b*. Hence there was no trace of the unilateral sterility which the members of series 235 displayed with *P. axillaris* ♂ ♂. These plants had one entire genome that was derived directly from *P. integrifolia*. The reactions prove that the genetical half of the diploid female constitution was not sufficient to effect unilateral sterility.

Data for AI: S 3.b. Five members of series 238 were identified as *S 3.b* and their chromosome numbers were 14, 15, 15, 18, and 18. The data for three of these, indicated in figure 54, are typical for the reactions of all five members. The resultants of the tests were conforming for incompatibility reactions of the factors *S 3* and *S b*, and these included all relations with the ♂ of both parent species. There were no reactions of unilateral sterility with pollen of *P. axillaris* and hence these plants did not possess that part of the diploid female constitution of *P. integrifolia* that effected hybridization sterility with pollen of *P. axillaris*.

It is to be noted that one of the two plants of this group which had *18* chromosomes was very decidedly like *P. integrifolia* in appearance (figure 52 B) and that another which had *15* chromosomes was much more like *P. axillaris*. Yet both lacked the diploid genic component of *P. integrifolia* ♀ ♀ which effected unilateral sterility with *P. axillaris* ♂ ♂.

Data for Six Members of Series 238. The reactions of six members with each other and with the two parental diploid species were mostly sterile. All had flowers that appeared to be normal and pollen was abundant. The chromosome numbers ranged from *16* to *20* and all the plants which had *19* or *20* chromosomes were in this group. Possibly these plants had three *S* factors and were triallelic. The reactions of sterility with the pollen of both *P. axillaris* and *P. integrifolia* were, in part at least, non-conforming in comparison with the behavior of plants of series 170 which were *S 1.3.b*. Most members of this group had robust habits of growth and were strongly like

FIG. 52. Plants of a back-cross progeny of AAI: *S 1.3.b* × *P. integrifolia*: A, No. 238–8, was *sn: 16*, but evidently triallelic, and was very much like *P. axillaris*; B, No. 238–7, was *sn: 18*, *S 3.b*, and very near *P. integrifolia*; C, No. 238–10, was *sn: 15*, *S 3.b*, and much like *P. axillaris*. FIG. 53. Plants of a back-cross progeny series 242 of AAI × *2n P. axillaris*: A, *sn: 18*; B, *sn: 18*; C, *sn: 20*; D, *sn: 20*; E, *sn: 17*.

P. axillaris even when the number of chromosomes was *16* (see A in figure 52). It is to be noted that the members of this presumably triallelic group produced no capsules and seeds to any intra-series cross-pollination.

Summary. 1. The two progenies, 235 and 238, had different seed parents that were believed to be *S 1.3.b*. Yet the two progenies were different in character, in range of *sn* chromosomes, and in genotypic constitution.

2. Series 235 was composed of twenty-two members that were *S a.b* and eight that were *S b.b*. Presumably all of the egg-cells that functioned in their production carried only *S b*, and the greater proportion of the pollen tubes that functioned had carried *S a*, but the gametic chromosome numbers ranged

			Ch.	1	3	8	5	19	28	1	4	3	2	10	7	11	14	12	8	9	6	S a.b S a.a S b.b	S 1.1 S 3.3 S 1.2 S 1.3 S 2.3
A<I	S a.b	235-1	15	S	S	S	S	S	S		S											S S	0 0 0 0 0
		3	16	S	S	S	S	S	S		S											S S S	0 0 0 0 0
		8	17	S	S	S	S	S	S													S S S	0 0 0 0 0
	S b.b	5	16		F	F	S	S	S	F	F		F		F							F F S	0 0 0 0 0
		19	16	F	F	F	S	S	S	F	F	F	F	F	F		F		F			F F S	0 0 0 0 0
		28	17	F	F	F	S	S	S	F	F	F	F		F							F F S	0 0 0 0 0
AI	S 1.b	238-1	15	F	F		S	S	S	S	S	S	F	F	F	F	F		F			F F S	S F F F F
		4	15		F		S	S	S	S	S											F F S	S F F F F
		3	18	F	F		S	S	S	S	S	S										F F S	S F F F F
AI	S 3.b	2	14	F	F	F	F			F		F	S	S	S					F	F	F F S	F S F F F
		10	15	F			F	F					S	S								F F S	F S F F F
		7	18		F		F	F		F		F	S	S	S							F F S	F S F F F
	Triallelic?	11	18	?	S	?				S	S	S	S		S	S			S	S	S	F F S	F F
		14	20							S		S	S	S	S		S	S	S	S		F F	S S F
		12	20							S	S	S	S	S	S		S	S	S			F S	0 0 0 0 0
		8	16	S	S		S	S	S	S		S	S	S	S	S	S	S	S	S	S	0 0 0	0 0 0 0 0
		9	19	S	S		S	S	S	S	S	S	S		S	S		S	S	S	S	0 0 0	0 0 0 0 0
		6	20	S			S	S	S	S	S	S	S		S	S			S	S	S	0 0 0	0 0 0 0 0

FIG. 54. Chart that indicates the reactions of back-cross progenies of triploids AAI × *P. integrifolia*. The six that were *S a.b* and *S b.b* had unilateral sterility with *P. axillaris*.

from *7* to *10*. The functioning of eight pollen tubes of *S b* in the normal pollinations of *S 1.3.b* × *S a.b* was nonconforming to the behavior of incompatibility factors, but it has been noted that stigmatic secretion was somewhat delayed on pistils of AAI plants. There was definite segregation and recombination in all plants of series 235 of the diploid complement of *P. integrifolia* ♀ ♀ which effected unilateral sterility with pollen of *P. axillaris*.

3. The genotypes definitely identified in series 238 were *S 1.b* and *S 3.b*; but there were six numbers that were presumably triallelic. Three of these were cross-sterile with all genotypes of both *P. axillaris* and *P. integrifolia* which were nonconforming reactions for either incompatibilities or unilateral sterility of hybridization.

4. Of the 49 members of AAI × II that were tested for *S* factors, 41 possessed only two *S* factors, and hence each of the female gametes that had functioned in their production had carried only one *S* factor. But the chromosome numbers determined in the 28 members ranged from *sn:14* to *20;* in these only one female gamete that was *gn:7* had functioned; 10 that were *gn:7 + 1* and 7 that were *gn:7 + 2* had functioned. Only six egg cells that carried two *S* factors had functioned in the production of viable zygotes of the *S 1.3.b* × *S a.b* relations (*gn* = gametic number).

5. In the progeny of 235 there were 22 members that were *S a.b* and for the production of these, pollen tubes that were *S a* must have functioned. But in the progeny of 238 no member, of those evaluated, possessed *S a;* all had *S b*. That *S b* pollen should function in the relation of *S 1.3.b* × *S a.b* is nonconforming both to incompatibility and to unilateral hybridization.

Data for the AAI Hybrids × 2n P. axillaris. *Data for Series 242.* No figure is here presented for the resultants of the tests of the plants of this series but the summary of the data for them is as follows. The parentage was *S 1.3.b* × *S 1.2*. Twenty-six plants were grown. Only four strongly resembled *P. integrifolia*. Most of the series were much like *P. axillaris* (figure 53) and several had white flowers and yellow pollen. But in only two plants were the corolla tubes as narrow as in *P. axillaris*. Each plant received an entire genome from its *P. axillaris* parent but a few plants (A in figure 53) were more strongly like *P. integrifolia* than any F_1 plant of the AI hybrids. But this plant was *sn:18* and the 11 chromosomes that it received from the *AAI* parent probably included more than one set of *P. integrifolia*.

The chromosome numbers were determined for ten members; one had *15,* one had *16,* one had *17,* two had *18*, one had *19* and a fragment, and four had *20*. Collectively, these plants probably received from *8* to *13* chromosomes through the gametes of their female triploid parent.

Each of the twenty-six plants was self-incompatible. The ten whose chromosomes were counted were also cross-incompatible in all of their reciprocal relations that were tested and hence appeared to be one genotype. Forty-three of the fifty possible relations of these plants with pollen of *S 1.1*, *S 1.2*, *S 1.3*, *S 3.3*, and *S 2.3* were tested and found fully sterile. Hence these plants appeared to be *S 1.2.3*. But none of the ten whose chromosomes were counted was triploid and one of these was *sn:15*. Thus ♀ gametes of the triploid *S 1.3.b* which have as few as nine chromosomes may carry two *S* factors and be heterogenic for them.

Six of these ten members of 242 were fertile with pollen of *P. integrifolia S a.b*, *S a.a*, and *S b.b* which is the normal reaction of hybridization in relations of AA and AAA *P. axillaris* × *P. integrifolia*. But three of the ten members were definitely sterile with pollen of *S a.b*, *S a.a*, and *S b.b* which were nonconforming reactions that could not be due to normal behavior of either incompatibilities or unilateral sterility. Apparently all ten of the members that were tested and whose chromosome numbers ranged from *sn:15* to

sn:20 were triallelic and *S 1.2.3*. In this case the female gametes that functioned must have been *S 1.3*.

Data for Series 243. The seed parent was 170–5 (*S 1.?.b*) × *P. axillaris S 1.3*. The production of seeds by this particular relation was nonconforming to the normal reactions of the *S 3* factor which other reactions seemed to indicate was present in the plant 170–5 (figure 47).

Thirty-six seedlings were obtained but they were noticeably irregular in vigor and several that were weak died early. Twelve were kept until they

243		Ch.	3	2	12	11	5	25	14	S 1.1	S 3.3	S 1.3	S 1.2	S 2.3	S a.b	S a.a	S b.b
3	S 1.3	14	S	F	F					S	S	S	F	F	H	H	H
2	S 3.b	"	F	S	S	S	S	S	S	F	S		F	F	H	H	S
12	"	"		S	S	S			S	F	S	F	F	F	H	H	S
11	"	15		S	S	S	S	S	S	F	S	F	F	F	H	H	S
5	"	16		S		S	S		S		S	F	F	F	H	H	S
25	"	19		S				S	S	F	S	F	F	F	H	H	S
14	"	20		S	S	S	S	S	S		S		F	F	H	H	S

P. axillaris	S 1.1	F	F		F	F	F	
	S 3.3	F	F	F	F	F	F	F
	S 1.3	S	F	F	F			
	S 1.2	F	F	F	F	F	F	
	S 2.3	F	F	F	F	F	F	F
P. integrifolia	S a.b	0	0	0	0	0	0	0
	S a.a	0	0	0	0	0	0	0
	S b.b	0	0	0	0	0	0	0

FIG. 55. Pattern of the reactions of seven members of a back-cross progeny of AAI: *S 1.?.b* × *2n P. axillaris S 1.3*. As males all had unilateral sterility with *P. integrifolia*.

had flowers. All strongly resembled *P. axillaris* and were quite like plant D that is shown in figure 53. The data for the seven plants of this progeny which were tested most fully are indicated in figure 55.

Three plants had *14* chromosomes, one had *15*, one had *16*, one had *19* and one had *20*. Evidently egg-cells that carried *7*, *8*, *9*, *12*, and *13* chromosomes had functioned. All the members were self-incompatible. The cross-reactions that were tested indicated that one plant was *S 1.3* and that six were *S 3.b*. Evidently each of the egg cells that functioned had carried only one *S* factor, and in six cases this had been *S b*. The one plant that was *S 1.3* could have received its *S 3* factor from its pollen parent, hence there was no definite evidence regarding the condition of an *S 3* factor in the parent plant

170–5. Such data could more readily be obtained by the analysis of a progeny of 170–5 (*S 1.3.b*) × *P. axillaris S 4.5*. But the six plants that were *S 3.b* presumably obtained the *S 3* factor from the *S 1.3* pollen parent.

As females the members of 243 were fertile with both *P. axillaris* ♂ ♂ and *P. integrifolia* ♂ ♂ except for the normal reactions of the *S* factors which they possessed. There was also fertility with *P. axillaris* ♀ ♀ except for the one cross-incompatibility of *S 1.3* × No. 3. The fertility of the testers *S 3.3* and *S 2.3* as females with members of *S 3.b* probably involved hidden incompatibility for pollen tubes of *S 3* and the functioning of pollen tubes that carried *S b*. These should be classed as a hybridizing reaction.

As females these plants had intraspecific fertility with AA genotypes and interspecific hybridization with II genotypes except for incompatibilities when a relation was syngenic. In these respects the plants were comparable to pure *P. axillaris*. As male members these plants were fertile with AA ♀ ♀, but in numerous cases the relation was syngenic for *S 3* (as *S 3.3*, *S 1.3*, and *S 2.3* × *S 3.b*) and hence it was probably pollen that carried *S b* that functioned. In respect to the component of *S 3.b* which was derived from *P. integrifolia* the relation was one of hybridization fertility which was not in conflict with intraspecific action of the component that was *P. axillaris*. But all members of series 243 were sterile as pollen plants with all genotypes of *P. integrifolia*. These reactions correspond to that of unilateral sterility of II ♀ ♀ × A ♂ ♂. In these relations the class of *S b* pollen did not effect seed production by *S a.a* ♀ ♀ plants. Hence the *S b* pollen possessed that haploid constitution of A ♂ which was responsible for the unilateral sterility in the relation II ♀ ♀ × A ♂. Thus it seems that the factor or factors that effect this specificity of A ♂ are not *S* factors.

Potential Fertility in the 3n × 2n Progenies. Special evaluations were made of the potential fertility of two of the members that had *14* chromosomes and were *S 3.b*. The pollen was noticeably uniform in size, all grains were trilete, but about 25 per cent of the microspores were aborted. There was excellent germination of a high percentage of the grains which had contents in cultures of 5 per cent agar and 1 per cent sugar. Also the production of normal seeds to fertile relations was high. Potential fertility in terms of the proportion of macrospores (and ovules) and microspores was increased over that of the *3n* hybrids (AAI) and over that of the *2n* hybrids (AI). But there was somewhat more abortion of both microspores and macrospores than in the *2n* members of *P. axillaris* or *P. integrifolia*.

The pollen of a plant that had *20* chromosomes and was *S 3.b* was evaluated. About half of all microspores were shrivelled and empty, and those that had contents were irregular in shape and some were four-pored.

It was observed that certain of the aneuploids of these and of other progenies had high percentages of abortions of both pollen and ovules, the latter being judged by the scale ovules present in capsules obtained in non-incompatible relations. But in every case a plant was able to produce seeds

that were apparently normal except only the three plants listed at the bottom of figure 54.

SUMMARY

1. In both of the AAI × AA and the AAI × II progenies the number of chromosomes included all numbers from *14* to *20* inclusive and the frequencies were, in sequence (figure 59), 4, 12, 9, 4, 5, 3, and 8. Evidently the ♀ gametes of the triploid hybrids which had functioned carried *7, 8, 9, 10, 11, 12,* and *13* chromosomes and of these *8, 9,* and *13* were most frequent. The selfed progenies of the AAI triploids had chromosome numbers which indicated that the gametes that functioned possessed chromosome numbers of *7, 9, 10, 11, 12, 13,* and *14.*

2. The seedlings of *S 1.3.b* × *S a.b* that were identified included the genotypes *S a.b, S b.b, S 1.b* and *S 3.b.* It is to be noted that the relation *S 1.3.b* × *S b.b* was incompatible but that in *S 1.3.b* × *S a.b,* the *S b* pollen tubes functioned more frequently than did the *S a* pollen tubes. Also most of the egg cells that functioned carried only one *S* factor.

All members of *S 1.3.b* × *S 1.2* which were analyzed seemed to be *S 1.2.3,* in which case the egg cells which functioned must have carried two *S* factors even when the number of chromosomes in the zygote was as low as *15.*

Of the seven members of *S 1.?.b* × *S 1.3,* one was *S 1.3* and six were *S 3.b.* In the production of these, evidently egg-cells that carried *S b* and pollen tubes that carried *S 3* had functioned most frequently.

3. Every member of all these progenies was self-incompatible and there were numerous cross-incompatibilities that conformed to normal reactions. But there were unconforming resultants, especially in the reactions of plants that had a higher number of chromosomes.

4. Reactions of unilateral sterility were obtained in the relations between certain members of these progenies and *P. integrifolia.* Certain members of the progeny of AAI × II, especially of the members of series 235, had the component of *P. integrifolia* ♀ which effected unilateral sterility with *P. axillaris.* The triploids of AAI did not have this complement. The seven members of series 243 derived from AAI × AA had the component of *P. axillaris* ♂ which effected unilateral sterility with *P. integrifolia* ♀. The triploid hybrids of AAI also had this complement. The conclusion is that the unilateral sterility of hybridization in *Petunia* involves reactions between diploid pistils and haploid pollen tubes and that this reaction and its genic determinative are independent of *S* factors.

Progeny of 3n Hybrids × *4n P. axillaris*

The relations of the triploid hybrids (*S 1.3.b*) × *4n P. axillaris* gave fine capsules which frequently contained as many as one hundred plump seeds of large size of which few had an embryo. All the seeds, which totaled 128, in three capsules were planted but only three germinated and one of these died in the cotyledon stage.

One plant of *S 1.3.b* × *S 1.1.2.2* had *20* chromosomes, was self-incompatible and fully sterile with ♂ ♂ of *S 1.1, S 3.3, S 1.2, S 1.3,* and *S 2.3,* but it produced capsules that had mostly pseudoseeds with ♂ ♂ of *P. integrifolia S a.b, S a.a,* and *S b.b.* Evidently this plant was *S 1.2.3,* in which case it had obtained the *S 3* from the ♀ ♀ parent.

The other seedling was derived from *S 1.3.b* × *S 1.1.3.3.* This plant had twenty-six chromosomes and it was fully self-fertile. It was sterile with pollen of *S 1.1, S 3.3,* and *S 1.3* but there were parthenocarpic capsules and many pseudoseeds to pollen of *S 1.2* and *S 2.3* and there were some capsules and seeds to pollen of *P. integrifolia S a.b, S a.a,* and *S b.b.* Evidently this plant had only *S 1* and *S 3* factors, but its self-fertility indicates that it was probably *S 1.1.3.3.*

Chapter 12. Hexaploid Double Triploids of Petunia

The First Double Triploid. *Pedigree, Origin, and Character.* The apical vegetative buds of vigorously growing rooted cuttings of several of the triploid hybrids of series 170 (AAI: *S 1.3.b*) were treated, during March, with 1 per cent colchicine in lanolin. In only one case did the terminal bud continue growth and it was hexaploid. No hexaploid laterals appeared on the other plants whose treated terminals died. The young leaves that were treated became abnormal in shape but the later leaves on the hexaploid stem were more normal in shape but definitely coarser than the leaves on the triploid parts of the same plant. Also the stem was noticeably thicker (figure 45 B).

The first flowers on the hexaploid stem opened in July and they produced capsules and viable seeds to self-pollination. Thus an inactivation of *S* factors was effected by the change from triploidy to double triploidy. The anthers of a flower bud were examined by smear stains of aceto-carmine. There were *42* (*6n*) chromosomes in the pollen mother-cells and frequently there were distributions which showed *21* chromosomes at each of the two poles of the first division and in each of the equatorial plates of the second division. There were twelve flowers in the terminal inflorescence, which was a bostryx. Seven flowers were selfed; all formed good capsules, but the number of seeds in a capsule did not exceed 88. The anthers of the flowers were noticeably larger than the anthers of any of the triploids of AAI as shown in figure 45.

Cuttings were made of the lateral branches that appeared below the hexaploid bostryx, but when these flowered in the following year all but one ramet had flowers that were self-incompatible and otherwise like the flowers of the triploid from which the hexaploid was derived. One ramet had a few flowers that were hexaploid and self-fertile but the rest of the flowers above these in the same bostryx were triploid. All further propagations were also triploid. The treatment had effected hexaploidy in the apical stem and in a segment of the lateral next below and otherwise the triploid cells had outgrown those that were hexaploid. It has rather frequently been observed (especially

by Levan, 1939) that mixtures of diploid and polyploid tissues may occur in the branches of a plant and that the diploid tissues tend to outgrow those that are polyploid.

The First Generation of Seedlings. *General Data.* Thirty-nine seedlings of the first generation of hexaploids were obtained from 81 plump seeds of three selfed capsules of the first hexaploid branch. These seeds were planted in November but germination was irregular and some seedlings did not appear above the soil until February. Several seedlings were weak and died early. An effort was made to grow all to flowering age. Thirty-one flowered.

The growth of the best of these seedlings was slower than that of the typical seedlings of any other cultures of *Petunia* that had been grown. Figure 57 shows plants of the second generation but they are representative of the appearance of the seedlings of hexaploids during May when diploids of the same age from seed planting were in good bloom. Some of the seedlings of the different *6n* progenies were so slow in growth that they did not flower until the summer of their second year of life. But the best of the plants became fine plants with leaves and stems that were much coarser than those of the triploids. Axial growth tended to predominate but some were well-branched. The best plants were obtained from cuttings that were rooted in late summer and were given good care during the following winter. Figure 36 shows a group of such ramets which had been kept in four-inch pots to facilitate the manipulations of controlled pollinations.

The first flowers on plants of the first generation appeared in late May several weeks after *2n* seedlings of seed plantings in December were in heavy bloom. The flowers of all these seedlings had some shade of purplish rose but there was much diversity in the shades of color. Except for flower coloring there was almost no trace of the characters of *P. integrifolia.* The flowers were coarser than those of the triploids of series 170 (figure 45) and the corolla was often more lobed and recurved. In no plant of this generation did the longer pair of stamens extend above the stigma but this condition appeared in some of the second generation.

Chromosome Members. The chromosome members were determined in the somatic cells of twenty-five of these plants (Sullivan 1947). Six were *sn:40,* three were *sn:41,* and sixteen were *sn:42.* Evidently the egg and sperm-cells that functioned most frequently in the self-fertilizations had *21* chromosomes but some gametes which had *19* and *20* also functioned. This indicated that there was a high degree of bivalent autosyndesis in which there was pairing of identical chromosomes.

Character of the Pollen. The pollen of five sister plants, each of which was *sn:42,* was evaluated by Mr. Alfred Owczarzak and his data are as follows:

217– 5: abortions 53%; range in size, 34.2–57.0 μ.
217–11: abortions 58%; range in size, 41.8–57.0 μ.

217–14: abortions 54%; range in size, 38.0–51.3 μ.
217–19: abortions 74%; range in size, 34.2–51.3 μ.
217–20: abortions 59%; range in size, 41.8–55.1 μ.

The mode for the size of all plump grains was 47.5 μ. In shape the grains were mostly trilete with some that were quadralete and a few that were pentalete. Thus the pollen grains of the hexaploids ranged to larger sizes than did pollen grains of tetraploids.

Potential Fertility in Seed Production. Figures 42 N, O and P show seeds and scale ovules that are typical of the selfed hexaploids and para-hexaploids. The data for 19 capsules which were representative of those obtained by selfing are as follows:

Plant with 40 chromosomes: 40 seeds
Plant with 40 chromosomes: 61, 67.
Plant with 41 chromosomes: 28, 30, 38, 39, 45, 104.
Plant with 41 chromosomes: 64.
Plant with 42 chromosomes: 33, 47, 50, 62, 81, 96.
Plant with 42 chromosomes: 87.
Plant with 42 chromosomes: 16, 71.

The seeds were plump and of good size but dissections showed that about one-fourth of the plump seeds were empty. Every capsule contained numerous scale ovules which evidently represented ovules that were unable to function in any relation. In terms of the number of seeds produced in a capsule these plants produced fewer seeds than did the tetraploids of *P. axillaris* to self-pollination. Also a larger proportion of seeds were empty and pseudospermic. There were numerous scale ovules in the selfed capsules of both the *4n* of AAAA and the *6n* of AAAAII. That the latter had the smaller capsules and the fewer ovules of all kinds was presumably due to the complement of the constitution that was *P. integrifolia*.

Self- and Cross-Fertility. All the thirty-one plants of the first hexaploid generation that lived to blooming age produced capsules that contained at least some viable seeds to self-pollination and also there was fertility for every cross-relation that was adequately tested.

But there was a reduced potential fertility. Many scale ovules were present and the resultants of pollinations were irregular to a degree not observed in other petunias. Frequently there were some pollinations that failed and these appeared to be due to irregularities in the maturity of stigmas especially in respect to the production of secretion. When the first flower of a relation failed, as many as ten further test pollinations were made and at least one good capsule was always obtained. For example: six flowers of 217–15 were selfed; three failed and three produced good capsules. Of the seven flowers which were selfed on 217–4, only one failed. All the seven flowers of 217–5 which were selfed produced good capsules. During periods of hot dry weather there were days when few pollinations were effective on

these *6n* plants, while at the same time the pollinations of *2n, 3n,* and *4n* plants were fully effective.

The Second Generation of Seedlings. *Viability of Seeds.* One progeny (279) was grown from the selfed seeds of 217–4 which was *sn:41.* One hundred and four seeds were planted; 17 germinated and 12 seedlings were grown to maturity. Two were *sn:40;* one was *sn:41;* seven were *sn:42;* one was *sn:43;* and one was *sn:44.*

Five progenies had parents that were *sn:42* and the seeds in one selfed capsule of each were planted. There were 206 seeds; 73 seedlings were ob-

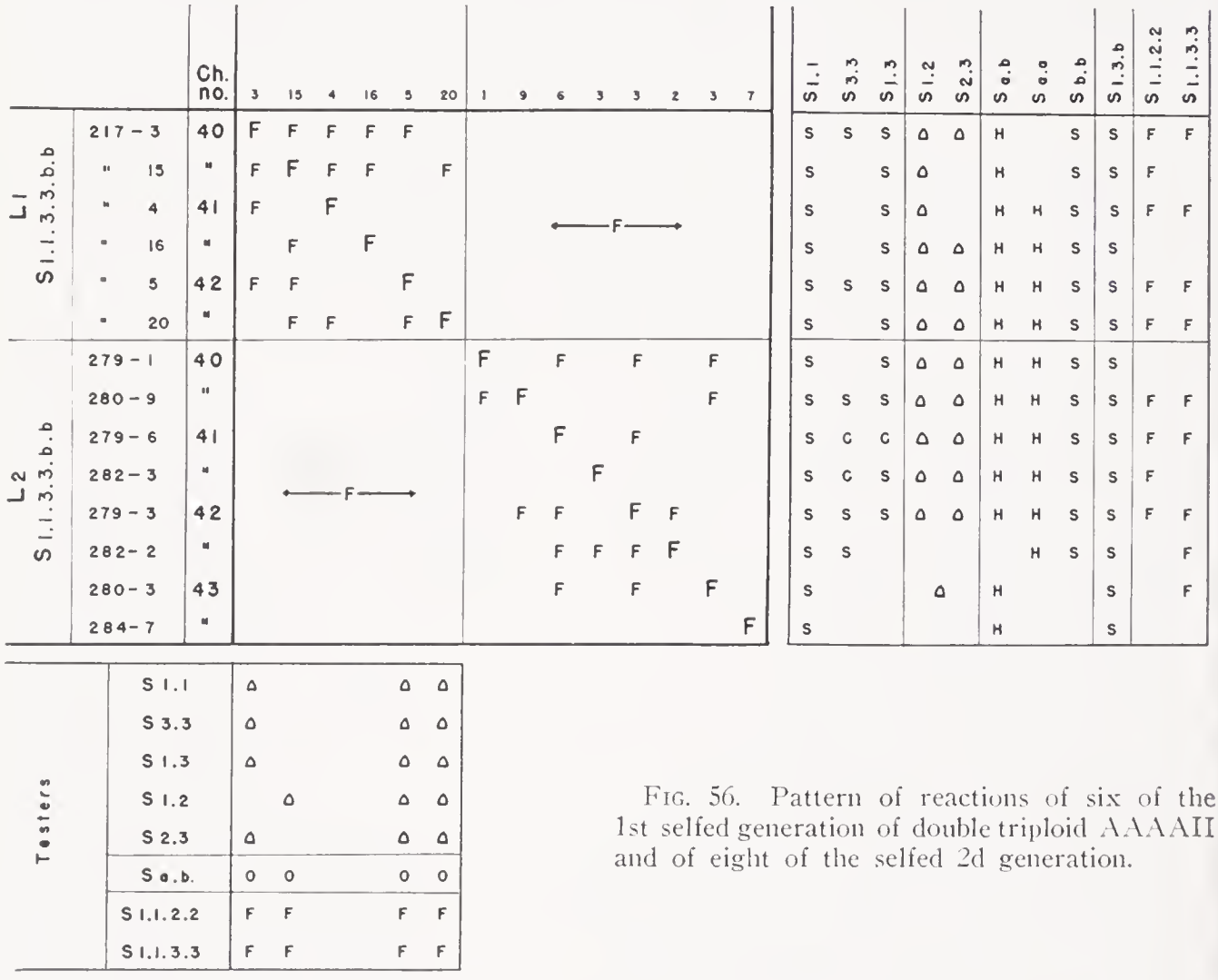


FIG. 56. Pattern of reactions of six of the 1st selfed generation of double triploid AAAAII and of eight of the selfed 2d generation.

tained of which 38 were grown to flowering age. Of these eight were *sn:40;* four were *sn:41;* nineteen were *sn:42;* and six were *sn:43.* Thus the chromosome numbers in the plants of the second generation included the numbers *43* and *44* which indicates that some gametes with as many as *22* chromosomes had functioned. It was certain that some seeds which had embryos did not germinate. No doubt special methods of stimulating germination would have increased the percentage of germination.

Character of the 2nd Generation. In appearance and habits of growth these plants were like the first generation. The fifty that were grown to

flowering age included some that had been weak and slow in their early life. Figure 57 shows the extremes in the appearance of the plants on May 15. Forty seedlings were discarded in the seed pans merely because the experimental greenhouse was overcrowded at the time. There was greater diversity in this generation than in the first generation especially in the size and color of flowers and a few had the longer pair of stamens above the stigma. The plant which had *44* chromosomes had the smallest flowers. Some plants had flowers that were larger than any produced by the F_1 and the corollas were frequently somewhat deeply lobed and recurved. Those that were well-branched made fine plants especially when cuttings were made in late summer and grown with good care over winter (figure 36).

Self- and Cross-Fertility. Forty-nine members of this second generation produced capsules and seeds to self-pollinations, but several gave the irregular resultants already noted for the first generation. One plant failed to produce capsules to any self- or cross-pollination and it appeared to be fully impotent for the anthers were shrivelled, they dehisced poorly, and pollen was either lacking or aborted. Also the pistils were poorly developed.

Seed Production. Thirty-three selfed capsules obtained from nineteen different plants were evaluated. The maximum number of seeds in a capsule was 79 and the average was 36. For plants which had unbalanced numbers of chromosomes (*41* and *43*) the number of seeds per capsule ranged from 22 to 42. The highest number observed was 79 in a capsule of a plant that was *sn:42*. These were rather low numbers of seed in comparison with *2n* and *4n* of *P. axillaris*. It seems that the seed production per capsule was even less than that of plants of *P. integrifolia* of *S a.a.* × *S a.b* relations. But the reduction in seed in the hexaploids and parahexaploids involved abortions of ovules which, apparently, were unable to function in any relation.

Character of the Pollen. The pollen of seventeen of this second generation was evaluated including some plants which had *40*, *41*, *42*, and *43* chromosomes. In shape, the grains which had contents were mostly trilete or quadrilete but some were pentalete (figure 50 L). In size, the composite range was 34.2 μ to 57.0 μ. The largest grains were of a plant that had *42* chromosomes. One plant that had *40* chromosomes had the least abortion of pollen (28%) that was observed and the range in size was 38 μ to 49.4 μ, which suggests that there may have been a very constant *20–20* meiotic distribution of chromosomes. In most plants at least 50 per cent of the pollen was plump and appeared to be normal.

The Cross-Reactions of the Hexaploids. *Cross-Fertility.* The resultants obtained for the two generations of hexaploids in cross-relations and in the relations with the parental species and with other polyploids are indicated in figure 56. Approximately two hundred cross-relations between members of the two generations were tested. Capsules which contained some apparently normal seeds were obtained except in several relations of which

FIG. 57. Young seedlings of the 2d generation of AAAAH. Chromosome numbers ranged from *sn: 40* to *sn: 44*. For best well-grown old plants of these hexaploids see figure *37*. FIG. 58. Seedlings of a progeny of an *sn: 41* plant that was derived of AAAAH × AAAA. A was *sn: 34*; B was *sn: 36*; C was *sn: 35*; D was *sn: 36*; E was *sn: 33*; F was *sn: 32*.

only one flower was tested. There were irregular results quite as in the selfing but whenever several tests were made, at least some pollinations were fertile.

Reactions with 2n P. axillaris. From ten to twenty-five of the members of each of the two generations were tested with pollen of *S 1.1, S 3.3,* and *S 1.3.* There was complete sterility in all except a few tests which gave small capsules that had no seeds of any kind. But with pollen of *S 1.2* and *S 2.3* there were usually capsules but there were mostly pseudoseeds and only a few seeds had an embryo. The complete failures were evidently of syngenic relations and the parthenocarpic capsules with their pseudoseeds were evidently of disgenic relations and the resultants were comparable with those of *4n* × *2n* of *P. axillaris.* The tests of the reciprocal relations of *2n P. axillaris* × *6n* involved about half of the first generation in a total of at least ten tests with each of the genotypes *S 1.1, S 3.3, S 1.2, S 1.3,* and *S 2.3.* Capsules were obtained in each relation and for most of the pollinations but there were seldom as many as fifty seeds of good size in a capsule and apparently all, or nearly all, seeds were empty. The resultants were comparable to those of the *2n* × *4n P. axillaris,* except that the number of pseudoseeds was less. In both cases the *2n* members had high potential fertility and the large number of pseudoseeds resulted from failures of fertilization or from seed abortion after fertilization.

Reactions with the Triploid Parent, S 1.3.b. As indicated in figure 56 every test of *6n* × *3n* (*S 1.3.b*) was a complete failure. These reactions were presumably syngenic in which case they indicate that all of the *6n*'s were probably *S 1.1.3.3.b.b.* Possibly the proportion of heterogenic grains was too few in the *S 1.3.b* pollen to effect any development of capsules even if the *S* factors were inactivated in such pollen.

Reactions with 4n P. axillaris. Approximately fifty of the members of the two hexaploid generations were tested as males with the genotypes *S 1.1.2.2* and *S 1.1.3.3* and a few tests were also made with *S 1.1.2.3.* There were capsules to every relation that was tested by three or more flowers, and the number of well-formed seeds was as high as 156, and a considerable number of these seeds had embryo and endosperm. In addition to the seeds there were at least 200 scale ovules. Two plantings of the seeds of these relations will be reported later. The reciprocal relations of *4n* × *6n* also gave capsules and seeds in each of twenty-five tests with pollen of plants of the first generation. The reciprocal reactions of *6n* with *4n* may be classed as fertile even in syngenic relations but the number of normal seed produced by *4n* plants was obviously much less than that produced by self-fertilization.

Reactions with P. integrifolia. The tests were adequate to demonstrate that members of both generations of the hexaploids produced no capsules to pollen of H: *S b.b* but did produce some capsules which contained a few apparently normal seeds to pollen of H: *S a.b.* The failure with *S b.b* may be attributed to the incompatibility reaction of *S b* pollen tubes in pistils that also possessed *S b.* The limited fertility of the hexaploids, that were strongly

P. axillaris in character and constitution, with pollen of diploid *S a.b* may be considered a hybridization reaction that is comparable with the resultants of *2n* and *4n P. axillaris* × *P. integrifolia*.

SUMMARY

Chromosome Members. Forty-two members of the generations derived from a single *6n* plant were also *6n*. The others had from *40* to *44* chromosomes each. Evidently gametes that had three sets of chromosomes functioned most frequently but some that had *19*, *20*, and even *21* chromosomes must also have functioned.

Genetic Constitution. The original hexaploid was obtained by the somatic duplication of the chromosomes in a triploid known to be *S 1.3.b*. The original hexaploid was a double triploid and certainly *S 1.1.3.3.b.b*. The reactions of all of the members of both generations of selfed progenies suggest that most of them were also *S 1.1.3.3.b.b* but some that were parahexaploid may have lost a chromosome that carried an *S* factor. If so the reproduction was fully heterogenic for *S* factors as it was in the tetraploid progenies.

Potential Fertility. With one exception these hexaploids and parahexaploids were able to function in seed reproduction to selfing. But the abortions of microspores and of macrospores and ovules was rather high even in plants that had six sets of chromosomes. There were at least two conditions which contributed to the low numbers of seeds in any capsule. First, the capsules were smaller than those of *2n P. axillaris* and they contained fewer ovules, but not as few as in the ovaries of *P. integrifolia*. And second, there was abortion of macrospores and ovules, as there was of microspores, which were due, undoubtedly, to incomplete bivalency in meiosis. Evidently there were also seed abortions after fertilization.

Self- and Cross-Fertility. The original hexaploid and all of both generations derived from it, with the exception of one member that was impotent, were self-fertile and intra-cross-fertile. The sterilities of these same plants in syngenic relations with *2n P. axillaris* and with *S b.b* of *P. integrifolia* show that the *S* factors in the hexaploid pistils still had incompatibility interactions when the pollen tubes were haploid and syngenic. It is to be noted that the hexaploids were cross-incompatible to pollen of the *S 1.3.b* triploids which undoubtedly comprised some pollen that had two different *S* factors. It has been considered that this condition of itself provides "competition" which prevents the incompatibility reaction of both *S* factors (Lewis 1947). The self-fertility of *6n* as well as the *4n* supports this hypothesis, but the self-sterility of the triploids and the cross-sterility of *6n* × *3n* do not, unless it is considered that such pollen was too limited in quantity.

Chapter 13. Progenies of the Double Triploids × Tetraploids

Data for the Progenies. *Series 285.* The seed parent had *41* chromosomes and was presumably AAAAH and *S 1.1.3.3.b.b*. The pollen parent

was 139–12 which was AAAA and *S 1.1.3.3*, and an original somatic tetraploid. The relation was syngenic for A components but the pistils had two of the factors of *S b* not present in pollen and also two genomes of chromosomes of *P. integrifolia*, and this component was one that was involved in unilateral sterility with *P. axillaris* ♂ ♂. Hence the fertility of the relation involved the inactivation of the *S* factors common to the *6n* pistils and the pollen tubes and an inactive status of the component of *P. integrifolia* in the pistils. Under these conditions the *2n* pollen tubes of the *4n P. axillaris* were able to function in fertilization in pistils of the hexaploid and para-hexaploid plants. Thus the *6n* constitution of itself did not set up a specificity barrier to crossing with the tetraploids of *P. axillaris*.

All of the 110 seeds of good size in a capsule of controlled pollination were planted. Thirty-two seedlings appeared above the soil of which seven were weak and soon died. Twenty-five flowered. The chromosome members were determined for sixteen; one was *sn:29*, three were *sn:31*; three were *sn:32*; four were *sn:33*, three were *sn:34*; and two were *sn:35* (figure 59).

Series 286. The seed parent was *sn:42*. The pollen parent was *S 1.1.2.2*. The relation was disgenic for the *S 2* factor. Sixty-eight seeds were planted in December 1945. There was irregular germination. A total of fifty seedlings appeared but several soon died. Twenty-five were grown to flowering age and these included several that were late in germination and so slow in growth that they did not flower until the summer of 1947 (see figure 59). Chromosome members were determined in sixteen; two were *sn:32*, four *33*, two *34*, six *35*, and two *36*.

Series 287. The seed parent of this series was a *4n* plant of *S 1.1.2.3*. The pollen parent was a member of the first generation of hexaploid progeny which had *42* chromosomes and was *6n*. The capsule had only five plump seeds of which one germinated and this seedling had *35* chromosomes.

Chromosome Members in Gametes. If it is assumed that the tetraploid parents in these *6n* × *4n* relations contributed gametes that had *14* chromosomes, then the gametes of the *6n* and the *6n* – *1* parents, that functioned to give series 286 and 287, carried some of each of the numbers from *15* to *22*. This means that some of the gametes that were agenomic, and of such low number as *15*, may have had only chromosomes of AA, that others probably had combinations of AA and I, and that gametes that had *21* chromosomes probably were trigenomic for AAI.

Character of the Progenies. There were extreme differences in vigor of growth (figure 58) of the members of these progenies and this was greater than the diversity in the selfed progenies of the hexaploids. Many had axial growth with few or no laterals. Two that were weak and slow in growth were given special care and in their second year they bore seven and twelve flowers respectively. Each was self-fertile and not distinguishable from others of the series. Both had *36* chromosomes which was the highest number found in these progenies. Possibly each received *22* chromosomes from the *6n* seed parent.

The flowers of these seedlings were variable in size. In some cases they were larger than the flowers of any *6n*. All flowers had stamens of three lengths but in only three plants was the pair of longest stamens above the stigma, as in *P. integrifolia*. Four plants had entirely white flowers and yellow anthers and three of these were less than *sn:42* and one was *sn:29*. The others had flowers of various shades of pale and dull purple. There were none of the attractive shades of rose and lavender that were seen in the F_2 hybrids of *2n P. axillaris* × *P. integrifolia* and in the back-cross progenies of *3n* hybrids × *2n* parental species.

Self- and Cross-Fertility. The fifty-one members of these progenies that flowered were self-fertile. Many cross-relations were tested and the resultants indicated that any member was cross-fertile with any other one. There were fewer irregularities in the results than were experienced in the testing of the hexaploids. For many of the relations every pollination gave a capsule.

Potential Fertility. The capsules were usually of good size and frequently they were as large as those of the *4n P. axillaris* and hence larger than capsules of the *6n*. The highest number of seeds observed in any capsule was 241. As a rule these seeds were plump and almost spherical (figure 43 Q). A considerable proportion of these seeds had endosperm and embryo. There were always several hundred ovules that did not function and which became scales.

The anthers of the flowers of all members of these progenies dehisced normally and there was pollen but frequently it was less abundant and granular than the pollen of diploids. The highest abortion of about 70 per cent was observed in the pollen of 286–26 which had *36* chromosomes and was a plant of poor and feeble growth. But all plants of all the chromosome members, balanced or unbalanced, had some functional pollen and produced capsules and some seeds that appeared to be normal.

Data for the Pentaploids. *Origin and Status.* There were nine pentaploids in these progenies. Two of series 285 had the parentage *S 1.1.3.3.b.b (?)* × *S 1.1.3.3;* six of series 286 were *S 1.1.3.3.b.b* × *S 1.1.2.2;* and one was of *S 1.1.2.3* × *S 1.1.3.3.b.b*. Hence eight of these pentaploids arose from the fusions of sperms that were purely AA with egg cells that were presumably trigenomic and that were probably AAI. Presumably their constitution was AAAAI and hence unbalanced for the I genome.

Self- and Cross-Fertility. From three to seven flowers per plant were selfed and every pollination gave a capsule. There were also capsules and seeds for every one of the twenty-nine cross-relations which were tested. The inactivation of *S* factors in pollen continued in these *5n* plants as it did in *4n* and *6n* plants and in the aneuploid sisters.

Potential Fertility. Special studies were made of the pollen of five of these pentaploids. The percentages of aborted grains were 27, 37, 57, 57, and 59. The size of the pollen grains that had contents ranged from 32.3 μ to 53.2 μ with a mode at 53.2 μ which was slightly larger than the pollen of the

tetraploid *P. axillaris*. Trilete grains were numerous, quadrilete grains were frequent, and there were some pentalete grains (figure 51 W). In one preparation of pollen of 286–7 there were seventeen pentalete grains. Abortion of pollen was greater than in *4n P. axillaris*. The number of plump seeds of good size (figure 42 Q) in eighteen selfed capsules ranged from 19 to 231 with an average of 89. One hundred and eight seeds taken at random from eight capsules were dissected and 87 had an embryo. There were at least two hundred scale ovules in a capsule.

Reactions with 2n P. axillaris. All of these pentaploids, and also all others of these progenies that were tested, were cross-incompatible with the haploid pollen of *S 1.1*. The two *5n* plants of series 285 were also cross-incompatible with pollen of plants of *S 1.3* and *S 3.3*. The six members of series 286 were cross-incompatible with *S 1.1* and *S 1.2* as males but produced capsules with the pollen of *S 1.3* and *S 3.3*. Six of the best of these capsules were evaluated. There was an average of about 100 seeds of good size per capsule. Forty-two of the best seeds in one capsule were dissected and an embryo was found in nine of them. Evidently the $5n \times 2n$ reactions were incompatible when the relation was syngenic but parthenocarpic with some good seeds when the relation was disgenic.

In the reciprocal relations of *2n P. axillaris* × *5n* there were, in every relation tested, capsules with pseudoseeds or possibly an occasional good seed. This resultant was quite comparable to that of $2n \times 4n$ *P. axillaris*.

Reactions with AAI (S 1.3.b) Triploids. Fourteen different combinations were tested which involved seven of the *5n* plants as females with four of the triploids of AAI. Every pollination failed in that capsules did not start to form. But every one of the thirty-nine reciprocal pollinations ($3n \times 5n$) that were made gave capsules and seeds of good size of which some had endosperm and embryo.

Reactions with 4n P. axillaris. Thirteen different combinations of $5n \times 4n$ and eleven of $4n \times 5n$ were tested which included some of each of the genotypes *S 1.1.2.2*, *S 1.1.3.3*, *S 2.2.3.3*, and *S1.1.2.3*. In all relations there were capsules and the number of plump seeds per capsule ranged from 39 to 214 and some of the seeds had an embryo. These tests were sufficient to show that the *5n* and the *6n* plants of these progenies were fertile reciprocally with the *4n* of *P. axillaris*.

Reactions with P. integrifolia. As pollen members these pentaploids were completely sterile with the Kew clone (*S a.b*) which is comparable to the hybridization sterility of the diploid parent species (*P. integrifolia* × *P. axillaris*).

As seed members the pentaploids were feebly fertile with pollen of *S a.b*. There were failures to some of the pollinations but the relation did give several small-sized capsules with a few seeds that had embryos. A reduced fertility was characteristic of the unilateral hybridization seen in the relations of *2n*, *3n*, and *4n P. axillaris* × *P. integrifolia*. The five relations that

were tested of *5n* × *P. integrifolia*, *S b.b* gave no capsules which may be considered as due to incompatibility reactions in pistils of pentaploids that carried a single *S b* factor.

SUMMARY AND EVALUATIONS

1. All members of these back-cross progenies of *6n* with *4n* were self- and cross-fertile. Thus there was inactivation of *S* factors in at least certain of the pollen in all relations of self- and inter-reproduction and also in relations with *4n* and *6n* plants.

	Chromosome numbers														
Parents	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
4n, P. axillaris															∞
4n x 2n "	9	–	–	–	1	–	–	17							
2n x 4n "					1	–	–	–	–	–	–	–	–	–	1
4n P.a. x 2n P.i.							1	6							
3n AAI						1	4	–	–	2	1	3	1	–	1
" " x 2n, P.a.	3	2	2	1	2	2	5								
" " x " P.i.	1	10	7	3	3	1	3								
" " x 4n							1	–	–	–	–	–	1		

	Chromosome numbers																
Parents	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
4n, P. axillaris	∞																
6n, AAAAII													6	3	16		
" 2d. gen.													10	6	26	7	1
6n x 4n					2	4	2	6	2								
6n-1 x 4n		1	–	3	3	4	3	2									
4n x 6n								1									

FIG. 59. Summary tabulation of the chromosome numbers in all progenies except those exclusively of diploid parentage.

2. The chromosome numbers in these progenies ranged from *sn:29* to *sn:36* with all intervening numbers represented except *sn:30*. It may be assumed that the *4n* parents contributed gametes that were *gn:14* and bigenomic. Hence it appears that the gametes of *6n* and *6n – 1* that functioned were as follows: one had *15* chromosomes; 3 had *17*; 5 had *18*; 8 had *19*; 6 had *20*; 9 had *21*; and 2 had *22*. In the selfed generations (figure 59) the somatic numbers ranged from *sn:40* to *44*. Of all progenies, 42 plants were

sn:42 and 9 were *sn:35* and in the production of these a total of 93 gametes that were undoubtedly *3n* had functioned. But the total number of aneuploids was 57, and in the production of each of these at least one gamete had either less or more than three genomes. Thus it was demonstrated that the double triploid constitution of *aaa'a'ii* was somewhat unstable and gave rise to aneuploid offspring. Some agenomic gametes and zygotic combinations of them were viable but some of these were weak in growth.

3. With one exception all seedlings of these progenies had some degree of potential fertility that effected seed production and admitted of self-reproduction. A goodly number of the aneuploids were as vigorous in growth and as fine in appearance as any of the plants that were *6n*. Had there been opportunity, the progenies of plants which were *sn:32, 34, 36, 40,* and *44* would have been grown to determine the patterns of their reproduction and the possibility of obtaining dysploid races. It is postulated that this is possible.

Chapter 14. Evaluations of Data on Reproduction in Petunia

The Methods of Reproduction. *Biscxuality.* The species and most horticultural varieties of *Petunia* have perfect and homomorphic flowers and each flower functions as a male and a female. Certain cultivated double flowered types do not have functional pistils and are unable to produce seeds.

Pollination. Plants of *P. parodii* and *P. parviflora,* which have no self- and cross-incompatibilities, produce numerous capsules and seeds to autonomous self-pollination. The flowers of *P. integrifolia* are strongly zygomorphic but the two longest stamens extend above the stigma and the flowers are readily self-pollinated. The pistils in flowers of *P. axillaris* extend slightly above the anthers. But incompatibilities make proper cross-pollinations necessary for production of seeds by flowers of *P. axillaris* and *P. integrifolia* and this is accomplished by insects.

Meehan (1870) observed that night-flying moths effect pollinations of petunias and that diurnal bees cut holes at the base of the tubular corollas in obtaining nectar. In England, Darwin (1876) never observed insects visiting the flowers of petunias and he states that his gardener had only seen one bee among such flowers. Over nearly twenty years the writer observed during the daytime only one butterfly and one honey bee inside of the large greenhouse which contained many petunias in good bloom from April until September and only once was a hawk moth seen during the hours of daylight. For several weeks during the early part of the spring the ventilators of the greenhouse were closed at night and during this time plants of *P. axillaris* produced no seeds unless properly pollinated by hand. When warmer weather arrived and ventilators were open at night, hawk moths effected proper cross-pollinations and numerous capsules developed. In the writer's home garden hawk moths were frequently observed at dusk and later as

visitors to flowers of petunias and of night-blooming *Hemerocallis*. The flowers of *Petunia parviflora* are so limited to a diurnal anthesis that, in the greenhouse at least, the flowers were seldom open for as much as an hour at midday, but there was no self-incompatibility and capsules and seeds were produced to autonomous selfing.

During the summer of 1950, the writer spent several hours almost daily in the experimental plots of petunias grown by the W. Atlee Burpee Company at Floradale Farms near Lompoc, California. Bumblebees were rarely seen among petunias as incidental and transient visitors. Honeybees were regular and abundant as visitors to a bed of flowering *Nicotiana*, that was situated in the midst of the petunias, where they obtained nectar that was flowing in a thin and, to the eye, imperceptible film out of the throat of deflexed flowers. Frequently such insects wandered about for a few moments among the flowers of adjacent petunias but they rarely alighted on a flower and when they did there was no attempt to gather either pollen or nectar. Humming-birds were occasional but brief visitors to flowers of petunias. But at dusk, especially during midsummer, hawk moths were often abundant in sustained visits to petunia flowers and as many as three different species were observed.

Sexuality and Agamospermy. Redinger (1938) has reported that plants of cultivated petunias which he called *P. nyctaginiflora* produced some pseudogamous seeds to the stimulus of the pollen tubes of certain plants of *Nicotiana* and *Salpiglossus*. The cytological studies indicated that the haploid eggs had a mitosis without cell division and hence became diploid, after which a diploid embryo developed. In one instance he reported that two successive mitoses occurred before there was a cell division and that this gave rise to a tetraploid embryo. Hesse (1938) also reported that he obtained both diploid and tetraploid seedlings of pseudogamous seeds of *P. axillaris* that were induced by pollen tubes of *Salpiglossus*.

Possibly induced agamospermous seeds may sometimes be formed in diploid and polyploid petunias in connection with intraspecific self- and cross-incompatibilities, and also in the *4n* with *2n* relations even when there are no incompatibilities. Steere (1932) obtained 1,348 diploids from a *2n* × *4n* cross. Of the same relation Matsuda (1935) obtained 85 diploids out of 89 seedlings and Levan (1937) obtained 6 diploids in a total of 16 seedlings. The writer obtained 9 diploids in a progeny of 27 members of *S 1.1.2.2* × *S 1.3* of *P. axillaris*, but since four of the diploids were *S 1.3* it is certain that the tetraploid parent had produced some haploid egg cells which carried *S 1* and that these had been fertilized with a sperm that carried *S 3*. It is certain that there were very few, if any, cases of pseudogamous seeds in the cross-relations reported in this paper. There were cases of non-conforming behavior of *S* factors and some of these may have involved agamospermous reproduction.

There were no cases of autonomous somatic polyploidy either in the

branches of a plant or in the primary cell of a zygote in any of the petunias studied by the writer.

The Extent and Character of Self- and Cross-Incompatibilities. A survey of the extensive studies which involved reproduction in cultivated petunias reveals that in most instances incompatibilities were either absent, or of a weak grade, or were disregarded and seeds were obtained by crossing. The few cases in which accurate data were obtained demonstrated that the incompatibilities may be either of the simple personate type or of a complex associate type.

It has generally been considered that the early hybrids of *Petunia* arose from the two very distinct types for which the proper names are *P. axillaris* and *P. integrifolia*, or possibly the type that does not have deflexed pedicels and which has been designated as *Petunia inflata* (Fries 1911). Definite mention was made by Lotsy (1914) of a white-flowered type that had a long, thin corolla tube which was not at all inflated or had only a trace of enlargement. This description suggests the character of *P. parodii* which was first specifically designated by Steere (1931) but was previously obtained from Argentina and described by Ferguson and Ottley (1932). But Lotsy states that he did not find any plants in his extensive cultures (as many as 2,000 F_2) that were self-fertile, but he provided no definite data on the patterns of the cross-incompatibilities.

In the list of "self-sterile" plants by Darwin in 1868 there was no mention of a *Petunia*. But in 1876, he stated that "*Petunia violacea*" was "quite sterile as far as I have observed." Yet in his studies of the cultivated race, which he incorrectly called *P. violacea*, seeds were obtained to self-pollinations, but never a full complement, and from such seeds five successive generations of seedlings were grown. A higher proportion of capsules and more seeds per capsule were obtained when cross-pollinations were made. But Darwin did not believe that cross-incompatibilities ever occur among the members of a variety or species that has homomorphic flowers. Evidently the techniques of his pollinations and the tabulations of results did not sufficiently reveal cases of cross-incompatibility to convince him that they existed.

Correns (1901, 1912) made studies of the inheritance of flower coloring in the hybrids of cultivated petunias which he called *P. nyctaginiflora* and *P. violacea*. He grew progenies in considerable number of *reciprocal* crosses, which is evidence that he did not have the true species. There is little information regarding incompatibilities in these plants but it is stated that of eleven sister plants six were self-fertile, two were feebly self-fertile, and three were self-incompatible. The cross-relations did not give uniform reactions. Correns decided that the reactions in these plants were not favorable for an evaluation of the heredity of self-fertility and self-sterility which he then believed should be simple and Mendelian in respect to dominance.

Tarao (1923) also recognized self-fertile and self-incompatible individuals among cultivated petunias and a progeny of a cross between the two was

composed of 26 self-fertile and 22 self-incompatible members. His evaluation was that a factor of self-fertility was allelic and dominant to one of self-sterility, which was the view that prevailed previous to Prell's hypothesis of oppositional factors. Several other brief considerations of self-incompatibilities in petunias during this period contribute no important data.

Ferguson and Ottley (1932) report that their plants of *P. parodii* (called *P. axillaris* at that date) were self-fertile. The use of such plants in breeding and in hybridizations provided many plants that were self-fertile. But self-sterility was reported (Brooks, Walsh & Ferguson 1930) in at least one pink-flowered cultivated type that was propagated vegetatively. Later, however, members of these clones produced seeds to selfing; the members of the first selfed generation were self-fertile but the members of the next generation were self-incompatible. The basis of this unstability was not determined. Also the term "cross-sterility" was applied to the unilateral failures of hybridization when plants of *P. parodii* (here called *P. nyctaginiflora*) were the male members in crosses with "other strains."

Several of the more recent studies with petunias have indicated a simple personate type in which all plants were self-incompatible and there were no more than four mating genotypes in a pedigreed progeny (as Harland & Atteck 1933; Sears 1937). Other studies demonstrated more complex patterns of reactions that involve an association of, and interaction between, an allelic series of *S* factors and another allelic series of factors which favor fertility. One such study by Wergin (1936) also considered a factor for half-sterility. Another study (Stout 1938) demonstrated the associate type and patterns of reactions in cultures of the diploid Rosy Morn petunia. The characteristic features of this type are that a pedigreed progeny may comprise more than four genotypes, that there are both self-incompatible and self-fertile plants, that the self-fertile plants possess *S* factors, and that there are numerous reciprocals that are different in reaction. Selection does not, as a rule, give a progeny that breeds true to either self-fertility or self-incompatibility.

Information concerning the fertilities and sterilities observed in the extensive breeding with petunias done by the W. Altee Burpee Co., has been supplied by Mr. Jerome H. Kantor and may be summarized as follows: Several expressions of sterility have been observed. There are some "sterile" individuals in nearly all varieties and often this involves a loss of potential fertility. In certain cases the failure to produce seed to autonomous pollinations is overcome by proper self- or intra-varietal pollinations. In some cases there is self-incompatibility. Certain seed-grown varieties produce few seeds to ony intravarietal cross-pollinations but the number of seeds is definitely increased by intervarietal pollinations. The amount of seeds per capsule decreases as flower size increases. Mr. Kantor states that "in all cases there is considerable work to be done to correlate the expressions of ploidy with other factors such as incompatibility." Thus it appears that several conditions have been observed that may be somewhat nonconforming

with present experimental data and that the analysis of such behavior may extend the knowledge of reproduction in petunias.

Vegetative Propagation. Evidently most of the wild species of *Petunia* and the cultivated varieties are essentially annuals without means of autonomous vegetative propagation. Plants of *P. parviflora* have prostrate stems that freely take root to a degree that they could become perennial wherever they can survive winter temperatures. But plants of both the wild and the cultivated petunias may be propagated from cuttings. For the studies of this report numerous individuals representative of various genotypes were maintained as pedigreed clones and used as testers.

The Chromosome Numbers in Petunia. *The Wild Species.* Chromosome numbers have been determined in only five of the twenty-eight species of *Petunia* that are recognized as valid taxonomic units. Four of these (*P. parodii, P. axillaris, P. integrifolia, P. inflata*) are *sn:14* and one (*P. parviflora*) is *sn:18*. No polyploid species are known. There is one case of dysploidy. Until the chromosome numbers are more fully determined for the other species no further evaluation can be made of the chromosomal constitutions in the wild members of this genus.

The value of *sn:14*, in four species of *Petunia*, is a low level of diploidy and also it is the lowest now known in the Solanaceae. The lowest numbers in the other genera of this family are *sn:16, 20, 22, 24,* and *28*, but there is some evidence that the *sn:24*, especially in *Solanum*, is itself a multiple of the genome number of *6*. These numbers indicate that the processes of selective reproduction within the Solanaceae have effected dysploid generic differentiations. Also of the two most populous genera, one (*Nicotiana*) has a series of dysploid species that includes the genome numbers of *8, 9, 10, 11,* and *12*, and the other (*Solanum*) has a series of polyploids that includes *sn:24, 36, 48, 72, 96*, and *144*.

The Cultivated Petunias. That certain cultivated petunias were *sn:14* was determined by Ferguson (1924). Soon thereafter Vilmorin and Simonet (1927) reported that some of the garden petunias had *28* chromosome (*4n*) and in 1931 Derman obtained triploids (*sn:21*) as seedling progenies of crosses between tetraploids and diploids. Other publications on chromosome numbers in cultivated *Petunia* have been summarized by Levan (1937) and by Sullivan (1947). From these, it appears that a somatic chromosome number higher than *sn:35* has not been reported in this genus previous to the present report (figure 59) except for two octoploid plants obtained by Levan (1939). But data on the chromosome numbers, and also on self-fertility and self-incompatibility, have been reported for relatively few of the numerous horticultural varieties of *Petunia* that breed true from seed and which now comprise several very distinctly different classes or types that are recognized in seed catalogs.

The writer has received from Mr. Jerome H. Kantor of the W. Atlee Burpee Company a survey of the extensive but unpublished data on culti-

vated petunias that have been obtained by this Company. The data have been summarized in the following statements that have been approved by Mr. Kantor. All varieties in the classes of small-flowered bedding petunias are diploid (*2n = 14*). These include the Gem, Dwarf-Bedding, Bedding, and Balcony types of an aggregate of thirty-two varieties. In this list is the Rosy Morn variety studied by the writer (Stout 1938) which has a complicated associate type of self- and cross-incompatibilities. The extremely large-flowered petunias that are classed as Giant Ruffled and Superbissima are invariably tetraploid. But it is emphasized that a mere doubling of chromosomes of a diploid variety rarely produces plants of the Superbissima class. There are several classes of flower type, as seen in Hybrida Grandiflora, Dwarf Giant, Giant Fringed, and Double Fringed, that include some varieties that are diploid as well as some that are tetraploid. Thus in several cases "it has been impossible to find any great difference between the lines that are *sn:14* or *sn:28*. In some cases there is no way of determining, other than by pollen measurements or chromosome counts, that a strain has *14* or *28* chromosomes." Mr. Kantor reported to the writer that triploids were obtained among the progenies of *4n* × *2n* varieties and that by colchicine treatment of their buds some branches that were *sn:42* were obtained. But he stated that "other than this, we have been unable to demonstrate a chromosome number above *28* in petunias." Hence a special interest centers in the status of the triploids and tetraploids and in their role in the further development of higher grades of ploidy in the genus.

Thus it appears that none of the cultivated petunias of today has more than the tetraploid of the basic haploid number of seven found in *P. axillaris*, *P. parodii*, and *P. integrifolia*. It is certain that the species *P. parviflora* was not involved in the hybridizations that gave rise to the cultivated petunias. The attempts which the writer has made to hybridize plants of *P. parviflora* with ramets of the Kew clone of *P. integrifolia* were complete failures.

The Petunias of the Author's Studies. The progenies of the intraspecific reproduction of each of the three diploid species, *Petunia axillaris*, *P. parodii*, and *P. integrifolia* all had *14* (*7+7*) somatic chromosomes. All of the hybrid progenies grown of these diploids were also diploid. Somatic autotetraploids were obtained of *P. axillaris* and all of their selfed progenies were also tetraploid. Autotriploids (AAA) were obtained from *4n* × *2n P. axillaris*. Triploid hybrids (AAI) were obtained of *4n P. axillaris* × *2n P. integrifolia*, and by colchicine treatment of these, one somatic hexaploid hybrid that were AAAAII was obtained and the selfed progenies were grown. In the aggregate of progenies that were grown and studied (figure 59) there were four polyploid groups (*3n*, *4n*, *5n*, and *6n*) and one or more seedlings of twenty aneuploid somatic chromosome numbers of which the lowest was *sn:15* and the highest was *sn:44*.

Most of the determinations of the chromosomes in these cultures were made by Dr. T. D. Sullivan whose publication (1947) presents twenty-nine

photomicrographs of the chromosomes in somatic tissues of the three diploid species (*P. parodii, P. axillaris, P. integrifolia*) and of various of the polyploids and heteroploids. About two hundred other determinations were made by Dr. Clyde Chandler and Miss Selma Kojan, with the writer checking the preparations, which were smear-stains of stages of meiosis. The evaluations of the *2n*, *4n*, and *4n* × *2n* cultures of *P. axillaris* were mostly completed before Dr. Sullivan began his studies and hence his report includes data for only three triploids of *P. axillaris* and also it does not include other data presented in figure 59. The techniques employed in these studies did not allow the identification in the hybrids of the chromosomes that were derived from the parental species. Even the sa-chromosome, first observed by Sullivan in a plant of *P. axillaris*, was identified in only one triploid and one hexaploid of the hybrid progenies.

Reproduction in the Petunias of Earlier Experimental Studies. *The Data Reported by Kostoff and Kendall.* The recognition that certain of the "gigas" types of petunias, which, according to Steere (1932), had been in cultivation since about 1888, may be tetraploids (Vilmorin & Simonet 1927) was soon followed by the observation (Kostoff 1930) that a tetraploid seedling had appeared among the progeny grown from the seeds of a diploid petunia. Kostoff and Kendall (1931) reported that this tetraploid seedling was fully "self-sterile" even to premature selfing but that its diploid parent was self-sterile to normal pollinations but gave seeds to premature selfing. This tetraploid had abortions of 50 per cent of the pollen but produced seeds to pollen of diploids from which twenty plants were grown. Of these sixteen were *4n*; two were *4n – 1*; one was *3n*; and one was *3n – 1*. It was suggested that the tetraploid progeny of the *4n* × *2n* relation may have arisen by a fusion of a *2n* egg-cell with two haploid nuclei of a pollen tube or by a fusion of a *2n* egg cell with a *2n* nucleus from a pollen tube. The tetraploids of this progeny produced seeds to selfing but seedlings from their seeds were not reported. The somatic chromosome numbers obtained in these studies were *20*, *21*, *27*, and *28*.

The Data Reported by Dermen. Dermen (1931) observed a tetraploid among cultivated diploids of *Petunia*. This plant was selfed and thirty-eight seedlings were grown of which twenty-seven had large flowers and eleven had small flowers. The chromosome numbers were not reported except that one of the small-flowered seedlings was found to be tetraploid. Some studies of the meiosis of the original tetraploid were made. In late diakinesis, *14* bivalents were seen; but there were some non-disjunctions of chromosomes and premature separations of chromatids and distributions of *16 + 12* and *15 + 13* were observed. The original tetraploid was crossed reciprocally with diploids "with apparent success both ways," but of numerous seeds only a few had contents or were "full" and only four seedlings which were triploid were reared and these were of the *2n* × *4n* relation.

A selfed progeny from seedlings of *3n* was obtained: one plant had *26*

(*4n* – *2*) chromosomes and three had *27* (*4n* – *1*) chromosomes. Of *2n* × *3n* parentage, thirty-nine plants were *sn:14* and fifteen were *sn:15*. Of *4n* × *3n* parentage, eleven were *sn:29*, two were *4n*, and one was *5n* (*35* chromosomes). No progeny was obtained of either *3n* × *2n* or *4n* × *2n*. Thus the numbers of somatic chromosomes in the plants obtained by Dermen were *14*, *15*, *20*, *21*, *26*, *27*, *28*, and *35*.

Data Reported by Steere (1932). Steere obtained tetraploids of the cultivated, large-flowered, gigas forms known as California Giants and Diener's Monsters. Large numbers of seeds were obtained of *2n* white-flowered × *4n* dark-flowered and a total of 1,348 seedlings were obtained all of which were diploid. Since the *2n* × *4n* relation does not, as a rule, give viable seeds the large number of diploids obtained by Steere was a non-conforming resultant not fully explained by the view that pseudogamy may have occurred. Of a *4n* × *2n* relation all sixteen members were triploid and there were nine other triploids produced by Dr. E. E. Dale of another *4n* × *2n* cross.

Data Reported by Tjebbes and Levan. Tjebbes (1932) obtained fifteen stocks of cultivated petunias among which there were tetraploids of the superbissima type and diploids that were incorrectly called *P. violacea* and *P. nyctaginiflora*. He observed much self-incompatibility among the plants which he called "*P. violacea*." Evidently selfed progenies were obtained of some of the tetraploids. He found that the *2n* × *4n* relation gave no viable seeds but forty-two seedlings were grown of the *4n* × *2n* cross.

In 1933 Levan took over the cultures of *Petunia* which Tjebbes had grown and he extended the studies to the analysis of chromosome numbers in various progenies of *4n*, of *4n* × *2n*, and of *2n* × *4n*. His publication (1937) includes discussions and tabulations of the data obtained by others on *Petunia*. Levan makes no definite mention of self- and cross-incompatibilities in either the *2n* or the *4n*. He states that he had diploids of a stock of "*P. nyctaginiflora*" that had been selfed for several generations but this may refer to intra-breeding. Evidently at least some of the tetraploids of his cultures were more or less self-fertile. He states that "self-fertilization of triploids succeeds very seldom in *Petunia*" which suggests that there may have been factors for self-incompatibility in both the *2n* and the *4n* parents. It may also be noted that Levan states that the anthers of the petunias which he studied dehisced in the bud stage and that "early pollinations has always proven to give the best results." The writer has already reported that the shedding of pollen before anthesis is characteristic of the self-fertile *P. parodii* and its hybrids but not of the flowers of *P. axillaris*. In the survey by Levan all *4n* seedlings were derived from *4n* types already in cultivation.

Levan's tabulations provide data on the chromosome numbers in the various progenies that involved tetraploid parentage and these may be summarized as follows. The so-called "tetraploid" types of *Petunia* in cultivation in 1933 included plants that had chromosome numbers of *27*, *28*, *29*,

and *30*. Selfed and cross-bred progenies of tetraploid and near-tetraploid plants had been grown to a number of 96 plants: twenty-three had *27* chromosomes; sixty-four had *28*; eight had *29*; and one had *30*. Levan found that the $4n \times 2n$ relation succeeded much more readily than the reciprocal did. But his data show that no more than seven viable seeds were obtained in any cross, which may refer to the seeds in a capsule. He obtained a total of twenty-six seedlings: one had *18* chromosomes; four had *20*; eighteen had *21*; one had *22*; one had *29*; and one had *31*. The summary of data reported by three others for the progeny of $4n \times 2n$ in *Petunia* is as follows. Kostoff and Kendall (1931) grew twenty plants: one had *20* chromosomes; one had *21*; two had *27*; and sixteen had *28*. Steere (1932) reported a progeny of twenty-four, all of which had *21* chromosomes. Matsuda (1935) grew thirty seedlings of which three had 14 chromosomes, one had *20*, twenty-three had *21*; one had *22*; one had *23*; and one had *28*.

For progenies of $2n \times 4n$ petunias there were four reports that were tabulated by Levan. Dermin (1931) grew three plants that were *3n*. Levan grew sixteen plants: six were *2n*; two were *3n*; one was $4n-1$; four were *4n*; one was $4n+1$; one was $4n+2$. Matsuda (1935) grew eighty-nine plants: eighty-five were *2n*; one was $4n-1$; three were *4n*. Steere (1932) reported that 1,348 seedlings obtained of a $2n \times 4n$ relation were all diploid. Steere considers that these diploids did not arise by parthenogenesis but that each one received a set of seven chromosomes from the ♂ parent. That such gametes were produced in considerable numbers suggests that if there was only one plant of the ♂ parentage it may have been a chimera that had some diploid tissue.

The data summarized by Levan, include diverse, miscellaneous, and heterogeneous stocks of both diploid and tetraploid petunias that were in cultivation. The numbers of the progenies of any one relation are few and the number of plants of a progeny is often low. The individual plants that were involved in nonconforming resultants were not propagated as clones and evaluated in reciprocal relations and in other relations that would contribute critical data on the character of the plants and their gametes.

Data for Experimental Tetraploids of Petunia. During the years 1937–1939 several reports were published on the production of somatic tetraploids by the colchicine treatment of diploid cultivated petunias. The citations for these are Blakeslee & Avery 1937; Nebel & Ruttle 1938a; Simonet & Dansereau 1938; Nishiyama 1938; Levan 1939; Perak 1939.

The report by Levan (1939) is of special interest in that two octoploid ($8n = 56$) plants were obtained by a repetition of the doubling induced by colchicine treatment of diploid seedlings. These had robust stems that were three times as thick as in the tetraploids, but the flowers were not as large. The plants were frail and fragile. There was an increase in the irregularities of meiosis: from 10 to 36 per cent of the pollen had contents but the plants produced *no* seeds to selfing or intra-crossing.

The various diploids used in these experiments were diverse cultivated types. The reports demonstrated that tetraploid petunias may readily be obtained by the colchicine technique. The tetraploids which were obtained were usually giant plants in comparison with the parental diploid stock, but the *4n* seedlings grew more slowly (see especially Levan's summary 1939). Only one report is known to the writer which is in disagreement with these data on petunias. Kostoff (1930) and Kostoff and Kendall (1931) observed that a spontaneous tetraploid seedling of *Petunia* and its seed progeny grew more rapidly and flowered sooner than the diploids of the cultures. Varrama (1947) has recently stated that the tetraploid petunia reported by Kostoff together with the tetraploid black currants, which Vaarama obtained, constitute "the only plants where the autotetraploid forms possess a quicker seed germination and are earlier flowering than the parental forms."

The reports cited above are in general agreement that the tetraploids of *Petunia* had a degree of potential fertility that was sufficient for seed reproduction. Steere (1932) and Levan (1939) report that sporogenesis in polyploids of *Petunia* may be quite regular. In general, however, the amount of seeds per capsule was found to be less than in diploids, as is the general rule for autotetraploids.

The data on self- and cross-incompatibilities in these experimental polyploids of *Petunia* provide very little data on either the presence or the absence of self- and cross-incompatibilities in the tetraploids or in the diploids from which they were derived. The reports by Kostoff (1930) and Kostoff and Kendall (1931) state that a diploid plant was self-incompatible to normal selfing but not to premature selfing, and that the tetraploid that appeared among the progeny was self-incompatible to both normal and premature pollinations.

Blakeslee and Avery (1937), who obtained somatic autotetraploids of several diploid plants including cultivated *Petunia,* stated that "tetraploidy has changed a self-sterile to a self-fertile form"; but in the following year Blakeslee (1938) stated that "in one case at least preliminary tests seem to indicate that doubling of chromosomes neither increases nor decreases the degree of self-sterility."

In a later publication by Blakeslee (1941) the following statements were made: "Doubling chromosome number of self-sterile plant may increase slightly the production of seed to selfing. Such an increase was observed in *Petunia.*" According to the summarized data, both *2n* and *4n* plants of *Petunia* produced capsules but a higher percentage of the *4n* capsules contained seeds, but in one variety neither the *2n* nor *4n* contained a full set of seed. But "there was no striking difference in the set of seeds from self-pollinating *2n* and *4n* plants of the following forms that are predominantly self-sterile: *Portulaca parana, Cosmos sulphureus, Rudbeckia hirta.*" It was concluded that the effect of tetraploidy on self-incompatibility in the species which were studied "cannot be great."

Reproduction in the Autotetraploids of Petunia axillaris. *New Specificity.* The data for these plants presented in Chapter 8 fully demonstrate that all members of the generations that were grown had a high degree of potential fertility, were self- and cross-fertile, and were evidently uniform for the tetraploid constitution. There was also an almost complete sterility barrier to crossing with the diploids of *P. axillaris*. Without exception each of the numerous members of the seed-grown tetraploids was readily to be distinguished from the parental diploids and their intra-bred progenies. In comparison with the latter, the tetraploids had thicker stems, larger leaves, larger flowers with conspicuously larger anthers and pistils, larger pollen grains that were often quadrilete, larger capsules, and larger seeds. Also the seeds of the tetraploids were slow in germination and the seedlings grew slowly and reached flowering age after a longer period of vegetative growth. Hence it is doubtful that such tetraploids could be established naturally in ecological habitats where the parental diploids thrive as annuals. All the tetraploid plants were self-fertile, while their somatic diploid parents were self-incompatible. In comparison with the diploids, the autotetraploid plants showed discontinuous variation to the degrees just noted. That they should be considered a new species is questionable. In this report they are called tetraploids of *Petunia axillaris*.

Potential Fertility. All the tetraploids of *P. axillaris*, of original somatic branches and of seedlings, possessed a high degree of potential fertility which provided for sexual reproduction by means of viable seeds. There were, however, abortions of approximately one-fourth of both pollen and ovules. Cytological studies revealed that unequal distributions of chromosomes were frequent in meiosis, owing to multiple pairing, nondisjunctions, and some bridge connections. But there were associations of bivalents, especially in the late stages of diakinesis, which effected distributions of the $2n$ number of chromosomes to many spores and gametes that were functional. The mature capsules of selfed and cross-bred relations contained both well-formed viable seeds and ovule scales and the two were sharply distinct in size and appearance, as is shown in figure 42 L and M. The evidence indicates that the abortions of the ovules which became scale ovules were chiefly absolute in status in that such ovules were unable to function in any relation. The examination of sections of capsules at various stages of development showed that the ovule scales were usually scattered at random on the placenta, that few contained an embryo and endosperm at any stage, and that their size increased to the extent indicated in figure 42 L. As the capsules enlarged the true seeds soon surpassed the scale ovules in size and the latter became compressed. There was, however, evidence that a few of the ovules which possessed haploid egg cells did not function in producing viable zygotes in selfing or in intra-breeding but may do so in the forced fertilizations of certain other relations, as disgenic $1n \times 2n$. Such ovules were evidently few in number.

Numerous studies indicate that autotetraploids have greater proportions of abortions of spores than their parental diploids have and that there are fewer viable seeds per capsule. There are relatively few autotetraploids in which there is no potential fertility. The evidence indicates that this abortion is chiefly a resultant of multiple pairing. It has been established that allotetraploids that are derived from sterile hybrid diploids may have high potential fertility and functional diploidy due to the selective pairing of each pair of the duplicated chromosomes. The abortions, which decrease the potential fertility of autotetraploids that are derived from diploids that have high potential fertility, are resultants of the extension of homologous pairing to tetrasomes. The abortions of the spores of many diploid hybrids result from non-pairing or from lack of harmony after pairing. When tetraploids, and especially somatic tetraploids, are derived from sterile hybrid diploids the duplication of each chromosome provides identical partners whose bivalent pairing may establish an amphidiploid status in the entire chromosomal constitution.

A fundamental feature that affects the degree of potential fertility of both auto- and allotetraploids is the extent to which preferential pairing of chromosomes establishes functional diploidy. Many natural species that are called diploid have had an origin through polyploidy. In evaluating such plants the determinations of both the number and the behavior of chromosomes are of significance.

The Stability of the Tetraploid Constitution. The evidence is reasonably conclusive that all members of all seed-grown progenies derived from the somatic autotetraploids of *P. axillaris* by self- or intra-cross pollinations were tetraploid. These were, however, the survivals of germination in soil, and not all seeds germinated. Every plant that was grown was self-fertile and had large anthers and pistils and all of the other features that characterized the original somatic tetraploids. Chromosome counts in pollen-mother-cells were made for more than 100 plants and the number of twenty-eight chromosomes was found in every one. But some of the members that were self-fertile may have been paratetraploid for it was found that some of the segregates in the selfed progenies of triploids which had *23–28* chromosomes were self-fertile (figure 48).

It has already been noted that the aggregate of somatic chromosome numbers found in the " tetraploid " cultivated petunias ranged from *25* to *30* inclusive, but that the tetraploid number of *28* was most frequent (Levan 1937). Evidently there was less stability in this reproduction in heterogeneous cultivated petunias of hybrid origin than in the autotetraploids of the pure stock of *P. axillaris*.

Diallelic Status and Reproduction. The original somatic tetraploids of *P. axillaris* were obtained of diploid plants that were heterozygous for *S* factors and hence each one must have been a balanced diallelic for *S* factors (as *S 1.1.2.2*). The line of selfed progenies derived from any one of these

plants appeared to maintain the same diallelic genotype. This was revealed by the differential reactions in syngenic and disgenic relations with the different diploid genotypes and especially with those that were homozygous (see figures 38 and 39). The resultants of all the tests definitely proved that no tetraploid, or paratetraploid, or triploid plant was homoallelic (as *S 1.1.1.1*). But the tests did not distinguish between balanced diallelics (as *S 1.1.2.2*) and the unbalanced diallelics (as *S 1.1.1.2*) that may also have been present.

The members of all the diploid parental stock and of all progenies obtained of normal cross-pollinations, but not of premature self-pollinations, were heterozygous for *S* factors. These factors operated as alleles in the diploids in a simple personate type of reaction and the plants were definitely diploid for the *S* factors. The duplication of the seven homologous pairs of chromosomes in the tetraploids produced six groups of four chromosomes, or tetrasomes, that were practically alike and which carried no *S* factors and one tetrasome of which two duplicated chromosomes carried the same *S* factor while the other two duplicates carried a different *S* factor. Thus in the meiotic pairing of such chromosomes there may be (a) the pairing of identical duplicates or (b) the pairing of homologous chromosomes that are not duplicates. The type of pairing in the tetrasome that carries *S* factors can be evaluated by the reactions of *S* factors in the members of the different progenies derived from the tetraploids. The extent to which multiple pairing occurs may be determined by cytological studies; and the extent to which abortions occur in microsporogenesis may be determined by the evaluations of pollen and of the production of seed. The two main features to be determined are (1) the effect which the doubling of chromosomes has on potential fertility and (2) the effect which it has on the pairing of the chromosomes which possess the *S* factors.

A diallelic status of an entire progeny that is derived from a balanced diallelic could be maintained by either of two processes: (a) The selective pairing of the two chromosomes that carry the same *S* factor would produce one class of heterogenic *2n* spores and gametes and the progeny would then comprise one genotype that would be a balanced diallelic; while such a condition is possible in autotetraploids as well as in allotetraploids it is considered to be rare in experimental autotetraploids and in others of recent origin (Little 1945); (b) a random pairing of the chromosomes or chromatids that carry the *S* factors would produce all three of the possible *2n* classes of pollen in the theoretical proportions of 1:4:1; then, if *only* the heterogenic pollen functions with all three classes of eggs the formula for gametic fusions would be (1, *S 1.1*; 4, *S 1.2*; 1, *S 2.2*) ♀ × *S 1.2* ♂ and the progeny would be composed of three diallelic genotypes of which two are unbalanced and one is balanced.

The Inactivation of S Factors in 4n P. axillaris. In the first report (Stout & Chandler 1941) that the somatic tetraploid branches on fifteen different self-incompatible diploid plants were self-fertile, it was stated that "at

least one, if not more, of the classes of pollen that segregated from the tetraploid complex is able to function in the production of seed after self-pollination." This statement was prompted by the observation that many of the pollen tubes remained in the apical part of the self-pollinated pistils. Two months later Straub (October 1941) reported that self-fertile tetraploid branches had been obtained on diploid self-incompatible plants of wild races of *Antirrhinum glutinosum* and *A. molle*, but evidently no reports on the behavior of seedling progenies have been made. Straub considered that the self-fertility of the tetraploids may be due to a dilution of an inhibiting substance in the enlarged tetraploid cells of the pistils or to a change in the character of the pollen tubes that was effected by the presence of two different *S* factors. A few months later (January 1942) Lewis and Modlibowska made the postulate that the increased degree of self-fertility of the tetraploid bud-sport, known as the Improved Fertility Pear, involved the selective inactivation of *S* factors in the pollen tubes that were heterogenic (as *S 1.2*) and that the homogenic (*S 1.1* and *S 2.2*) pollen tubes retained incompatibility reactions.

The self-fertility of all plants of *4n P. axillaris* demonstrated that the *S* factors, which were active in the diplont-haplont reactions of pollen-tube growth in the parental diploids, became inert in at least some one of the classes of pollen involved in the self- and cross-relations of the derived tetraploids. Two features of the reproduction suggest that the inactivation is selective for classes of pollen produced by the tetraploids; (a) The pollen tubes in self-pollinated pistils of the *4n* plants were distributed at various levels in the styles and (b) no homoallelic members were obtained in any selfed progenies. One hypothesis for explaining such resultants is that the inactivation of *S* factors occurs only in heterogenic pollen tubes as proposed by Lewis and Modlibowski. The other hypothesis is that only gametes that are heterogenic are produced.

The Conception of Antigen-Antibody Reactions in Incompatibilities. That intraspecific self- and cross-incompatibility may be similar to antigen-antibody reactions has been recognized by Stout (1916), East (1929, 1934, 1935), and Sears (1937). Lewis (1943a) applied this conception in detail and proposed that when two different *S* genes are together in the nuclei of *2n* pollen tubes there is a *competition* that results in the failure of each of the two *S* factors to produce its specific antigen. The application to diploids is that the incompatibility reaction is one of agglutination; that the antigen is produced in a pollen tube; that the antibody is formed in the tissues of the pistil; and that a pollen tube that grows in a style that has the homologous antibody absorbs this antibody and then there is a precipitation reaction that checks or stops the growth of pollen tubes. It is assumed that the antigens are polysaccharides and that antibodies are proteins and that the homologous specificity of the two is determined by the same *S* factor. It was considered that the presence of two different *S* factors in the nuclei of a pollen tube of

a tetraploid automatically results in a competition that weakens or prevents the incompatibility reaction of that tube in syngenic relations. It was also assumed that homogenic pollen tubes (as *S 1.1*) will retain their incompatibility action in syngenic relations.

Further Experimental Evidence in Petunias. Conclusive evidence on the point mentioned above could undoubtedly be obtained in *P. axillaris*, and in other plants also, from an analysis of the behavior of monoallelic tetraploids that are produced by the colchicine treatment of homozygous diploids. In the hope of obtaining such evidence, during 1945 many vegetative buds on ramets of *S 1.1* and *S 3.3* genotypes were treated, but no tetraploid branches were obtained. The earlier treatments that had readily effected tetraploid branches were applied to the first laterals of young and vigorously growing seedlings. Also the tests which proved that all of the *4n* plants which were obtained were diallelic did not distinguish between the balanced and the unbalanced diallelics that may have been present. If monoallelic pollen (as *S 1.1* and *S 3.3*) retains incompatibility reaction the monoallelic tetraploids of *P. axillaris* should be self-incompatible.

Degrees of Inactivation of S Factors. The data on natural and experimental tetraploids indicate conclusively that there are extreme differences in the extent to which *S* factors are inactivated.

(1) There are inactivation and self-fertility, as noted above, for somatic tetraploids of *Antirrhinum glutinosum* and *A. molle;* and for all somatic tetraploids of *Petunia axillaris* and for all of their selfed seedlings. But in these cases there may still be cryptic incompatibility for the homogenic pollen.

(2) In contrast to the above cases, there may be continued self-incompatibility of all tetraploids; as reported by Howard (1942) for somatic *4n* branches of self-incompatible plants of *Brassica Rapa, B. campestris,* and *Raphanus sativus;* by Hecht (1944) for *4n* members of *Oenothera rhombipetala;* by Lewis (1942, 1943a, 1947) for all *4n* seedlings of *Oenothera organensis,* in which, however, the degrees of incompatibility were somewhat reduced; by both Warmke (1942, 1944, 1945) and Bauman (1945, 1946) for tetraploid seedlings of *Taraxacum Kok-saghyz;* and by Kerns and Collins (1947) for induced somatic autotetraploids of the Cayenne clone of pineapple.

(3) Of special interest are the resultants reported by Atwood (1944 a and b) for the induced tetraploids of *Trifolium repens* and their seed-grown progenies. Tetraploids were obtained which were considered to be diallelic and these were self-incompatible. A progeny of 29 members was obtained of two of the self-incompatible *4n* plants that had no common *S* factor, as *S 1.1.2.2* × *S 3.3.4.4*. Three of this progeny were self-incompatible but 26 were self-fertile. It was considered that the self-incompatible members were diallelic (as *S 1.1.3.3*) and that those that were self-fertile were either triallelic (as *S 1.1.3.4*) or quadriallelic (as *S 1.2.3.4*).

It is obvious that the most adequate evaluations of self- and cross-incom-

patibilities depend on a knowledge of the degrees of the reactions and of the patterns of the cross-reactions, not only in the *4n* but also in the respective parent or parents. Experimental tetraploids provide material for such analyses. But at the present time such studies are few in number, limited in scope, and often incomplete. The somewhat provocative statement has been made that "all natural autotetraploid races of self-incompatible species are self-compatible" (Lewis & Modlibowski 1942; Lewis 1943a). The inference is, it seems, that either the *S* factors have been inactivated permanently or that they were eliminated by selective reproduction, and that this condition always occurs in autotetraploids but not necessarily in allotetraploids. Little (1945) remarks that "this conclusion is too broad, for there are some exceptions as the North Temperate autotetraploid species of *Tradescantia,* which are self-incompatible as the diploids from which they arose." There is evidence that various wild species that are obviously polyploid have self- and cross-incompatibilities but many of these may be alloploids.

Reproduction and Sterility Barriers in the Relations between Diploids and Autotetraploids. *The Earlier Data.* The data for autotetraploids of *Oenothera, Datura,* and *Solanum* were summarized by Jørgensen (1928) with the addition of new data from his own experimental studies with *Solanum.* He reported that autotetraploids usually have potential fertility that is less than that of diploids, that they seldom cross with the diploids of the species, and that, when a cross relation gives some seed, the progenies are usually triploids that have low potential fertility. It is to be noted that all the species which Jørgensen reviewed, and the others which were involved in his own experimental studies, had no intraspecific self- and cross-incompatibilities. Hence, the failure of the autotetraploids to cross with the diploids involved no feature of intraspecific incompatibilities but was an expression of a new relative specificity.

In 1933 Müntzing surveyed the evidence that had accumulated in regard to the extent and nature of the failure of crosses between *4n* and *2n* chromosomal species and races. He concluded (a) that the $4n \times 2n$ relation gives some progeny more frequently than does the $2n \times 4n$ relation; (b) that the failures most frequently involve seed abortions as especially recognized by Watkins (1932) for hybridizations; and (c) that the progenies of such crosses (Gairdner 1926; Kostoff & Kendall 1931; Dermin 1931, for *4n* with *2n*) may possess chromosome numbers that range from *2n* to *4n.* Muntzing developed the conceptions that there is a selective functioning of gametes and also a zygotic abortion that arises during the development of embryo and endosperm because of relative differences in the number of chromosomes. The term "incompatibility" was applied to the failures of reproduction in the relations between *2n* and *4n* and this was included with phenomena of "hybrid incompatibility."

In 1936, Müntzing published an extensive evaluation of the data on the reproduction and the specificity of autotetraploids. It was emphasized that

purely quantitative and numerical doubling of chromosomes will effect changes in the processes of reproduction to the extent that different chromosome races of a single species are prevented from crossing with each other by "barriers of incompatibility and sterility." Müntzing made no mention of the intraspecific self- and cross-incompatibilities that are known to operate in some of the diploid species that are closely related to certain of the tetraploids which he discussed. He emphasized that the barrier in question is most frequently effected by the abortion of zygotes after fertilization. Hence the so-called incompatibility is not a reaction between pistils and pollen tubes, as in intraspecific incompatibilities, but is a nutritional disharmony between the embryo, endosperm and nucellus in the ovules of the female member. It is a resultant of relations between two groups of plants that differ in ploidy and not of self- and cross-reactions within a single group. Also, since it occurs in true autotetraploids purely as a resultant of quantitative chromosome doubling, it is distinct from the steriliters of hybridity that are frequently called "hybrid incompatibility." This term was, however, used in the title of Müntzing's 1933 publication which chiefly discussed the cross-relations of autotetraploids.

The Tetraploids of Petunia axillaris. The diploid plants of *P. axillaris* had intraspecific self- and cross-incompatibilities. The autotetraploids derived from such plants were all self-fertile. But the evidence is conclusive that their pistils retain incompatibility reactions with diploid pollen in syngenic relations. The data show that the expressions of the new specificity of the *4n* in relations with the *2n* can only be judged by the resultants of disgenic relations or by those relations in which the *S* factors are inactivated in the pollen. The data for the $4n \times 2n$ and the $2n \times 4n$ may now be evaluated in this respect.

There were always fine capsules in any $2n \times 4n$ relation, either syngenic or disgenic. But seed abortions developed in both of these relations. The *2n* female parents had a high degree of potential fertility hence the abortions of their ovules were relative and not obligate. In the $2n \times 4n$ relations seeds of good size were formed, presumably to the full extent of the potential fertility of the ovules, but when the capsules were ripe very few of the seeds contained a viable embryo. From a total of over 12,000 seeds only three seedlings were obtained and one of these which was *4n* was probably from a stray seed. It is considered that pollen tubes of the *4n* functioned in fertilization in the syngenic $2n \times 4n$ relations, as they did in selfing, and that in the disgenic relations any class of pollen may function. Thus, in all $2n \times 4n$ relations the superimposed mechanism of incompatibilities was inoperative and the basic mechanism of reproduction in the *2n* female and the *4n* male was operative up to the point of seed development. Then, in accord with the evaluations by Müntzing, there is a shift from the normal ratios of *2n* embryo: *3n* endosperm: *2n* nucellus to a ratio of *3n* embryo: *4n* endosperm: *2n* nucellus that involves disharmony in development and effects zygotic abortion.

In the *4n* × *2n* relations in *P. axillaris* the incompatibilities of the haploid pollen continued to operate in all syngenic relations and when both *S* factors of the diploid pollen were syngenic the incompatibility was complete and the ovaries did not begin to enlarge. In diploids this condition involves the failure of haploid pollen tubes to effect fertilization and it is considered that this is also the condition in syngenic *4n* × *2n* relations.

The early reports (Stout & Chandler 1941, 1942; Stout 1945a) referred only to syngenic relations of *4n* × *2n* and were in error in considering that the degree of this sterility was an expression of specificity. Further extensive tests revealed that in all disgenic *4n* × *2n* relations, for either one or both *S* factors of the ♂ ♂ *2n*, there were large capsules which contained numerous pseudoseeds. From several thousand of such seeds only twenty-four seedlings were obtained. Thus when there were no incompatibilities for either one or both *S* factors of a *2n* ♂ ♂, there were many embryo abortions after fertilization and relatively few survivals of zygotes.

That seed abortions may be due to other causes than purely quantitative and numerical differences in the chromosome numbers of two parent plants of a cross-relation has been recognized (especially Brink & Cooper 1947). But the data that have accumulated since the evaluations by Müntzing (1936) have very fully demonstrated that embryo abortion constitutes a very general and effective barrier to the crossing of *4n* and *2n* plants in which there are no *S* factors.

The Seedlings of 4n × *2n P. axillaris.* Twenty-seven seedlings were obtained of *4n* × *2n* relation that was disgenic for one *S* factor: seventeen were triploid (*sn:14+7*), one was *sn:18*, and nine were diploid (*sn:14*). These seedlings were derived from parents whose *S* genotypes were known and their reactions with testers revealed their respective genotypes for *S* factors.

All of the thirteen triploids that were evaluated were triallelic (figure 44) and *S 1.2.3*. Only disgenic pollen tubes had functioned in the production of the plants that survived embryo abortion. The female gametes that functioned were, without a doubt, *2n* and heterogenic. No homogenic egg cells had functioned which suggests that such gametes may not have been produced.

Seven of the eight diploids were analyzed (figure 43). All were heterozygous. Those that were *S 1.3* certainly arose from gametes that were ♀ *S 1* and ♂ *S 3*. Those that were *S 1.2* arose from *S 1.1.2.2* × *S 1.3* and these could have arisen from ♀ *S 2* × *S 1*, or from an *S 1.2* egg cell that was parthenogenetic. But there has been no conclusive evidence of parthenogenesis in any of the petunias that were studied.

The evidence is conclusive that the *S 1.3* members of this *4n* × *2n* progeny arose from haploid eggs. Hence the tetraploids may produce some haploid egg cells which function in reproduction when pollen tubes are haploid and disgenic. But no triploids or diploids were obtained in any of the numerous

selfed or intracrossed progenies of the tetraploids, and hence any haploids that were among the spores and gametes either did not function in fertilizations or did not produce viable offspring. The controlled *4n* × *2n* pollinations enforced the action of only haploid pollen tubes; the *4n* selfings involved competition between large numbers of *2n* pollen tubes and the relatively few haploid pollen tubes that were sometimes present, and also there was competition which eliminated *2n* and *3n* zygotes. A large number of *4n* zygotes regularly survived.

It is a fact that no egg cells that were homogenic (*S 1.1*, *S 2.2*, or *S 3.3*) functioned in the production of any of the seedlings obtained of *4n* × *2n*. But the number of the seedlings is too few to demonstrate that such egg cells were not present in the ovules of *4n* diallelics. Here, as elsewhere in the studies of tetraploids, it would be of value if known monoallelic *4n* genotypes were obtained for comparisons of reactions, for use as testers, and as parents of progenies that could be fully evaluated.

Sexual Reproduction in the Triploids *The Role and Status of Triploidy.* Without question the chief significance in the evaluation of triploids is the role which they may have in the origin of new associations of chromosomes that are, or that may become, functional dysploids, secondary polyploids, and amphitriploids. The survey of the somatic chromosome numbers in flowering plants (Darlington & Janaki Ammal 1945) unmistakably reveals that many cases of dysploidy and secondary polyploidy have arisen during the origin and differentiation of genera and of species within genera. That the patterns of sexual reproduction in triploids may provide material for the origin of such species is evident. That they provide exceptional opportunities in experimental selective breeding for the development of true-breeding dysploids, aneuploids, and double triploids is also evident.

The extensive studies of triploids in flowering plants are in general agreement on three main features. (1) Triploids arise by the fusion of gametes that are unequal in the number of genomes. (2) The processes in the developments of zygotes and endosperm in seeds limit the survival of triploid embryos in the ovaries of *2n*, *3n*, and *4n* plants. (3) In the sexual processes of triploids there are (a) many abortions of spores, gametes and embryos that are often selective, (b) a decided failure to employ the limited potential fertility in the sexual production of offspring that are also triploid, and (c) the production of many numerically new organizations of chromosomes in the survivals of spores, gametes and zygotes.

There are few experimental studies in which the number of the members obtained in the selfed progeny of a triploid has been large enough to provide adequate data on the complete pattern of the sexual reproduction. Frequently the evaluation of the potential fertility of a triploid is based on the progenies of crosses between it and diploids or tetraploids. The data that have been obtained reveal noteworthy differences in the character of the offspring of the triploids of different genera and even of species of the same genera. The

progeny may be predominantly diploid, or tetraploid, or decidedly aneuploid, or there may be still higher levels of ploidy. Nearly always there are some aneuploids especially for those that are trisomic for one or more members of the basic genome. The evaluations of the survivals in such progenies have provided much significant data on the stability and balance of genomes in spores, gametes, and zygotes in relation to abortions and survivals. In general, the emphasis has been placed on the preferential survival of genomes over agenomes.

By definition triploids possess three genomes. In simple autotriploids of AAA constitution there are trisomes for each member of a primary genome. In one class of allotriploids the tetraploid parent may be an autoploid (AAAA) and the other parent a distinct species (BB). In another class the tetraploid parent may be an amphidiploid, as AABB, and the diploid parent may be either a species directly related to the amphidiploid (as A′ A′ or B′ B′) or a distinct species (as CC). Hence in the meiosis in various classes of triploids, as well as in their progenies, there are many differences in the extent of the relative homologies among the members of the three genomes that are balanced in number. But only two of the three genomes of a triploid may have the same number or all three of them may have a different number. In such cases there are numerical as well as structural and genic features of homologies that have direct effects on potential fertility and the patterns of reproduction.

It should be noted that the triploid constitution in the sporophytic stage in the life cycle of flowering plants is often a most favorable one in respect to vigor of growth, adaptation to survival under natural conditions either by vegetative reproduction or by agamospermy, and to combinations of qualities of value in horticulture. Among perennials many triploids have become established as clonal so-called " species " and the list of triploid clones among the flower and fruit crops is a long one (Crane & Lawrence 1947). Such plants provide evidence that the triploid constitution is, of itself, not necessarily a lethal one. Such a clone is, however, the multiplication of an individual triploid seedling that escaped the abortions that perdominate when triploid zygotes arise in the ovules of either diploids, triploids or tetraploids.

The basic mechanism of sexual reproduction in triploids involves the same mechanism of sexuality that operates in the intraspecific sexual reproduction of diploids which centers in the two critical features of meiosis and fertilization. The data for the AAA and the AAI triploids of *Petunia* (cf. chapters 9 to 12) demonstrate the extent to which the superimposed mechanism of intraspecific self- and cross-incompatibilities may mask the potential fertility of triploids and of their progenies. When such reactions occur they must be determined before the pattern of the sexual reproduction in a triploid can be evaluated correctly.

Data for the Triploids of Cultivated Petunias. There are, it appears, no records of the sponstaneous origin of triploids among the seedlings of dip-

loid petunias. That certain giant races of cultivated petunias are tetraploid, was determined in 1927 and soon thereafter there were reports that 5 triploid seedlings had been obtained of $2n \times 4n$ relations, and that 67 others had been obtained of the $4n \times 2n$ relation (Dermen 1931; Kostoff & Kendall 1931; Steere 1932; Matsuda 1935; and Levan 1937). Levan has tabulated the data for the progenies that were obtained of $3n$ selfed, $3n \times 2n$, $2n \times 3n$, $3n \times 4n$, and $4n \times 3n$. The aggregate number of seedlings was 322: of these 101 were $2n$; 55 were $4n$; one was $3n$; and one was $5n$. In these there were presumably multiples of the genome number of 7. But there were also 154 members that were aneuploid and these included some of all the somatic numbers of 15, 16, 17, 18, 19, 20, 24, 25, 26, 27, 29, and 31. Thus there were survivals of a wide range of agenomic spores and gametes and of their combinations in zygotes. The triploids of these studies were of diverse hybrid origins and the pedigrees of the various progenies were not indicated.

There were several studies of meiosis in these triploids. Dermen determined the frequencies of the distributions of chromosomes to sporogenous cells and his data for the number of cells that received from 7 to 14 chromosomes are: 1 with 7; 12 with 8; 32 with 9; 40 with 10; 41 with 11; 20 with 12; 5 with 13; 1 with 14. This is approximately a binomial frequency distribution that ranged from the genome number of 7 to the double of that number. But there were also monads, diads, and triads that were formed from a single mother-cell and some of these had more than $2n$ chromosomes. It is obvious that the zygotic progenies in petunias, including those reported by Dermen, do not correspond closely to the distributions observed in meiosis. There were relatively few spores that received either one genome or two genomes. But a total of 158 of the seedlings were diploids or polyploids and only 154 were aneuploid. Hence there were either excessive abortions of agenomic spores or of the zygotes of their fusions. The spores and gametes that had 9, 10, and 11 chromosomes functioned less often than did those that were $n + 1$, $2n + 1$, and $2n - 1$.

It appears that only Levan has tested the patterns of reproduction in the offspring of triploids of petunias and his studies were confined to plants that were $2n + 1$. Of two progenies of $2n + 1$ selfed there were 7 and 5 members; of one $2n + 1 \times 2n + 1$ relation there were 13 members; of three $2n + 1$ "free flowering" progenies there were 4, 2, and 13 members. Of these plants, 31 were *sn:11* and 13 were *sn:15*. Of the $2n + 1 \times 2n$ relation there were 9, 10, and 18 plants of which 28 were *sn:14* and 9 were *sn:15*. Of $2n \times 2n + 1$ there were 81 members of which 80 were *sn:14* and 1 was *sn:15*. It was concluded that the egg cells of $2n + 1$ plants may carry some $n + 1$ chromosomes as well as n chromosomes but that $n + 1$ pollen grains rarely contributed to a viable embryo. There were 24 plants that were *sn:16* in Levan's progenies of $3n \times 2n$ and two others of $3n \times 3n$ parentage, but these were probably $2n + 1 + 1$ $(2n + a + b)$ and trisomic for two members of the genome. At any rate no $2n + 2$ $(2n + a + a)$ plant was obtained of a selfed

2n + 1 parent. Such a plant should arise from the fusion of two gametes that were *n + 1* and which carried the same extra chromosome. The balanced and mated condition in the chromosome complement should effect increased potential fertility and true-breeding stability of a genotype that was dysploid.

Levan collected seeds of open-pollinated capsules of some 15 or more plants of various parentage (*3n × 2n, 3n × 4n, 4n × 3n, 2n × 4n, 4n,* and *4n – 1 × 2?*) and grew a total of 116 plants. Of these 55 were *2n*, 35 were *4n*, 17 were *2n + 1*, 1 was *2n + 2*, 2 were *4n – 1*, 4 were *4n + 1*, and 2 were *4n + 2*. These data together with those obtained from progenies of *2n + 1* parentage were interpreted as evidence that a mixed population of *2n, 4n, 3n*, and their aneuploid progenies will, in a few generations of natural seed reproduction, eliminate the aneuploid forms including "all members in the proximity of the triploid number." It was emphasized that, in such populations, "balance is not acquired in nature until all aneuploids have been eliminated and only diploids and tetraploids remain." It may be granted that the evidence indicates that most aneuploids are trivalents and hence partly triploid and that they, as well as triploids, cannot breed true to their chromosome numbers. But it must be recognized that new dysploids may originate both in nature and in appropriate experimental breeding.

The information concerning the self-fertility and the self-incompatibility of the triploids of cultivated petunias may be reviewed. Kostoff and Kendall (1931) state that the one triploid that they obtained was "self-sterile" but it produced seeds to the pollen of *4n* plants. Steere (1932) states that his triploids were self-fertile and cross-fertile, but that they were derived from self-fertile diploids. Levan (1937) states that "self-fertilization in triploids succeeds very seldom in *Petunia*," but he makes no definite mention of either self- or cross-incompatibilities in any other of his petunias. If Levan's triploids were truly self-incompatible they must have obtained *S* factors from both of their *2n* and *4n* parents. According to Tjebbes (1932) there were self- and cross-incompatibilities in some of the stocks of the petunias which he turned over to Levan.

The Autotriploids of Petunia axillaris. The 13 seedlings of this group were obtained from disgenic relations of AAAA × AA. Seven seedlings were of *S 1.1.2.2 × S 1.3*, and six were of *S 1.1.3.3 × S 1.2*. These AAA triploids were all self-incompatible and cross-incompatible in all relations. The tests of them as females with pollen of known diploids revealed that all were *S 1.2.3* (figure 44). They were derived of relations that were disgenic for only one *S* factor and only those pollen tubes that carried this factor had functioned. Thus the *3n* plants continued to have incompatibility reactions with syngenic haploid pollen but had no such action with their own heterogenic pollen in self-fertility. The pistils of these triploids whose somatic cells possessed three different *S* factors (*S 1.2.3*) had incompatibility reactions with any syngenic haploid pollen. They produced no capsules to normal selfing even though they produced some heterogenic pollen but it may be

that the functional heterogenic pollen tubes in selfing were too few to effect the formation of capsules.

The studies of meiosis of the AAA triploids indicated that the sporogenous cells collectively received chromosome numbers of from 6 to 15 with still lower numbers in the small supernumerary cells and that there were many abortions of spores. No triploids were obtained of fully disgenic constitutions, as *S 4.5.6*, that could be used to determine the degree of seed production possible in disgenic relations between triploids. But the seeds obtained of *S 1.2.3* × *S 4.5* indicated that approximately 10 to 20 per cent of the ovules were able to function in the formation of large seeds with embryos. This is a rather high potential fertility for autotriploids, but the genome number of 7 is a low one and the chromosomes are short (Sullivan 1947) which are conditions that are considered favorable (Darlington 1937) to complete disjunctions in the meiosis of polyploids.

It seems that the fully disgenic relations of AAA × AA (*S 1.2.3* × *S 4.5*) were more highly productive of good seeds than were the relations of AAAA × AA. There were, however, no fully disgenic tetraploids, as *S 4.4.5.5*, that could be used in relations with the *S 1.2.3* triploids. The reciprocal relations of *S 1.2.3* with *S 1.1.2.2* and *S 1.1.3.3* (figure 45) were tested. These gave induced parthenocarpic capsules when *S 1.2.3* was ♀ and no capsules when *S 1.2.3* was ♂. The failure of heterogenic pollen to effect viable embryos in these *3n* × *4n* relations corresponds to the resultants of *2n* × *4n*. Since the same failures are characteristic of the same crossing relations in which no *S* factors are involved, these failures in the petunias may be considered as expressions of the barrier of specificity that is independent of *S* factors.

The resultants of the tests of *S 1.1.2.2* and *S 1.1.3.3* × *S 1.2.3* were definite that there were either no capsules or a few shrivelled ones of small size. Hence the disgenic pollen and the heterogenic pollen had not been in sufficient number to effect development of capsules. Thus it appears that the AAA triploids were less able to make use of their potential fertility in relations with *4n* than in relations with *2n*. These resultants are, however, not in complete agreement with the behavior of cultivated petunias in which a total of 87 seedlings were obtained of the reciprocal relations of *3n* with *4n* (tabulation by Levan 1937).

No progenies were grown which had any of the AAA triploids as a parent and hence there are no data for the pattern of their sexual reproduction or for the chromosome numbers in the gametes that were able to function.

Reproduction in the AAI Allotriploids. Six triploids and one sister seedling that was *3n* − *1* were obtained from the seeds in one capsule of *4n P. axillaris* (*S 1.1.3.3*) × *2n P. integrifolia* (*S a.b*). This hybridization did not involve any incompatibility reactions betwen *S* factors that were common to both parents. But each parent had *S* factors of the same personate type and in the F_1 and F_2 of the diploid hybrids these factors had allelic associations

and a high degree of homologous allosyndesis. It is, however, to be noted that in the *S 1.3* × *S a.b* and the *S 1.1.3.3* × *S a.b* hybridizations the *S a* factor did not appear in any of the progenies that were grown. The basis of this non-conforming resultant was not tested further. The tests of these AAI triploids indicated that all were *S 1.3.b*. Hence the egg cells that had functioned were heterogenic (*S 1.3*). But in the AAI *S 1.3.b* × II (*S a.b*) the *S a* factor appeared in 22 of the progeny.

The AAI triploids were self- and cross-incompatible but these relations were fully syngenic. As in the case of the AAA triploids the heterogenic pollen that may have been formed did not function in self- or intra-genotype relations. But also no capsules were obtained by any cross-relations between AAA and AAI triploids which differed in one *S* factor (*S 1.2.3* and *S 1.3.b*).

Progenies of certain of the AAI triploids were obtained of premature selfing and of crosses with diploids and tetraploids. The determinations of the *S* factors and the chromosome numbers in the plants of these progenies make possible the evaluations of the patterns of reproduction in respect to potential fertility, to the range and frequency of the chromosome numbers in gametes and zygotes, to the selective action of *S* factors, and to the reactions that were expressions of unilateral sterility and fertility of hybridization.

Chromosome numbers were determined in 13 members of the premature selfing of AAI, in 23 seedlings of AAI (*S 1.3.b*) × II (*S a.b*), of 26 seedlings of AAI × *S 1.1.2.2* and *S 1.1.3.3*. The summary for all of these plants (figure 59) reveals that one was *4n*, 4 were *2n*, none was *3n*, and 59 were aneuploid. Of the latter, 13 were *3n − 1*, 12 were *2n + 1*, and all other aneuploid numbers between *2n* and *4n* were represented except *21*, *22*, and *27*. Thus in relatively few cases did genomic gametes of the triploids function and evidently some egg cells functioned which had each of the agenomic numbers between *n* and *2n*. Also in the progeny of premature selfing there was no member less than *sn:19*, hence there were no survivals of any fusions between gametes that were *n*, *n + 1*, or *n + 2*. But since there had been premature selfing the offspring may not indicate the full range of potential fertility.

Of the progeny of the premature selfing of *S 1.3.b* the two members that were *sn:20* and the one that was *sn:19* were self-incompatible and *S 1.3.b* (figure 48). Hence in their origin one gamete had been heterogenic and the other had carried a different *S* factor and the chromosome numbers in the gametes could collectively have been *7* and *12*, *7* and *13*, *8* and *12*, *9* and *11*, and *10* and *10*. The eight numbers that were collectively *sn:23*, *24*, *25*, *26* and *28* were all self-fertile and triallelic. Since the triallelic triploids were self-incompatible, it is assumed that each of these self-fertile triallelics had four *S* factors of which one was duplicated (as *S 1.1.3.b*). Some of the known self-fertile tetraploids of *P. axillaris* were triallelic (as *S 1.1.2.3*). If this assumption is correct, the two gametes that fused to give each of these self-

fertile aneuploids (*sn:23* to *26*) were heterogenic and had one *S* factor in common. Also at least one of the gametes was agenomic and less than *2n*. The evidence seems to indicate that in petunias the presence of four *S* factors of which two or more are different (as *S 1.1.3.3*, *S 1.1.2.3* and *S 1.1.3.b*) is necessary in a plant to insure inactivation of *S* factors in at least one class of its pollen. This supports the view that the inactivation occurs within pollen tubes that are heterogenic. But it appears that this constitution must be present in *both* pistils and pollen to effect self-fertility and that then a plant will be self-fertile even if its chromosome number is as low as *sn:23*.

Each of the two progenies (17 and 28 members) of AAI (*S 1.3.b*) × II (*S a.b*) and AA (*S 1.2*) contained one or more members of each of the classes from *sn:14* to *sn:20* (figure 59). It is assumed that the diploid pollen parents contributed only gametes that were *n* and that the functional female gametes of the triploids collectively carried some of each chromosome number from *7* to *13* inclusive. There were only four known diploids and hence it may be considered that only four egg cells that were *n* had functioned. Two of these carried *S b* (figures 54, 55) and one carried either *S 1* or *S 3*. All of the 25 members that were tested were self-incompatible and six of them, of AAAI × II, which were *sn:16*, *18*, *19*, and *20* were triallelic. Thus gametes which possessed as few as *9* chromosomes had carried two *S* factors. A plant that was *sn:16* was trisomic for the chromosome that carried *S* and hence must also have been trisomic for one other chromosome. But of the seven members that were tested of AAI × AA all were self-incompatible and apparently each possessed only two *S* factors. In the production of these, no gamete had been heterogenic for *S* and none of the progeny that was tested was trisomic for a chromosome that carried *S*.

The *S 1.3.b* triploids produced fine capsules with pollen of either *S 1.1.2.2* or *S 1.1.3.3*. The number of plump seeds was usually less than 100 which possibly indicates the number of the ovules that had some degree of potential fertility. But when a capsule was ripe there were few viable seeds, due it is believed to abortions of zygotes. Since capsules and pseudoseeds were the rule in all *2n* × *4n* and *3n* × *1n* relations it appears that incompatibility reactions did not occur in these relations unless they were cryptic. The one seedling of *S 1.3.b* × *S 1.1.2.2* that was obtained was *sn:20*, self-incompatible, and *S 1.2.3*. Evidently the egg cell had carried *S 3* and the sperm had carried *S 1.2*. The one seedling of *S 1.3.b* × *S 1.1.3.3* was *sn:26*, self-fertile, and evidently *S 1.1.3.3*. Presumably an egg cell that had *12* chromosomes and was *S 1.3* had fused with a sperm that had *14* chromosomes and was *S 1.3*.

The evidence concerning the status of unilateral sterility in the reproduction of the AAI hybrids and their progenies may now be evaluated. The relation II ♀♀ × A ♂ is one of unilateral sterility. But the relation AAI ♀♀♀ × A ♂ was one of fertility in disgenic relations. Hence the I com-

ponent in association with the AA component did not effect unilateral sterility. But the seven members of AAI were all sterile as males with II ♀ ♀ (*S a.b* × *S 1.3.b*), and hence their pollen tubes possessed the haploid complement of *P. axillaris* that effected the sterility in the hybridization relation of II ♀ ♀ × A ♂. The relation of II: *S a.a* × AAI: *S 1.3.b* gave no capsules and hence the pollen that was *S b* was either too few in number to effect capsules or it had the specificity of A ♂. Six of the members of the progeny of AAI × II (*S 1.3.b* × *S a.b*) that were either *S a.b* or *S b.b* in constitution and *sn:15, 16, 16, 16, 17,* and *17* (figure 54) were all cross-sterile with A ♂. Hence the constitution of their pistils was sufficiently *P. integrifolia* to effect unilateral sterility. But six sister seedlings that were *sn:14, 15, 15, 15, 18,* and *18* and either *S 1.b* or *S 3.b* in constitution were cross-fertile with disgenic A ♂. Hence there was segregation and recombination in some but not in others of those factors in pistils which effect the unilateral sterility of II ♀ ♀ × A ♂. It seems certain that this II ♀ ♀ constitution is not a single gene but is a pair of alleles or possibly a diploid association of complementary genes that can be reassembled in some of the members of back-cross progenies. This same behavior was observed in the F_1 hybrids and the back-cross progenies of *P. parodii* × *P. axillaris* (chapter 6).

The association of chromosomes and their disjunction in the AAI triploids is a matter of special interest. It is certain that in the diploid F_1 and F_2 of AI hybrids there was allosyndesis and a high degree of potential fertility. There was much diversity among the F_2 with intermediate expressions of most specific characters and few members closely resembled either of the parents. There was evidently much interchange of genes followed by random assortment of chromosomes in the genomes.

In the AAI triploids there were two genomes of A and A and one of I and the respective chromosomes were pure for the species. There was chance (a) for the normal homologus pairing of A with A and the random distribution of the members of the I genome or (b) for trivalent associations and competition between allosyndesis and "autosyndesis" within each of the three homologs.

In his discussion of gene ratios in triploids Lindstrom (1936) states that "because of the great amount of sterility in triploids it is not profitable to discuss genic inheritance" and that theoretical ratios for a duplex trisomic, as AAa, "are never realized in breeding experiments because the male gametes with extra chromosomes are rarely functional in competition with normal microspores." This emphasizes those cases in which the survivals among the progenies of triploids are *2n, 2n + 1,* or *2n + 1 + 1* and have received extra chromosomes from female gametes. But the progenies of the AAI triploids had various recombinations of the three chromosomes which carried the *S 1, S 3,* and *S b* factors and these could be identified with certainty. The analysis of the combinations of *S* factors in the progeny may be made in respect to the pattern of meiosis.

Of the intrabred progeny of AAI (*S 1.3.b*) there were 1 plant of *sn:19*, 4 of *20*, 2 of *23*, 1 of *24*, 3 of *25*, 1 of *26*, and 1 of *28*. All but the one tetraploid member were aneuploid and at least one gamete in their origin had been agenomic. Three of these plants were *S 1.3.b* and hence one gamete had carried one *S* factor and the other had carried two different *S* factors and either *S 1.3*, *S 1.b* or *S 3.b*.

The progenies grown of *3n* × *2n* comprised 4 plants of *sn:14*, 12 of *5*, 9 of *16*, 4 of *17*, 5 of *18*, 3 of *19*, and 8 of *20*. The *S* factors were identified in most of these plants. Some gametes which carried each of the three *S* factors functioned. Gametes that carried two *S* factors functioned and evidently these were heterogenic, which is expected of *S 1.3.b* in which all three *S* factors are allelic. The plant 238 No. 4 (figure 54) was derived of *S 1.3.b* × *S a.b*; it possessed *S 1.b* and hence the egg-cell if its origin had been *S 1*. It would seem that the selective pairing of *S 1* with *S 3* in plants that were *S 1.3.b* would give gametes that were either *S 1.b* or *S 3.b*. There were, however, no diallelic constitutions (as *S 1.1.3*, *S 1.3.3*, or *S 1.1.3.3*) among the progeny of *S 1.3.b* that had either three or four *S* factors. The continued action of *S* factors in syngenic *3n* × *2n* relations would limit the appearance of such members in a progeny. A technique of identifying all *S* factors and of determining the chromosome numbers in the progenies of fully disgenic allotriploids, as *S 1.2.b* × *S 3.4.a*, would provide more definite data on the associations of chromosomes in meiosis.

According to the criteria of the evaluations of biosystematics recently emphasized by Clausen, Keck and Hiesey (1945) and Stebbins (1947) the status of the AAI hybrids of *Petunia* would be classed as autoploidy. The basis for such a designation is that the F_1 and F_2 of *P. axillaris* × *P. integrifolia* are highly fertile and have a high degree of homologous pairing that effects bivalent meiosis and genic interchanges in diploid reproduction. On the basis of hybridization these two species would be classed as ecotypes of one ecospecies. But these two species are so distinct in numerous specific features that they are very properly placed in different subgenera (Fries 1911). They certainly exemplify an extreme case of genic differentiations that have arisen within genomes of the same number which have retained the structure and the physiological properties that effect bivalent homologies. These are not the typical ecotypes of one species or ecospecies. Their differentiations far exceed those that would be accorded varietal status and in addition the hybridization is unilateral.

Reproduction in the Simple Triploids in Other Solanaceae than Petunia. *General Status.* It appears that, except for *Petunia*, there are no triploids in any Solanaceae that have a lower present genome than *12*. The genome number of *12* in the *sn:24* species probably arose from the more primary number of *6*. But in a number of the genera, the lowest genome number of any species is *12* and the differentiation of the chromosomes has acquired nonhomologous status and the genome has unity of at least secondary basic status. At the present time simple triploids or autotriploids are

known in certain of the *gn:12* (*sn:24*) species of *Datura*, *Lycopersicum*, *Capsicum*, and *Solanum*. The patterns of reproduction in such triploids provide important information, in comparison to the reproduction of the low-ploid (3 × 7) triploids of *Petunia*, on the relative stability and balance of genomes, on the survival of agenomes, and on the fate of the triploid complex. The principles or rules of reproduction in autotriploids will serve as a basis of the evaluations of the more complex hybrid triploids that arise from the hybridizations of distinct *2n* and *4n* species.

The Triploids of Datura stramonium. All of the eleven species of *Datura* whose chromosome numbers have been counted are *sn:24* and polyploids and aneuploids have appeared only in experimental studies of *Datura stramonium*. The constitutions of the autotriploids and tetraploids, with respect to origin and to genome numbers of *12*, were SSS and SSSS.

There were no intraspecific self- and cross-incompatibilities in any of the plants of *Datura* that were studied by Blakeslee and his associates. But the relations of selfing and of crossing within each of the *3n* and the *4n* members were called "compatible." In contrast, the relations of *2n* and *4n* were called "incompatible" and this term was also applied to failures in certain of the hybridizations between species. These "incompatible" reactions do not involve *S* factors and are entirely distinct in nature and status from intraspecific self- and cross-incompatibilities.

There are no records of spontaneous triploids among the inbred seedlings of diploid *D. stramonium*. In 1916, a plant was observed which was called a mutation and its selfed progeny was considered to have the status of a new species (Blakeslee & Avery 1919). Soon this plant was found to be tetraploid (Blakeslee, Belling & Farnham 1920) and other similar tetraploid seedlings were observed among the cultures of diploids. Colchicine treatments effected somatic tetraploids (*sn:48*) and also somatic aneuploids that were collectively *sn:23, 42, 43, 44, 45, 46, 47, 56, 67*, and *89* (Blakeslee & Avery 1937; 1938). This wide range of somatic aneuploidy was obtained from the diploid constitution of *sn:24* and, to the knowledge of the writer, these results have not been duplicated in any other diploid species. Such a series would provide valuable materials for critical studies of the potential fertility and the patterns of reproduction in somatic aneuploids that arose from a single diploid constitution by somatic duplication of chromosomes.

The triploids that were obtained and studied by Blakeslee and his associates were autoploids derived by crossing *4n* × *2n* of *Datura stramonium*. These are comparable to the AAA triploids of *Petunia axillaris*. The data for the reciprocal relations of *2n* with *4n D. stramonium* have been summarized and evaluated by Sansome, Satina, and Blakeslee (1942). In the *2n* × *4n* relation the pollen tubes usually burst in the styles previous to fertilizations and only one seedling, which was triploid, was obtained of many such pollinations. During twenty years, a total of 103 seedlings were grown of *4n* × *2n*. Of these, 35 were *2n*, 57 were *3n*, and 11 were *4n*. Thus it seemed

that the egg cells of the *4n* which had functioned were either *n*, *2n*, or *3n*. It was considered, however, that the seedlings that were *2n* may have been produced by parthenogenesis. The ovaries of the *4n* plants contained an average of 537 ovules, but in 18 selfed capsules that were evaluated an average of only 107.6 large seeds was produced. This indicated that only one of five of the ovules was able to function in seed production. But the 33 capsules obtained of $4n \times 2n$ contained an average of only 5.1 large seeds. The examinations revealed that this low production of seeds was due to abortions of zygotes rather than to failures of fertilization.

The potential fertility and the pattern of the sexual reproduction in the autotriploids were very fully evaluated. In meiosis (Belling & Blakeslee 1922), chiefly trivalent grouping and random distributions gave to sporogenous cells all chromosome numbers from *12* to *24*. Some stray chromosomes which formed microcytes were observed and there were some giant pollen grains that presumably had *36* chromosomes. The percentage of aborted pollen grains was estimated at 43.6 (Blakeslee & Cartilege 1926).

Of the selfed progenies of the triploids, 216 seedlings were grown: 53 were *sn:24;* 121 were *sn:25;* 31 were *sn:26;* 5 were *sn:48;* and 6 were undetermined (Blakeslee 1927). Thus gametes that were either *n*, $n + 1$, or $n + 1 + 1$ were chiefly involved in the production of viable zygotes. Of the $2n \times 3n$ relation only 7 plants were obtained but evidently only one pollination of this relation was made: 6 were *sn:24;* 1 was *sn:25*.

The extent of the potential fertility of the *3n* ovaries was evaluated by the proportions of fertilizations and embryo abortions that occurred in the $3n \times 2n$ relation. The number of ovules in the ovaries of *3n* plants averaged 815, which was almost the same as the average of 813 in diploids (Blakeslee & Cartiledge 1926). The extent to which fertilizations occurred in the $3n \times 2n$ relation was studied in sections through ovaries fixed at 6–8 days after pollination (Satina, Blakeslee & Avery 1938). In a large number of the embryo sacs there was no sign of fertilization. In one ovary, 85 ovules had proembryos and endosperm but several hundred ovules were unfertilized and more or less disintegrated. In 75 capsules of $3n \times 2n$ there was an average of 61 seeds of which about half were either defective or empty. Hence only about 30 of the average of 815 ovules in an ovary of a *3n* plant were able to function in the production of embryos when the pollen was uniformly *n*.

The data for the germination of seeds and the character of the selfed seedlings are as follows: for 388 "good" seeds, 11 did not germinate; 106 seedlings died; 57 were *2n;* 134 were $2n + 1$; 71 were $2n + 1 + 1$; and 9 were $2n + 1 + 1 + 1$. For 48 "defective" seeds: 24 germinated and 14 seedlings lived; 1 was *2n;* 4 were $2n + 1$; 8 were $2n + 1 + 1$; and 1 was $2n + 1 + 1 + 1$ (Satina, Blakeslee & Avery 1938). An earlier report (Belling & Blakeslee 1922) stated that of 75 seedlings of $3n \times 2n$, 24 were *sn:24*, 33 were *sn:25*, and 10 were *sn:26*.

Thus the sexual reproduction in the selfed autotriploids was decidedly

limited to progenies that were *2n*, *2n + 1*, *2n + 2*, and *2n + 3*. It is obvious that the majority of gametes that functioned were *n* or *n + 1* and that none higher than *n + 3* could have functioned except for an occasional *2n* and *3n* that was involved in producing a plant that was *4n*. There were either abortions of the microspores and macrospores that received more than *15* chromosomes, or abortions of the gametes with such numbers, or abortions of the zygotes produced by such gametes. The expected or theoretical distributions of chromosomes, as observed in meiosis and sporogenesis, should give as many *2n* as *n* spores. But either the *2n* spores were not viable, or *2n* pollen tubes failed to grow in *3n* pistils, or there were no survivals of *3n* zygotes. Regarding this resultant, Satina, Blakeslee, and Avery (1938) remark that "It is not clear why *3n* types have not appeared in the offspring from the cross *3n* × *2n*. . . . We know of no reason why there should be selection against *3n* zygotes. That they may be at a disadvantage in comparison with *2n* and *4n* zygotes, however, is suggested from the results of the *4n* × *2n*, which gives relatively few seeds and these have poor germination."

Thus the data for *Datura* are in close agreement with the observations made by Müntzing (1933, 1936) and by others that there are extensive abortions of the *3n* zygotes that are formed after the controlled pollinations of *4n* × *2n* relations in which the fertilization of *2n* eggs by *n* sperms is forced without competition. The data for *Datura* also conform to the general rule that the few triploid zygotes that are formed in the ovules of triploids to selfing or to *3n* × *2n* relations usually abort.

Extensive and critical studies were made of the patterns of reproduction of *2n + 1* plants of *Datura* which revealed that there were segmental reorganizations of chromosomes. The progenies obtained of *2n + 1* plants included over 60,000 seedlings. Twelve primary and fourteen secondary trisomics were identified among the *2n + 1* members. In representing the new constitutions the two halves of the twelve chromosomes are indicated as **1–2, 3–4, 5–6,** etc. and the extra chromosomes that are unidentified are indicated by numerals in italic. It was found that the extra chromosome may be (1) any one of the twelve members of the genome (as *2n* + **1–2**, a primary trisomic); or (2) it may be composed of the doubled half of any one chromosome (as *2n* + **1–1** or *2n* + **2–2**; a secondary trisomic); or (3) it may be composed of halves of two different chromosomes (as *2n* + **1–3**, a tertiary trisomic); or (4) it may comprise three segments of either two or three different chromosomes (as *2n* + **2–11–12**); or (5) it may be composed of only a half of a chromosome. It is to be emphasized that frequent and extensive segmental exchanges and the reconstruction of chromosomes occurred during meiosis in *3n* and *3n + 1* plants which involved both exchanges between nonhomologous chromosomes and internal interchange between the parts of a single chromosome or of two homologous chromosomes. It has been recognized (literature by Darlington 1937) that there are numerous cases of interchange constitutions in diploids, triploids and tetraploids of both natural and

experimental origins and that these "structural hybrids" have, as a rule, sterilities of abortions in sporogenesis, and that they seldom breed true in immediate progenies.

Thus new reconstructions of chromosomes were obtained by Blakeslee in *2n + 1* plants that were derived from triploids which were in turn obtained from known diploids and tetraploids that belonged to one species. The reconstructions in a single extra chromosome effected new expressions of characters many of which were identified as properties of a particular chromosome or segment of a chromosome (Blakeslee 1934; Blakeslee & Avery 1938).

But in the studies by Blakeslee and associates no trisomic (*sn:25*) of *Datura stramonium* bred true in sexual reproduction. On account of the unbalanced agenomic constitution this is to be expected. Hence the critical interest is in whether further selective reproduction in such progenies can give rise to balanced and matched constitutions that are 2 (*n + 1*) and true breeding.

In the selfed progenies of *2n + 1* plants there were (a) a large proportion of *2n* members, (b) many members that were *2n + 1* and like the parent, (c) some members of a different *2n + 1* type, (d) a very few plants that were *2n + 2*, and (e) frequently there were some *4n* plants. Of the 60,000 seedlings reported in table 2 of the 1938 report, 18 were haploid and one was triploid. It was stated that "in the reduction divisions of a *2n + 1* plant the chromosome of pollen-mother-cells segregate in such a way that equal numbers of *n* and *n + 1* daughter cells result. Presumably a similar condition holds for megaspore mother cells." But the progenies did not include *2n*, *2n + 1*, and *2n + 2* classes in the proportions of 1 : 2 : 1. There was a great deficiency of the *2n + 1* class and almost complete elimination of plants that were *2n + 2*. But there were some plants that were *sn:26*. Such plants may be double trisomics (*2n + x + y*) that would not breed true or they may be a balanced double of *n + x* and the extra chromosomes may be any one of the reorganizations already noted above. Among such balanced agenomics there are excellent chances that selective and differential pairing will effect functional diploidy. It appears that this feature was not thoroughly tested and the detailed data for the breeding that was done with *2n + 2* segregates were not presented. On the basis of the results obtained it was stated that "Simple tetrasomic types (*2n + 2*) do not breed true. In their offspring from selfing are found *2n + 2*, *2n + 1*, and *2n* individuals. In back-crosses *2n + 2* plants fail to appear." The failure of *sn:26* plants to breed true may have been because no zygotic combinations were obtained and tested in which the two extra chromosomes were matched in a dysploid constitution.

But two true-breeding extra chromosomal and dysploid types that were *sn:26* were obtained after x-ray treatments (Blakeslee 1934), and their origins and constitutions were fully evaluated. In one of these, two pairs of chromosomes had been reconstructed from one of the primary pairs. The

two arms of the largest chromosome (**1–2**) of the genome had been reorganized to give a chromosome that was **2–2** and another that was **1**. Then the fusion of two gametes which were alike gave a functional diploid. In the other new dysploid each genome of *13* chromosomes was composed of ten members of the original genome, and three new chromosomes that were reconstructed from the parts of the other two of the primary set together with a segment of one of the other members. The constitutions of these three new chromosomes as designated by Blakeslee were **2–14, 13–23,** and **14**.

It should be mentioned that a genome number of *12* may comprise one reconstructed chromosome and that two such genomes which were identical could be obtained in *sn:24* which bred true to both chromosome number and a new expression of characters.

Thus it was demonstrated that true-breeding types which had new differentiations in specific character arose in both *sn:24* and *sn:26*. These were considered to have the status of new " synthetic " or " artificial new species " and the following statements were made: " The pure-breeding types are more distinct from the original form from which they arose than some of the species of *Datura* which have been founded on simple factor differences. Our types we have ventured to call artificial or synthesized new species. They differ from the ancestral form not by a single factor but by a whole group of factors " (Blakeslee 1934).

It is to be noted that the prerequisites for the appearance of extra chromosomal dysploids, as *sn:26* in *Datura*, are (1) that the agenomic constitution must survive in both pollen and egg cells, (2) that the zygote of such fusions must survive, and (3) that there must be a functional diploidy in the sexual reproduction that provides stability to the new genome in successive generations.

The experimental evidence that *2n + 2* individuals do not breed true has neither been extensive nor based on persistent selective breeding. The aneuploids that arise in considerable number in the progenies of triploids provide excellent material for such studies. The writer greatly regrets that he did not have time to make such studies of the extrachromosomal aneuploids obtained in the cultures of *Petunia*. There was a wide range of chromosome numbers in the aneuploids of *Petunia* which indicated the survival of agenomes of *8, 9, 10, 11, 12,* and *13* chromosomes. Thus there was less selective unity in the genome number of *7* in *Petunia* than there was in the genome number of *12* in *Datura*. The aneuploids of *Petunia* which had these agenomes were as a rule vigorous and profusely flowering, and all had some degree of potential fertility. The extensive breeding of such plants would certainly produce some zygotic combinations of gametes that possessed matched agenomes. That these may not be obtained in all members of a first selfed progeny of a trisomic is obvious. When aneuploids have even numbers of total chromosomes some of the members of the selfed progeny which have the same number should have matched chromosomes.

The Triploids of Lycopersicum esculentum. (1) Spontaneous autotriploids (*sn:36*) of cultivated tomatoes were observed and studies of their sterility and fertility were reported by Lesley and Mann (1925), Margaret M. Lesley (1925), J. W. Lesley (1928), and M. M. and J. W. Lesley (1930). These plants were without fruit and were noticeably more robust than diploid plants. No tetraploids were observed in the fields but segments of tetraploid tissue were found in the meristem of certain branches of otherwise diploid plants. It was considered that the triploid seedlings arose from the fusion of unreduced *2n* gametes with gametes that were *n*.

Two triploids of the Dwarf Aristocrat tomato produced no seeds to numerous self-pollinations. In the earlier tests no fruits were obtained of *2n* × *3n* relations but later two seeds were thus obtained from which diploid seedlings were grown. About 25 per cent of the pollen of the triploids appeared to be normal but was not functional in producing viable seeds in selfing and it rarely thus functioned in the *2n* × *3n* relation. The studies of meiosis in pollen mother cells indicated much trivalent pairing with disjunction of two homologs and random distribution of the third member. Chromosome numbers were counted in 94 pollen cells: 1 had *12*; *22* had *18*: 1 had *24*; and there were some of all numbers from *19* to *23* except *22*. There were lagging chromosomes, premature division of univalents, and the formation of supernumerary nuclei and cells.

As females these triploids produced with pollen of *2n* a total of 56 fruits which had an average of four seeds. Fruits of diploids had an average of 50 seeds. Sixty-six seedlings of *3n* × *2n* were grown: 10 were *2n* (*sn:24*); 35 were *2n* + *1*; 5 were *2n* + *1* or *2*; 14 were *2n* + *1* + *1*; and 2 were *2n* + *1* + *1* + *1*. Hence the egg cells that had functioned had from *n* to *n* + *3* chromosomes. There are no self- and cross-incompatibilities in these cultivated tomatoes and hence the "self-sterility" of these triploids and the feeble cross-fertility of *2n* × *3n* were evidently due entirely to poor pollen and abortion of zygotes. The reproduction in the *3n* × *2n* was limited and decidedly selective for gametes that were haploid or that had no more than three extra chromosomes, as was the case in the triploids of *Datura stramonium*.

(2) Jørgenson (1928) had a triploid clone of the Balch's Fillbasket tomato that was obtained in England from a callus of a decapitated stem of a plant whose chromosome number was unknown but which was probably triploid itself. Plants of this clone did not produce fruits and seeds to self-pollinations. Jørgensen also obtained a somatic autotetraploid (*sn:48*) branch of the diploid Danish Export Tomato by the decapitation method and then obtained *sn:36* seedlings by crossing this *4n* with *2n*. The seeds of this relation that gave triploids were of small size and poorly developed but they were germinated by special means of culture. The number of such seedlings was not stated but it was reported that they produced no seeds to selfing. The *3n* × *2n* relation gave fruits that contained few seeds. The self-fruitless-

ness of these triploids was presumably due to the low potential fertility of both ♀ and ♂ gametes. Evidently the application of an abundance of good pollen from *2n* plants provided sufficient fertilizations and embryos for the formation of fruits with a few seeds.

(3) Huskins (1934) obtained ramets of the same triploid clone of Balch's Fillbasket Tomato that Jørgensen had. These also failed to produce seeds to selfing until two fruits appeared which had 22 seeds. Eleven seedlings grown from these seeds were all tetraploid (*su:48*) and hence all the ♀ and ♂ gametes that had functioned were presumably *2n*. Huskins noted that this reproduction did not conform to the selective reproduction expected of triploids. He suggested that some change in the somatic number of chromosomes may have occurred in the branch that produced the two fruits and the 22 seeds, but a further evaluation of the condition was not determined, nor were seedlings grown.

(4) Rick (1945) has recently surveyed the nature and the extent of unfruitfulness in the fields of three canning varieties of tomato grown commercially in the lower Sacramento Valley in California. Of the 66 unfruitful plants that were studied, 45 were triploid, 14 were diploid, three were tetraploid, two were trisomic, and two were haploid. It was reported that in all cases the unfruitfulness involved "gametic sterility." Triploids were the "commonest of all unfruitful types" and these "set very little seed." The evaluations of eleven of the unfruitful diploids revealed that abortions of gametes was complete for both pollen and ovules in five plants, that there was male sterility in two plants, and that three plants were both sterile and aberrant in gross morphology. These were considered to be the effects of gene mutations, of which thirteen were later indicated (Rick 1948). Rick noted that the unfruitful plants made excessive vegetative growth and continued to produce flowers indefinitely due to the "close correlation between vegetative growth and absence of fruit" as was demonstrated experimentally by Murneek (1926). It is to be recognized that Rick's analysis of the spontaneous origin of gametic sterility in diploids suggests that some type of loss of maleness or femaleness may be present in triploids in addition to the abortions due to triploidy itself.

The studies of the autotriploids of *L. esculentum* indicate that the triploids have a very limited potential fertility that almost precludes further sexual reproduction. How this condition would be influenced by further somatic doubling that gives double triploids is not known.

Triploids of Solanum melongena. Several abnormal plants were observed (Janaki Ammal 1931, 1934) in cultures of this species. These plants were unfruitful. The somatic chromosome number was determined in one of the plants and this was triploid and *su:36*. In meiosis, there were trivalents, bivalents, and univalents and there was much abortion of pollen. After many selfings one fruit that contained 14 seeds was obtained. Thirteen seedlings were grown; 2 were tetraploid (*sn:48*); 11 were "nearly tetraploid" with

somatic numbers of *44*, *45*, and *46*. Thus there was survival of gametes that were *2n* and not less than *2n* – *4* and that ranged from *gn:20* to *gn:24*. The progeny was strongly tetraploid and hence in sharp contrast to the reproduction in triploids of *Datura* which was decidedly diploid.

(3) *Data for Tuberous Solanums.* Of the tuberous members of *Solanum* that have received specific names (Darlington & Janaki Ammal 1945), 61 are exclusively *su:24*, 13 are *su:36*, 21 are *su:48*, 4 are *su:60*, and one is *su:72*. There are also complexes that include *su:24* and *36* (one), *su:24* and *48* (two), *su:36* and *72* (one), and in the complex called *S. tuberosum* there are *su:24*, *48*, *96*, and other constitutions of experimental status. The data for these may here be evaluated not only in reference to the triploids but also in regard to intraspecific incompatibilities and to the status of the complex included in *Solanum tuberosum*. There is evidence (Lawrence 1931; Cadman 1942, 1943) that the basic number of chromosomes in *Solanum* is *6* and that the lowest somatic number of *24*, now known in this genus, is tetraploid in origin but functionally diploid in meiosis. But in this consideration the present basic number will be considered to be *12*.

Self-incompatibilities have been reported in ten different diploid species of the tuberous group of *Solanum*: *S. chacoense* and *S. jamesii* (Stout & Clark 1924); *S. caldasii* (Clark 1927); *S. bulbocastanum* (Livermore & Johnstone 1940); *S. rybrnii*, *S. boyacense*, *S. lanciforme*, and *S. phureja* (Carson & Howard 1942); *S. aracc-papa* and *S. subtilis* (Pal & Bushkar 1942). The authors last named presented data which indicate that clones and seedlings of *S. caldasii* (*su:24*) probably have the personate type of self- and cross-incompatibility. Members of one diploid species, *S. polydenium*, were found to be self-fertile by Clark (1927).

Johnstone (1939) reported that tetraploid (*su:48*) seedlings which were obtained from colchicine-treated seeds of *Solanum jamesii* and *S. chacoense* produced seeds to their own pollen. Members of these species were found self-incompatible by Stout and Clark (1924) and the diploid plants which Johnstone had of these species may have been self-incompatible. In 1940 Livermore and Johnstone provided data for experimental tetraploids of another species. "The authors obtained no seeds by selfing diploid *S. bulbocastanum*; approximately twenty-five seed balls with a good supply of seeds have been produced by selfing *4n* lines produced from these diploid lines." A letter to the writer from Professor Livermore stated that four different tetraploid seedlings were selfed, that the seeds were plump and assumed to be viable, but that no attempt was made to germinate them.

The clonal status of the so-called species of *Solanum* that are *su:36* is definitely known (Anon. 1936). Twenty-one of these were found in cultivation locally in southern Peru where *su:24* and *su:48* types were also in cultivation. All of these *su:36* "species" appear to be so highly sterile from loss of potential fertility that they can only be propagated vegetatively as clones. Stout and Clark (1924) found that ramets of *S. maglia* (*su:36*)

were so highly pollen-sterile that no reliable data on self-incompatibility could be obtained. Clark (1927) found that vegetative propagations of *S. commersonii* (*sn:36*) had a high degree of pollen abortion. Evidently the clonal species that are *sn:36* have the steriliters of abortions that are characteristic of true triploid hybrids. Possibly some of them also have self- and cross-intraspecific incompatibilities. Presumably some progenies of the solanums could be obtained experimentally of $3n \times 3n$ or of $3n$ with $2n$ and $4n$ that would reveal the patterns of meiosis and reproduction, but at the present time such data are evidently lacking.

For the tuberous species of *Solanum* that are *sn:48* and tetraploid for the genome number of *12*, there is evidence that members of *S. fendlerii* (*Navajo potato*) have high potential fertility and no self-incompatibilities (Stout & Clark 1924). Pal and Bushkar (1942) state that several tetraploid species were "self-compatible" but no names were provided.

The clones of potatoes widely cultivated in the North Temperate zone have mostly been classed as *Solanum tuberosum* and most of them are *sn:48*. It is considered (Anon. 1936) that this group arose directly from the tetraploid type *S. andigenum*, which is widely grown in cold altitudes in the Andes, by the selection of characters that effect good vegetative growth and formation of tubers over a wide area of the warmer Temperate zone. As a group, these clones are grown in areas where conditions of temperature and length of daylight effect non-blooming, the blasting of flowers, and the abscission of ovaries. Of 30 clones grown at the New York Botanical Garden in 1920 and 1921, none produced flowers that opened normally. Members of the same clones bloomed in profusion at Presque Isle, Maine. At both places good crops of tubers were produced.

Studies were made at Presque Isle, Maine, of 132 of the cultivated clones and 78 seedlings (Stout & Clark 1924). Few of these produced viable pollen to the extent that they were able to function as good pollen parents in selfing or in crossing. But most clones and seedlings had functional pistils that would produce seed balls and viable seeds when there were proper hand-pollinations. Thus in these clones the sterility is chiefly a male sterility and certain of its features are independent of, and in addition to, the abortions of meiosis that involve polyploidy and hybridity. Most of the cytological studies of the abortions in the pollen of potatoes have not recognized this condition.

There are a few clones and seedlings of the cultivated potatoes that have high potential fertility of ovules and also pollen that is about 20 per cent viable and functional. Considerable data obtained for the crosses of these clones over a period of nine years (Stout & Clark 1924) show that in most cross-relations seed balls and viable seeds were obtained. There was no definite evidence of any cross-incompatibilities. The clones which were both male and female fertile, as McCormick, Bursola, and Clio, produced few fruits irregularly to open-pollinations, but presumably will produce fruits when proper self-pollinations are made by hand. Ellison (1936) reports

that in England certain "self-fertile" clones (Shetland Black, Ballydon, and Sharpe's Victor) may flower freely and set a berry for nearly every flower.

The view that there is a genetical basis for the different grades of pollen abortion in potatoes has been emphasized by Kranz, Becker, and Fineman (1939). They note that the percentages of pollen abortion in numerous seedlings ranged from 0 to 60 and that there were wide differences in the degrees of abortion among seedlings of pedigreed lines. They suggest that there is segregation of four genotypes based on the influence of a tetrasomic gene which has lethal effects in pollen when it is homozygous. The non-flowering of seedlings and clones, and the abscission of fruit after proper pollination are viewed as antagonisms between fruit production and vegetative vigor that have genetical values.

For the one species, *S. demissum,* which is *sn:72*, Clark (1927) reported self-fertility and this status was verified by Pal and Bushkar (1942). There are various reports of hybridization between this species and *S. tuberosum* (*sn:48*), *S. maglia* (*sn:36*), *S. pendleri* (*sn:48*), and *S. commersonii* (*sn:36*) (Schnell 1948) but these provide no data on self-incompatibility. The recently introduced Essex clone is a selection derived from crosses of *S. demissum* with cultivated clones that are *sn:48* (information in a letter from Dr. Donald Reddick).

As a group, the tuberous species and clones of potatoes possess extreme reductions in sexual reproduction which are maintained by vegetative propagation. Fertilities that effect fruit and seed formation have not been important factors in the selection of cultivated types and clones. Intraspecific self- and cross-incompatibilities are definitely known in ten of the diploid species while self-fertility is known in one diploid species. In three instances the experimental tetraploids obtained of self-incompatible diploids were self-fertile. At least one tetraploid species (*S. pendleri*) has given seedlings that were self-fertile. No self- and cross-incompatibilities have been demonstrated in the numerous clones that are cultivated in United States and Europe. But the extent to which these tetraploids have arisen from self-incompatible diploids and the present status of the tetraploids, in regard to the inactivation or the loss of *S*, factors, are at present inferential.

There is, it appears, no experimental information regarding double triploids of either autoploid or alloploid status in the tuberous Solanums. In this group there is only one species (*S. demissum*) and two derivatives of another species (*S. vallis-mexici*) that are now known to be *sn:72*. The effects that somatic doubling of the three sets in triploids may have could be determined and there are numerous triploid clones of the tuberous solanums available for such studies.

Evaluations. (1) Both the frequency of the occurrence of simple triploids and the level of their sexual reproduction vary greatly according to species and genera. In *Lycopersicum* the triploids were, as a rule, impotent

either as males or as females or as both. The selfed progenies of the triploids of *Datura stramonium* were almost exclusively *2n, 2n + 1,* or *2n + 1 + 1.* Those of AAI in *Petunia* ranged from *3n – 1* to *4n* but the chromosome numbers in the progenies of *3n* × *2n* revealed that egg cells that carried chromosome numbers from *n* to *2n – 1* were functional. The seedlings of *Solanum melongena* were *4n – 4* to *4n.*

(2) That the triploid constitution may survive in functional gametes has frequently been demonstrated and when the functional gametes range from *2n* to *3n* the progeny may range from *3n+* to *4n* and even to doubled triploids. Such gametes are the survivals of distributions of chromosomes that follow limited bivalent pairing and the *3n* gametes are formed by "restitution."

An example of this pattern of sexual reproduction was reported and evaluated by Thompson (1931). Simple triploids were obtained of *Triticum turgidum* (*4n* and *sn:28*) × *T. monococcum* (*2n* and *sn:14*) and the selfed progeny included *sn:26, 27, 28, 29, 30, 35, 36,* and *42.* In the meiosis of the triploids the number of bivalent allosyndetic pairing ranged from 3 to 7 with 5 as the most frequent number. When there were only 3 bivalents the distributions could be from *3* to *18.* But 98 per cent of the pollen aborted. Yet there were functional gametes that had from *13* to *18* chromosomes and also there were some that had *21* chromosomes. Selfed F_2 progenies were grown of plants that were *sn:28, 29, 30, 35, 36,* and *42.* The number of plants in a progeny ranged from 3 to 12, which is too few to indicate definitely the patterns of reproduction. Collectively, the chromosome numbers ranged from *sn:26* to *sn:42* and hence the reproduction continued to be from *4n – 2* to *6n.* In this case, as in many others, adequate tests were not made to determine the possibility of obtaining true-breeding dysploids, which have such chromosome numbers as *sn:30* and *sn:36,* from derivatives of triploids that are *sn:21.* It is to be noted that the pattern of sexual reproduction in these triploids of *4n* × *2n* wheats was different from that of the AAI (*sn:21*) triploids of *Petunia* which had the same genome number of *7.* In the latter there was much trivalent grouping and evidently no viable gametes with more than *14* chromosomes.

(3) It appears that simple triploids seldom reappear in the progeny of triploids even when some functional *n* and *2n* gametes are produced. Abortion of the triploid zygotes appears to be highly selective in the ovules of plants that are *2n, 3n,* or *4n.*

(4) At the present time the data for the wide range of aneuploid derivatives of triploids that have a low *n* indicate that true-breeding dysploids are difficult to obtain. Most evaluations have considered that they are impossible to obtain. It may be emphasized that the experimental evidence does not demonstrate that dysploid derivatives are impossible in ranges between *2n* and *3n* and between *3n* and *4n.*

(5) The survival of a triploid complex in gametes that function in sexual reproduction preserves and continues that complex in sexual progenies that

include high-level aneuploids and balanced double triploids of hexaploid and amphitriploid status.

Reproduction in Doubled Triploids. *The Recognition of Amphitriploidy.* The early evaluations of polyploid sequences by Tahara (1915) and Winge (1917) recognized that the series of genome numbers in a genus is typically arithmetical, as *a, 2a, 3a, 4a, 5a,* and *6a,* and not exclusively a sequence of doubled numbers, as *a, 2a, 4a, 8a,* and *16a.* The earlier discussion by Strasburger (1907) considered that abnormal mitosis may effect tetraploidy especially in connection with apomixis. The publications by Winge (1917), Rosenberg (1917) and Ernst (1917, 1918) contributed important data and presented significant hypotheses. Rosenberg found semi-heterotypic meiosis and restitution nuclei in parthenogenetic types of *Hieracium* but it was not until 1925 and 1927 that he explained that the failure of reduction may result in *2n* gametes that can function in producing polyploids. Ernst emphasized the role that hybridization may take in effecting apogamous plants that may then give rise to tetraploids. Winge postulated that in the primary cell of a hybrid zygote the two disharmonious genomes find a necessity for congenial partners that induces a somatic duplication of each chromosome without an immediate nuclear division and that this gives rise to a tetraploid. If sexual reproduction continues in that plant the progeny is tetraploid. If sexuality is replaced by apomixis the progeny is also tetraploid. In 1924 Winge extended his hypothesis to include the doubling of chromosomes in a hybrid zygote that is derived by hybridizing a species that is *2n* with one that is *4n* and he notes that "then a hexaploid new species might similarly be imagined as the result."

Since 1924 it has been demonstrated experimentally and by cytological examinations that (1) somatic doubling of chromosomes may occur or be induced in the tissues of rather mature plants as well as in the cell or cells of a young zygote, (2) that doubling may also occur in the formation of gametes, and (3) that both these processes may occur in members of "good" species as well as in hybrids.

In 1925, Clausen and Goodspeed reported that a doubled triploid had been obtained experimentally as a derivative of a hybrid between a *2n* and a *4n* species of *Nicotiana.* The production of this plant was considered to be "an experimental verification of Winge's hypothesis." This doubled triploid was called a "tetraploid" and it was given the new specific name of *Nicotiana digluta.* Since 1925, numerous cases of doubled triploids have been included in the lists of "amphidiploids" (cf. Goodspeed & Bradley 1942), along with tetraploids that have arisen from low-level diploids. That the two have a different status must be recognized in the evaluations of reproduction. The term amphiploidy (Clausen, Keck & Hiesey 1945) includes all cases in which there is "the addition of all chromosomes of two distinct species" that is "effected through chromosome doubling either before or after fertilization." Such amphiploids may be polyploid or dysploid, and tetraploid or hexaploid.

Autoploidy is defined as the multiplication of the chromosome sets within the limits of one species and as being always a form of polyploidy, never as dysploidy. These useful terms have definite limitations. They depend on the basic conceptions of a species. They apply best to species that have a low *n* and their immediate derivatives. The doubling of chromosomes in a species that is already an allotetraploid is certainly not to be considered as autoploidy, at least of a simple type. At any rate the interpolation of triploidy that follows tetraploidy extends polyploidy to the level of hexaploidy and effects constitutions that provide increased possibilities for competition between autosyndesis, allosyndesis, and preferential syndesis which have profound effects on the patterns of reproduction.

The Experimental Double Triploids of Petunia. The intraspecific hexaploid constitution in AAAAII petunias was obtained by the induced somatic doubling of a known triploid hybrid that was AAI and *S 1.3.b.* This hexaploid constitution was therefore autotetraploid for AAAA and diploid for II. In the original somatic double triploid all chromosomes were pure for each of the respective parents for they had not been subjected to meiosis in the triploid seedling. This provided each chromosome with a duplicate. The evidence indicates that this effected a pairing of duplicated chromosomes (preferential pairing) and a distribution that gave to most gametes the triploid number of chromosomes. In the selfed and intra-bred progenies of the double triploids there were some aneuploids of $6n-2$, $6n-1$, $6n+1$, and $6n+2$. Thus there were some gametes in which there were losses of one or two chromosomes and gains of one or two chromosomes.

It was demonstrated that the experimental autotetraploids of AAAA were both self-fertile and true-breeding to the extent that they did not throw aneuploids in selfing but there was evidence that they may have produced some *n* gametes that did not function in selfing. The reproduction was definitely bivalent for identical chromosomes, at least for those that carried *S* factors. The double triploid constitution added to the tetraploid AAAA constitution a duplicated genome of *P. integrifolia* and the further reproduction showed some disjunctions of one or two chromosomes that survived in both gametes and zygotes. There were, however, abortions of both microspores and ovules which indicated further irregularities in meiosis that were lethal in either spores or zygotes. The potential fertility of the double triploids was relatively lower than that of autotetraploids. It may be assumed that the complete AAAAII complex could be stablized by selective breeding and the polyploid series in petunias would thereby be raised from *2n* and *4n* to *6n*. There is already the one natural dysploid species of *Petunia parviflora* that is *sn:18*. There is the possibility that new dysploids could be obtained as derivatives of some of the wide range of aneuploids (figure 59) that were obtained (a) in somatic numbers between *sn:14* and *sn:21* and (b) in numbers of *sn:32, 31, 36, 40,* and *44*. But the possibility that new dysploids may be obtained in *Petunia* and at low *n* levels is a matter for future experimental investigation.

Some Evaluations of Doubled Triploids. (1) Double triploids have six genomes and hence the complexities in their reproduction are greatly increased over those that exist in tetraploids that have only four genomes. If the main types of tetraploids are *aaaa, aaab, aabb*, etc., to *abcd*, the corresponding double triploids would be *aaaaaa, aaaabb, aabbcc*, etc., to *abcdef*. Thus far there has been no very extensive and adequate evaluation of the patterns of reproduction in hexaploids in distinction to tetraploids. Most frequently the term amphidiploid, or merely the term amphiploid, is used to designate any polyploid of hybrid status. While this has certain advantages it is often an oversimplification that masks significant differences in respect to the number and character of genomes.

(2) The wide range of constitutions in tetraploids that have only four basic genomes has been a matter of much discussion and analysis. As Stebbins (1947) has recently recognized, tetraploids include autoploids, a wide range of segmental alloploids, and true alloploids. Any one of these constitutions may be carried into a double triploid where it is associated with two other genomes that may have any one of many different degrees of relationship. Stebbins has recognized that double triploids may be autopolyploid with respect to one genome but collectively allopolyploid because of the presence of a different genome and he cites two such cases that are presumably *aaaabb*. That double triploids include many more complications is recognized for they provide for the association of three different genomes from as many different species. Stebbins cites, as an example of this, the synthetic *Nicotiana digluta* which is a double triploid of two species ($4n \times 2n$) of which one is of remote hybrid origin.

(3) In respect to the levels of polyploidy higher than tetraploidy, Stebbins considers that "a large proportion, if not a majority, of hexaploids, octoploids and higher polyploids represent some variant of the autoallopolyploid condition, in other words, they have resulted from autopolyploids, segmental allopolyploids, and true allopolyploids combined in different ways. The complete cytogenetic and phylogenetic analysis of such higher polyploids will probably be made in only a few clear and important cases." Stebbins cites a case of a fertile *sn:70*, or *10n* derivative, which was obtained of parents that were *sn:42* (*6n*). From this and other data he makes the following statements. "It is possible, therefore, that some of these higher polyploid F_1 hybrids may give rise in later generations to several distinct species all descended from the same hybrid combination. The limitless possibilities and complexities of such a situation can only be imagined. In many genera containing these high polyploids we must, therefore, be content with assuming that most of these species contain various combinations of autopolyploidy, segmental allopolyploidy and true allopolyploidy, and that their phylogenetic relationships will be difficult or impossible to unravel."

(4) Much attention has been directed to the cytological features that contribute to sterility and fertility, to stability in reproduction, and to the genetical character of polyploids and their derivatives. An excellent evalu-

ation of the data for these features in "amphidiploidy" has recently been presented by Goodspeed and Bradley (1942). These authors include under the term "amphidiploidy" all allotetraploids that have four genomes, all double allotriploids of *6n*, and alloploids of higher levels. Several double triploids are included in the discussions and of a list of 16 fertile polyploids that arose from sterile F_1 hybrids there are at least four that arose from F_1 triploids that had one parent that was already allotetraploid. It was concluded that fertility in "amphidiploids," including amphitriploids and their derivatives, is usually correlated with bivalent pairing and that this is most likely to be complete when the F_1 hybrids (both *2n* and *3n*) are themselves sterile.

(5) Natural hexaploid species, that are presumably double triploid in origin, are rare in the Solanaceae. The *Solanum nigrum* includes a widely distributed and constant type that is *6n* and presumably autoploid and three others of the complex that are also *sn:72* have been given specific rank. Only *S. demissum* of the tuberous Solanums is *sn:72*. The two species of *Atropa* whose chromosomes have been counted are *sn:72*. The genera *Datura*, *Lycopersicum*, and *Solanum* and a large section of *Nicotiana* have evidently arisen as tetraploids of *gn:6* and have developed much speciation at that level. There are also some 9 species of *Nicotiana* and about 20 of *Solanum* that have advanced to *sn:48*. There are now no species of the Solanaceae that have a genome number of *6*, but there are the dysploid numbers of *7*, *8*, *9*, *10*, and *11*. Triploids in considerable number have arisen and been perpetuated as clones in the tuberous Solanums, and there are four clonal species that are *sn:60*. But the evidence seems conclusive that doubled triploidy has played a very minor role in the evolution of the genera and species of the Solanaceae. There has been little advance to the hexaploid level.

(6) The experimental and cytological studies of sexual reproduction in triploids demonstrate that triploids may be agents in the production of tetraploids and doubled triploids. The various studies have emphasized that the derivatives that have balanced genomes and functional diploidy are most easily obtained. But there is some evidence that dysploids and secondary polyploids may be obtained by experimental means. In the rather small population of the selfed *3n* and the *3n* × *2n* progenies of AAI triploids in *Petunia* there was every aneuploid between *2n* and *4n* except *sn:22* and *sn:27*. Experimental studies have not fully tested the possibilities that stabilized dysploids of chromosome numbers between *2n* and *4n* may be obtained.

Chapter 15. Unilateral Hybridization

Evaluations. *The Status of Unilateral Hybridization.* Definite recognitions of unilateral interspecific hybridization have been few and chiefly incidental and considerations of its significance have been superficial, especially

in the evaluations of the mechanism and of its role in speciation and biosystematics. That unilateral hybridization is one of several types of unilateral reproduction is to be recognized and this is well exemplified in the petunias.

In Petunias. Three distinctly different types of unilateral reproduction occur in the petunias studied by the writer.

(1) There were interspecific reactions of unilateral fertility and sterility in the reciprocal relations of any two of the three species, all of which had the same somatic number of chromosomes.

(2) In the intraspecific reproduction of the cultures that were studied of either *P. axillaris* or *P. integrifolia* there were cross-fertility and cross-incompatibility in reciprocals when one member was homozygous (as *S 1.1*) and the other was heterozygous (as *S 1.2*) and there was one *S* factor in common. In the normal diploid-haploid reactions of the personate type of intraspecific incompatibility, unilateral reactions do not occur. But in the associate type in both diploids and polyploids (Stout 1938) and in the diploid-diploid reactions, that are now known in few homomorphic flowering plants (Hughes & Babcock 1950), unilateral reactions are frequent and important.

(3) There are also differences in the reciprocals of relations between different levels of "ploidy," especially of the *2n* with the *4n*. There was a stronger and more complete barrier to reproduction in the $2n \times 4n$ relation of *P. axillaris* than in the reciprocal relation. But the feeble fertility of the $4n \times 2n$ relation was masked by the epistatic action of incompatibility when the relation was fully syngenic (as $S\ 1.1.2.2 \times S\ 1.2$). The evidence indicates that unilateral sterilities may operate at various levels of ploidy and be interspecific as well as intraspecific.

The Case of Mirabilis longiflora and M. jalapa. These two species are *sn:58* as is the one other species of this genus that has been studied. Counts of chromosome numbers have been made in only one species of another genus of the Nyctaginaceae and this case is *sn:20*. Evidently these two species of *Mirabilis* are high-level dysploids.

Kölreuter (1766) reported that he had made as many as 200 pollinations of *Mirabilis longiflora* × *M. jalapa* that had completely failed to yield seeds but that the reciprocal relation readily gave viable seeds. Correns (1903) also found that seeds were obtained only when *M. longiflora* was the pollen member. He found that the hybrids were potentially highly fertile and produced seeds to self-pollinations. He grew progenies and reported simple Mendelian ratios for the heredity of certain characters, and especially for the color of flowers. Evidently no tests were made for the reactions of these hybrids with the parents in respect to unilateral resultants.

These two species of *Mirabilis* differ greatly in the relative length of the pistils. The pollen of the short-styled *M. jalapa* does not function in the long pistils of *M. longiflora*. Evidently the locus of the sterility is in the style and the reaction has a diploid-haploid status. But the hybrids have potential

fertility. The F_1 hybrids were described as reasonably uniform and somewhat intermediate but much like *M. jalapa* in character. The F_2 presumably had certain extremes in the length of pistils that should make easy the tests for reactions that are comparable to those of the initial unilateral reactions. Since there are no intraspecific incompatibilities in these two species of *Mirabilis* the analysis of the reproduction in the hybrids should be relatively simple.

The Unilateral Hybridization of Corylus avellana with C. americana. A summary of nut breeding (Crane, Reed & Wood 1937) indicates that a large number of the cultivated clones of the European filbert, *C. avellana,* produce no fruits when pollinated with pollen of clones of the American species *C. americana,* but that the reciprocal pollinations yield viable seeds from which a large number of the F_1 hybrids have been grown. This appears to be a definite case of unilateral hybridization between two distinct species. But two species of Old World origin, *C. avellana* and *C. colurna,* do not hybridize in either of the reciprocal relations.

In the reports on production of nuts in the cultivated clones of the several species of *Corylus,* the interspecific failures of hybridization are confused with the intraspecific incompatibilities. It is stated that all known clones of *Corylus* are self-incompatible and that there are also interclonal cross-incompatibilities even when the decidedly synchronous dichogamy of clones is sufficiently reciprocating to allow proper cross-pollination (cf. Stout 1928).

The study of true self- and cross-incompatibilities in seedlings of any species of *Corylus* has not been made, hence there is no information on the type of the intraspecific incompatibilities. That these are entirely distinct from the failures of hybridization is certain. Presumably the F_1 hybrids of parents that are self-incompatible will also be self-incompatible.

It may be noted that chromosome numbers are known in nine species of *Corylus.* All are *sn:28* and presumably tetraploid (4×7) although no species that is *sn:14* is known. The species of two other genera of Corylaceae are *sn:16* with one complex of *sn:16* and *sn:64.* The species of *Corylus* are evidently examples of tetraploids that have intraspecific self- and cross-incompatibilities. The commercial importance of these species and of their hybrids is certainly sufficient to warrant a critical analysis of the patterns of reproduction.

Unilateral Intravarietal "Hybridization" in Maize. Demerec (1929) reported that in several intervarietal crosses of maize there was a perfect set of seeds when a white, rice pop corn was the male parent but that no seeds resulted from the reciprocal pollinations. Certain previous studies had assumed that the deficiencies of sugary seeds in F_2 generations of crosses between sugary and rice pop varieties were due to differential growth of pollen tubes in competitions and that there was a dominant factor linked with the sugary character that was responsible for such selective growth. But Demerec proved that the unilateral sterility occurred in controlled cross-pollinations where there was no competition. The reaction was absolute. The pop corn

variety was sterile to pollen of certain other varieties with which it was fertile as a pollen parent. Demerec recognized that the seat of the unilateral reaction was in the pistil. The reaction was between the pistils of the pop corn variety and the otherwise functional pollen tubes of the several varieties of flint, dent, and sugar corns that were tested. There was inability of the pollen tubes to grow in the pistils of the pop corn and reach the ovules. It was observed that in these relations, the female pop corn member was homozygous for a factor that was dominant for the endosperm character. Hence the question arises as to whether this is itself the factor that effects unilateral sterility in the pistils, or whether it is distinct from such a gene, and if so whether or not there is linkage.

The components involved in the reactions of the diploid pop corn pistils with haploid pollen of other varieties, as sweet corn, may be represented as PP: *X p.p* × SS: *Y s.s.* Certainly all pollen grains of a variety pure for sweet corn carry the haploid constitution that is involved in the sterility reaction with diploid pistils of the pop corn. Jones (1924, 1928) found that the F_1 hybrid plants of SP hybrids were fully fertile with the pollen of either pop corn or sweet corn when each was applied separately in controlled pollinations. This demonstrated that the half or haploid constitution that was pop corn in the pistils of F_1 hybrids was not sufficient to effect sterility with any pollen of the sweet corn. This resultant is comparable to that in which F_1 PA hybrids of *Petunia* are fully fertile with pollen of *Petunia parodii.*

The F_2 and the cross-bred progenies of SP hybrids of corn should include extreme segregates in which unilateral reactions reappear when tested with sweet corn pollen. On the basis of the results in PA hybrids of *Petunia* it can be predicted that the genic components of pop corn that effect unilateral sterility will be found to be independent of the endosperm gene. It is to be hoped that techniques of experimental analysis can determine if the mechanism of unilateral sterility in these varieties of maize involves components of the normal intravarietal reproduction or if it is a completely superimposed mechanism and also if the male component that is involved is identical in all the varieties that have unilateral sterility as males in reactions with pop corn.

A preliminary report on studies of the unilateral reproduction in crossing varieties of maize has recently been made by Nelson (1949) and a more complete report together with new data may be expected.

The cases of uilateral interspecific and intravarietal hybridization that are briefly discussed above are only a few of those that could be mentioned in hermaphrodites. They exemplify the limitation of critical data on the genetical status of the relative specificity that unilateral reactions of sterility and fertility involve.

The Constitutional Basis of Unilateral Hybridization. *The Status of Relative Specificity and Half-Speciation.* Unilateral hybridization reveals a condition of *half-speciation* in the differentiations of those organs and

elements of sexuality that effect reproduction. In hermaphrodites among flowering plants, this half-speciation is a differential in the relative specificity of pistils and stamens of an individual, either in its bisexual flowers or in the staminate and pistillate flowers of monoecious plants.

The relation of *Petunia parodii* × *P. axillaris* is one of unilateral fertility. The pistils of PP: ♀ ♀ and the pollen tubes of AA: ♂ ♂ react as do the comparable organs of one species or of the ecotypes of one ecospecies. There is absence of specific differentiation in some component of the diploid PP: ♀ ♀ constitution and some component of the AA: ♂ ♂ constitution.

But the relation of AA: ♀ ♀ × PP: ♂ ♂ is one of complete sterility. The pistils of *P. axillaris* and the diploid stamens and the haploid pollen tubes of *P. parodii* have an extreme differential specificity that is characteristic of two species that do not hybridize. It is obvious that the analysis of the basis of such unilateral specificities will provide basic information regarding the nature of speciation. Here is an isolating mechanism that operates in half of the hermaphrodite mechanism of reproduction.

There have been several outstanding treatments on the role that speciation has in "biosystematics." As far as the writer is aware, these make only incidental reference to unilateral hybridizations. Also it appears that no adequate experimental tests have been made to determine the genetic basis of unilateral hybridizations. It is difficult to understand why there has been this neglect, for several conspicuous cases of unilateral hybridization were known in the parents of hybrid progenies that were studied for the analysis of the genetics of other characters. Of these, the case of *Mirabilis longiflora* and *M. jalapa* has already been noted.

The Nature of Bisexuality and Relative Specificity. The male and female organs and elements in hermaphrodites arise by somatic differentiations of somatic cells that are *alike* in genetic constitution. In flowering plants sexual reproduction *begins* in the differentiations of pistils and stamens from diploid cells that are alike. This principle has had a very general recognition since the theory of qualitative somatic segregation proposed by Weismann was found to have very limited application. As Correns (1926, 1928) and Hartman (1929) have emphasized, a diploid hermaphrodite that is stabilized in its differentiations of sex organs is actually homozygous and diploid for both maleness and femaleness, and this can be expressed as ♀ ♀ : ♂ ♂ or as *FF: MM*. But Correns and Hartman recognized that there is a physiological mechanism which effects the somatic differentiations that produce pistils and stamens from somatic cells that are alike. They emphasized the obvious fact that any special genic components that are active in the differentiation of pistils must be present but inactive in the stamens. Also the genic factors that effect differentiation of stamens must be present but inactive in pistils. Hence both the female and the male gametes carry a haploid genic dosage of both maleness and femaleness. Correns and Hartman proposed that there are realizer genic components which operate as inhibitors and activators in

somatic differentiations. This postulate has been rather vaguely recognized in discussions of sexuality in flowering plants. Usually somatic differentiations of pistils and stamens have been treated as if they involved genetic segregations. For the present considerations it will be recognized that the pistils of diploid hermaphrodite flowering plants are ♀ ♀ (♂ ♂) and that the stamens are ♂ ♂ (♀ ♀); that the meiotic segregations that occur give pollen haploid constitutions of ♂ (♀), and that both egg cells and sperms possess ♀ and ♂ genic components.

The Multipart Constitution of P. parodii. In this species the mechanism of differentiation in flowers is stabilized, the flowers are uniformly hermaphrodite, and there is coordination of the physiological reactions, especially between pistils and pollen tubes, in intraspecific reproduction. There are no intraspecific incompatibilities; *S* factors are absent. But when hybridization tests are made, unilateral specificity is revealed. There is some component of the PP: ♂ ♂ complex which segregates uniformly in the pollen (P: ♂) and effects a sterility reaction with *P. axillaris* ♀ ♀ but not with PP: ♀ ♀.

The ♂ ♂ complex, in the reactions of its haploid pollen tubes, has three reactions or functions. (1) There is intraspecific fertility with PP: ♀ ♀; (2) there is unilateral sterility with *P. axillaris* ♀ ♀ and (3) with *P. integrifolia* ♀ ♀. It seems obvious that either *one* genic component of haploid P: ♂ has two, or possibly three, different functions or that the P: ♂ constitution is *multipart* and composed of different genic components each of which has one function. Of the entire PP: ♀ ♀; ♂ ♂ constitution the only reactions of sterility are those of unilateral interspecificity that involve some diploid component of unilateral relative specificity of pollen and pollen tubes. This specialized component may be designated as *Y p.p.*

But the PP: ♀ ♀ constitution and its expressions in the differentiations of pistils also have three functions. There is participation in (1) intraspecific reproduction and in unilateral interspecific reproduction with (2) *P. axillaris* ♂ ♂ and (3) *P. integrifolia.* The question arises as to whether all these functions involve one genic component (as PP: ♀ ♀, *F p.p*) or two or more different nonallelic components.

The Multipart Constitution of P. axillaris. The aggregate functions of the entire AA: ♀ ♀ complex involve participation (1) in intraspecific reproduction, (2) in self- and cross-incompatibilities when there is syngenic relations of an *S* factor, (3) in unilateral interspecific sterility with PP: ♂ ♂, and (4) in unilateral interspecific fertility with *P. integrifolia* ♂ ♂.

The AA: ♂ ♂ complex also has four functions: (1) intraspecific reproduction, (2) self- and cross-incompatibility, (3) interspecific fertility with PP: ♀ ♀, and (4) interspecific sterility with H: ♀ ♀. Thus the AA: ♂ ♂ has only one reaction of unilateral sterility and the component involved in this may be designated as *Y a.a.* The component of AA: ♀ ♀ that is involved in the one unilateral sterility with PP: ♂ ♂ may be designated as *X a.a.*

The Multipart Constitution of P. integrifolia. Each of the II: ♀ ♀ and the II: ♂ ♂ complexes has four functions but they differ from those of *P. axillaris* (cf. figure 19). The II: ♂ ♂ has unilateral fertility with AA: ♀ ♀ and PP: ♀ ♀, but it is a *reduced* reproduction that is relatively lower in the PP: ♀ ♀ relation. The II: ♀ ♀ complex has complete unilateral sterility with AA: ♂ ♂ and PP: ♂ ♂ and the component involved in this may be called *X i.i.*

It may be noted that many of the characters and properties of pistils and stamens involve adaptations that influence pollination, such as monoecism, dicliny, and dichogamy. These are in no sense direct physiological interactions between ♀ and ♂ elements. The physiological interactions of unilateral sterility and fertility in plants like the homomorphic petunias, operate after there are proper pollinations and, in the cases under present consideration at least, before there is fusion of gametes. There are in these petunias no special organs exclusively devoted to either intraspecific incompatibilities or to the sterilities of unilateral hybridizations, as there are in heteromorphic somatic differentiations of both stamens and pistils of dimorphic and trimorphic plants (see especially Lewis 1949).

The Problems of Evaluations. The extent to which the diverse reactions that are noted above involve independent genic components of the ♀ ♀ and ♂ ♂ complexes may, it seems certain, be determined if adequate techniques can be devised for evaluations. The evaluation of the behavior of the *S* factors is relatively simple, for an *S* factor has syngenic action of sterility, unless there is the feature of inactivation, and the presence and role of a single *S* factor in a diploid F_1 hybrid can readily be tested. The evaluation of those genic components that are involved in a unilateral sterility, as AA: ♀ ♀, *X a.a* × PP: ♂ ♂, *Y p.p*, should be much simpler where both of the parents have no intraspecific self- and cross-incompatibilities. No doubt there will some day be sufficient analyses of such cases to establish the principles that may be applied to the cases in which one or both of the parental species have self- and cross-incompatibilities.

The Data for, and the Evaluations of, the PI Hybrids Grown by Mather. *Nomenclature.* Three reports by Mather, by Bateman, and by Mather and Edwards (1944) concerned plants that were called *Petunia axillaris.* These plants were grown from seeds that were obtained from Professor Margaret C. Ferguson and it was stated that they were all self-fertile and that they had the characters that were described and illustrated by Ferguson and Ottley (1932) under the name *Petunia axillaris.* But Steere (1931) had already recognized that the long slender corolla-tube, the two lengths of the stamens, and other features of difference made this type so distinct from the true *P. axillaris* that it merited distinction as a different and new species which he designated as *Petunia parodii.* Steere, Ferguson, and the writer all obtained seeds of *P. parodii* directly from Professor Lorenzo Parodi in Argentina.

Mather and Ferguson obtained cuttings of the only clone of *Petunia*

integrifolia that was being grown at Kew Gardens and the writer obtained cuttings from Professor Ferguson. According to the international rules of botanical nomenclature the epithet *Petunia violacea* is a synonym of *Petunia integrifolia* (cf. chapter 4).

A further correction should be made in regard to a statement by Lewis (1949, page 481). In referring to a preliminary report by Stout and Chandler (1941) on *2n* and *4n* members of *Petunia axillaris*, it is stated that "the species used was probably the garden form, *Petunia violacea*." The assumption that this was a case of confusing the widely different types of *P. axillaris* and *P. integrifolia* (*P. violacea*) is, to say the least, somewhat gratuitous. It has already been noted at several points in this present paper that there has been much careless and indiscriminate use of the names *Petunia axillaris*, *P. nyctaginiflora* and *P. violacea*, but the writer knows of no case in which a white-flowered petunia with large flowers has been called *P. violacea*.

Mather's strain of *P. parodii* was a different one from that which the writer had, but both strains had no *S* factors of self- and cross-incompatibility and both had complete unilateral sterility as males with the Kew clone of *P. integrifolia*.

Mather's data for the reactions of reproduction in these F_1 hybrids, and in the further progenies of them, differ in several important features from the comparable reactions obtained by the writer for the P.A hybrids. From the date of the earliest experimental studies by the writer with *P. axillaris*, the symbols *S 1*, *S 2*, *S 3*, and *S 4* have been applied to this species while the symbols *S a* and *S b* were applied to the Kew clone of *P. integrifolia*. Hence to avoid confusion in the following discussion it is necessary to change Mather's terms for *P. integrifolia* from "*S 1*" to *S b* and from "*S 2*" to *S a*; and his use of "*S a*" in *P. parodii* to *S p*.

Mather's Interpretations. Mather reduced the genetic components that were directly involved in the unilateral sterility of *P. integrifolia* × *P. parodii* to the simple formula of (II) *S a.b* × (PP) *F p.p* = 0 and 0. The formula for the reciprocal was (PP) *F p.p* × (II) *S a.b* = F and F. But he assumed that the fertility factor (*F p*) of *P. parodii* became allelic to *S a* and *S b* in the hybrid offspring and that then the three factors were integrated as segregating units of one allelic series (*S p.a*, *S p.b*, *S p.p*, and *S a.b*).

The interpretations that were made include other postulates: (1) That pollen tubes that carry the *S p* factor do not grow efficiently in pistils that have only one *S p* factor. (2) That pollen tubes that possess *S p* never grow efficiently down any style that is *S a.b* or that has either *S a* or *S b*. (3) That the factor *S p* in a pistil never inhibits the proper growth of pollen tubes that carry either *S a* or *S b*. (4) That *P. parodii* possesses a gene, or genes, not allelic to *S a*, *S b*, or *S p* (*F p*) that modify *S a* and especially *S b* in both the styles and the pollen, and (5) That the factor *S b* strengthens *S a* when the two are together in the tissues of styles.

The Interpretation of "Switch Action." An important interpretation

was made regarding the self-fertility of the F_1 hybrids of *P. parodii* (*S p.p*) × *P. integrifolia* (*S a.b*). Five members of this progeny were highly self- and cross-fertile and these were considered to be (PI) *S p.a*. It was considered that the self-fertility of these plants was due to the functioning of *S a* pollen tubes while *S p* pollen tubes failed in a hidden or masked reaction of sterility. Thus it was considered that the *S a* factor in the diploid cells of the pistils was inactivated by association with *S p* and that then the *S a* pollen tubes did not have any reaction of incompatibility. Also *S p* pollen tubes failed because there was only one *S p* factor in the hybrid pistil. Thus there was a change or "switch" in the respective action of *S a* and *S p* factors. The *S a* factor in pollen of an *S p.a* became, temporarily, a fertility factor and the *S p* factor became a sterility factor.

It is to be noted that the assumptions are that one factor, "*S p*," is active in both pistils and pollen tubes and can have a different function in the two, and that also an *S* factor may switch from an incompatibility action to a positive fertility action. The writer considers that the evidence obtained from PA hybrids (chapter 6) indicates that the *S* factors have only reactions of syngenic incompatibility and that they are not inactivated in diploid hybrid pistils.

Nonconforming Reactions. In the reactions that were tested of F_1 and later generations, Mather frequently found "nonconforming reactions that did not conform to any simple scheme based on the assumptions." Nonconforming reactions were attributed (a) to the different influences of the assumed fertility factor (*S p*), (b) to changes in the status of *S a* and *S b* when they became separated, and (c) to the modifying action of other genes. "It was necessary to postulate non-allelemorphic genes" which influenced at least six different reactions in the PI hybrids (Mather 1944, page 230).

The Evaluations. Mather's methods of testing, his data, and his interpretations for the PI hybrids merit critical examination, especially in view of the differences in the comparable reactions of the PA and AI hybrids studied by the writer.

The adequate indentifications of the genic constitutions and components in the parental species, in the F_1, and in the segregates of later generations depend (a) on the reactions with known testers that provide definite identification of the various allelic components, and (b) on the analysis of those pedigreed collateral progenies that are necessary to identify those factors or components that have hidden reactions, inactivation, switch behavior, modifying influence, mutations, or nonconforming behavior. It is obvious that the identification of allelemorphic integrations and of associations of genic and cytoplasmic components that have complementary and epistatic or hypostatic relations becomes difficult when mechanisms of intraspecific incompatibilities and of unilateral interspecific sterility and fertility are superimposed upon or integrated with basic fertility. The reader of these lines is advised to examine Mather's data and evaluations, for the following dis-

cussion is chiefly devoted to matters that seem most critical both in agreements and disagreements with the behavior of PA and AI hybrids.

The F_1 Hybrids of P. parodii × P. integrifolia. (1) Mather considered that the two known *S* factors of *P. integrifolia* Kew clone and one fertility factor of *P. parodii* were the principal genic components that were involved in the reactions of unilateral sterility and fertility in the reciprocal relations of hybridization and in the fertilities and sterilities of the subsequent progenies. According to his symbols the unilateral fertility involved *F p.p* × *S a.b* and the unilateral sterility involved *S a.b* × *F p.p*. The segregations and recombinations gave an aggregate of only four genotypes: *S p.a*, *S p.b*, *S p.p*, and *S a.b*. These symbols may be continued in the present considerations, except when attention is directed to other of the ♀ ♀ and ♂ ♂ components.

(2) There were only nine of these F_1 hybrids and several were not fully tested (Mather 1944, figure 1). The reactions with testers indicated two groups or genotypes; five members were classed as PI: *S p.a* and four were considered to be PI: *S p.b*. Hence pollen that carried either *S a* or *S b* had functioned, which was to be expected since there was no opportunity for either factor to have syngenic incompatibility. Mather considered that the *S a* and *S b* factors were also active agents in the reactions of fertility. The writer considers that the evidence indicates that the *S a* and *S b* factors have *only* the property of syngenic incompatibility and that in this hybridization relation there were other genic components that effected the unilateral fertility. That such components may have segmental linkage with *S a* and *S b* in homologous chromosomes is to be recognized.

(3) The F_1 PI: *S p.a* members of the F_1 were highly self-fertile and were called "self-compatible." The *S p.b* members "often failed to set seed after self-pollination, but sometimes set a small capsule and even occasionally a full capsule." But the two groups were fully cross-fertile in reciprocal pollinations.

The analysis of the F_2 progenies in respect to the identification of the genotypes provide data for a judgment (a) of the extent to which *S a*, *S b*, and *S p* genic components participated in the fertilizations and (b) of the extent to which they were involved in sterilities.

The F_2 Progenies of Selfing. (1) Mather's data for the 20 members of these F_2 are assembled and rearranged in figure 60. The assignments of the genotypes, in terms of *S a*, *S b*, and *S p* factors, are, it is believed and intended, in accord with Mather's evaluations.

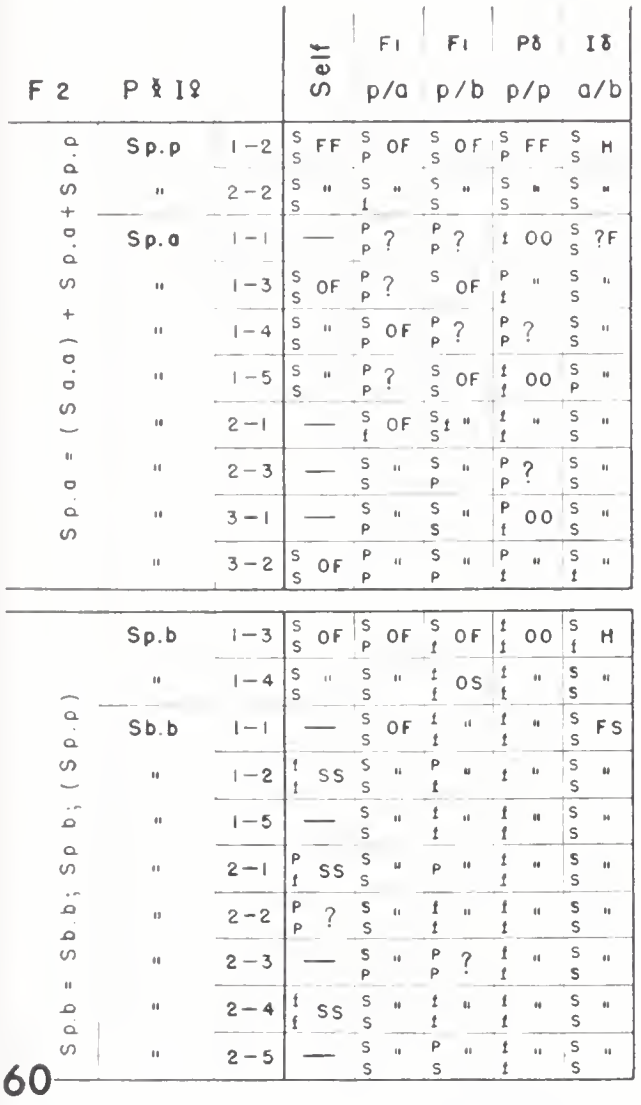
Usually two pollinations were made in a test and the results of these are indicated in small letters as given by Mather; "s" is for "successful pollination"; "p" indicates "partially successful"; and "f" is for a "failure". Large-sized letters indicate total resultants: F indicates fertility; S indicates failure due probably to syngenic incompatibility; O indicates probable failure of *S p* pollen; FO and FS indicate fertility with hidden sterility. Certain of the results appear to have hybridization (H) status. Verification of many

of the assignments of genotypes would involve the analysis of further progenies. No attempt is made to assign "switch" actions or to designate the various modifying factors that were involved in nonconforming resultants.

(2) Eight of the 20 members were male sterile. Frequently members of the cross-bred F_2 and of back-cross progenies were also male sterile. Such plants could not be tested for self-fertility or for reactions as males. These frequent cases of male abortions during the stages of development of stamens by somatic differentiation indicate that certain recombinations of PP: *MM* and II: *MM* components together with the recombinations of realizer factors effected the abortions. No doubt techniques may be devised and employed that would identify the factors for maleness and femaleness and for the realizer factors that were important in the differentiation of sex organs in these hybrid hermaphrodites. It is to be noted that there was only one case of male sterility in the many PA and AI hybrids grown by the writer. There were vegetative abortions of pistils, stamens and petals in the progenies obtained by the premature selfing of the Kew clone and the genetic basis of these was analyzed (chapter 4). The frequent male sterilities in PI hybrids and the absence of these in the PA hybrids indicate that the specific differentiations of *P. parodii* and *P. axillaris* are much less than are those of *P. parodii* and *P. integrifolia*, as noted in Chapter 1.

(3) The reactions with testers indicated that two of the ten members of the selfed progeny F_1 PI: *S p.a* were *S p.p* and eight were *S p.a*. No member was *S a.a*. Evidently two pollen tubes that carried *S p* had functioned, which does not conform to Mather's postulates. The eight members of this F_2 that were *S p.a* could have arisen from either *S p* or *S a* pollen tubes. If *S a* pollen tubes had functioned, as assumed by Mather, there had been (a) inactivation of *S a* in diploid pistils by association with a haploid constitution that was *P. parodii* and also (b) the selective fertilization of only *S p* eggs by *S a* sperms. If, however, only *S p* pollen tubes had functioned, the reactions were fully comparable to those that occurred in the selfing of PA: *S 0.1*, etc. Two members were cross-fertile as females with *P. parodii* ♂ ♂, and hence there was a recovery of an intraspecific reproduction.

(4) None of the ten members of the selfed progeny of PI: *S p.b* was *S p.p* and hence no *S p* pollen tubes had functioned unless they had fertilized *S b* eggs to give rise to the two members that were *S p.b*. Eight members were classed as *S b.b*, and their origin would involve the inactivation of *S b* in the diploid pistils of PI: *S p.b*. These pistils were not tested with II: *S b.b*. Also it would appear that there was some selective fertilization of *S b* sperms with *S b* eggs in preference to *S p* eggs. This may involve selective intraspecific reproduction of *P. integrifolia* components over both unilateral hybridization of P ♀ and I ♂ components and intraspecific reproduction of P ♀ with P ♂. Mather has recognized that pollen tubes of *P. parodii* do not grow well in pistils that have only a haploid constitution of *P. parodii*, but this could be due to the presence of factors derived from *P. integrifolia*, as



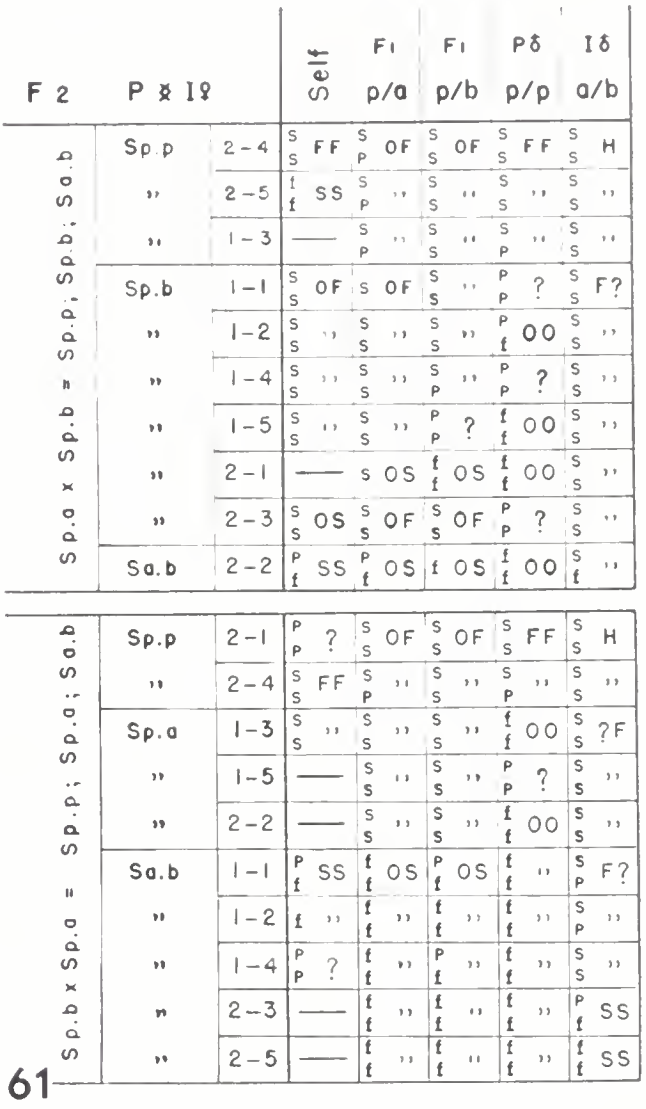


FIG. 60. Transposition of Mather's data for two F_2 progenies of selfed F_1 genotypes of *P. parodii* × *P. integrifolia*. The small letters are those used by Mather for the results of pollinations: s = successful; p = partially successful; f = failure. The O symbol refers to failures of pollen that carried *S p*; large *S* refers to failures due to a syngenic action of the incompatibility factors *S a* and *S b*. FIG. 61. Transposition of Mather's data for two F_2 progenies of the reciprocal crosses of the two F_1 genotypes *S p.a* and *S p.b*. Most F_1 and F_2 members were sterile with *P. parodii* ♂. The comparable relations of F_1 and F_2 of the PA hybrids were fertile (cf. figures 22 and 25).

♀ *N i*, that effect unilateral sterility. Several of the results indicate selective reactions between gametes after pollen tubes reach ovules.

The F_2 of Intergenotypic Crosses. (1) Twenty members comprising ten each were grown of the two reciprocal progenies of F_1 PI: *S p.a* with *S p.b*. Both of these relations were disgenic for the factors, *S a* and *S b*, and hence the reactions did not involve any inactivation of these factors in pistils. The reproduction that did occur involved the integrated reactions of components that effected unilateral hybridization, unilateral sterility, and intraspecific reproduction. Mather considered that all these were effected by the factors P: *F p* and I: *S a* and *S b*, and an evaluation may be attempted on that basis.

Six of the 20 members were male sterile and could not be tested for reactions of selfing. The twenty members were tested only as female parents (figure 61).

(2) Five members were fertile with *P. parodii* ♂ ♂ and hence were considered to be *S p.p.* In the origin of these plants, *S p* pollen tubes had presumably functioned in pistils that were PI: ♀ ♀, *S p.a* or *S p.b.* One of these F_2 *S p.p* members failed to set capsules to two self-pollinations, but these failures may have been incidental. All five of the *S p.p* segregates were cross-fertile with II: ♂ ♂, *S a.b* which was presumably a reaction of unilateral hybridization. Further progenies of these *S p.p* segregates were not grown.

(3) Six members of this F_2 progeny were classed as *S a.b* and these were either self-incompatible or male sterile. All were cross-sterile as females with F_1 *S p.a* ♂ ♂ and *S p.b*, as was II: ♀ ♀, *S a.b.* Thus there was a recovery of unilateral sterility in which *S a* and *S b* were either the factors that effected the action, as Mather considered, or that were linked or associated with other factors (as *X i.i*) that had this function.

Two of the *S a.b* segregates were cross-incompatible with *P. integrifolia* ♂ ♂ as expected. But four others had unexpected and nonconforming reactions of cross-fertility with II: ♂ ♂, *S a.b.* Hence the *S a* and *S b* factors in the pistils of these six members of the F_2 had lost power to effect syngenic incompatibility in crossing with *P. integrifolia* ♂ ♂ but had retained it in selfing. Mather states that these *S a.b* segregates "were out of phase" with the original *S a.b* component in *P. integrifolia* and that "we must assume that this was due to modifying genes." The identity of the components that effected the epistatic reactions of sterility and fertility in these cases could no doubt be determined by the analysis of the reciprocal and the collateral progenies that may be obtained from them.

The Sterility Reactions of the F_1 Hybrids. The F_1 hybrids of PI were completely sterile as males with *P. integrifolia S a.b.* As females the *S p.b* were fully sterile and the *S p.a* almost sterile with *P. parodii.* In the comparable relations, the PA hybrids were fully fertile except for hidden syngenic incompatibilities. This noticeable difference in the reproductions of PI and PA hybrids indicates different degrees in relative speciations and cenospecific status. The adequate analysis of the PI hybrids should provide critical data on the genetic status of the two types of sterility that are involved: (a) the incompatibility factors obtained from *P. integrifolia* and the unilateral sterility of which the only genic components came from the male differentiations of *P. parodii.*

(2) As male members all F_1 plants that were tested were cross-sterile with II: ♀ ♀, *S a.b.* except for one pollination that was "partially successful" (Mather 1944, table 1). In this relation the pollen that carried either *S a* or *S b* presumably had syngenic incompatibility. Evidently all pollen that did not possess *S a* or *S b* also failed to function, and this could only be due to unilateral sterility. Mather's evaluation that either *S a* or *S b* in ♀ ♀ constitutions has unilateral sterility with *S p* pollen describes the conditions, but may not identify the genic components that are involved, especially if such

genic elements are merely linked with *S a* or *S b*. The total sterility is evidently an integration of syngenic incompatibility with unilateral sterility that is epistatic over any intraspecific influence between any complements of *P. integrifolia* ♂ ♂ in haploid pollen with the diploid pistils of *P. integrifolia* *S a.b*.

(3) The three PI: *S p.b* members that were tested as females with PP: ♂ ♂ were entirely sterile. There could be no syngenic incompatibility. Hence the haploid component that was *P. integrifolia* in the pistils was sufficient to effect unilateral sterility with P: ♂. The writer suggests that the genic components that were involved may be represented as I: ♀, *X i* and P: ♂, *Y p*. Mather considers that the I: ♀, *S b* factor is itself a factor that inhibits the growth of all pollen tubes of *P. parodii*.

(4) There were partial and irregular reactions of feeble fertility and sterility in the relation PI: ♀ ♀, *S p.p* × PP: ♂ ♂, but as no progenies were grown an analysis of the fertilizations cannot be made.

(5) There were numerous cases of hidden syngenic reactions of sterility on the part of true *S* factors and hidden reactions of unilateral sterility which included Mather's considerations of switch actions. The extents to which these operated selectively but concurrently in relations that give capsules and seeds, are subject to evaluations by the analysis of the various collateral progenies that may be grown.

The Back-Cross Relations That Were Fertile. (1) The relations of II: ♀ ♀, *S a.a* × PI: ♂ ♂, *S p.a* and *S p.b* were all "successful." When *S p.a* was a pollen parent there was, presumably, syngenic incompatibility for pollen that carried *S a*. But if all other pollen was "*S p*" and was functional, there was a nonconforming reversal in the action of *S p* pollen. Possibly some of the pollen that did not have *S a* also did not have the component of P ♂ that effected unilateral sterility but did have factors of *P. integrifolia* that effected intraspecific reproduction. No progenies were grown of these fertile relations. The analysis of such progenies, and of other of the collateral progenies obtainable from the two genotypes that were the pollen parents, should indicate whether two or more classes of pollen were produced.

(2) Mather tabulated data for the character of the progenies of six back-cross relations that were fertile (his tables 6 to 11 inclusive). On the basis of the three factors, *S a*, *S b*, and *S p*, the analyses of these six progenies are as follows:

	S pp :	*S pa* :	*S pb* :	*S ab* :	*S aa* :	*S bb*
(6) PP: *S p.p* × PI: *S p.b* =	9	–	1	–	–	–
(7) PP: *S p.p* × PI: *S p.a* =	10	E	–	–	–	–
(8) PI: *S p.b* × II: *S a.b* =	–	6	E	4	–	E
(9) PI: *S p.b* × II: *S a.a* =	–	4	–	1	–	–
(10) PI: *S p.a* × II: *S a.b* =	–	1	E	9	E	–
(11) PI: *S p.a* × II: *S a.a* =	–	6?	–	–	4	–

(6 and 7) The relations between the two parents in these two cases did not involve syngenic incompatibilities, unilateral sterility, or any switch actions. Each pollen parent possessed one genome of *P. integrifolia* which included the chromosome that carried *S a* or *S b*. This genome favored a very weak grade of unilateral hybridization fertility. Each pollen parent also possessed a genome of *P. parodii* which included not only "*F p*" but one of all other factors which effect fertility and sterility in *P. parodii*. The segregations in the pollen of the PI: *S p.b* plants involved reassortments of the seven pairs of homologous chromosomes as well as cross-over exchanges between the chromosomes that carried *S a* or *S b* and its homolog. The analysis is on the basis that there were two classes of pollen. The character of the two progenies indicate that only one *S b* pollen tube had functioned and that no *S a* pollen tubes functioned. The *S a* and *S b* pollen tubes had lost out in competition with *S p* tubes in pistils that were pure *P. parodii*. The only functions that were involved in the reactions of the pollen were (a) intraspecific reproduction by pollen that carried sufficient components of *P. parodii* and (b) interspecific reproduction by pollen that had the necessary components of *P. integrifolia*. The latter, at best, always had a weak grade of reproduction and in these progenies of only 10 members each the competition gave a selective action of intraspecific reproduction.

There were nineteen segregates that were cross-fertile as females with *P. parodii* and hence they were classed as P<>I: *S p.p*. They had received one genome pure for *P. parodii* from their female parent and from their hybrid male parent they received a genome that did not possess either *S a* or *S b*.

It is clear that the *S a* and *S b* pollen tubes failed in these two back-cross relations because the *S a* and *S b* were themselves factors that effected unilateral fertility or were linked or associated with other factors that had this function. For the analysis of linkages and unbalanced ratios of segregates a much larger number of plants should be grown and also the progenies of the "*S p.p*" segregates should be grown and adequately tested.

(8, 9, 10, and 11) These back-cross relations did not involve any unilateral sterility. In each case the haploid constitution of *P. parodii* in the pistils (including *S p*) favored unilateral fertility and the haploid constitution that was *P. integrifolia* favored intraspecific reproduction except for syngenic incompatibilities. Hence the analyses of these four back-cross progenies provide data on the behavior of *S a* and *S b* factors which are alone in pistils that have one complete haploid genome of *P. parodii*.

(8) This progeny consisted of four members that were considered to be *S a.b* and six that were *S p.a*. The eliminations of *S p.b* and *S b.b* genotypes demonstrate that the *S b* in the pistils continued to have syngenic incompatibility, that the accompanying *S p* had no switch influence, and that only the *S p* pollen had functioned. But with the *S b* pollen there had been a complete genome of *P. integrifolia*. This had unilateral fertility in relation with

the haploid complement of *P. parodii* in the F_1 pistils and also intraspecific fertility with the other genome which was *P. integrifolia*. Thus the fertility of the relation involved an integration of two types of fertility that masked the hidden syngenic incompatibility which was only revealed by the character of the progeny.

(9) Only five plants were reported of the back-cross of PI: ♀ ♀, *S p.b* × II: *S a.a*. Neither syngenic incompatibility nor unilateral sterility was involved in this relation. Four members were considered to be *S p.a* and one was *S a.b*. The reproduction involved an integration of intraspecific fertility and unilateral fertility, which is evidence that the two different genic components which effect these in II: ♂ ♂, are present in any haploid genome. The number of plants that were grown in these progenies is too few to give an evaluation of the relative values of the competition.

(10) Nine members of the progeny of F_1 PI: *S p.a* × II: ♂ ♂, *S a.b* were judged to be *S a.b* and one was *S p.a*. No member was *S a.a*. Evidently nine pollen tubes that carried *S b* had functioned with eggs that carried *S a*. Thus syngenic incompatibility was almost complete, the *S a* factor in the pistils had not been inactivated, and intraspecific reproduction had been epistatic over unilateral reproduction. No relation of unilateral sterility was involved. The reproduction had involved 9 egg cells that carried *S a* and 9 sperms that carried *S b*. If these factors had only the function of superimposed syngenic incompatibility the factors that effected the fertility were associated with them or possibly linked in the same chromosome.

(11) The relation of F_1 PI: ♀ ♀, *S p.a* × II: ♂ ♂, *S a.a* was tested by two pollinations on each of three members. Three pollinations failed, and three were partially successful. Thus there was the same feeble grade of reproduction that was seen in the unilateral hybridization of *P. parodii* × *P. integrifolia*. The relation involved syngenic incompatibility for all *S a* pollen. This pollen class had failed, with one exception, in the same *S p.a* pistils in case 10. Here there was no competition with any other pollen tubes, the action of *S a* pollen tubes was forced and the incompatibility reaction was in competition with both interspecific hybridization with the *S p* genome, and intraspecific reproduction with the genome that carried *S a*. To this extent the fertility of this integration may be considered as an inactivation of *S a* in the pistils of *S p.a*.

All of the 10 members of this progeny were fertile as females with II: ♂ ♂, *S a.b*, except for one "odd failure." All failed with PP: ♂ ♂. None completely failed with PI: ♂ ♂, *S p.a* and *S p.b*. Thus this back-cross progeny had sufficient *P. integrifolia* constitutions to have reactions of unilateral sterility with PP: ♂ ♂.

The F_1, F_2 and back-cross segregates that were classed as *S p.a* had irregular reactions in tests with *P. parodii* pollen. An *F p* (or *S p*) factor in pistils did not have its assumed switch to a factor of unilateral sterility. This was considered to be due to the presence of modifiers that favored intra-

specific fertility between components of *P. parodii*. Mather lists six reactions of pollen tubes that strongly indicate a number of non-allemorphic modifiers. These reactions involve certain relations of *S p*, *S a*, and *S b* pollen tubes and pislets of *S b.b*, *S p.b*, *S p.a*, *S a.a*, and *S a.b* genotypes.

The members of the progenies of cases 4 to 11 were not tested as males, and no progenies were grown of segregates that were *S p.p*, *S a.b* and *S a.a*. Such progenies should provide critical data on the nature and the status of modifying factors, on the question of the superimposed status of the true *S* factors, and on features of competition, epistatic relations, and inactivation of factors. All of these are involved in the multipart functions of pistils and pollen tubes in the integrations of intraspecific reproductions, of intraspecific sterilities and fertilities of hybridization, and of the superimposed mechanism of intraspecific syngenic incompatibilities. The data for the PA and the AI hybrids may be reviewed in respect to these matters.

Sterilities and Fertilities in the Reproduction of PA Hybrids. *The F_1 and the Selfed F_2.* All F_1 hybrids of *P. parodii* ♀ ♀ × *P. axillaris* ♂ ♂, and all F_2 obtained by selfing F_1 members were self- and cross-fertile except one member that was *S 1.1* (figures 22 and 25). The critical questions that arise are: (1) to what extent did hidden syngenic incompatibilities occur; (2) were the *S* factors that were derived from *P. axillaris* inactivated in hybrids by the association with components of *P. parodii;* (3) were any fertilities effected by double or switch properties of the known *S 1* and *S 3* factors; (4) were there any reactions of hidden unilateral sterility; and (5) were the fertilities expressions of intraspecific fertility, or of intraspecific reproduction, or of integrations of both.

(1) The resultants of tests, which included *S 1.1* and *S 3.3* factors, and the analyses of the progenies that were grown were conclusive that the one *S* factor that any hybrid possessed continued to have syngenic incompatibility. The hybridization of PP: ♀ ♀, *S 0.0* × AA: ♂ ♂, *S 1.3* gave PA: *S 0.1* and *S 0.3*. Then PA: *S 0.1* selfed gave F_2 PA: *S 0.1* and *S 0.0*. If Mather's symbols are applied, PA: *S p.1* selfed gave *S p.1* and *S p.p*, and it was only the *S p* pollen that functioned.

(2) Thus there was no inactivation of an *S* factor in pistils of hybrids.

(3) Also there was no switch in the function of any *F p* factor that was possessed by the chromosome of *P. parodii* that was homologous to the chromosome of *P. axillaris* that carried *S* factors.

The definitely personate status and the single function of each of these factors (*S 1*, *S 3* and *F p*) greatly simplify the further analysis of the fertilities that are involved in the reproduction of these hybrids.

(4) Were there any hidden reactions of unilateral sterility? It is obvious that any F_1 member of these hybrids obtained a haploid dosage of the AA: ♀ ♀, *X a.a* component and a haploid dosage of the PP: ♂ ♂, *Y p.p* component that reacted in the unilateral sterility. In an F_1 hybrid the *X a* component would have expression in all pistils and *Y p* would have expression in

half of the pollen tubes. If these assumed components were carried by the homologous chromosomes that possessed *S* factors they would be subject to eliminations when there was syngenic incompatibility.

A critical test of the ability of the haploid constitution (including *S 1* or *S 2* and *X a*) that was *P. axillaris* to function in PA pistils is to test PA ♀♀ with PP ♂♂. This relation was fully fertile. Hence the A ♀ component did not effect unilateral sterility, and the P ♂ haploid component which favored intraspecific reproduction had epistatic status. It was demonstrated in back-cross progenies that the diploid *X a.a* constitution could be segregated independently of the true *S* factors. But in the back-cross relations of PA ♀♀ × PP ♂♂ the hybrid pistils reacted as do pistils of *P. parodii.*

The fertility of F_1 PA hybrids with pollen of the *P. parodii* is in sharp contrast to the sterility of F_1 PI hybrids to pollen of *P. parodii. P. axillaris* and *P. parodii* are closely related and highly fertile in a unilateral hybridization. The diploid constitution, at least of *X a.a* components, is necessary to effect unilateral sterility. *P. parodii* and *P. integrifolia* are so different that they are classed in different subgenera. They have a very feeble unilateral hybridization. This unilateral sterility is so strong that a haploid genome of *P. integrifolia* in pistils of F_1 PI: *S p.a* hybrids effects unilateral sterility.

(5) Any F_1 PA hybrid certainly had a complete genome that was *P. parodii* and this included a haploid dosage of the ♀ genic component that was involved in unilateral fertility. That such a component is not identical to the component that determines intraspecific reproduction in these plants must be recognized. Also any F_1 member had a haploid ♂ component, which was present in any pollen grain of *P. axillaris* and reacted in unilateral fertility. Hence when any F_1 of PA was selfed there was opportunity for haploid doses of P ♀ and A ♂ to react together in unilateral fertility. But if this A ♂ component was a single factor (as *F a*) that segregated, the unilateral fertility would be confined to half of the pollen. The F_1 and the F_2 progenies that were grown were self-fertile, reciprocally cross-fertile, and reciprocally fertile in back-cross relations with both *P. axillaris* and *P. parodii.* It seems certain that there were coordination and integration of unilateral interspecific fertility with intraspecific fertilities of one or of both parents in various of these reproductions.

The Back-Cross Progenies. The progenies of *P. axillaris* ♀♀ × F_1 PA hybrids included a total of thirty members that were cross-sterile as females with haploid pollen of *P. parodii.* Each member had received a haploid complement of A: ♀, *X a* from the female parent. Those that also received another haploid complement of *X a* from the segregations in the pollen of the PA: ♂♂ parent were unilaterally cross sterile with *P. parodii* ♂♂.

The members of series 341 (figure 26) were derived from AA: ♀♀, *S 1.1* × PA: ♂♂, *S 0.1.* There was syngenic incompatibility for all *S 1* pollen tubes and only *S 0* pollen functioned to give only *S 0.1* genotype in

the progeny. Hence the *X a* component (which had been dormant in anthers and pollen) was carried by *S 0* pollen. In F_1 and F_2, all *S 0.1* (or *S p.1*) plants were fertile with pollen of PP: ♂ ♂. But in these back-cross progenies approximately half of such genotypes were sterile in this relation.

Series 344 (figure 27) was obtained from AA: ♀ ♀, *S 1.2; X a.a* × PA: ♂ ♂, *S 0.3; Y p.0.* There was no syngenic incompatibility, but pollen that carried *Y p* presumably had hidden unilateral sterility. The PA: ♂ ♂ complex also possessed a haploid dosage of *X a* which was inactivated in stamens by somatic differentiation, and hence it would be carried by some of the pollen tubes. Of the twelve members of this back-cross progeny, eight had a reaction of unilateral sterility with PP: ♂ ♂ and hence these were ♀ ♀, *X a.a.*

The tests indicated in figures 26 and 27, demonstrate that segregates that were *S 0.1, S 0.2,* or *S 2.3* could be either cross-fertile or cross-sterile with *P. parodii* ♂ ♂. It is concluded that the evidence is sufficient to establish that an AA: ♀ ♀, *X a.a* component exists and can be segregated independently of the true *S* factors and that an *X a* component when alone in PA pistils cannot effect an action of unilateral sterility with *P. parodii* ♂ ♂. Since these *X a.a* components were segregated in diploid pistils that were *S 0.1; X a.a,* the two allelic series could be in one pair of homologous chromosomes and have segmental linkage. Possibly the dual functions and switch actions ascribed in the behavior of PI hybrids may be due to linkage of non-allelic factors and segmental segregations.

It is to be noted that plants of *P. parodii* possess no ♀ component that is involved in unilateral sterility with either *P. axillaris* or *P. integrifolia* and hence the pistils of an F_1 PA plant will be *X a.0.*

Since certain segregates that were *S 0.1* and *S 0.2* were also *X a.a,* it would be possible to obtain segregates in both selfed and crossed progenies of these plants that were ♀ ♀, *S 0.0; X a.a.* Such plants would have no self- and cross-incompatibilities and this would simplify the analysis of unilateral fertility and sterility in their relation to intraspecific reproduction.

Unilateral Sterility in the AI Hybrids. *The Status of the S Factors.* Both parents contributed *S* factors to these hybrids. The evidence seems conclusive that the two series of *S* factors were personate in status and allelic in the hybrids. Hence there was no chance for any fertility factor to be allelic to them.

Every member of the F_1 and F_2 of the diploid PI hybrids was self-incompatible. There were also very effective syngenic reactions in cross-relations that involved *S 1, S 3,* and *S b,* but there were nonconforming reactions that involved *S a* which was known to have a weaker degree of reaction than that of the *S b* factor. The identifications of the *S 1.b* and *S 3.b* genotypes in the F_1 were definite but the combinations of *S 1* and *S 3* with *S a* were not satisfactorily determined.

The Genic Components of Unilateral Sterility of P. integrifolia × *P. axillaris.* The genic components of this reaction may be represented as II: ♀ ♀,

X i.i for a genic component that is activated in pistils and as *A.A*: ♂ ♂, *Y a.a* for the genic component that is active in pollen tubes. It is considered that these are features of relative specificity that are expressions of the somatic differentiations of MM: FF complexes. The two most critical tests for the presence and the functional reactions of these components in hybrid progenies are the reactions of the AI hybrids (a) as females with testers of *P. axillaris* ♂ ♂ and (b) as males with *P. integrifolia* ♀ ♀.

The F_1: figure 28. As female members, all F_1 hybrids that were tested were fertile with both parental species except for syngenic incompatibilities and for three nonconforming reactions that involved *S a*. Each F_1 AI hybrid received an I: ♀, *X i* component from its pollen parent but this was not sufficient to effect unilateral sterility with *P. axillaris* ♂ ♂. This corresponds to the comparable reactions of PA hybrids but not to those of the F_1 PI hybrids.

As males all F_1 AI hybrids were sterile with *P. integrifolia S a.b* and *S a.a* but fertile with *S b.b*. In the relation of II: *S a.a* × AI: *S 3.b* and *S 1.b* there were no syngenic incompatibilities. An F_1 of the AI hybrids could possess only one genic component that was involved in uilateral sterility and it would be A: ♂, *Y a*. Hence only half of the pollen could have unilateral sterility. These reactions of sterility do not conform to the expected reactions of *S* factors or of any assumed components that effect unilateral sterility.

As female members, all F_1 AI hybrids were sterile with *P. parodii* ♂ ♂. This is expected since both *P. axillaris* and *P. integrifolia* had unilateral sterility with *P. parodii* ♂ ♂. The F_1 would be *X a* + *X i* and the complete sterility would be an integration of two unilateral sterilities. It is to be noted that one dosage of *X a* in F_1 PA: ♀ ♀ was not sufficient to effect sterility with P: ♂.

As males all AI hybrids were fertile with *P. parodii* ♀ ♀ which would involve an integration of the unilateral fertility of both *P. axillaris* and *P. integrifolia* as males with *P. parodii*. No reaction of unilateral sterility was involved for *P. parodii* ♀ ♀ has no such action.

The F_2: figure 29. An F_2 progeny was obtained of AI: *S 3.b* × AI: *S 1.b*. None of the members possessed the *S a* factor which gave nonconforming reactions in the F_1. As female members these plants had no reactions of sterility with either parental species except for the syngenic reactions of *S* factors. Hence there had been no segregations of an *X i.i* ♀ ♀ component that could effect unilateral sterility with *AA*: ♂ ♂. This conforms to the fertility of all F_2 of PA: ♀ ♀ with PP: ♂ ♂. As males the F_2 members had nonconforming reactions with the three genotypes of *P. integrifolia*.

It is to be noted that all members of the AI F_1 and the F_2 had complete and uniform reactions of unilateral sterility as females with *P. parodii*. Hence as females all F_2 appeared to be *X a.i*, *X a.a*, or *X i.i*. As males, all F_2 were fertile with *P. parodii* ♀ ♀, but since this species had unilaterol fertility with both *P. axillaris* ♂ and *P. integrifolia* ♂, no genic components of unilateral

sterility were involved in this relation. The fertility of this relation was solely a reaction of components of fertility.

Unilateral Reactions by Triploids, AAI: figure 47. These plants had the ♀ ♀ ♀ components of *X a.a.i* and the ♂ components of *Y a.a.i.* As male members they were sterile with *P. integrifolia S a.b, S a.a,* and *S b.b.* These reactions appear to be a unilateral sterility. At any rate the haploid component that was *P. integrifolia* in the AAI ♂ had no influence in effecting fertility. Since this component carried *S b* there may have been hidden syngenic incompatibility for pollen that was *S b.*

As female members, all AAI hybrids were cross-fertile with diploid *P. axillaris* ♀ ♀ except for syngenic incompatibilities. Hence the haploid component of *X i* ♀ evidently had no action of unilateral sterility in the AAI × AA relation. The basic intraspecific fertility of AAI: *F a.a* × AA: *F a.a* was epistatic.

Unilateral Sterility in Members of a Back-Cross Progeny: figure 54. Six members of a progeny of AAI × II had sterility as females with all genotypes of diploid *P. axillaris.* Hence these members were *X i.i.* These plants were *su:15, 16, 16, 16, 17,* and *17* and either *S a.b* or *S b.b.* But six other members that were either *S 1.b* or *S 3.b* did not have unilateral sterility with *P. axillaris* ♂. Hence they were probably *X a.i* ♀. These resultants are comparable to the segregation and recombination of the *X a.a* component in the back-cross progeny of AA × PA. It appears the *X i* component is distinct from the *S a* and *S b* factors but is linked with them. There were three members of this progeny (figure 54) that were self-incompatible and cross-sterile as females with all sisters and with both parents.

The most significant of the reactions is that certain segregates had sterility with *P. axillaris* ♂ ♂. These plants were either *S a.b* or *S b.b.* But the tests did not demonstrate that an II: ♀ ♀, *X i.i* component was present and independent of the true *S* factors.

Unilateral Sterility in the Progeny of AAI × AA: figure 55. All seven members of this progeny were sterile as males in all relations with *P. integrifolia S a.b, S a.a,* and *S b.b.* The chromosome numbers ranged from *su:14* to *20.* Six numbers were *S 3.b.* Evidently all these plants were ♂ ♂, *Y a.a,* in which case the one *S* factor of *P. axillaris* in the ♂ ♂ *S 3.b* could not itself be the one factor that effected the unilateral sterility of all pollen. None of these plants was cross-sterile as females in disgenic relations with *P. axillaris* ♂ ♂ and hence none were *X i.i.* But such genotypes would not be expected of ♀ ♀, *X a.a.1* × ♂ ♂, *X a.a.* The AA: ♀ ♀, *X a.a* component necessary to effect unilateral sterility was segregated independently of *S* factors in certain members of back-cross progenies. In the six members of series 243 (figure 55) that were *S 3.b,* that were derived from AAI × AA, the AA: ♂ ♂, *Y a.a* component was obtained. These six plants were fertile as females with II: ♂ ♂, *S a.b* and *S a.a.* Hence these plants had the unilateral fertility and sterility in reciprocal relations with *P. integrifolia* which corresponded to those of *P. axillaris.*

Relative Specificities and Biosystematics. *The Status of the PA, AI, and PI Hybrids.* The noteworthy differences in the reactions of the hybridizations, in the degree of potential fertility, and in the reactions of reproduction in these three groups of hybrids are obviously expressions of different grades of relative unilateral specificity. As taxonomic units the three species, *P. parodii*, *P. axillaris*, and *P. integrifolia* possess different degrees of speciation in respect to visible characters and especially in the differentiations of flower organs. But each species is homomorphic. In addition to these differences there are those physiological properties that effect unilateral reactions of sterility in the reciprocal relations of hybridization. These reveal unsuspected multiple functions of both pistils and pollen tubes.

P. parodii and *P. axillaris* are noticeably similar in many characters, but the two are so different from *P. integrofolia* that they must be classed as members of different subgenera. The hybridization of *P. parodii* ♀ ♀ × *P. axillaris* ♂ ♂ is more fertile in the formation of capsules and seeds than is the hybridization of *P. parodii* ♀ ♀ × *P. integrifolia* ♂ ♂. Also functions of unilateral sterility continue to operate in PI hybrids, especially in the critical tests with PP: ♂ ♂; but such reactions did not occur in the F_1 or the F_2 that were grown of the PA hybrids. Thus the reactions of reproduction in the PA hybrids and in their relations with both parents were most complete. Hence *P. parodii* and *P. axillaris* and their hybrids have a higher degree of ecospecific status than have *P. parodii* and *P. integrifolia*, and their hybrids.

The Genic Components of Unilateral Sterility. The three species of *Petunia* under discussion differ in the seat or locus of the relative differentiations that are involved in the unilateral sterilities. Obviously, the three reactions of unilateral sterility in hybridization involve four relative specificities that may be represented as follows:

P. axillaris, ♀ ♀, *X a.a* × *P. parodii*, ♂ ♂, *Y p.p*;
P. integrifolia, ♀ ♀, *X i.i* × *P. parodii*, ♂ ♂, *Y p.p*;
P. integrifolia, ♀ ♀, *X i.i* × *P. axillaris*, ♂ ♂, *Y a.a*

The presence of any one of these genic components is not revealed in the intraspecific reproduction of either of the species. *P. axillaris* is the only species in which both ♀ and ♂ differentiations are involved in a reaction of unilateral sterility.

The reactions of unilateral fertility in the hybridizations involve the following relations:

P. parodii ♀ ♀ × *P. integrifolia* ♂ ♂
P. parodii ♀ ♀ × *P. axillaris* ♂ ♂
P. axillaris ♀ ♀ × *P. integrifolia* ♂ ♂

No attempt is here made to assign definite genic values to the constitutions that are involved in the fertilities of hybridization and of reproduction in the hybrids.

P. axillaris is the only one of these three species in which both the ♀ ♀

and the ♂ ♂ organs are involved in the double functions of both unilateral sterility and fertility in addition to their functions in intraspecific fertility and in syngenic incompatibility. Which one of the four functions of the ♀ ♀ is realized in a relation depends on the genic composition of the haploid pollen tubes, and the ♂ ♂ function that is in action depends on the composition of the ♀ ♀ of the other members of a relation. Hence the entire genotypic constitution of any hybrid or back-cross segregate can only be determined by its reactions as female and male with all critical testers and also by the adequate analyses of all of the collateral progenies that can be obtained. The data for the PA, PI, and AI hybrids, and especially for the back-cross segregates, are incomplete and inadequate.

Mather's data partially test the assumption that all functions of fertility and sterility in *P. parodii* and *P. integrifolia,* and in their hybrids also, may be determined and controlled by one allelic series of genes that consists of two true *S* factors of *P. integrifolia* and one fertility factor of *P. parodii.* The reactions were such that it was postulated these factors effect dual functions in both pistils and pollen tubes and can switch from a function of fertility to one of sterility. At least six important nonconforming reactions were recognized that were attributed to other and nonallelic genic factors. Mather did not have the true *P. axillaris* and his PI hybrids received true *S* factors from one parent only. His F_2 and back-cross progenies were tested as females only.

The Role and Status of S Factors. 1. Any of the incompatibility genotypes of *P. axillaris* had reactions of unilateral fertility as males and unilateral sterility as females with any member of *P. parodii* which possessed no *S* factors. There was no syngenic relation for any *S* factor, and it is believed that the *S* factors had no function in the reactions of hybridization.

The extensive tests of the PA hybrids demonstrated that all the *S* factors continued to have effective incompatibility reactions of a strictly personate and independent status in any syngenic relation, as they had in cultures of *P. axillaris.*

2. But the *S* factors of the Kew clone of *P. integrifolia* had irregular and nonconforming reactions in both the PI and the AI hybrids. There were cases of partial or irregular inactivation of *S* factors and of nonconforming behavior of the *S p* factor. Mather considered that the *S a* and *S b* factors may be entirely inactivated in respect to incompatibility reactions by association with polygenes of *P. parodii.*

It must be recognized that there are meagre data on the behavior and status of *S* factors in the intraspecific reproduction of *P. integrifolia.* The studies of the two *S* factors of the Kew clone involved progenies of premature pollinations (chapter 4). Possibly the *S a* and *S b* factors have a partial or incomplete personate status and are readily influenced by associations with fertility factors. The associate type of behavior in incompatibilities is known in both diploid-haploid and diploid-diploid reactions (Stout 1938, 1945). In the diploid-diploid type, now known in few flowering plants the reaction of a pistil is determined by the relative strengths of the two *S* factors and both

classes of pollen are alike in a reaction that is determined for a pollen tube by the association of the two factors previous to reduction. It is possible that hybridization may change the status of certain *S* factors, especially of *P. integrifolia*, from a personate to an associate value.

3. The mechanism of intraspecific incompatibility, in *P. axillaris* and *P. integrifolia*, has one feature of similarity to that of unilateral sterility in the hybridization of these species with *P. parodii*. Both reactions appear to involve the failure of haploid pollen tubes to grow in diploid pistils. It is well established that an *S* factor has a function in both pistils and pollen tubes and that the reaction is personate and syngenic. In contrast to this, the genic factors in *P. parodii* ♂ ♂, that are involved in unilateral sterility with AA ♀ ♀ and II ♀ ♀, appear to be active only in pollen tubes when they are growing in pistils of another species. They do not effect sterilities in intraspecific reproduction. It may be postulated that their reactions of sterility are disgenic.

In petunias these two mechanisms of sterility have a limited and superficial role. They are accessory to, and superimposed upon, the fundamental basis and functions of reproduction which involve further stages of reaction and development such as fusion of gametes and the formation of viable seeds.

CONCLUDING REMARKS

1. Nonconforming resultants are those that do not correspond to what have previously been obtained or that are expected on the basis of experience, belief, and theory. Nonconforming reactions were few in the behavior of the PA hybrids; they were frequent and drastic in the reproduction of the AI hybrids; and they were numerous in the reactions of the PI hybrids, grown by Mather, especially in respect to the modifying influences of factors of fertility. Except for the data on the petunias, there is little information regarding the heredity and the reactions of unilateral sterilities in hybrids. The situation is much like that which existed some thirty years ago regarding the knowledge about intraspecific incompatibilities.

2. We may be reminded that each important advance in the knowledge of intraspecific incompatibilities, since the early conceptions of "self-sterility," has involved the recognition of a nonconforming resultant, the proper evaluation of it by new experimental techniques, and often a radical change in belief and theory. For example: for at least thirty years after Darwin's statements in 1876, the belief that "self-sterility" had an individual status was so firm that intraspecific cross-incompatibilities were not considered possible and the rather simple experimental tests which reveal such behavior were not made. For a time after the recognition of cross-incompatibilities (de Vries 1906; Correns 1912, 1913) it was believed that all of the pollen of a plant had the same reaction. Much of the data for the reactions did not conform to this belief and such reactions were not properly evaluated until Prell's (1921) explanation of a diploid-haploid reaction was applied and demonstrated by definite tests.

3. The nonconforming reaction of self-fertility of the Kew subclone, the one case of self-fertility of a seedling of *P. axillaris* (figures 8 and 10), and the status of the one member of a PA progeny (figure 25) that was self-incompatible were all resolved by experimental analysis which involved collateral progenies.

Without doubt many of the nonconforming reactions that are reported in the reproduction of the PI and the AI hybrids may be analyzed in ways that will reveal further principles regarding the nature, scope, function, and genic basis of unilateral hybridizations. Without doubt certain of the components of basic fertility which Mather recognized as having modifying effects could be stabilized and identified in segregates.

4. The experimental studies that were made of the unilateral specificities, sterilities, and fertilities in the reproduction of three species of *Petunia* and their hybrids contribute much new data and reveal some of the tests and the experimental techniques that are necessary in such studies. There were few attempts in the studies of the petunias to test the members of back-cross progenies for genic components that were linked in the homologous chromosomes that carried *S* factors. That there were segregations of genic components that effected unilateral sterility from those that effected syngenic incompatibility was demonstrated in both the PA and the AI hybrids. Members of the genotypes of PA: *S 0.1* and *S 0.2* that were cross-sterile with *P. parodii* ♂ ♂ would no doubt give inbred progenies that would be pure for ♀ ♀, *X a.a* and be without any *S* factor. The analyses of linkage and segmental transmissions of genic components that are involved in multiple physiological functions of male and female organs in hermaphrodites, especially when three or more species are involved in unilateral sterilities, are indeed a challenge to future investigators.

5. The genic components that effect unilateral specificity are automatically transmitted to the F_1 progenies along with the genic components that effect fertility in the reciprocal relation. They are inactive in the reaction of unilateral hybridization but are, nevertheless, transmitted and will later have reactions that may be identified. The *S* factors of intraspecific incompatibilities are also inactive in the disgenic relations in hybridization but are transmitted to members of the hybrid progenies. These two mechanisms of sterility are superimposed upon those that effect intraspecific and interspecific reproduction and together they provide multiple functions to the somatically differentiated *MM: FF* complexes in hermaphrodites.

When these conditions exist in the reproduction and the unilateral hybridizations of two or three species, they provide an almost unexplored field for experimental and genetical studies of critical features of reproduction that may be listed as follows: (1) What is the genetical basis of half-speciation; (2) what genic components are active in both pistils and stamens (and pollen tubes) and what ones are active in only one of these organs; (3) what genic components have syngenic reactions with themselves, as a personate *S* factor has, and what ones have a disgenic action; (4) which one of any two

or more of the functions of an organ has epistatic power; (5) which, if any, of the genic factors may have dual functions that "switch" from a sterility to a fertility reaction; (6) how many of the genic components that are involved in fertilities and sterilities of reproduction are carried in one series of homologous chromosomes of two, or three, or more species; (7) what are the basic patterns of biosystematic reproduction in unisexual, and in bisexual organisms in which unilateral hybridizations are involved and how does complete speciation arise from half-speciation.

It is obvious that an important step in the evaluation of unilateral speciation is to obtain complete and adequate experimental data for the hybrids of those species that have only unilateral sterility and no complications of incompatibilities, and to extend such studies to numerous cases in both plants and animals. Then the role of the superimposed self- and cross-incompatibilities in both homomorphic and heteromorphic hermaphrodites may be evaluated in regard to speciation.

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CHROMOSOME STUDIES ON CALIFORNIA MOSSES

By
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CHROMOSOME STUDIES ON CALIFORNIA MOSSES

William C. Steere,[1] Lewis E. Anderson and Virginia S. Bryan

Introduction. The chromosomal behavior of bryophytes has been relatively little studied, compared with higher groups, perhaps because of the slight economic importance of these plants and the technical difficulties in making preparations for cytological research. A fnrther impediment to cytological studies of bryophytes arises from the very considerable difficulty that may be experienced in identifying with certainty the species investigated. Outstanding exceptions, of course, were the first experimental production of polyploid series by the Marchals (1907, 1909, 1911), in mosses, and the first discovery of sex chromosomes in plants in an hepatic, *Sphaerocarpos*, by Allen (1917). The long-continued work of Allen (1945) on the genetics and cytology of *Sphaerocarpos*, the many brilliant experiments of Wettstein (1923, 1924, 1928, 1942) and his collaborators, (Barthelmess 1938, 1941) on apospory, hybridization, and genetics of mosses, and the recent comprehensive work of Burgeff (1943) on the genetics of *Marchantia* illustrate the richness of experimental material available among bryophytes.

The present investigation has had two principal aims: (1) to ascertain the chromosome numbers of native populations of Californian mosses, in order to relate these data to questions of phylogeny and taxonomy, insofar as possible, as Manton (1950) has done for pteridophytes, and (2) to study chromosomal behavior during meiosis, with emphasis on such criteria as the multiple association of chromosomes, prematnre and lagging disjunction, the so-called accessory chromosomes, sex chromosomes, and other phenomena that may influence the speciation and evolution of mosses. Careful studies of the meiotic process in mosses, with the aid of modern techniques, are very rare indeed. Notwithstanding the discovery of heterochromatin in mosses by Heitz (1928), and the investigation of meiosis in the Grimmiaceae (Vaarama 1949), many problems remain untouched and unsolved.

Early investigators of the chromosome behavior of mosses found the exact determination of chromosome numbers to be exceptionally difficult if not impossible, partly from the small size of the nuclei and partly from the characteristic tendency of the chromosomes to clump, thus apparently reducing their number (cf. Allen 1912, p. 123–128). The outstanding work of Heitz in developing new and more suitable techniques for the study of the nuclei of bryophytes enabled him (1928) to estimate the chromosome number of many mosses with reasonable accuracy, and later, to report with

[1] Grateful acknowledgment is made to the National Science Foundation for a grant of research funds to Stanford University (NSF-G186) which has not only made the present investigation possible, but will enable it to be continued.

some certainty the chromosome numbers of several further species (1932, 1942). Since the pioneer efforts of Heitz (1926, 1928), reports of chromosome studies of wild populations of mosses have appeared with increasing frequency, among which the most important are those by Jachimsky (1935), Kurita (1937, 1939), Lowry (1948), Shimotomai and Kimura (1936), Shimotomai and Koyama (1932), Sinoir (1952), Tatuno (1951), Yano (1951, 1952), and especially the recent and exceptionally important works of Vaarama (1949, 1950a, b, 1953a, b). At the present time, the chromosome number of about 150 species of mosses has been reported, with varying degrees of accuracy, out of approximately 20,000 species that have been described. Tabular summaries of published chromosome numbers have appeared recently in the works of Lowry (1948) and Sinoir (1952). Except for scattered earlier works, for example those of Allen (1912) on *Polytrichum juniperinum*, and of Scheuber (1932) on *Timmia cucullata*, most of the chromosomal studies on North American mosses have been made very recently, especially by Lowry (1948, 1953) and by Vaarama (1949, 1950b, 1953a, b). The total number of species whose chromosome number has been reported from American material perhaps reaches 25 and certainly does not exceed 30. Consequently, the present investigations, covering the behavior of meiotic chromosomes in 55 species, have doubled the number of American species so far studied. We are able to report chromosome numbers and behavior in 2 families, 15 genera, 37 species, and 7 varieties whose chromosomes have not been studied previously, as well as in 7 species that had been investigated earlier, primarily in European populations. The emphasis of the great majority of the investigators just cited has been on problems concerned with heterochromatin, sex chromosomes, and on chromosome number for its own sake. The only serious attempt to correlate chromosome numbers and behavior with the taxonomy of mosses is that of Lowry (1948), in his thorough cytotaxonomic investigation of the genus *Mnium*. In view of the relatively restricted attention paid to the use of cytological data in systematic problems in mosses in the past, and with a very considerable further body of new data available from our investigations, the time seems appropriate for an analysis of previously reported chromosome counts with special reference to their bearing on problems of phylogeny and taxonomy.

Materials. Admittedly, the choice of Californian mosses as material for this investigation was determined largely by the residence of the senior author. Nevertheless, the Californian moss flora presents several advantages for such studies, among them the facts that it is a rich and interesting one, consisting of over 300 species (Koch 1950); that many species become conspicuously abundant under favorable conditions; that it presents a good deal of significance in the families and genera that comprise it, in terms of

patterns of geographic distribution and floristic migrations (Lesquereux) 1865, 1868); and that most species produce their sporophytes and undergo meiosis during a few months in winter and spring, thus insuring abundant experimental material during the proper season.

One of the most troublesome problems encountered in this investigation was identification of the species with some degree of certainty. The standard practice of applying European names to many species of North American bryophytes is open to considerable doubt and suspicion, because of the clear differentiation of the floras of the two continents (Fernald 1931). Furthermore, the names of species of the eastern United States are too often applied to populations of the West Coast that appear to be specifically distinct. Of course, the corollary is true: significant chromosomal differences between populations of Europe or of the Eastern United States and those of the West Coast may prove to be very helpful in clarifying taxonomic problems.

Material of more than 60 species and varieties, not all of which yielded preparations suitable for cytological study, was collected in the general vicinity of San Francisco Bay, in Santa Clara, San Mateo, Santa Cruz, Marin, and Contra Costa counties. A few collections came from as far north as Sonoma County, at the Hedgpeth Ranch, and Mendocino County, at Ukiah. Some further collections were obtained in the Sierra Nevada, in Yosemite National Park, Mariposa County.

Each population studied cytologically is represented by a voucher specimen deposited in the Dudley Herbarium of Stanford University. These specimens, each referred to in the following report by the initials of the author who collected it and numbered in a consecutive series, will furnish a permanent record of our species concepts and identifications (cf. Sauer 1953), and the detailed locality data will make possible the recovery of the populations at any time. In the absence of permanent cytological preparations, this sort of documentation may prove useful to others working on the chromosomes of mosses, especially if they wish to duplicate our results.

Methods. As the technical difficulties in studying the chromosomes of bryophytes have presented one of the greatest handicaps to research in this field in the past, the methods used in the present investigation will be outlined in some detail. When mastered, the techniques are relatively easy, and thereby open to investigation a group of plants that have remained too long neglected.

At meiosis in the spore mother cells (SMC) or sporocytes of mosses, the chromosomes are especially satisfactory for determination of their number. During the meiotic divisions the chromosomes tend to be greatly condensed, deeply staining and, in the early stages, conjugated in pairs, presenting the reduced number in a compact, easily-resolved state.

A further advantage of the dividing SMC is the correlation that usually exists between the stage of its development and the external appearance of the sporophyte. Maturing sporophytes of a few species can be found throughout the year in some regions of the United States, although in California, most species produce capsules only during the winter and early spring months. At the time of meiosis sporophytes are characterized by their bright green appearance and by their attainment of mature size. Mosses collected when the capsules are approaching mature size will continue normal development if kept in a cool laboratory with diffuse sunlight, in covered glass dishes or in plastic bags. A large collection was made whenever possible since the capsules within a population may represent a wide range of development. A collection that includes dark green or brown mature capsules, and also completely green capsules of mature size, will almost certainly contain all the meiotic stages.

The best chromosome preparations are made as soon as possible after collecting mosses in active metabolic condition. Plants collected under conditions of excessive cold or dryness, however, usually give better results if watered and stored in the laboratory for several hours to several days. When most of their capsules appear to be near the meiotic division mosses may be stored in a refrigerator, where the reduced temperature will arrest or delay the development of the sporophyte and maintain the plants in good condition until they can be studied, especially if supplied with light for several hours each day.

For squash preparations, the most promising capsules are selected under the binocular dissecting microscope. As a general rule meiosis occurs when the capsule is mature in form and size, but still green and slightly translucent; the cells of the annulus may show some brown or red coloration and the operculum may be tan. However, in some species the SMC divide when the entire capsule is bright green and in others when the capsule wall itself has become mostly red-brown.

The capsule, severed from the seta, is transferred to clean slide. Under a binocular dissecting microscope the operculum is removed with a fine needle or spear, and pressure applied at the base of the capsule. In most species the contents of the capsule can be squeezed out; in others, the spore-bearing tissues must be dissected out.

A saturated solution of orcein in 45 per cent acetic acid (acetic orcein) gives adequte fixation in many species, but not in all. The solution itself or the weight of the cover slip on inadequately fixed cells may cause the SMC to disintegrate. Preliminary fixation in Carnoy's solution is advisable as a precaution in the preparation of squashes of moss capsules. A drop of fixative is added as soon as the SMC are removed from the capsule and care must be taken to prevent the SMC from drying. Any Carnoy-type fixing

solution (3:1; 3:6:1; 4:3:1) may be used and little difference will be noted in the resultant preparations.

A second and even a third drop of fixative may be required for delicate cells or those with large obscuring inclusions.

An acetic-orcein staining solution, prepared by adding 1.5 gm. of orcein (National Aniline synthetic) to 100 cc. of 45 per cent acetic acid and shaking until the solution is saturated, gives excellent results with moss chromosomes. After filtration this solution produces an intense stain in the chromatin but is taken up only slightly by the cytoplasm. Periodic filtrations may be necessary to remove a fine iridescent precipitate. Natural orcein gave a less intense stain than the synthetic dye. A drop of aceto-orcein is added when the fixative has almost entirely evaporated and a cover slip placed over the stain. Heating the slide almost to the boiling point over a small flame intensifies the stain in the chromosomes and clears the cytoplasm. Following the heating process, the slide should be checked under the microscope to determine in a general way the condition of the cells. Stages with spores or with SMC that are obviously too young may then be discarded without further treatment. An attempt should then be made to distribute the cells in a single layer between the slide and the cover slip. When the slide is re-heated and placed on a flat surface, repeated gentle tapping on the cover glass with the handle of a dissecting needle causes the cells to flow apart into one layer. The progress of this operation may be checked with the compound microscope, under low power, and the pressure of the tapping decreased or increased as the fragility of the cells indicates. The slide, re-heated, is inverted on absorbent paper when the cells are well spread; gentle pressure at the edges will force out surplus stain. If the cells were still intact, pressure may be applied over the cover slip itself to flatten the preparation further.

Since capsules with early tetrad stages sometimes include a few cells with earlier stages, they are occasionally useful for estimates or for verifications of chromosome numbers. In preparations including cells in which cytokinesis has started, the flattening of metaphase figures is likely to be difficult. Cells in early prophase are sometimes accompanied by a few prematurely dividing SMC that may furnish first metaphase counts. Diakinesis figures are helpful in obtaining counts in species whose chromosomes tend to clump at first metaphase and for verifying the presence of accessory chromosomes or fragments. Naturally, the most useful preparations include stages from late prophase through second anaphase.

When the metaphase chromosomes do not stain intensely, more stain should be added and the preparation re-heated. The chromosomes of most mosses stain sufficiently for study immediately but some preparations may need to stand for a few hours.

To seal the completed preparations, the slide is warmed and vaseline drawn around the edge of the cover slip with a needle. By heating gently the vaseline will flow around the edge of the cover glass. Temporary mounts remain in good condition for at least one day, ample time for the study of the SMC of one capsule. If necessary, storage in a damp chamber at a reduced temperature will preserve the preparations longer.

Certain hazards exist in the interpretation of the division figures. In cells which have been strongly flattened, first anaphase or second metaphase plates may flow together and appear as a single figure. The tendency for homologous chromosomes to separate precociously in meiosis of mosses, observed previously by Vaarama (1949), may give an erroneous impression of aneuploidy or polyploidy. Similarly, chromatids anticipating the next division may be observed as early as first metaphase in some species. The secondary association of bivalents at first metaphase, discovered in our investigations, may lead to the appearance of fewer chromosomes in the first metaphase plate than in the succeeding stages.

The number of chromosomes is best ascertained through the study of many figures at diakinesis, metaphase I and anaphase II. Otherwise, several possible anomalies of meiosis, as the multiple association of bivalents, their premature dissociation, the premature division of anaphase chromosomes, the lumping of chromosomes at any stage, or the presence of polyploid areas in the archesporium, may result in an incorrect chromosome count if a single stage of meiosis is observed. The authors generally studied as many meiotic stages as possible in as many sporocytes as could be found in suitable condition in each population.

All three investigators made separate chromosome counts of each population reported upon here. When these independent studies were not in agreement, observations were continued until the differences in opinion could be reconciled. If unanimous agreement could not be reached on the basis of the available material of some populations, no report has been included here. All drawings have been made (largely by the senior author) to the same scale, under standard conditions, with the aid of a 1.4 mm. oil immersion lens, a 20× compensating ocular, and a camera lucida. The magnification achieved with this equipment, in all drawings (figs. 1–183, is ×2800. The magnification of all the published drawings is ×2160.

Results. Our data will be reported under the heading of the various families to which the populations belong, arranged in the order established by Brotherus (1924–1925). Insofar as possible, our results are coordinated with those of previous workers, for the sake of completeness. A brief summary of the results, with some interpretation, is given at the end of each family.

Fissidentaceae

Fissidens (*Bryoidium*) *limbatus* Sull. (figs. 1–4): $n = 5$. This count is of especial interest for two reasons, for the small number of chromosomes, and also because this number does not seem to be related closely to any series of chromosome numbers reported for other species of *Fissidens*, here or elsewhere. The meiotic chromosomes of *F. limbatus* are very similar in size and shape and behave regularly in both divisions. As seems to be not uncommon in mosses, but more conspicuous here than in most species, the bivalent chromosomes tend to dissociate prematurely, producing 10 univalents at the first metaphase (figs. 3 and 4). This species is a common and variable one, occurring on moist shaded soil, especially on vertical banks, where it is usually one of the first plants to appear. The population studied here grows on the east bank of San Francisquito Creek, Santa Clara County, at the Stanford University Golf Course (LEA 1, Feb. 10, 1953).

Fissidens (*Aloma*) *pauperculus* Howe (figs. 5–7): $n = 12$. This extremely rare and local species has remained almost completely unknown until its rediscovery a few years ago (Koch 1948), and still seems to be restricted in its geographical distribution to the range of the coast redwood, *Sequoia sempervirens* (Koch 1951). The chromosome complement differs markedly from that of *F. limbatus*, not only in number, but also in morphology, since the chromosomes of the present species fall very clearly into several size and shape classes. The premature dissociation of a single bivalent before the first division provides a further feature of interest in the chromosome behavior at meiosis. Our material came from the slopes of Mt. Tamalpais in Marin County, near the Muir Woods National Monument, from bare soil at the very base of a coast redwood (LEA 53, April 8, 1953).

Summary. The Fissidentaceae, consisting almost entirely of one very large genus, presents one of the finest assemblages of material for cytotaxonomic study available among mosses. The genus *Fissidens*, with nearly a thousand described species, occurs abundantly in all parts of the world, although not as richly in California and the West Coast in general as in most other parts of the United States. Towards the tropics, the species of this family become progressively more numerous. The grouping of the species of *Fissidens* presents some interesting problems. Grout (1943), in the most recent monograph of the North American species, recognizes eleven sections. Other authors consider that some sections actually deserve the categorical rank of subgenera, and they may be raised eventually to the status of independent genera (Steere 1947), especially if cytotaxonomic evidence supports such a change in concept. However, a surprisingly small number of species have had chromosome counts reported, and none of them from American populations. On the basis of European material, Heitz (1928) reported that *Fissidens adiantoides* Hedw. has "(19)21" chromosomes, and

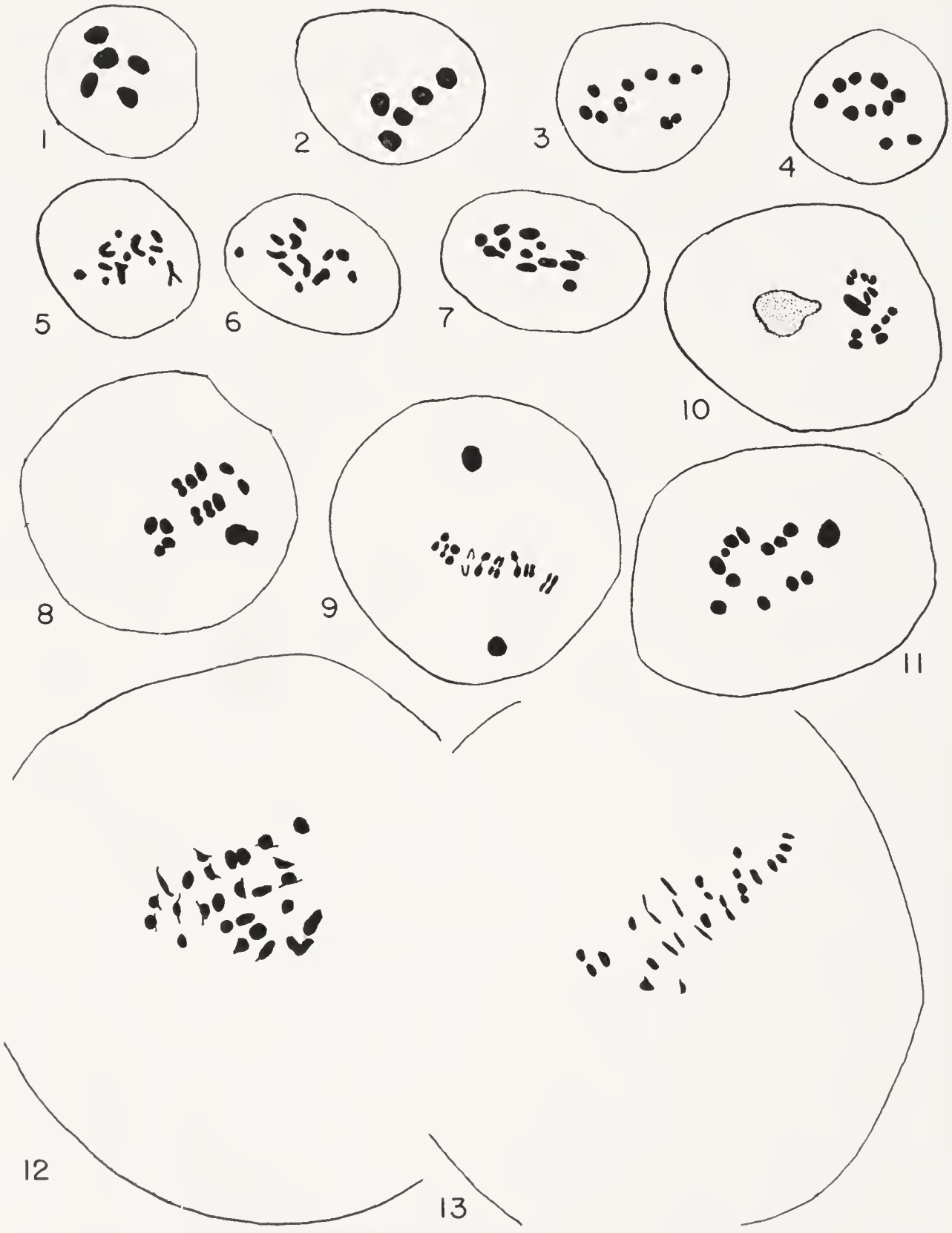

PLATE I. Figs. 1–4. *Fissidens limbatus* ($n = 5$). Figs. 1 and 2. Polar views of first metaphase, with five bivalents. Figs. 3 and 4. Sporocytes in which all bivalents have dissociated precociously. Figs. 5–7. *Fissidens pauperculus* ($n = 12$). Figs. 5 and 6. Bivalent chromosomes just before first meiotic metaphase. Fig. 7. Polar view of first metaphase. Figs. 8–10. *Ceratodon purpureus* ($n = 13$). Fig. 8. First metaphase, early. Fig. 9. First anaphase, early. Fig. 10. First telophase, one chromosome group consolidated. Fig. 11. *Ceratodon purpureus* fo. *xanthopus* ($n = 13$), polar view of first metaphase. Figs. 12 and 13. *Ditrichum schimperi* ($n = 26$). Fig. 12. Polar view of first metaphase, early. Fig. 13. Side view of first metaphase. All figures $\times 2160$.

Sinoir (1952) very recently indicated that in *F. taxifolius* Hedw., $n = 9$. By coincidence, these species belong to the same section, Sect. *Serridium*, and are reasonably closely related. Yano (1951) has reported the number, $n = 16$, for *F. cristatus* and *F. japonicus*. Fortunately for our survey of sections, the two Californian species studied represent two further sections, Sect. *Bryoidium* and Sect. *Aloma*. It is interesting to note that the sections so far studied possess very different chromosome complements, although it is still too early to draw any serious conclusions on the significance of this fact.

Ditrichaceae

Ceratodon purpureus (Hedw.) Brid. (figs. 8–10) : $n = 13$. Our findings, the first to be based on American populations, agree with previous reports on European material, even to the presence of a bivalent that is much larger and more conspicuous than the others. The association of bivalents at and just before the first meiotic metaphase, as well as their appearance of duplication (fig. 8), would seem to furnish evidence that this is a polyploid species in terms of the autosomes and that the much larger "bivalent" may perhaps represent a multivalent structure. A careful and detailed study of the prophase stages of meiosis, as well as of the gametophytic chromosomes of this species would be well merited, to obtain further information concerning these points. Our material of this common and weedy species came from a sandy roadside at the upper entrance to Big Basin State Park, Santa Cruz County (WCS 43, March 18, 1953). A further check was made on material from Yosemite National Park, Mariposa County (VSB, 55, April 5, 1953).

Ceratodon purpureus (Hedw.) Brid. fo. *xanthopus* (Sull.) Britt. (fig. 11) : $n = 13$. Originally described in the category of a variety, this geographical race is very common in California, and differs from the species by its longer seta which is yellow rather than red. We found no significant difference between the chromosomes of this form and of the species, at least in our preliminary studies. The material investigated came from a roadside cliff in Marin County, between Tomales and Petaluma (WCS 57, April 11, 1953).

Ditrichum schimperi (Lesq.) Paris (figs. 12 and 13) : $n = 26$. No chromosome numbers for this genus have been reported previously. The well-recognized relationships of *Ditrichum* to *Ceratodon* may be reflected by the closely related chromosome numbers, even though the appearance of the chromosome complement is very different, indeed. Several classes of chromosome size and shape may be distinguished, but the single, very much larger bivalent so characteristic of *Ceratodon* is lacking, at least in this species. Three chromosome pairs tend to be so closely associated before the first meiotic division that occasional counts, $n = 24$, were made, until this anom-

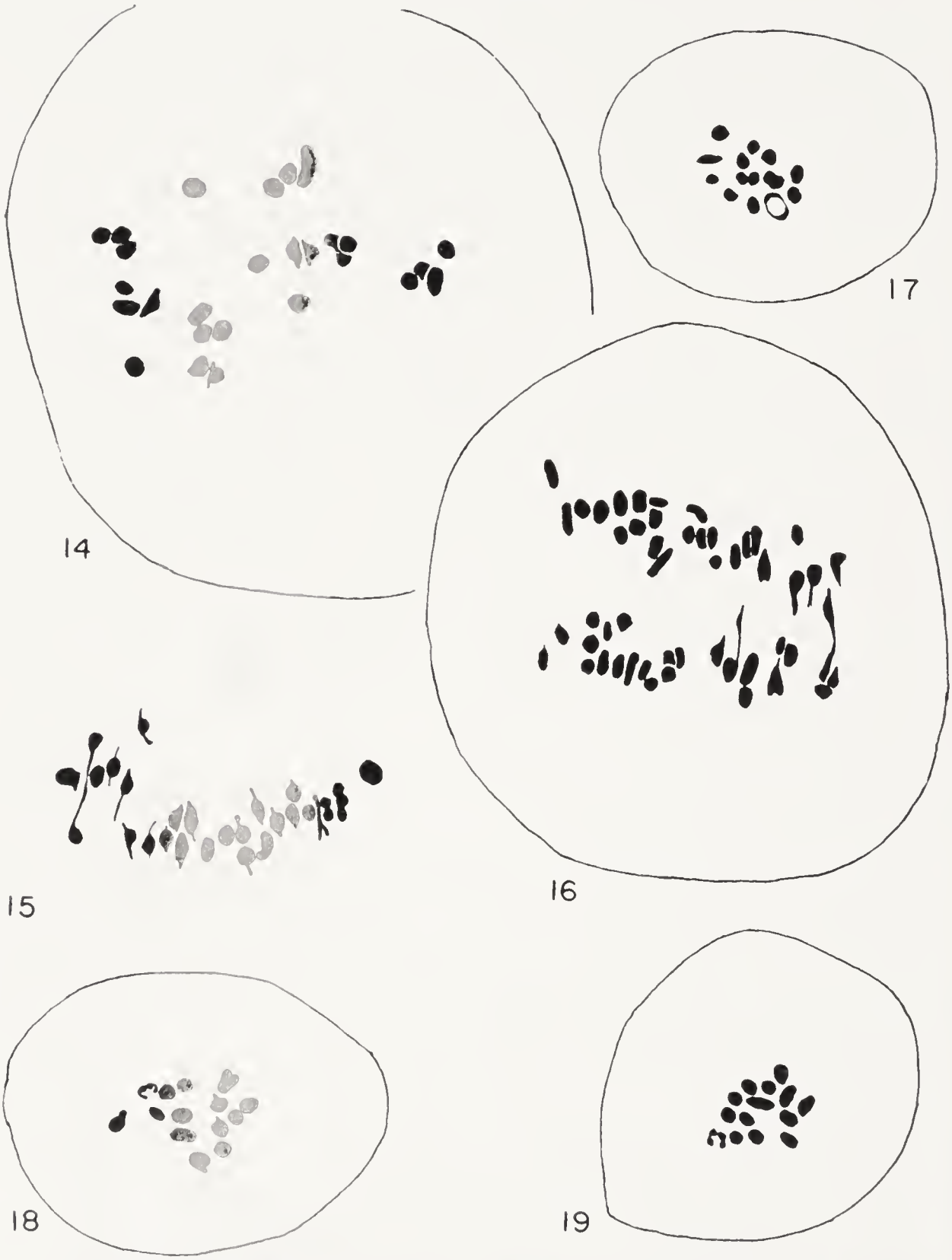

PLATE II. FIGS. 14–16. *Pleuridium bolanderi* ($n = 26$). FIG. 14. Polar view of first metaphase, early. FIG. 15. Side view of first metaphase. FIG. 16. First meiotic anaphase. FIGS. 17–19. *Anisothecium varium* ($n = 14$), polar views of first meiotic metaphase showing behavior of "accessory" chromosomes. All figures $\times 2160$.

alous behavior was understood. The population studied occurs on soil under redwoods, Mt. Tamalpais, just above Muir Woods National Monument (LEA 59, April 8, 1953).

Pleuridium bolanderi C. Müll. (figs. 14–16) : $n = 26$. This appears to be the first chromosome count reported for the genus. The behavior of the chromosomes at meiosis is such that an accurate count was very difficult to achieve, since bivalent and univalent chromosomes may not always be distinguished easily on the basis of size alone. As a result of differences in the time of disjunction of the different chromosome pairs, counts ranging from 26 to 30 chromosomes were made. However, a few very clear preparations of spore mother cells just prior to the first meiotic metaphase show definitely that 26 is the basic number and that any number over this represents disjoined pairs rather than bivalents. Diakinetic figures in this material showed chiasmata distribution with unusual clarity. The population studied was collected near Pepperwood Creek, on the Hedgpeth Ranch in western Sonoma County (WCS 12, Feb. 22, 1953).

Summary. Until now, only one genus and species of the Ditrichaceae has been studied cytologically although the family contains nearly 20 genera. *Ceratodon purpureus* has received an unusual amount of attention, partly because of its great abundance and wide distribution in the northern hemisphere, and partly because of its remarkable chromosome complement, which includes a very large "sex" chromosome. This species has been studied by Heitz (1928), who reported the chromosome number, $n = 11–12$, and by Jachimsky (1935), Shimotomai and Kimura (1936), and Vaarama (1950b), all of whom reported that $n = 13$. Although it may eventually turn out to be in part only a coincidence, it is interesting to note at this point that in the three genera of Ditrichaceae so far studied, the basic haploid chromosome number is 13.

Dicranaceae

Anisothecium varium (Hedw.) Mitt. (*Dicranella varia* Schimp.) (figs. 17–19) : $n = 14$. This is the first species of a relatively large genus to have its chromosome behavior investigated. A feature of unusual interest is found in the closed (fig. 17) or open (figs. 18–19) ring of 4 very small chromosomes, of the type that Vaarama has called accessory chromosomes. Since these are usually associated, they are considered here as one bivalent chromosome that has undergone premature division. This is a situation where the study of somatic chromosomes would be especially enlightening as a means of showing the relationship between mitotic and meiotic chromosome behavior, something at present very little understood in mosses. The material was collected from a population growing on the steep east bank of San Francisquito Creek, at the Stanford University Golf Course, Santa Clara County (LEA 2, Feb. 10, 1953).

Dicranoweisia cirrata (Hedw.) Lindb. (figs. 20–22): $n = 11$. This count, observed in many sporocytes, seems to be beyond reasonable doubt. One figure, at a late diakinesis stage (fig. 20), seems especially conclusive. The meiotic chromosomes fall into several distinct size classes, with four large pairs, of which one is dimorphic, three pairs of medium size, three small pairs, and one pair so small that it might well be classed in the category of accessory chromosomes, were it not for the very regular pairing and the presence of other chromosomes almost as small. Although no other chromosome counts have been made in this genus, the number reported here fits well within the chromosome range known for the family. The material investigated came from the fire-scarred base of a large redwood at Big Basin State Park, Santa Cruz County (WCS 31, March 18, 1953).

Summary. The Dicranaceae, a large and interesting family consisting of some 50 genera, have received very little chromosome study, and chromosome counts have been reported earlier from only four genera. Heitz (1928) reported the number, $n = $ ca.12, for *Rhabdoweissia fugax;* Vaarama (1950b) has studied *Cynodontium strumiferum* ($n = 15$) and *Paraleucobryum longifolium* ($n = 12$); and several species of *Dicranum* have been investigated by various workers, giving chromosome counts of $n = 10$–12 (Heitz 1928, Shimotomai & Koyama 1932, Vaarama 1950a, b, Yano 1951). The chromosome numbers of these six rather unrelated genera, although representing a rather small percentage of the family, nevertheless would seem to indicate that low chromosome numbers may be expected throughout.

Encalyptaceae

Encalypta vulgaris Hedw. var. *mutica* Brid. (figs. 23–25): $n = 13$. From the relatively large size of the sporocytes, among the largest found in this investigation, and from the well spread out chromosomes, there could be no doubt about the number reported here. A point of cytological interest is the distinctive shape and size of several of the bivalent chromosomes, especially one that is clearly dimorphic. The regular behavior of the chromosomes in meiosis gives no clue to the extensive and widely recognized variability of this species. The population studied grows on the north slope of Mt. Hamilton, Santa Clara County, on bare soil in chaparral vegetation (WCS 39, March 24, 1953).

Summary. The Encalyptaceae consisted of the single genus, *Encalypta*, with about 30 species, until recently, when the previously incompletely known genus, *Bryobrittonia*, was discovered to belong here (Steere 1953). No chromosome numbers are previously reported for members of this family. The systematic position of the Encalyptaceae has been the source of some debate, because of the unusual range of variation in the structure of the peristome, which, depending on the species, may be lacking, single, or

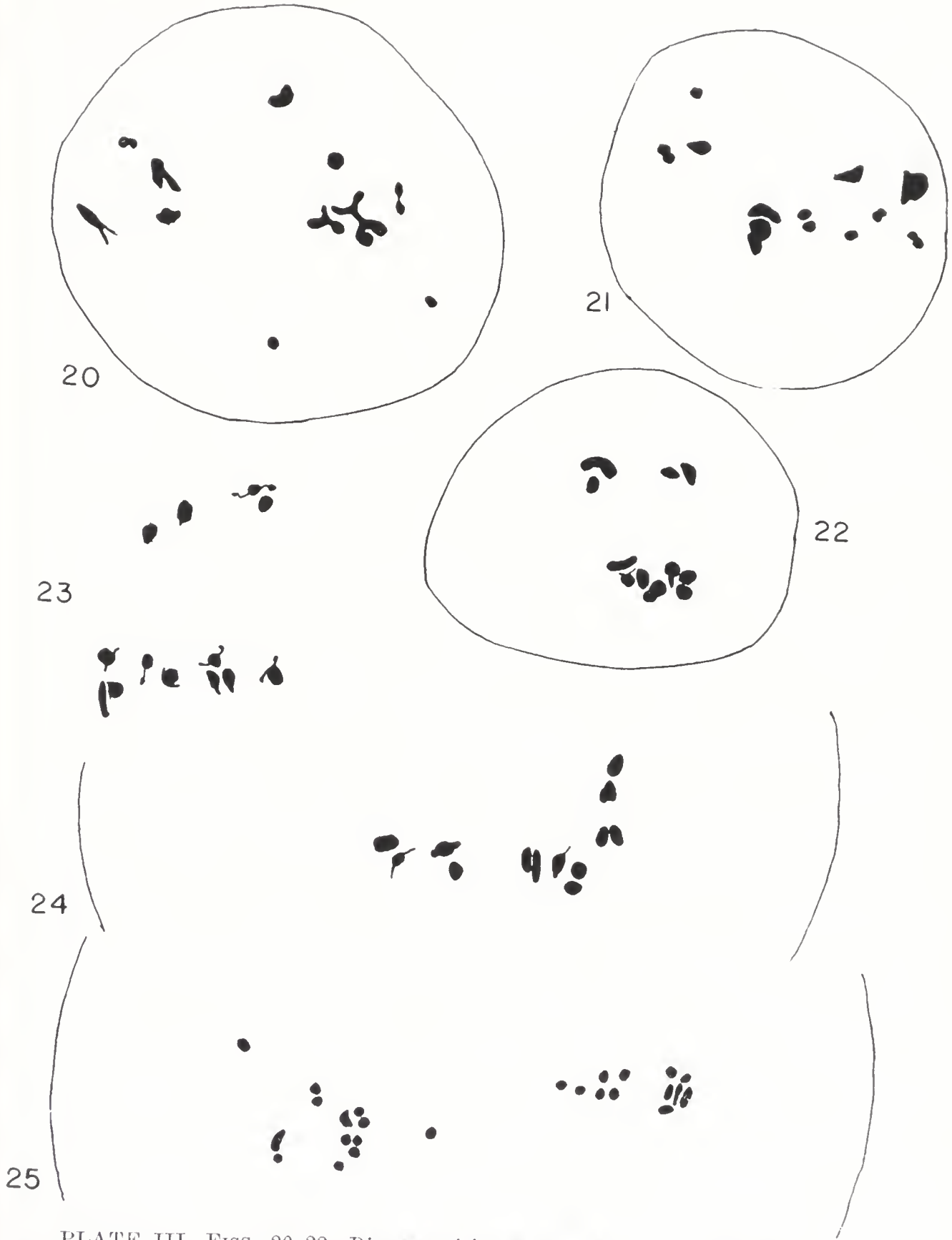

PLATE III. FIGS. 20–22. *Dicranowcisia cirrata* ($n = 11$). FIG. 20. Sporocyte at diakinesis. FIG. 21. First metaphase, early. FIG. 22. First metaphase, polar view. FIGS. 23–25. *Encalypta vulgaris* var. *mutica* ($n = 13$). FIGS. 23 and 24. Side views of first meiotic metaphase. FIG. 25. Polar view of second metaphase. All figures $\times 2160$.

double. However, rather general agreement exists that the Encalyptaceae are related to the Pottiaceae, regardless of which one may be the derived group. No feature of the chromosomes of the species of *Encalypta* investigated would disprove any such relationship, but on the other hand, their morphology strongly resembles that of several members of the Pottiaceae, *Phascum*, for example.

Pottiaceae

Aloina ambigua (BSG.) Limpr. (figs. 26–28) : $n = 24$. Since no species of *Aloina* has previously been studied cytologically, this chromosome number cannot yet be interpreted with relation to the cytotaxonomy of the genus. Premature disjunction of chromosome pairs gives an apparently larger number of chromosomes, but counts made just before the metaphase of the first meiotic division, and at metaphase of the second division (fig. 28), where all the chromatic elements are visible and well separated, substantiate the number reported here. Where higher numbers were encountered, up to 30, some adjacent chromosomes, obviously univalent, could easily be paired by inspection. For example, figure 27 can be interpreted as $n = 26$. An interesting feature of this species is the very small "accessory" chromosomes segregating prematurely, that because of their otherwise regular behavior, are to be considered as a single pair. The material investigated came from a large population growing near Sequoia Hall, on the Stanford University Campus (LEA 6, Feb. 19, 1953).

Barbula brachyphylla Sull. (figs. 29–30) : $n = 12$. The status of this species has been questioned from time to time (Steere 1938, 1939), because of the possibility that it might represent only a reduced ecological form of *B. vinealis*. The rarity of the species (Koch 1950) and the lack of field observations have prevented a full solution of the problem. However, the difference in chromosome number established in this population would seem to suggest a specific distinction. Two conspicuously larger bivalent chromosomes characteristic of the several species of genus *Barbula* (Jachimsky 1935, Vaarama 1950a, 1953b) occur in this material, collected on the north slope of Mt. Hamilton, Santa Clara County (WCS 45, March 26, 1953).

Barbula convoluta (figs. 31–32) : $n = 11$. This semi-weedy species, very widely distributed and often abundant in areas disturbed by man's activities, presents a chromosome set of unusual interest in the Pottiaceae because of the conspicuous differences in size and shape among the meiotic chromosomes. In addition to two large pairs and one very small pair there is a bivalent clearly dimorphic in side view. This chromosome number does not agree well with that reported for other members of the genus *Barbula*. As more species are studied, it will be of interest to determine if the different sections of the genus, some of which have themselves been raised to

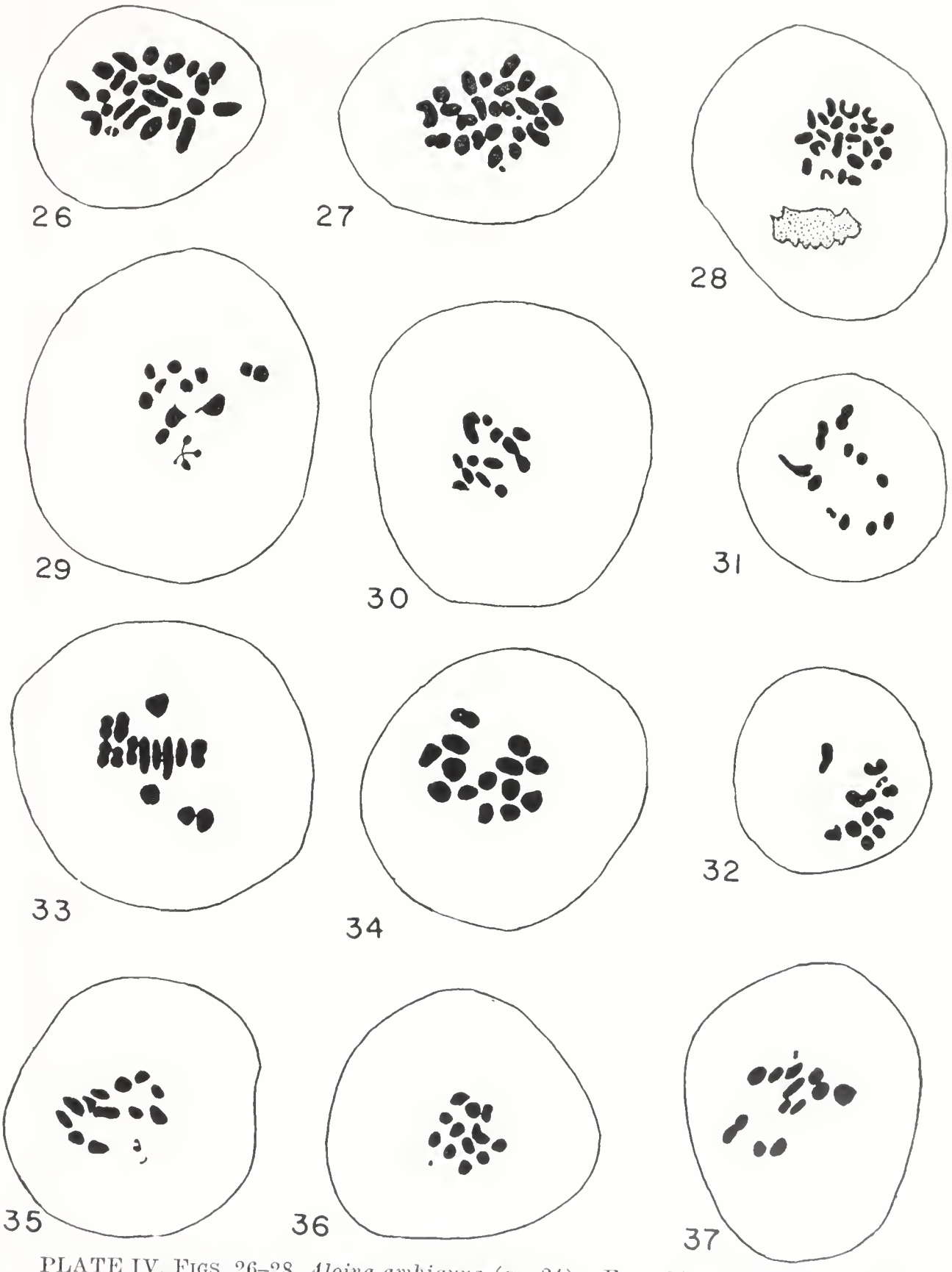

PLATE IV. FIGS. 26–28. *Aloina ambigua* ($n = 24$). FIGS. 26 and 27. Polar view of first meiotic metaphase. FIG. 28. Polar view of second metaphase, one plate indicated diagrammatically in side view. FIGS. 29 and 30. *Barbula brachyphylla* ($n = 12$). FIGS. 29 and 30. First meiotic metaphase, early and median, respectively. FIGS. 31 and 32. *Barbula convoluta* ($n = 11$), polar views of first metaphase. FIGS. 33–34. *Barbula vinealis* ($n = 14$). FIG. 33. Early metaphase, first division. FIG. 34. Polar view of first metaphase. FIGS. 35–37. *Desmatodon hendersonii* ($n = 13$), polar views of first meiotic metaphase, showing "accessory" chromosome. All figures $\times 2160$.

generic status (Hilpert 1933, Chen 1941), are characterized by their own individual chromosome series. The population studied grows on soil at a picnic area near Searsville Lake, San Mateo County, southwest of the Stanford University Campus (WCS 36, March 10, 1953).

Barbula vinealis Brid. (figs. 33–34) : $n = 14$. *Barbula vinealis* and the closely related *B. cylindrica* form one of the most perplexing complexes of mosses on the West Coast, which has been the source of numerous superfluous specific names, although possibly only several races of the same species may be involved, as pointed ont several years ago (Steere 1938, 1939). Vaarama (1953b) has reported the chromosome number, $n = 13$, for *Barbula cylindrica,* from a population growing on the Stanford University Campus, so that it is certainly significant that we should have found 14 pairs very clearly in our study of many division figures in the capsules of *B. vinealis.* Cytological study of the species of *Barbula,* as already indicated, may be very helpful in furnishing further evidence bearing on their relationships. The chromosomes of *Barbula vinealis,* in several capsules studied, seem to behave very regularly during meiosis, and give no evidence of cytological irregularities that might result from recent hybridization with somewhat distantly related species, so that this species may be more distinct from *B. cylindrica* than previously believed. Our material came from two populations, from the rocky gorge of Stevens Creek, Santa Clara County (WCS 8, Feb. 12, 1953), and from San Mateo County Park, between La Honda and Pescadero (VSB 19, March 5, 1953).

Desmatodon hendersonii (Ren. & Card.) Williams (figs. 35–37) : $n = 13$. This species of *Desmatodon,* a genus for which no chromosome numbers have previously been reported, has long been confused, in California and elsewhere in the West, with an eastern species, *Didymodon tophaceus* (Brid.) Jur., because of the remarkable similarity of appearance and habitat (Steere 1954). Two noteworthy cytological features are the presence of two large bivalents at the first meiotic division, as well as the constant appearance of an extremely small pair, of the order of size of accessory chromosomes (Vaarama 1949). Since the minute bivalent becomes clearly double in nature and dissociates in a perfectly normal manner, it is better considered as an unusually minute pair. Further cytological work should be most helpful in determining more accurately the relationships within the genus *Desmatodon,* which at best is an artificial one consisting of several disparate elements. Our material was collected at the foot of a small waterfall in Alum Rock State Park, near San Jose, Santa Clara County (WCS 51, April 2, 1953).

Phascum cuspidatum Hedw. var. *americanum* Ren. & Card. (figs. 38–43) : $n = 26$, $n = 29$–30. Vaarama's (1953b) report of the relatively high chromosome number of $n = 52$ from a Finnish population of *Phascum cus-*

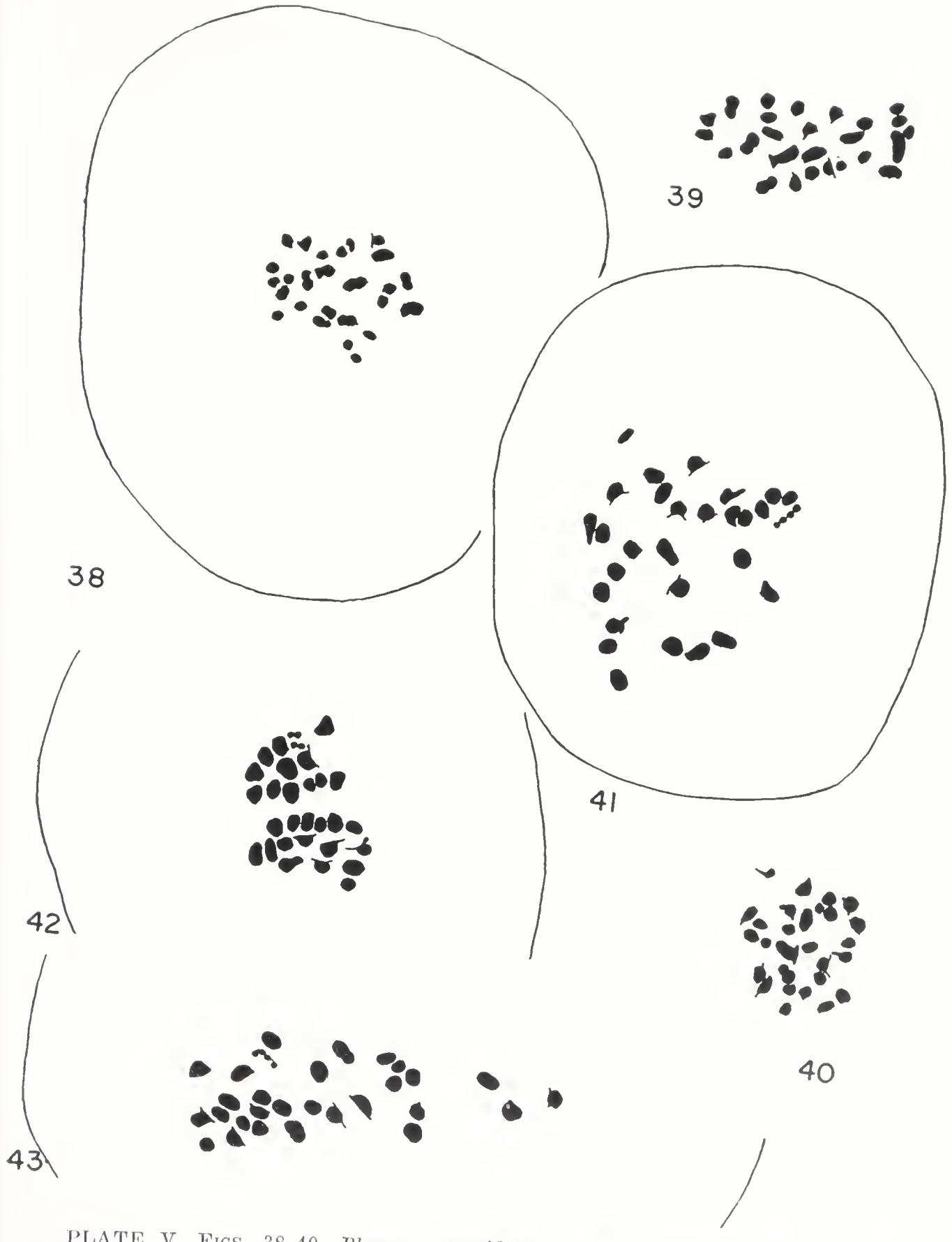

PLATE V. FIGS. 38–40. *Phascum cuspidatum* var. *americanum* (Mt. Hamilton race, $n = 26$), polar views of first meiotic metaphase. FIGS. 41–43. *Phascum cuspidatum* var. *americanum* (Sonoma County race, $n = 28 +$ "accessory chromosomes"), polar views of first meiotic metaphase. All figures $\times 2160$.

pidatum is of considerable significance in view of the obvious morphological differences between the European species and the American plants passing under the same name. Of special interest here was our discovery of two chromosome races in the California populations, which may reflect geographic isolation over a long period of time, in geological terms. Sporocytes of *Phascum cuspidatum* var. *americanum* in a population from western Sonoma County, on the west slope of the outer Coast Range, were found to have 28 pairs of normal meiotic chromosomes, plus four of the very minute chromatic bodies (figs. 41–43) that have been termed isochromosomes or accessory chromosomes by Vaarama (1949). These small chromosomes appear either as a chain of four, or as two separate pairs, and there is some question whether they should be considered as one pair that disjoins very early, or as two pairs. Another population of the same species from the north slope of Mt. Hamilton, in the Mt. Hamilton Range, shows clearly the chromosome number, $n = 26$, without any "accessory" chromosomes (figs. 38–40). Our counts of the chromosomes of these two races are based on a study of dozens of sporocytes, with the three collaborators in full agreement. It is difficult to reconcile these two chromosome complements without a study of the somatic chromosomes, which in their usually clearer morphological differences may shed some light on the relationship of what appear to be aneuploid races or populations. However, this situation probably represents the same type of aneuploidy through chromosome fragmentation that Vaarama (1953a) has described at length in *Orthotrichum tenellum*. The Sonoma County race came from a pastured hillside on the Hedgpeth Ranch (WCS 13, Feb. 22, 1953), and the Mt. Hamilton race occurs in an open area among chaparral vegetation (WCS 41, March 24, 1953). Aside from the very small chromosomes, both races show several bivalents of individual shapes, that may be recognized from figure to figure, and one very dimorphic pair may occasionally be seen, when properly oriented.

Pottia davalianum (Smith) Steere[2] (figs. 44–45): $n = 30$. Vaarama (1950b) reported the chromosome number, $n = 25$ for *P. truncata*, from Finland. The present species is quite unrelated to *P. truncata*, but there still appears to be some relationship in chromosome number, if the basic haploid number in the genus is considered to be five. As seems characteristic of the chromosomes of mosses at the first meiotic division, the bivalents are easily induced to dissociate by the application of pressure, and some counts as high as 50 were obtained from cells subjected to too much pres-

[2] *Pottia davalianum* (Smith) n. comb. *Gymnostomum davalianum* Smith, Kon. Sims Ann. Bot. 1: 577. 1805. The name, *Pottia rufescens* (Schultz) Warnst., much used by European bryologists, is a later homonym of *P. rufescens* (Hook.) C. Müll., and therefore illegitimate. *Pottia minutula* (Schleich.) Fürnr. a synonym, is also much used, but was originally described considerably later.

sure during the "squash" process. The identification of the present material is still open to some question, and we may be dealing with an undescribed species. In his monograph of the North American species of *Pottia*, Wareham (1939, p. 203) says, "*No American specimens of* Pottia *having echinate spores have been seen*" (italics his). The population reported upon here has very conspicuously spinose-papillose or echinate spores, and certainly represents some species not heretofore recognized in the United States. In view of the great variability of *Pottia davalianum* in Europe, it seems reasonably safe to assign this name to our population on a provisional basis, until further taxonomic studies can be made. Our material came from a large population on moist soil on the Stanford Campus, near Sequoia Hall (LEA 7, Feb. 19, 1953).

Timmiella vancouveriensis Broth. (figs. 46–48): $n = 14$. The chromosomes of this well-marked and very distinct genus are of unusual interest in that the bivalents are found in pairs (fig. 48) in some sporocytes, although they separate by the first metaphase. This feature is not too rare among the mosses we have studied, and would certainly seem to indicate some condition of ploidy. Furthermore, the bivalent chromosomes can often be paired up very clearly with other bivalents, by inspection, even when they are quite separate. The chromosome relationships of this genus will remain obscure until more genera of the Pottiaceae can be investigated. Our material came from a roadcut on the slopes of Mt. Tamalpais just above the Muir Woods National Monument, Marin County (WCS 58, April 8, 1953).

Tortula bolanderi (Lesq.) Howe (figs. 49–50): $n = 13$. This species, originally described from California, and still restricted to the West Coast, is an unusually clear-cut and distinctive one, easily distinguished from the other American species of *Tortula*. The chromosome number, $n = 13$, was determined with certainty at the first (fig. 49) and second (fig. 50) meiotic metaphases in a population growing at Searsville Lake, San Mateo County, southwest of the Stanford University campus (VSB 38, March 10, 1953). Another population, growing on roadside cliffs above Saratoga, Santa Clara County, near the eastern summit of the Coast Range (LEA 35, March 18, 1953) did not yield satisfactory results because of the consistent clumping of chromosomes in all sporocytes studied. The number, $n = 13$, differs somewhat from the other species in the subgenus *Syntrichia*, in which the basic number, $n = 12$, was generally observed. The chromosomal difference may well be related to the distinctiveness of this species within the genus, already mentioned.

Tortula laevipila (Brid.) Schwaegr. (figs. 51–52): $n = 12$. Although Vaarama (1953b) reported the chromosome number, $n = 15$ for this species, from a population growing on the Stanford University campus,

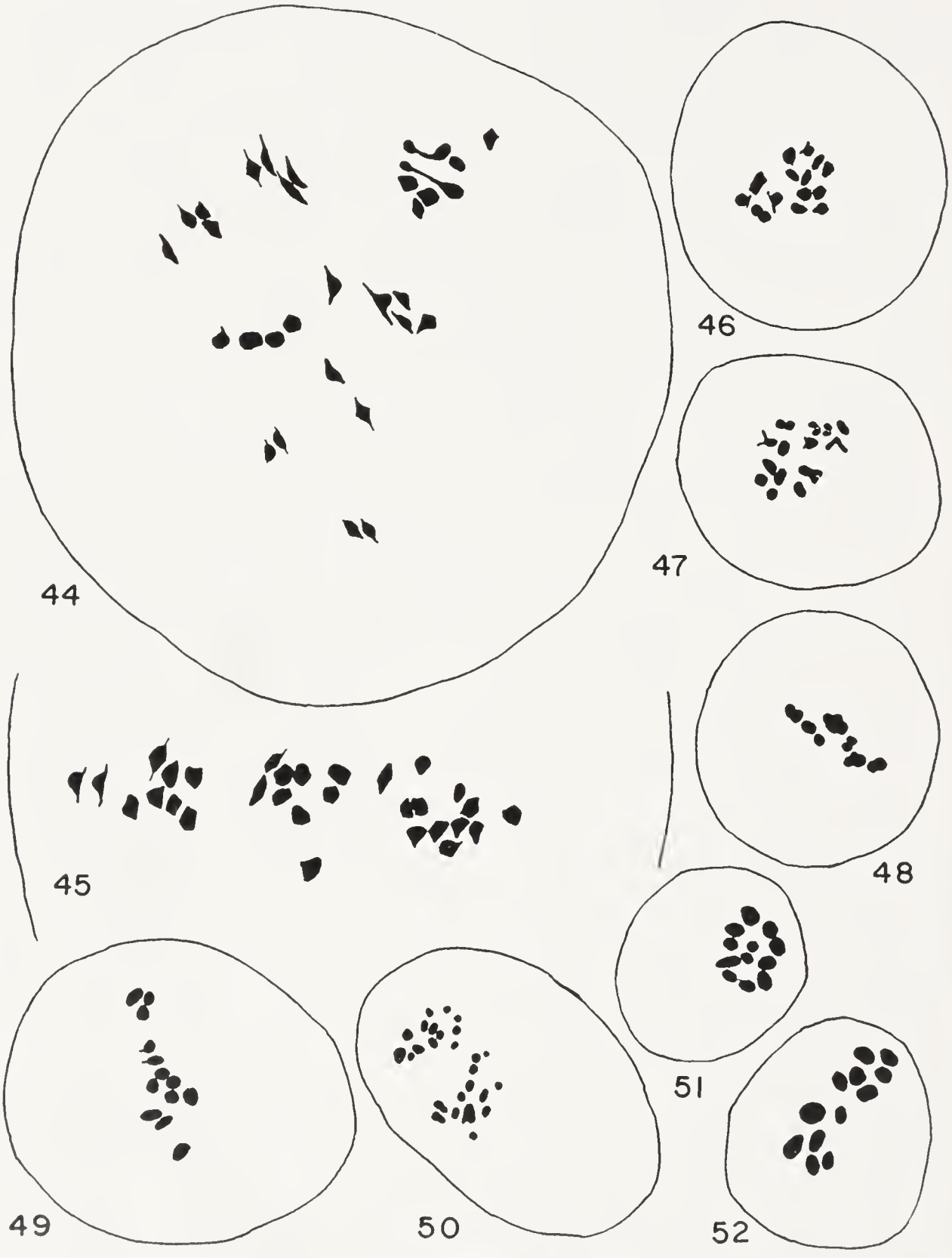

PLATE VI. FIGS. 44–45. *Pottia davalianum* ($n = 30$), first meiotic metaphase, early. FIGS. 46–48. *Timmiella vancouveriensis* ($n = 14$). FIGS. 46 and 47. First meiotic metaphase, early. FIG. 48. Multiple association of bivalents at first meiotic metaphase. FIGS. 49 and 50. *Tortula bolanderi* ($n = 13$). FIG. 49. First meiotic metaphase. FIG. 50. Second meiotic metaphase. FIGS. 51 and 52. *Tortula laevipila* ($n = 12$), polar views of first meiotic metaphase. All figures × 2160.

our material, collected on an oak near the summit of the Coast Range, on Alpine Road, in San Mateo County (VSB 47, March 31, 1953), clearly shows 12 bivalents at the first meiotic division. In view of the range of chromosome numbers reported for Californian populations of *Tortula muralis* by Vaarama (1953b), and elsewhere in this paper for *T. princeps*, we are well prepared to expect chromosome races in this clearly xerophytic species, whose chief habitat is on the bark of the California live oak, well above the ground. These races with different chromosome numbers that occur within aggregations of individuals commonly interpreted as species help to explain the variability and the taxonomic confusion of these same "species," and the reputation for being "difficult" held by the genus *Tortula*.

Tortula muralis Hedw. (figs. 53–54) : $n = 48$. This species, as its name indicates, characteristically grows on masonry walls, and since it receives moisture only from rain or fog, it passes much of the year, even during the winter, in such a state of desiccation that dry plants may be rolled into dust between the fingers. When moistened, however, the plants at once regain their bright, blue-green color and continue their life processes actively, as reflected by the production of a new crop of sporophytes during any extended wet period. Nevertheless, since the maturation of the sporophyte, sporogenesis, and meiosis must certainly be affected by such drastic environmental changes, it is not surprising that chromosome races should have developed in this species. We found that the population studied, growing on a north-facing masonry wall at Memorial Court, Stanford University (WCS 5, Feb. 14, 1953), showed in its meiotic divisions a very large number of chromosomes. At the first division the smallest number seems to be 48, although in some cells as many as 51 chromosomes were counted. As the chromosomes in excess of 48 appear univalent, on the basis of size and proximity to other univalents, our final interpretation is that $n = 48$. This interpretation agrees to some extent with the findings of Vaarama (1953b), who reports that two races of *Tortula muralis* from the vicinity of Berkeley, California, have chromosome numbers of $n = 60$ and $n = 66$. These data indicate that the basic chromosome number of the species is $n = 6$, or some multiple thereof, and that, furthermore, we may expect to find many other chromosome races within this highly variable and taxonomically puzzling species.

Tortula princeps De Not. (figs. 55–61) : $n = 12$, $n = 24$ (+1 accessory bivalent), $n = 36$ (+2 accessory bivalents). Three populations of this moss, the largest and handsomest species of *Tortula* in the United States, were investigated cytologically. Each population was found, by a curious coincidence, to have a different chromosome number, forming an interesting polyploid series. A population growing on the north slope of Mount Hamilton, near the summit, Santa Clara County (VSB 52, March 24, 1953) has the

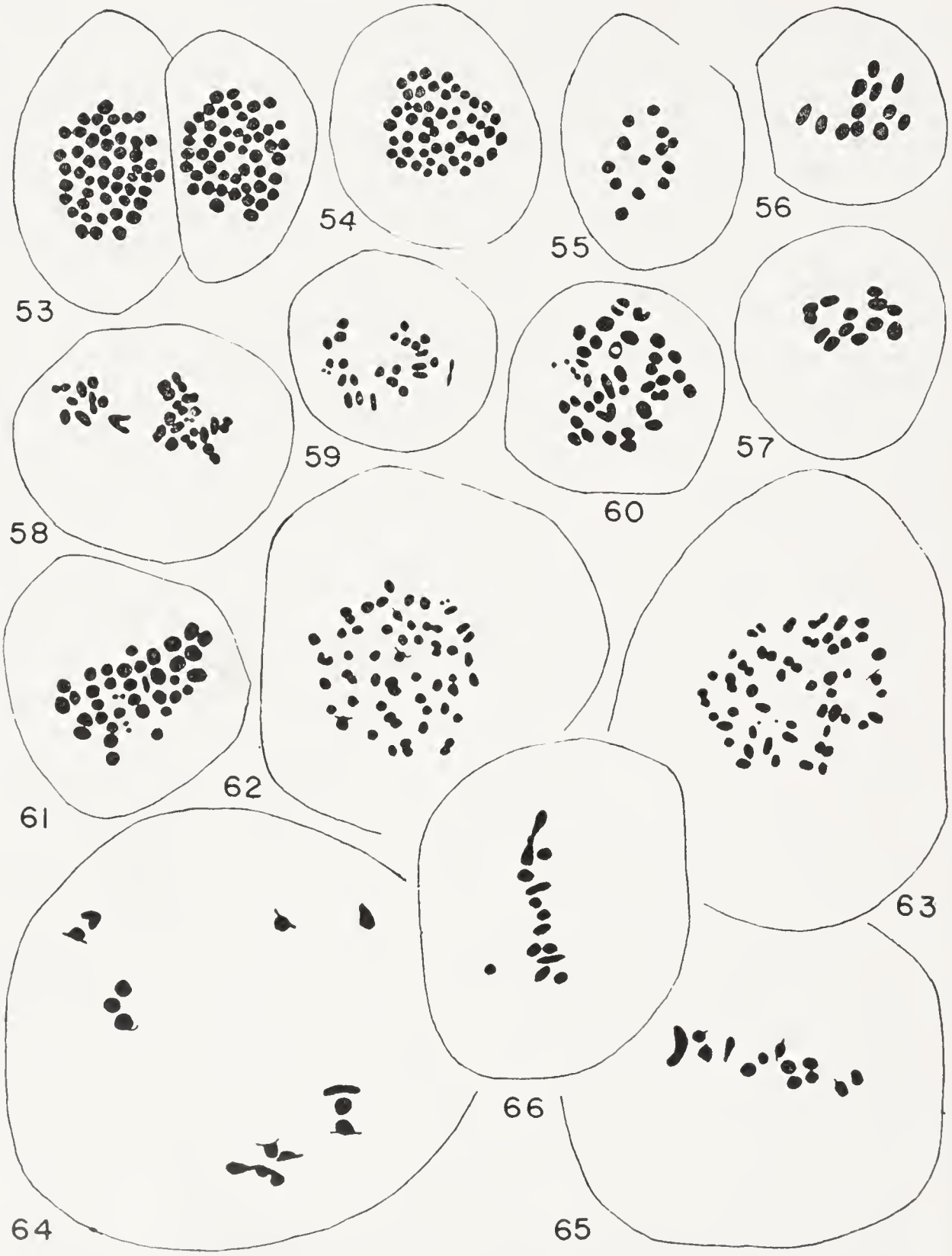

PLATE VII. FIGS. 53 and 54. *Tortula muralis* ($n = 48$), polar views of first meiotic metaphase. FIGS. 55–57. *Tortula princeps* ($n = 12$), polar views of first meiotic metaphase. FIGS. 58–59. *Tortula princeps* ($n = 24 + 1$ accessory pair), first meiotic metaphase. FIGS. 60 and 61. *Tortula princeps* ($n = 36 + 2$ accessory pairs), first meiotic metaphase. FIGS. 62 and 63. *Tortula subulata* ($n = 49$), polar views of first meiotic metaphase. FIGS. 64–66. *Weissia viridula* ($n = 13$). FIG. 64. Late diakinesis (Searsville Lake). FIGS. 65 and 66. Side view of first meiotic metaphase (Mt. Tamalpais). All figures × 2160.

chromosome number, $n = 12$, without question (figs. 55–57). No evidence appeared to support the possibility that bivalents were associated in such a way as to conceal the true chromosome number, as no quadrivalent chromosomes were seen. The chromosomes of this race behave regularly and give the impression of a well-stabilized meiotic mechanism, quite different from the situation found in the other races. Another population, from the rocky gorge of Stevens Creek, Santa Clara County, in the Coast Range (WCS 5a, February 12, 1953), proved to have 24 bivalent chromosomes of normal size and shape, plus one very tiny pair that gave evidence of precocious disjunction (figs. 58–59). The most interesting population grows at the base of oaks on the Hedgpeth Ranch in western Sonoma County (WCS 15, Feb. 22, 1953), and was found to differ greatly from the Mount Hamilton race in its irregular chromosome behavior. Many capsules and many sporocytes had to be studied before the chromosomes could be counted with any real confidence, because of the precocious dissociation of bivalent chromosomes, perhaps aided by the pressure on the cells required by the "squash" technique. The presence of numerous univalent chromosomes tended to give very high counts, but occasional early metaphase figures and the presence of two pairs of very tiny chromosomes would seem to give the keys to the chromosome number, $n = 36$ (+) (figs. 60–61). The presence of one pair of very minute chromosomes associated with 24 normal bivalents, and of two tiny pairs associated with 36 bivalents must be of some significance, in cytological behavior. No chromatic fragments or tiny chromosomes were seen in the race with 12 bivalents, but there is some possibility that such bodies could have been overlooked. The presence of three races with different chromosome numbers, in a taxonomically distinct although variable species, suggests that detailed studies of further populations from widely separated areas, as well as the investigation of somatic chromosomes, might shed valuable light on problems of speciation in mosses, as well as in other plants.

Tortula subulata Hedw. (figs. 62–63): $n = 49$. This large number of chromosomes was established without doubt in many sporocytes, although in some cells numbers as high as 52 occurred, because of the premature dissociation of bivalents. However, these disjoined homologous chromosomes could be identified not only by their proximity to each other but also by their orientation. This is one of the highest chromosome numbers reported for a natural moss population, exceeded only by *Tortula muralis* and a Finnish population of *Phascum cuspidatum* (Vaarama 1953b). It is of more than casual interest to note that these high numbers all occur in the same family. A very small single or double chromatic body was seen in most sporocytes (figs. 61–62), which seems to be a normal part of the chromosome complement. The chromosomes display unusual regularity in size,

shape, and in their meiotic behavior, indicating the relative stability of the species. Our investigation was based on a population growing on moist soil of steep banks along the highway just south of the Wawona entrance to Yosemite National Park, Mariposa County (WCS 71, April 20, 1953).

Weissia viridula Hedw. (*W. controversa* Hedw.) (figs. 64–66): $n = 13$. The chromosome number, $n = 14$, already has been reported for this variable, eosmopolitan, semi-weedy species, on the basis of material from Finland, by Vaarama (1950b). The Californian populations differ slightly in the chromosome number and also lack the conspicuous separation of chromatids in the first meiotic anaphase, reported by Vaarama, a phenomenon that may be influenced to some extent by temperature, moisture, or other external environmental conditions. The difference in chromosome number can be explained through the constant presence in the Californian populations of one chromosome pair that is conspicuously larger at the first meiotic metaphase. If this pair were to disjoin prematurely, under certain conditions, as large chromosomes may do, each half of the pair would almost certainly be taken for a bivalent chromosome, because of the comparable size. The populations studied were collected on soil on steep banks along a trail, at Searsville Lake, San Mateo County, southwest of the Stanford University campus (VSB 26, March 10, 1953), and on the middle slopes of Mt. Tamalpais, Marin County (WCS 75, April 8, 1953) (slides of the latter prepared by Grace Blanchard Iverson). The Searsville Lake population demonstrated a very considerable aberration in the spores, with relation to size and the number of nuclei. Since almost no work has been done on the percentage of sterile vs. viable spores in mosses especially as an indicator of hybridization, this observation cannot yet be properly evaluated.

Summary. The Pottiaceae, a large family of world-wide distribution, especially in dryer and more calcareous regions, consists of perhaps 80 genera and nearly 1500 species. In California, for example, this is by far the largest family of mosses, with some 20 genera and more than 60 species, comprising nearly one-fifth of the known moss flora of the state (Koch 1950, Steere 1951). In spite of the richness of species and genera, chromosome numbers have been reported previously from only five genera of Pottiaceae, from *Barbula* (3 species), *Phascum* (1 species), *Pottia* (1 species), *Tortula* (2 species) and *Weissia* (1 species). We are now able to furnish additional data for the genera just listed, in terms of further species studied, and also to add chromosome counts for members of the genera *Aloina, Desmatodon,* and *Timmiella.* In general, the meiotic chromosomes of this family seem to present less morphological distinctiveness and greater numbers than elsewhere among mosses, at least within our experience. Furthermore, there seems to be an exaggerated tendency for the bivalents to dissociate prematurely, and to clump, so that unusual difficulties were experi-

enced in counting chromosomes of several genera. Consequently, the need for counts of somatic chromosomes to substantiate and to correct the numbers reported from meiotic chromosomes is especially great in this family. There seems also to be a disproportionate amount of anomalous behavior of the chromosomes in the Pottiaceae for which the aneuploid races of *Phascum cuspidatum*, the polyploid races of *Tortula princeps*, and the widespread presence of accessory chromosomes may be cited as evidence. The several tendencies noted toward chromosomal irregularities may account for the fact that higher chromosome numbers occur in wild populations of the Pottiaceae than in any other family of mosses yet investigated. The only other generalization that seems possible at this time is that members of the subfamily Trichostomeae (*Barbula*, *Timmiella* and *Weissia*) tend to have consistently smaller chromosome numbers than members of the Pottieae (*Aloina*, *Desmatodon*, *Phascum*, *Pottia*, and *Tortula*). The most common basic number of chromosomes in the family appears to be 12, with a range from 11 through 14, complicated throughout by the high frequency of ploidy.

Grimmiaceae

Grimmia alpestris (Web. & Mohr) Nees (figs. 67–70): $n = 13$. This widely distributed and not uncommon species, both in Europe and in North America, does not seem to have had its chromosome number reported previously. The 13 bivalent chromosomes, as seen just before the first meiotic metaphase, fall rather clearly into several classes, in terms of their characteristic shapes (figs. 67–68). However, at the metaphase stage, as seen in polar view (figs. 69–70), the chromosomes became more similar in appearance. The chromosome behavior was very regular, unlike that in some other species of this large genus. The material studied came from a population growing on cliffs just below Wawona point, at more than 6000 feet altitude, Yosemite National Park (WCS 72, April 20, 1953). This is the only member of the section *Alpestris* to have received chromosomal study.

Grimmia apocarpa Hedw. (figs. 71–75): $n = 13$. The variability of this species is almost legendary, and the nomenclatural recognition of dozens of forms, varieties, subspecies, and species within its range of variation has resulted in a very considerable taxonomic confusion. In the most recent monograph of the species of *Grimmia* of North America, north of Mexico (Jones 1933), we find that many of the varieties ascribed to this species have themselves been given specific rank by earlier authors. The two earlier reports of the chromosome number of this species agree neither with each other, nor with the number ascertained from our investigation. Heitz (1928) reported the approximate number, $n = $ ca. 20, whereas Vaarama (1953b) has found the chromosome number of the Finnish population investigated by him to be $n = 12$. Our material, from two widely separated populations, represent-

ing two varieties, clearly shows the chromosome number, $n = 13$. Species that are confused taxonomically may consist of several chromosomal races; although cytological studies provide data helpful in taxonomic studies, they cannot necessarily solve taxonomic problems at the specific level. The two populations studied, with the same chromosome number, differ considerably not only in the size and shape of the chromosomes, but also in such morphological features as the shape of the capsules and the leaves. Material of these two populations was sent to Dr. Geneva Sayre for identification, because of her interest in this difficult genus (cf. Sayre 1946, 1952), and we are indebted to her for the following remarks: "The two *apocarpas* do not seem to me to fall into any named varieties. If *G. apocarpa* is not a species but a spectrum, which I sometimes suspect, then No. 40 is at one end and No. 70 somewhere past the middle in the other direction. Number 40, from Mt. Hamilton, closely approaches var. *alpicola* in its broad leaves, large spores and bright red teeth. However, the capsule is oblong, not ovate, and the calyptra is mitrate. Number 70, from Yosemite, is pretty good *apocarpa,* but approaches var. *stricta* (*gracilis*), the leaves being somewhat distant and secund. Typical *stricta* has narrower leaves, a wirier stem and usually a brownish color."

Since many botanists, especially in Europe, would place this species in the segregate genus, *Schistidium*, it is interesting that it should have the same basic chromosome number as the other Californian species of *Grimmia* that were investigated. The two populations were found growing (1) on rocks along a small stream on the north slope of Mount Hamilton, Santa Clara County (VSB 40, March 24, 1953) and (2) on large boulders moistened by spray from Bridal Veil Falls, Yosemite National Park (WCS 70, April 20, 1953).

Grimmia pulvinata (Hedw.) Smith (figs. 76–77) : $n = 13$. A common and variable species, the present one occurs in relatively dry and exposed places in a semi-weedy fashion. For example, it is one of the first mosses to colonize masonry walls, and exposed rocks in road cuts where it occurs in grayish cushions up to several inches across. The meiotic behavior of this species is strikingly irregular perhaps because of its xeric habitat, and its complete dependence on the occasional rains or fogs for moisture, as well as its probable hybrid ancestry. Some bivalent chromosomes obviously divide prematurely whereas others disjoin very late. As a consequence, occasional chromosomes lag behind in the cytoplasm after the formation of daughter nuclei, and sporocytes with flat metaphase plates are very difficult to find. A study of the percentage of sterility of spores in these populations would be highly desirable to determine if irregularities at meiosis result in the formation of sterile cells. Unlike flowering plants showing similar cytological anomalies, the tetrads of spores appear to be normal, and no groups of more or less

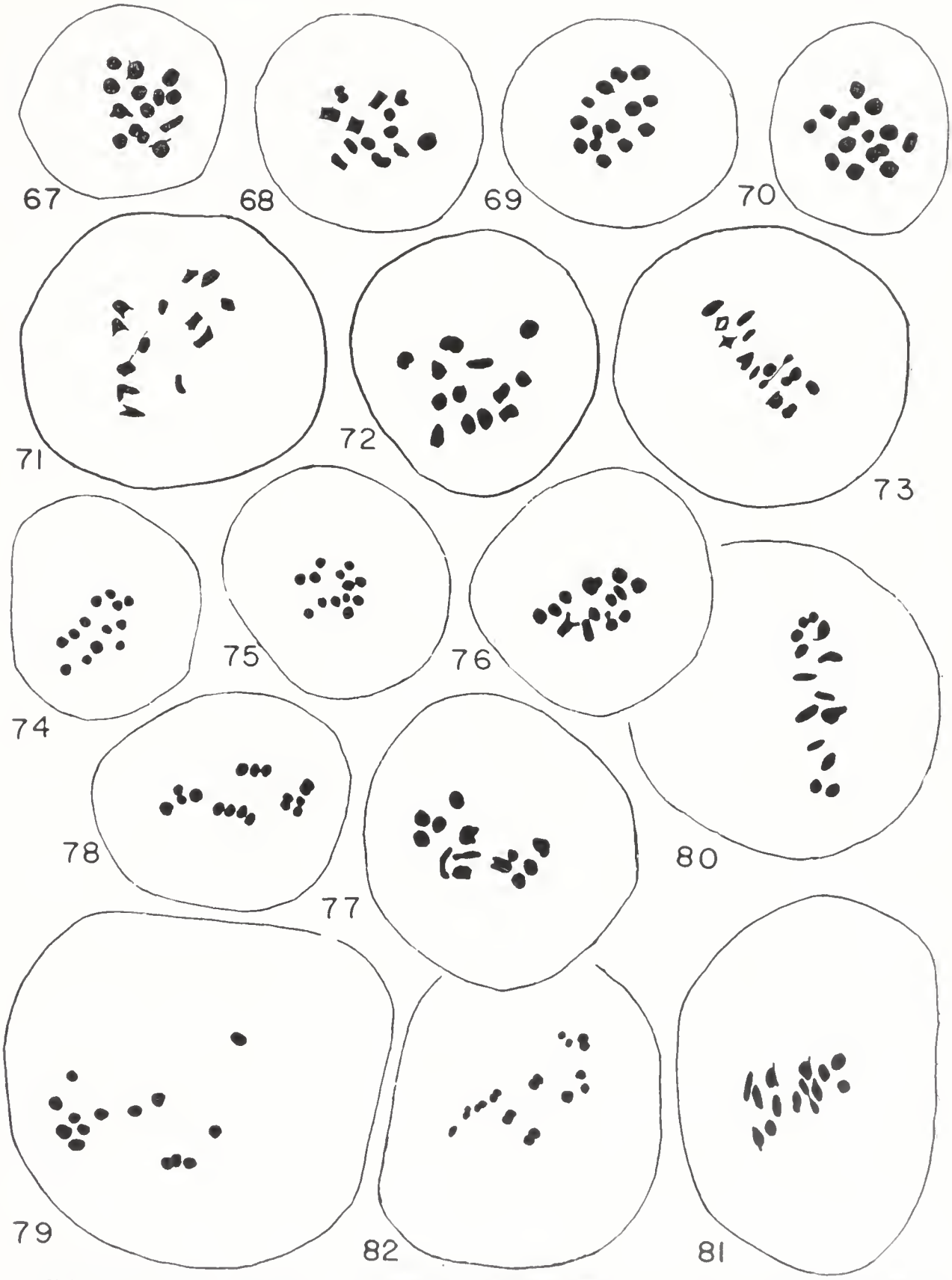

PLATE VIII. FIGS. 67–70. *Grimmia alpestris* ($n = 13$). FIGS. 67 and 68. Early stage of first meiotic metaphase. FIGS. 69 and 70. Polar views of first metaphase. FIGS. 71–73. *Grimmia apocarpa* near var. *alpicola* (Mt. Hamilton, $n = 13$). FIG. 71. Early stage in first meiotic metaphase. FIG. 72. Polar view of first metaphase. FIG. 73. Side view of first metaphase. FIGS. 74 and 75. *Grimmia apocarpa* near var. *stricta* (Yosemite, $n = 13$). Polar views of first metaphase. FIGS. 76–77. *Grimmia pulvinata* ($n = 13$). Early stages of first meiotic metaphase. FIGS. 78 and 79. *Grimmia trichophylla* ($n = 13$), first meiotic metaphase. FIGS. 80–82. *Rhacomitrium depressum* ($n = 14$). FIGS. 80 and 81. Side view of first meiotic metaphases. FIG. 82. Polar view of first metaphase. All figures $\times 2160$.

than four spores were observed. A further problem in counting the chromosomes of this species arises from the fact that the chromatids separate very early, so that at anaphase or even at the irregular metaphase of the first meiotic division, the second division of some chromosomes has already occurred. Consequently, the chromosomes fall into three distinct size classes, the large bivalents, the smaller, yet clearly divided univalents, and the very small chromatids or second division chromosomes. Four-parted chromosomes are not uncommon at anaphase and their premature division results in extremely complex division figures, that may be interpreted in terms of the size classes of chromosomes just outlined, to reach the original chromosome number, $n = 13$. In occasional cells, all the bivalents seemed to divide prematurely to give the appearance of a very large chromosome number. Although Vaarama (1949) reports polyploid cells in *G. muehlenbeckii,* we found that in *G. trichophylla* and *G. pulvinata* those cells seeming to have double the number of chromosomes expected could be interpreted convincingly as having experienced a precocious disjunction or division, respectively, of the first or second division meiotic chromosomes. No accessory chromosomes were found in this species, although a considerable difference in size exists between the bivalents. The two populations investigated grow on exposed boulders at Stevens Creek, Santa Clara County, on the east face of the Coast Range (WCS 9, Feb. 12, 1953) and on the southern slope of Mt. Diablo, Contra Costa County (LEA 28, March 13, 1953).

Grimmia trichophylla Grev. (figs. 78–79) : $n = 13$. This is without doubt one of the most common and widely distributed species of *Grimmia* in California, especially in the Coast Ranges, where it occurs in numerous forms that are undoubtedly more a reflection of environmental than of genetic variations. The only species of *Grimmia* in the subgenus *Eugrimmia* previously studied cytologically is *G. muehlenbeckii* (Vaarama 1949), which by a curious coincidence is the one species in a very large and traditionally difficult genus most closely related to the present one. In fact, in modern treatments, Dixon (1924) considers *G. muehlenbeckii* as a subspecies of *G. trichophylla,* whereas Mönkmeyer (1927) and Jones (1933) treat it as a simple variety. Consequently, it was of special interest to be able to compare the cytological behavior of *G. trichophylla* with that reported for a Finnish population of the very closely related *G. muehlenbeckii.* In the first place, we can report that *G. trichophylla* unquestionably has the chromosome number, $n = 13$, from a study of many preparations of capsules of a population growing on the south slope of Mt. Diablo, Contra Costa County (VSB 29, March 13, 1953), whereas Vaarama reported that the chromosome number of *G. muehlenbeckii* is $n = 14 + 2$ accessory chromosomes. Furthermore, we can report that the population of *Grimmia trichophylla* studied possesses no accessory chromosomes, and that

the chromosome complement was remarkably uniform in size and shape, in contrast to the related species, *G. pulvinata*, which seems to have larger and more differentiated chromosomes.

Rhacomitrium depressum Lesq. (figs. 80–82): $n = 14$. This chromosome number seems to have especial significance as it relates very well to the number, $n = 28$, reported by Vaarama (1949) for *R. heterostichum*, *R. hypnoides*, and *R. ramulosum*. Unfortunately for our purposes, however, the chromosome number of the most closely related species, *R. aciculare* Brid., has not yet been reported. *Rhacomitrium depressum* is restricted in its geographic distribution to the Pacific Coast, where it has been collected only rarely. Our material came from a population growing on wet cliffs at the entrance to Yosemite National Park, above the Merced River, Mariposa County, not far from the type locality (WCS 67, April 20, 1953).

Summary. Grimmiaceae is a relatively small family, consisting of about six genera adapted to xeric habitats, and most of the species grow on exposed rock or soil. The only genera of any magnitude are *Grimmia*, with more than 200 species described, and *Rhacomitrium*, with some 80 species. Although Heitz (1928) reported an approximate chromosome number for *Grimmia apocarpa*, all other chromosome numbers reported for members of this family seem to have been determined by Vaarama (1949, 1953b), whose excellent special study of this family will long remain the classic one, and by Yano (1951). The basic chromosome numbers in *Grimmia* and *Rhacomitrium* are 12, 13, or 14, or some multiple thereof, and show a remarkable constancy. Because of the long dry summers and the presence of extensive xeric areas, California is peculiarly rich in members of this family, and the genus *Grimmia*, with more than 20 species is, with the possible exception of *Tortula,* the largest genus of mosses in the state. The four species studied represent three sections of the genus, of which one (*Schistidium*) is widely recognized as a genus, yet the same chromosome number was encountered in all. The five species of *Rhacomitrium* whose chromosome numbers are reported by Vaarama appear to be, with one exception, tetraploid, in terms of the sporophyte, having 26 and 28 pairs. The exceptional species, *Rhacomitrium canescens*, is reported to have 12 pairs, and like *R. depressum* investigated by us, might be considered to represent the diploid condition. However, a study of the chromosome figures given by Vaarama, as well as those included here, show a considerable amount of duplication of bivalents at the first meiotic division, on the basis of size and shape. Thus, it is probable that those species of *Grimmia* and *Rhacomitrium* with 12, 13, and 14 chromosome pairs are already of a tetraploid nature.

Funariaceae

Funaria hygrometrica Hedw. (figs. 83–84): $n = 28$. This chromosome count is significant in that Vaarama (1953b) has reported the number, $n =$

14, from a population of this same ubiquitous species growing as a weed at Berkeley, in greenhouses of the University of California. The material from which this count was made came from a population growing in a pastured field under rather undisturbed conditions, accompanied by several ephemeral mosses, as *Pleuridium bolanderi, Phascum cuspidatum,* and *Ephemerum serratum*, at the Hedgpeth Ranch in western Sonoma County (WCS 11, February 22, 1953). The material was in excellent condition and showed no reduction in fertility and in the production of sporophytes in spite of its probable tetraploid nature. Furthermore, the meiotic divisions were found to be very regular. Vaarama (1950b) has reported the chromosome number, $n = 28$, from this species in Finland, but he did not indicate the source of his material, whether out-of-doors, or under glass. It would be of considerable interest to know if the weedy form so abundant in greenhouses, gardens, on burned soil, etc., represents a race different from the one which seems well established as a native plant in natural habitats. The only available evidence impinging on this question is favorable, and comes from the fact that the greenhouse race used by Wettstein (1923–24) in his important experiments also had the chromosome number, $n = 14$. It will be recalled that Wettstein was able to produce artificially, through apospory, polyploid races of *Funaria hygrometrica,* with chromosome numbers of $n = 28$ and $n = 56$, from a population in which $n = 14$.

Funaria muhlenbergii Turn. var. *patula* BSG. (fig. 85) : $n = 28$. American material of this species has not been studied previously, and this variety apparently has not been investigated at all. However, Griesinger (1937) has reported the chromosome number, $n = 26$, for the species (as *F. mediterranea*), from European material. The very great taxonomic and nomenclatural confusion over this species, and its intrinsic variability, may possibly stem from the existence of races with different chromosome numbers. A careful cytological study of these races might supply data helpful in clarifying the systematics of the species. Aneuploid races have been reported to occur in *Phascum cuspidatum*, earlier in this paper. We studied many sporocytes of this variety and can report the number, $n = 28$, with confidence. The chromosomes are remarkably uniform, not only in shape and size, but also in their behavior during meiosis. The population investigated occurs on calcareous soil near the stream in Alum Rock State Park, near San Jose, Santa Clara County (VSB 50, April 2, 1953).

Summary. The Funariaceae consist of about a dozen genera, of which only *Funaria* and *Physcomitrium* are of any size. This family has become especially well known through the notable researches of Wettstein and his collaborators on the genetics and cytology of several of its members. The artificial hybridization of species within and between the genera *Funaria, Physcomitrium*, and *Physcomitrella* have shed considerable light on the

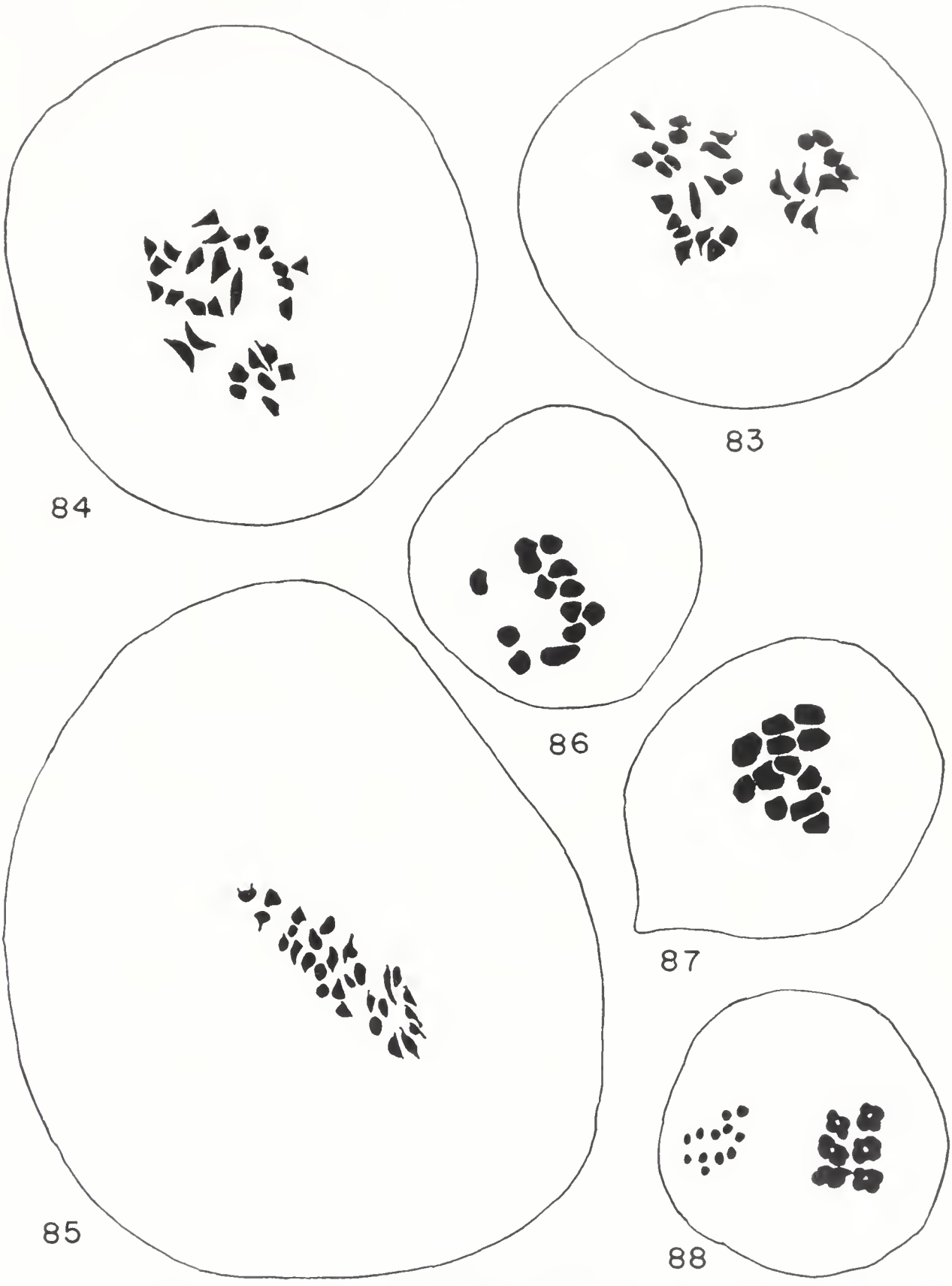

PLATE IX. FIGS. 83 and 84. *Funaria hygrometrica* ($n = 28$), polar views of first meiotic metaphases. FIG. 85. *Funaria muhlenbergii* var. *patula* ($n = 28$), first meiotic metaphase. FIGS. 86–88. *Bryum argenteum* var. *lanatum* ($n = 12$). FIGS. 86 and 87. Polar view of first meiotic metaphases. FIG. 88. Polar view of second metaphase, multiple association of chromosomes in one plate. All figures $\times 2160$.

nature of generic boundaries in these essentially weedy mosses, and pose some serious problems for taxonomists. The production through experimentally induced apospory of highly polyploid races and hybrids in *Funaria hygrometrica* and *Physcomitrium piriforme* (Wettstein and Straub 1942, Barthelmess 1938, 1941), ranks among the outstanding experimental investigations yet carried on with bryophytes. Reports of chromosome numbers in other species of *Funaria* have been n = ca. 10 in *F. flavicans* (Beardsley 1931), and n = 24 in *F. californica* (Vaarama 1953b). Wettstein (1924) reported the chromosome number, n = ca. 16, in *Physcomitrella patens,* and the basic number, n = 18, in *Physcomitrium*, in which he produced races with the chromosome numbers, n = 36 and n = 72, under experimental conditions (Wettstein and Straub 1942, Barthelmess 1938, 1941). With the exception of the chromosome number estimated for *Funaria flavicans,* n = ca. 10, which may well need correction, the basic numbers seem reasonably closely related in all the genera studied, n = 14, n = 16, n = 18, with multiples which would also indicate a basic number, n = 12. A careful study of the somatic chromosomes of the members of the Funariaceae may well furnish evidence for the original basic chromosome number, and indicate the derivation of the higher aneuploidic numbers.

Bryaceae

Bryum argenteum Hedw. var. *lanatum* BSG. (figs. 86–88) : n = 12. This chromosome count, determined with certainty at the metaphases of both first and second meiotic divisions, is at variance with the chromosome number, n = 10 reported previously for the species from European material (Marchal 1920, Jachimsky 1935), and furthermore does not agree with the basic number, n = 10, that appears to be characteristic of the genus *Bryum.* However, it is not remarkable that weedy species of this sort, known to occur in nearly every part of the globe, should have aneuploid races, perhaps of geographical significance. The relationships of populations of this species growing in Europe and in California must be extremely remote, in a historical sense—if we are even dealing with the same species. The association of chromosomes observed in other species of *Bryum* was noted here at the second meiotic division (fig. 88). A further anomaly was furnished by a small chromatic body observed in a few SMC at the first meiotic division (fig. 87). The population investigated came from an open, pastured area at the Hedgpeth Ranch in western Sonoma County, on the west slope of the Coast Range (WCS 4, Feb. 22, 1953).

Bryum capillare Hedw. (figs. 89–91) : n = 10 (+ 2–3 accessory chromosomes). The chromosome number, n = 10, has already been reported for this species (Marchal and Marchal 1911, Heitz 1928), but the presence of accessory chromosomes does not seem to have been noted previously either in this

species or in the genus *Bryum*. The Marchals (1911) were able to produce a tetraploid race by experimental means in which the chromosome number was $n = 20$, which they called "var. *bivalens*." One of the most interesting features of meiotic behavior in this species is the complete association of the bivalents into five groups (fig. 90) in some sporocytes, giving some indication that this species is already diploid through duplication of the chromosome set, even though we still have not encountered any species of *Bryum* with the expected basic chromosome number, $n = 5$. The accessory chromosomes are very distinct, and likewise remain closely grouped before the first meiotic metaphase, but their behavior during the first and second meiotic divisions was not observed in detail, except that they did not seem to divide. This species is extremely widespread over the world and is also unusually variable in its morphology and its habitat preference. Consequently, several chromosome races probably exist. The population investigated grows on soil on the Stanford University Campus, near Sequoia Hall (LEA 3, Feb. 19, 1953).

Bryum pseudotriquetrum (Hedw.) Schwaegr. (*B. bimum* Schreb.) (figs. 92–95) : $n = 11$. This species differs from the others investigated, *B. argenteum* and *B. capillare*, not only in the presence of a tiny chromosome pair, but also in the difference in size and shape among the chromosomes themselves. Two much larger bivalents elongate conspicuously at early anaphase I. Although the minute chromosome was too small to show its double nature before and during the first meiotic metaphase, it was seen clearly to divide after the other chromosomes had already reached the poles (fig. 95). This species is common and abundant through all the temperate regions of the world, where it exists in many geographical races. The population investigated grows in seepage zones on roadside cliffs in Marin County, between Tomales and Petaluma (LEA 61, April 11, 1953).

Epipterygium tozeri (Grev.) Lindb. (*Pohlia tozeri* Del.) (figs. 96–99) : $n = 11$. This is the first report of a chromosome number in the small genus *Epipterygium*, which has been maintained within the generic concept of *Pohlia* by Andrews (1935). The chromosome number, $n = 11$, does not agree with the basic number, $n = 5$, characteristic of the genus *Bryum*, nor with the single chromosome count reported for the genus *Pohlia*, since in *Pohlia nutans* (Hedw.) Lindb., $n = 14$ (Heitz 1928). The bivalent chromosomes of *Epipterygium tozeri* fall very clearly into three classes, with four large ones, four of medium size, and three small ones. One of the most conspicuous features of the first meiotic metaphase is the premature separation of one very small chromosome pair into two parts, which in turn sometimes appear to be double. Furthermore, one bivalent is very definitely and strongly dimorphic when seen from the side. The material studied came from a steep shaded bank along Foothill Road, on the Stan-

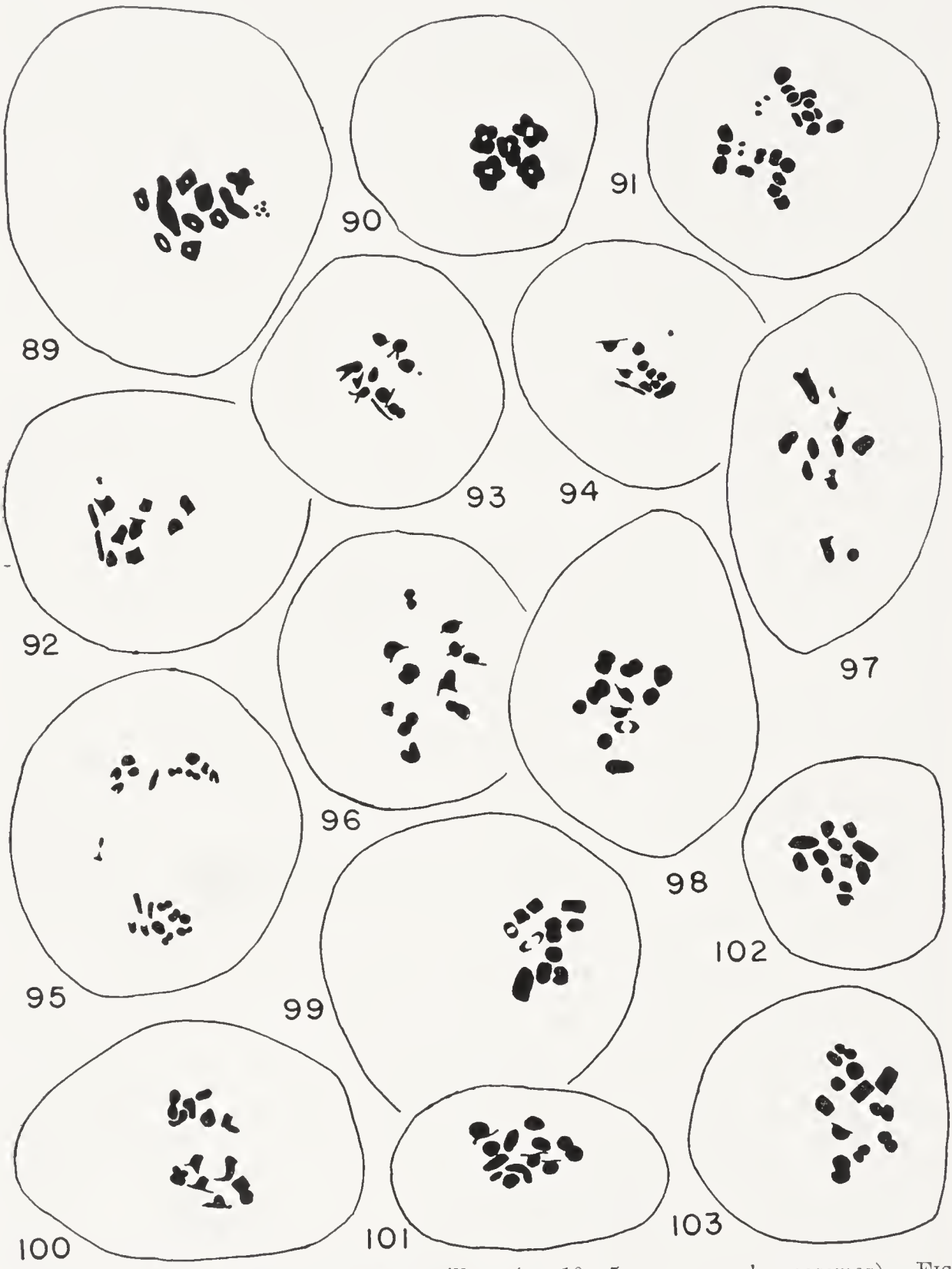

PLATE X. FIGS. 89–91. *Bryum capillare* ($n = 10 + 5$ accessory chromosomes). FIG. 89. Late diakinesis-early metaphase stage, with group of five accessory chromosomes. FIG. 90. Multiple association of chromosomes at the first meiotic metaphase. FIG. 91. First anaphase, showing apparent random distribution of accessory chromosomes. FIGS. 92–95. *Bryum pseudotriquetrum* ($n = 11$). FIGS. 92–94. Late diakinesis-early metaphase stages, showing small pair. FIG. 95. Late stage of first anaphase, showing dissociation of small pair. FIGS. 96–99. *Epipterygium tozeri* ($n = 11$), various metaphase stages, from very early to median, showing behavior of small pair. FIGS. 100–103. *Pohlia longibracteata* ($n = 12$), metaphase stages from early to median. All figures $\times 2160$.

ford Campus, Santa Clara County (WSC 17, March 4, 1953). The second population studied came from wet vertical banks, Alpine Road, near the summit of the Coast Range (WCS 48, March 31, 1953).

Orthodontium gracile Schwaegr. (figs. 104–107): $n = 12$. In its chromosome complement of ten large bivalents, this species agrees very well with the situation characteristic of the genus *Bryum*. However, the normal bivalents are much larger than those seen in any species of *Bryum*, and are accompanied by two very much smaller pairs, of the order of size of accessory chromosomes. However, as the minute chromosomes are obviously double, and divide at the first meiotic metaphase, they are perhaps better considered as minute bivalents. The anaphase of the first meiotic division is remarkable for the complete division of the chromosomes into their chromatids, although they remain in proximity, in the same manner as reported for *Weissia viridula* by Vaarama (1950b). This seems to be the first chromosome count reported from a small genus that has been recently revised by Meijer (1951). The material investigated grew on an old, burned redwood stump in a deep forest on the east side of the Coast Range, along the La Honda Crossing Road, San Mateo County (LEA 22, March 7, 1953).

Pohlia longibracteata Broth. (figs. 100–103): $n = 12$. Like the chromosome number of *Epipterygium tozeri*, this count does not agree either with the basic chromosome number, $n = 5$ (or $n = 10$), of the genus *Bryum*, nor with the chromosome count, $n = 14$, reported for *Pohlia nutans*, apparently the only species of the genus previously studied cytologically (Heitz 1928). *Pohlia* is a large and complex genus, taxonomically speaking, although the present species is a clear cut one, with its known geographic distribution restricted to California, Oregon and Washington. The chromosomes fall into several classes, in terms of shape and size. The specimens studied came from the San Mateo County Park, on the west slope of the Coast Range between La Honda and Pescadero (VSB 18, March 5, 1953).

Summary. Although the Bryaceae, with fewer than twenty genera, may appear small in comparison with families with more genera, such an appearance is deceptive because of the large size of several of the component genera. Nearly a thousand species have been proposed within the genus *Bryum*, alone, for example. Members of this family are found in all parts of the world, and because of the wide geographic distribution and the somewhat weedy nature of many of its species, the genus *Bryum*, especially, has been selected for considerable experimental study during the past half century. The original experiments on the development of gametophytic tissues through the regeneration of the setae of moss sporophytes (Pringsheim 1878) and the subsequent utilization of apospory in the production of polyploid races were based in part on several species of *Bryum* (Marchals 1911,

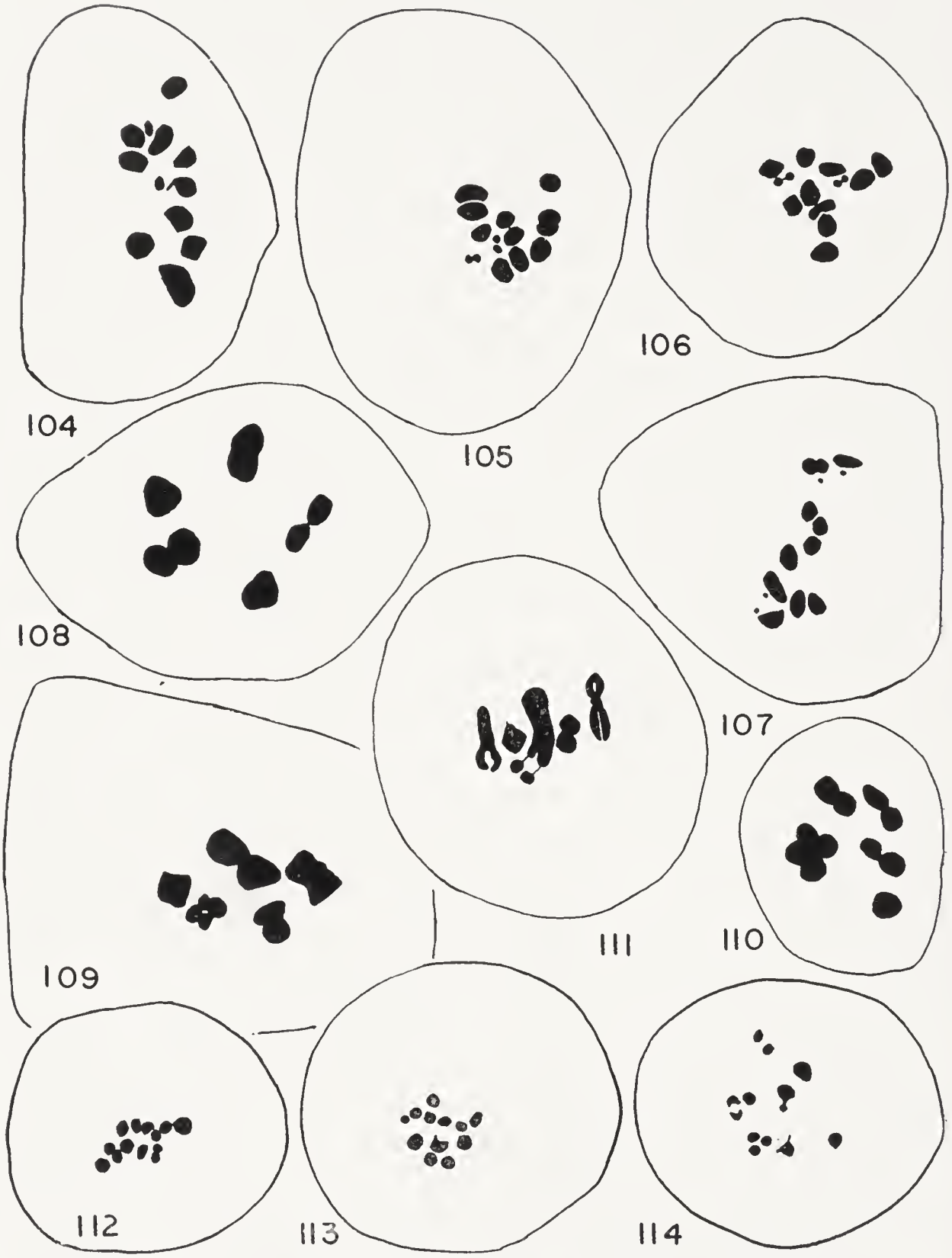

PLATE XI. Figs. 104–107. *Orthodontium gracile* ($n = 12$), various stages of first meiotic metaphase to show behavior of two minute pairs. Figs. 108–111. *Leucolepis menziesii* ($n = 5$). Figs. 108–110. Polar view of first meiotic metaphase. Fig. 111. Side view of first metaphase, showing heteromorphic bivalent with satellites. Figs. 112–114. *Aulacomnium androgynum* ($n = 12$), polar view of first meiotic metaphases. All figures $\times 2160$.

Wettstein 1924, Griesinger 1937). The present investigation tends to make the chromosomal situation in *Bryum* more complicated than realized earlier. Previous workers, almost all investigating European populations, report the chromosome number of *Bryum argenteum* as $n = 10$ (Marchal 1920, Jachimsky 1935), whereas we find twelve pairs; of *B. caespiticium* as $n = 10$ (Marchals, 1911, Wettstein 1924); of *B. capillare* as $n = 10$ (Marchals 1911, Heitz 1928), whereas we find 2–3 additional accessory chromosomes, not previously detected; and of *B. pseudotriquetrum* as $n =$ "9–10" (Heitz 1928), whereas we find an additional small bivalent, giving the count, $n = 11$. Of course, it must be realized that in species as widely distributed as those of *Bryum*, in their present interpretation at least, the presence of chromosome races may be correlated with geographical races. The only species of *Pohlia*, *P. nutans*, that has previously had a chromosome count reported, $n = 14$ (Heitz 1928) is only distantly related to the Californian *P. longibracteata*, so that it is not surprising for the latter to have a different basic chromosome number, $n = 12$. Two species of *Rhodobryum*, *R. giganteum* and *R. roseum*, have been reported to possess the chromosome number, $n = 11$ (Yano 1952). The genera, *Orthodontium* and *Epipterygium*, for which chromosome counts have not previously been reported, show basic numbers of $n = 11$ for *E. tozeri* and $n = 12$ for *O. gracile*. In summary, then, it can be seen that the basic chromosome numbers reported for members of the Bryaceae, $n = 10$, $n = 11$, $n = 12$, and $n = 14$, are reasonably consistent.

Mniaceae

Leucolepis menziesii (Hook.) Steere (figs. 108–111): $n = 5$. Lowry (1948) has reported the chromosome number, $n = 5$, for this species in his outstanding study of the somatic chromosomes of *Mnium*, from a collection made near Seattle, Washington. Lowry indicates a very considerable range of variation in the length of the somatic chromosomes, from 4.6 μ to 7.4 μ, and the meiotic chromosomes show one very large bivalent whose members tend to dissociate prematurely (figs. 108 and 109). This large chromosome may be dimorphic, although too few division figures of the proper stage were available to prove this beyond all doubt. However, since the plants are clearly dioecious, the presence of such a dimorphic pair ("sex chromosome") is highly probable. Unfortunately for any clear correlation between the somatic chromosomes of the gametophyte and the meiotic chromosomes, Lowry does not specify whether he investigated male or female plants of this species, or both. However, except for the large chromosome pair just described, the meiotic chromosomes seem to fall into the size classes established by Lowry, and no accessory chromosomes were seen. This is the only species of the present investigation in which satellites were seen in meiotic chromosomes (fig. 111). On the basis of its distinctive external morphology

alone, this species was segregated into a monotypic genus by Lindberg (1868), although retained in *Mnium* by Andrews (1940) and by Lowry (1948). However, since the chromosome morphology differs so markedly from that of any known species in *Mnium,* and a different basic number occurs, there seems to be abundant reason for recognizing the genus *Leucolepis* as valid. The population investigated occurs on the wet, rocky bank of a stream, under redwoods, in San Mateo County Park, between La Honda and Pescadero (LEA 21, March 5, 1953).

Summary. The Mniaceae is a relatively small family, consisting of very few genera, but the individual plants of many species are conspicuously large and handsome. Perhaps for this reason, more species in *Mnium* have been studied cytologically than in any other genus of mosses, even in much larger ones. The extensive cytotaxonomic study of *Mnium* by Lowry (1948) has been supplemented by the more recent work of Vaarama (1950b) and Tatuno (1951). The basic chromosome numbers in *Mnium,* in over 20 species studied, are $n = 6$ and $n = 7$, or, in a few species multiples thereof. Although *Mnium microphyllum* and *M. flagellare,* which have been segregated into the genus *Trachycystis* (Lindberg 1868) on the basis of morphological structure, the chromosome number, $n = 7$, reported for both species by Tatuno (1951) gives little cytological support for such a generic separation. With respect to other genera, Lowry (1948) reported the chromosome number, $n = 14$, for a single species of *Cinclidium, C. stygium,* and the number, $n = 5$, for *Leucolepis menziesii* (as *Mnium*), as already noted under that genus.

Aulacomniaceae

Aulacomnium androgynum Schwaegr. (figs. 112–114): $n = 12$. The chromosome number of this species was estimated as $n = 10$–11 by Heitz (1945). The study of many sporocytes of a Californian population resulted consistently in the determination of the chromosome number as $n = 12$, a discovery of considerable interest, since it agrees with the report by Vaarama (1950), of $n = 12$ for *A. palustre,* whose chromosome number had been estimated as $n =$ "(9)10" by Heitz (1928). The chromosomes differ considerably in size, with two very large bivalents (fig. 114), several very small ones, and at least one pair that is distinctly dimorphic in side view. This species is very common on rotting wood in the coniferous forests of California, both in the Coast Ranges and in the Sierra. The material investigated here grew on a rotting redwood log in the Big Basin State Park, Santa Cruz County (VSB 42, March 18, 1953).

Summary. The Aulacomniaceae consists of two widely distributed genera, *Aulacomnium* and *Leptotheca,* and the small total of approximately a dozen species. Nevertheless, we find published references to the chromosome numbers of two species of *Aulacomnium,* estimated for *A. andro-*

gynum and *A. palustre* by Heitz (1928, 1945), and a definite report for the latter species by Vaarama (1950b). Both species appear to have the same chromosome number, $n = 12$.

Bartramiaceae

Anacolia menziesii (Turn.) Paris (figs. 115–118) : $n = 8$. In sharp contrast to its variety *baueri*, which will be treated next, this species shows more regular cytological behavior, although occasional late anaphases show some variation in the rate of chromosome movement. The division figures studied showed very clearly the presence of seven large bivalent chromosomes, and a very small pair that dissociates precociously. The basic chromosome number, $n = 8$, agrees well with that reported for other members of the family, especially for *Bartramia*. This is the first member of the genus *Anacolia* to have its chromosome number counted. The material studied was collected near the summit of the Coast Range, above Saratoga, Santa Clara County (WCS 34, March 18, 1953).

Anacolia menziesii (Turn.) Paris var. *baueri* (Hampe) Paris (figs. 119–121) : $n = 7$. From the irregular behavior of the chromosomes at meiosis, especially in terms of precocious and laggard disjunction of chromosomes at the first meiotic division (figs. 120 and 121), there must be some genetic imbalance, perhaps due to hybridization. In addition to the presence of univalent chromosomes at a time when they would ordinarily not be encountered, accurate counts are made still more difficult by the premature division of anaphase chromosomes. Although originally proposed as an independent species, this moss is now considered to be only a variety of *A. menziesii*, from which it differs in the cylindrical or oblong, rather than spherical capsule, the longer seta, and the smaller spores which are ornamented differently. Furthermore, the variety seems to prefer distinctly drier habitats. It is perhaps significant that a marked chromosome difference should be correlated with morphological and ecological differences. The taxonomic problems in *Anacolia* have recently been reviewed thoroughly by Flowers (1952). The population studied here came from a steep shaded roadside bank on the south slope of Mt. Diablo, Contra Costa County (VSB 27, March 13, 1953).

Summary. The Bartramiaceae, although consisting of only about ten genera, is a relatively large family, since *Bartramia* and *Breutelia* each contain over 100 species, and *Philonotis* nearly 200 species. Furthermore, the family is represented in all parts of the world, from the coldest to the warmest climates, in all latitudes. In general, although some of the species tolerate very exposed situations, most of them seem to have a rather high water requirement. Previously reported chromosome counts in the Bartramiaceae are $n = 8$ for *Bartramia pomiformis*, from a Japanese popula-

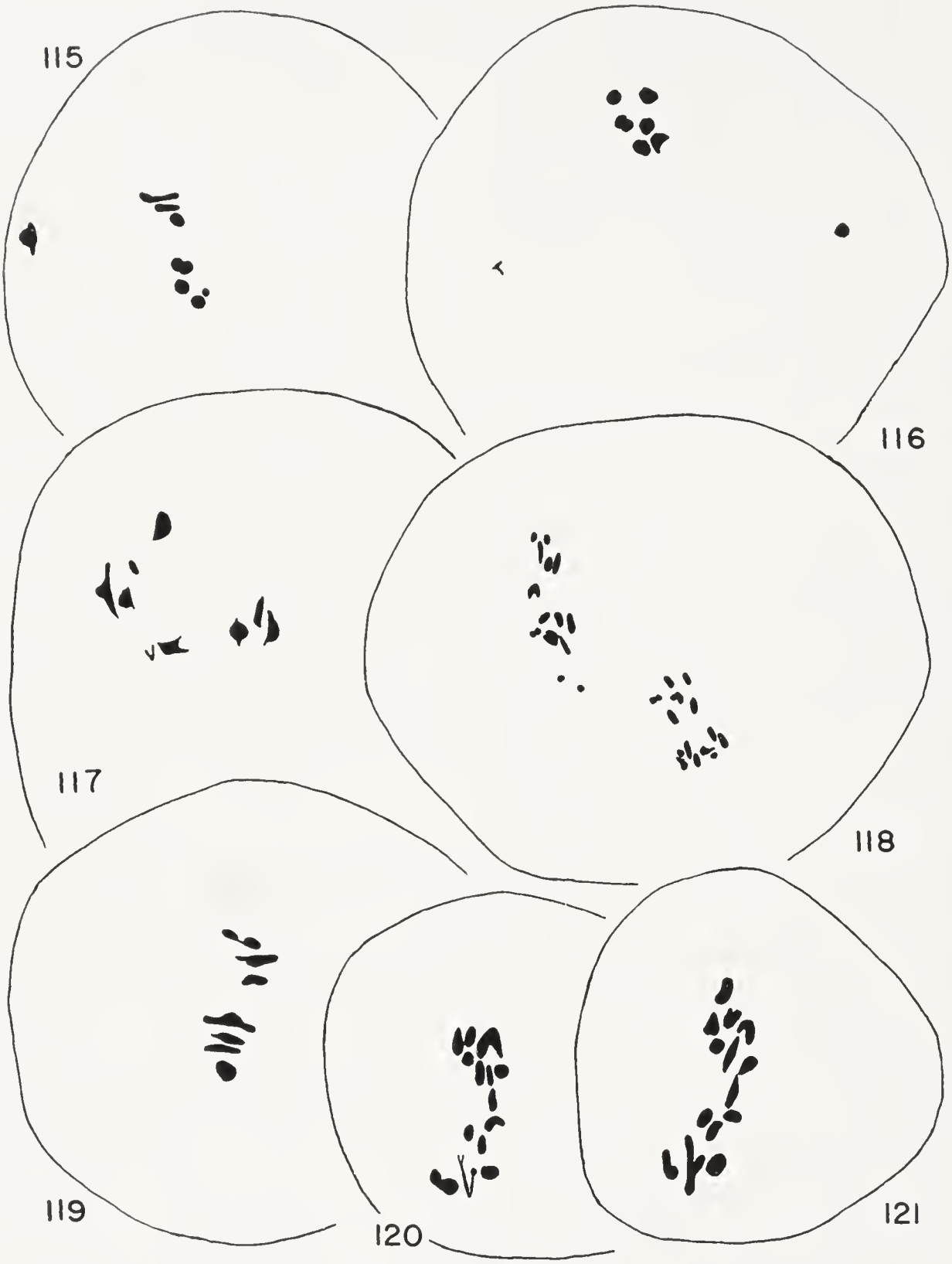

PLATE XII. FIGS. 115–118. *Anacolia menziesii* ($n = 8$). FIGS. 115–117. Very early stages of first meiotic metaphase. FIG. 118. Late anaphase of first division showing precocious dissociation. FIGS. 119–121. *Anacolia menziesii* var. *baueri* ($n = 7$) FIG. 119. Side view of first meiotic metaphase. FIGS. 120 and 121. Anaphase stages of first division. All figures × 2160.

tion (Kurita 1937) and for its var. *crispa*, in Finland (Vaarama 1950b); and $n = 12$ for *B. ithyphylla*, from Finland (Vaarama 1950b). For *Philonotis fontana*, Heitz (1928) estimated a chromosome number, $n = 7–8$, whereas Vaarama (1953b) definitely reports the number, $n = 6$, from a population in southern Finland. No American material of this family has been previously studied cytologically.

Hedwigiaceae

Hedwigia ciliata (Hedw.) Brid. (figs. 122–124): $n = 11$. The chromosome number we have established for a Californian population is of special interest in that it is just half the chromosome number, $n = 22$, reported by Vaarama from Finnish material (1950b). A small chromosome that disjoins prematurely presents another feature of interest in our material This species has, for mosses, an astonishingly wide geographical distribution, occurring in every continent, including Australia, and even extending throughout the tropics at higher altitudes. In view of its morphological variability and wide distribution, the presence of chromosome races is perhaps to be expected. Our material occurred on a large, exposed boulder along a stream on the west face of Mount Hamilton, Santa Clara County (LEA 46, March 24, 1953).

Pseudobraunia californica (Sull.) Broth. (*Braunia californica* Sull.) (figs. 125–126): $n = 11$. This is the first report of a chromosome number from a monotypic genus restricted in its geographic distribution to the Pacific Coast of North America, from California to British Columbia. Some significance should certainly be placed on the fact that the chromosome number agrees with that of Californian material of the related genus *Hedwigia*, in the same small family. The chromosomes at the first meiotic anaphase are remarkable for the distinct separation of the chromatids (fig. 126), as reported by Vaarama (1950b) for *Weissia viridula*, so much so that if it were not for their proximity, they might greatly confuse the counting of chromosomes. The chromosomes fall into several clear-cut classes on the basis of their size and shape. The population investigated was collected on a boulder at the Hedgpeth Ranch in western Sonoma County (WCS 20, Feb. 22, 1953).

Summary. Consisting of a half-dozen small genera, the Hedwigiaceae seem to reflect very ancient origins, in terms of the geographical distribution and specialized morphology of most members. Only one representative of the family, the single species of the genus *Hedwigia*, has been studied cytologically. Vaarama (1950b) found the chromosome number, $n = 22$, in a Finnish population, whereas in the Californian material, $n = 11$. The genus *Pseudobraunia*, also with a single species, *P. californica*, was found to have the chromosome number, $n = 11$. The agreement of the two genera

PLATE XIII. FIGS. 122–124. *Hedwigia ciliata* ($n = 11$), various stages of first meiotic metaphase, from early to median. FIGS. 125 and 126. *Pseudobraunia californica* ($n = 11$). FIG. 125. First meiotic metaphase, early. FIG. 126. First anaphase, late, showing premature division of univalent chromosomes. All figures $\times 2160$.

in their chromosome number may certainly be taken to some degree as positive evidence of their relationship.

Orthotrichaceae[3]

Orthotrichum affine Brid. (figs. 127–129): $n = 6$. The chromosomes of this species seemed to be unusually uniform in size and shape, among the group of species in which $n = 6$ The population studied occurs on moist shaded rocks and at the base of trees at Mirror Lake, Yosemite National Park, Mariposa County (WCS 64, April 20, 1953).

Orthotrichum bolanderi Sull. (figs. 130–133): $n = 6$. This well-marked species is endemic to California and is not uncommon in the San Francisco Bay area. The chromosome number, $n = 6$, seems very certain in both meiotic divisions, since it was established in large numbers of sporocytes from two populations. The only unusual behavior observed was the appearance in a single sporocyte of very small chromatin bodies in two of the four anaphase chromosome groups, in the second meiotic division (fig. 133). Material from both populations studied occurred on vertical rock faces, one from a rocky knob near Searsville Lake, San Mateo County, a few miles west of Stanford University (LEA 23, March 10, 1953), and from near the summit of Mt. Diablo, Contra Costa County (LEA 24, March 13, 1953).

Orthotrichum cylindrocarpum Lesq. (figs. 160–163): $n = 11$. This species is closely related to *O. tenellum* and considered as a variety of it by Koch (1950), but is maintained as a separate species by Grout (1946) in the most recent monographic treatment of the North American members of the family.

The chromosome behavior of the two forms at meiosis seemed very similar, in that both possess ten normal bivalents and one very small pair. The only difference noted was that the minute bivalent apparently lags in its disjunction, instead of dissociating precociously, since it showed no signs of doubleness at the first metaphase. The material was collected on the trunk of a roadside oak south of Olema, Marin County (WCS 74, April 28, 1953).

Orthotrichum lyellii Hook. & Tayl. (figs. 134–136): $n = 6$. The chromosomes of this species show more individuality and seem to be larger than those of the related species with six bivalent chromosomes. This species is a very common one on the West Coast from British Columbia to Mexico, and is the largest and most abundant *Orthotrichum* in California, where it forms large black tufts on trees or more rarely on rocks in regions in which fog is prevalent. Of the several populations studied, two may be

[3] Since a detailed account of the chromosome behavior and the cytotaxonomy of *Orthotrichum* is in preparation and will appear separately, the results are given here in abridged form, for the sake of completeness in the present report.

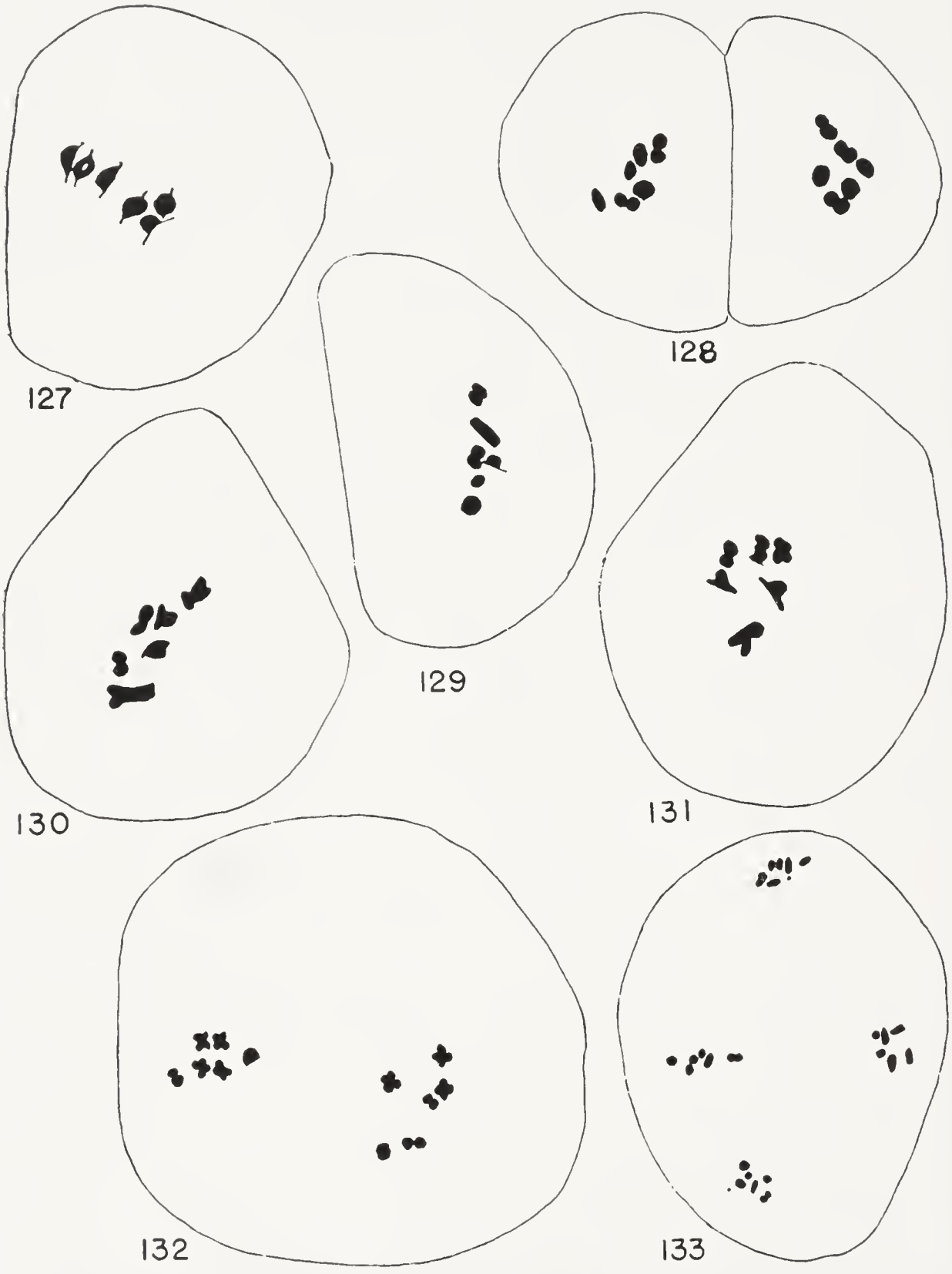

PLATE XIV. Figs. 127–129. *Orthotrichum affine* ($n = 6$). Figs. 127 and 129. Side view of first meiotic metaphase. Fig. 128. Polar view of simultaneous metaphases in sister sporocytes. Figs. 130–133. *Orthotrichum bolanderi* ($n = 6$). Figs. 130 and 131. Early stages of first meiotic metaphase. Fig. 132. Anaphase of first division, late. Fig. 133. Late anaphase stage of second division, with chromatic fragments in two nuclei. All figures × 2160.

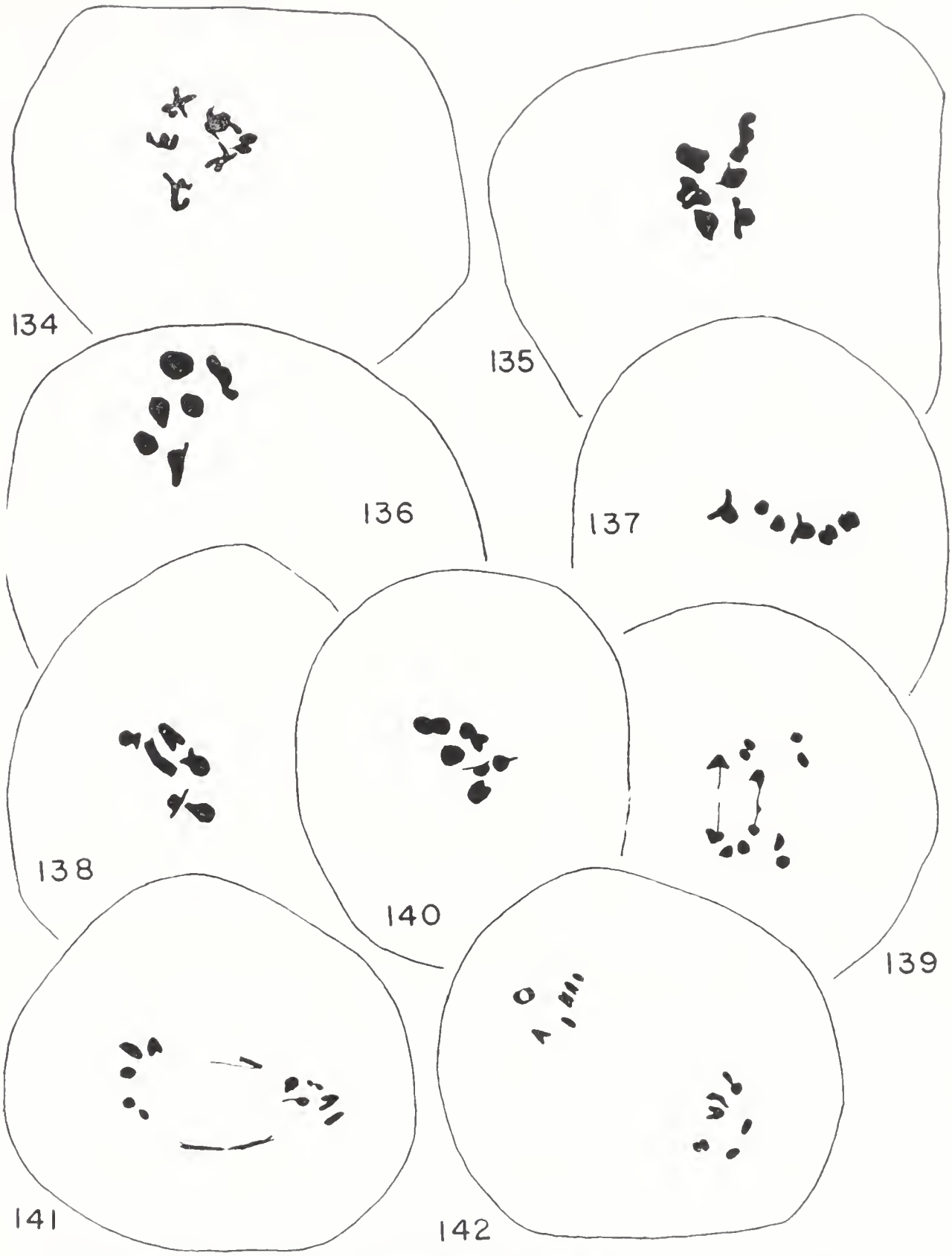

PLATE XV. Figs. 134–136. *Orthotrichum lyellii* ($n = 6$). Fig. 134. Late diakinesis stage, showing chiasmata. Figs. 135 and 136. Early and median metaphases, respectively, of first meiotic division. Figs. 137–139. *Orthotrichum rupestre* ($n = 6$). Figs. 137 and 138. Stages of first meiotic metaphase. Fig. 139. First anaphase, showing some irregularity of disjunction. Figs. 140–142. *Orthotrichum rupestre* var. *globosum* ($n = 6$). Fig. 140. Early metaphase, first meiotic division. Fig. 141. Anaphase of first division, with some irregularity of disjunction. Fig. 142. Telophase of first division. All figures $\times 2160$.

cited, one from the trunk of an oak on the south slope of Mt. Diablo, near the summit, Contra Costa County (WCS 25, March 13, 1953), and from tree trunks, Yosemite National Park, Mariposa County (VSB 54, April 5, 1953).

Orthotrichum rivulare Turn. (figs. 146–153): $n = 11$. In a genus characteristically found in xeric environments, usually growing on rocks and tree-trunks at some distance above the ground, this species is conspicuously atypical because of its amphibious habitat along streams, on rocks and tree bases that are submerged at high water. Nevertheless, the chromosome complement is very similar to that of the distantly related *O. tenellum*, in the presence of ten normal bivalents and one very small pair that dissociates precociously. A very considerable difference was noted in the appearance of the chromosomes, however, in the two populations studied, one from boulders in the rocky canyon of Stevens Creek, Santa Clara County (LEA 10, Feb. 12, 1953), and the other from the base of trees along Robinson Creek, a few miles southwest of Ukiah, Mendocino County (WCS 66, April 11, 1953). This difference may reflect either a minor chromosomal race or only metabolic variations in plants subjected to different degrees of desiccation.

Orthotrichum rupestre Schleich. (figs. 137–139): $n = 6$. This widely distributed species occurs in North America only in the Rocky Mountains and westward. The chromosome number, $n = 6$, observed in dozens of sporocytes in several capsules, can be recorded with confidence. One very large and elongated chromosome pair is conspicuous at the first meiotic metaphase, whereas the other bivalents are approximately equal in size. The material investigated came from large boulders, usually wet with spray, at the foot of Bridal Veil Falls, Yosemite National Park, Mariposa County (LEA 68, April 20, 1953).

Orthotrichum rupestre var. *globosum* (Lesq.) Grout (figs. 140–142): $n = 6$. The chromosomes of the variety are very similar in appearance to those of the species, but behaved much more irregularly during meiosis. Many sporocytes were observed to have lagging chromatids at the first meiotic anaphase (fig. 141). The population investigated grows on rather dry boulders in a ravine near Wawona, Yosemite National Park, Mariposa County (WCS 65, April 20, 1953).

Orthotrichum tenellum Bruch (figs. 154–159): $n = 11$. This species has already received detailed cytological study by Vaarama (1953a), who found different chromosome numbers in two capsules collected from the same population on the Stanford Campus. In one capsule, according to Vaarama, the sporocytes had 9 bivalents of normal size and two very small chromatic bodies, whereas in the other capsule, the sporocytes possessed 10 normal bivalents and one very minute chromatic body. In a half dozen capsules

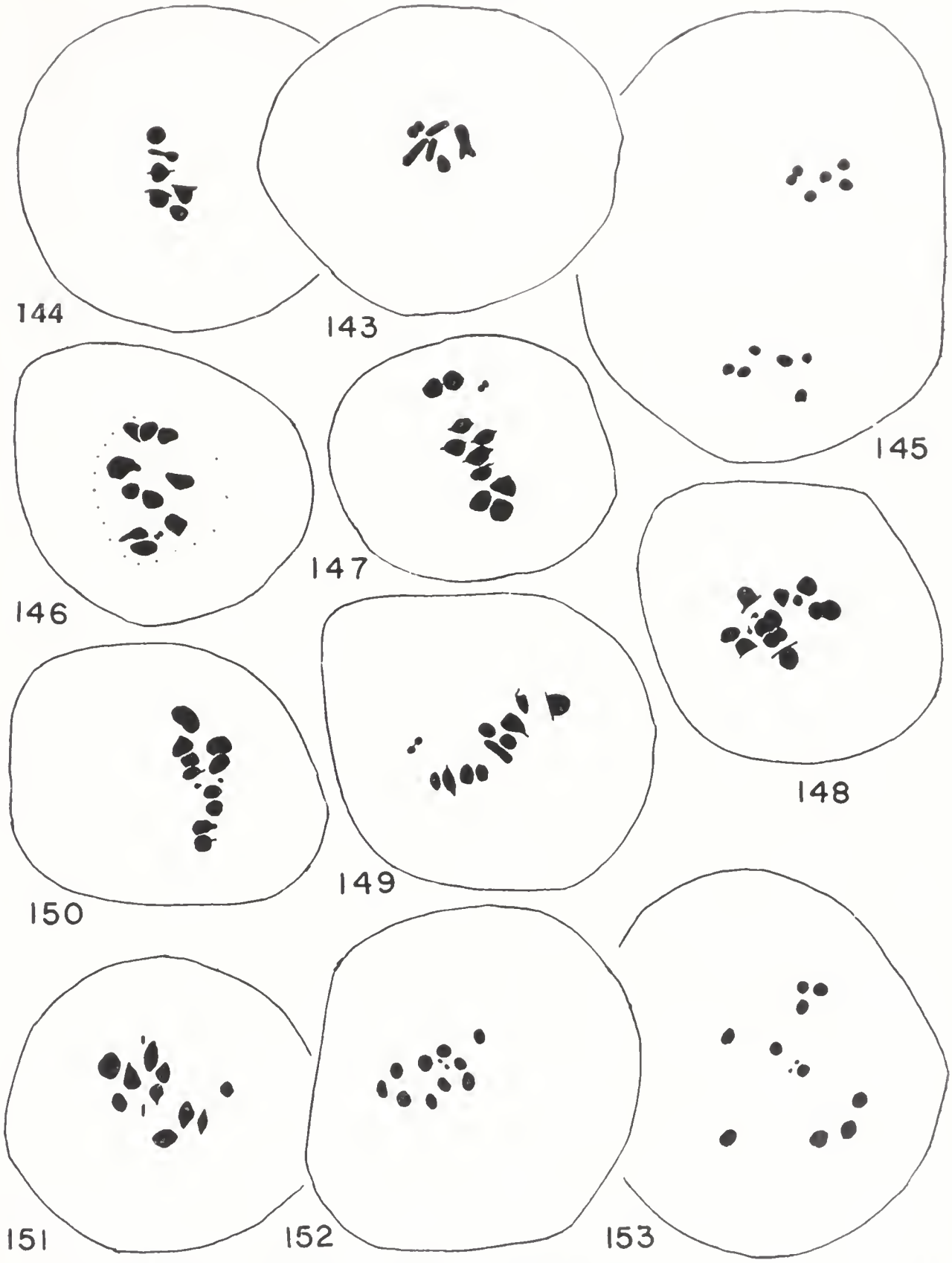

PLATE XVI. FIGS. 143–145. *Orthotrichum texanum* ($n = 6$). FIGS. 143 and 144. Early stages of first meiotic metaphase. FIG. 145. Metaphase of second division. FIGS. 146–151. *Orthotrichum rivulare* (Santa Clara County, $n = 11$). FIG. 146. Diakinesis stage, nuclear membrane still visible. FIGS. 147–151. Various stages of first meiotic metaphase, to show behavior of minute pair. FIGS. 152 and 153. *Orthotrichum rivulare* (Mendocino County, $n = 11$), polar view of first metaphases. All figures $\times 2160$.

investigated, and at least 50 sporocytes in each, we found the second situation described by Vaarama—ten normal bivalents and one minute pair. Vaarama interpreted the minute chromatic bodies to be accessory chromosomes, but because of their regular behavior, except for small size and precoeious dissociation, we prefer to consider them one pair. Their orientation at the first metaphase (figs. 157–158) and their regular behavior at the second metaphase (fig. 159) would seem to provide conclusive evidence for this interpretation. The material investigated occurred on the trunk of *Thuja*, near the summit of Mt. Tamalpais, Marin County (WCS 60, April 8, 1953).

Orthotrichum texanum Sull. (figs. 143–145) : $n = 6$. This western species is restricted in its geographic distribution to the Rocky Mountains, from British Columbia to Texas, and westward. The chromosome number, $n = 6$, was seen consistently in so many sporocytes that it can be accepted without doubt. We noted small differences between this and other species in the morphology of the chromosomes, of a sort that might very well be much more recognizable in the somatic chromosomes. The population investigated grows on rocks in Yosemite National Park, Mariposa County (VSB 56, April 5, 1953).

Summary. The Orthotrichaceae consists of some 15 genera which are primarily tropical in their geographical distribution and are rather remarkably specialized in their ecological preference for a habitat on the trunks of trees, or more rarely, on rocks or soil. *Orthotrichum,* with nearly 200 species, and *Zygodon*, with over 100 species, are widespread in the temperate regions of both hemispheres, and at higher altitudes in the tropics, whereas *Macromitrium*, with more than 400 species described, and *Schlotheimia*, with over 100 species, are essentially tropical and subtropical in their distribution. *Macromitrium* would make an especially interesting genus for cytological investigation, because of the great variation in morphology within the genus and because of the occurrence of so many species in any moist tropical region. Notwithstanding the large size of the genus, no chromosome counts have yet been reported, although interesting work on sexual dimorphism and heterospory done by Ernst-Schwarzenbach (1944), calls urgently for parallel cytological studies.

Most previous reports of chromosome numbers in the Orthotrichaeeae appear to be due to the excellent pioneer work of Vaarama (1950b, 1953a, 1953b), who gives the following counts: *Amphidium lapponicum,* $n = 16$; *Orthotrichum speciosum,* $n = 6$; *O. stramineum,* $n = 13$; *O. tenellum,* $n =$ 9–10, plus accessory chromosomes; and *Ulota curvifolia,* $n = 21$, plus accessory chromosomes. Although the present authors are reporting more fully elsewhere on chromosomal behavior in the Orthotrichaceae, it seems worthwhile to call attention to the fact that all Californian populations belong-

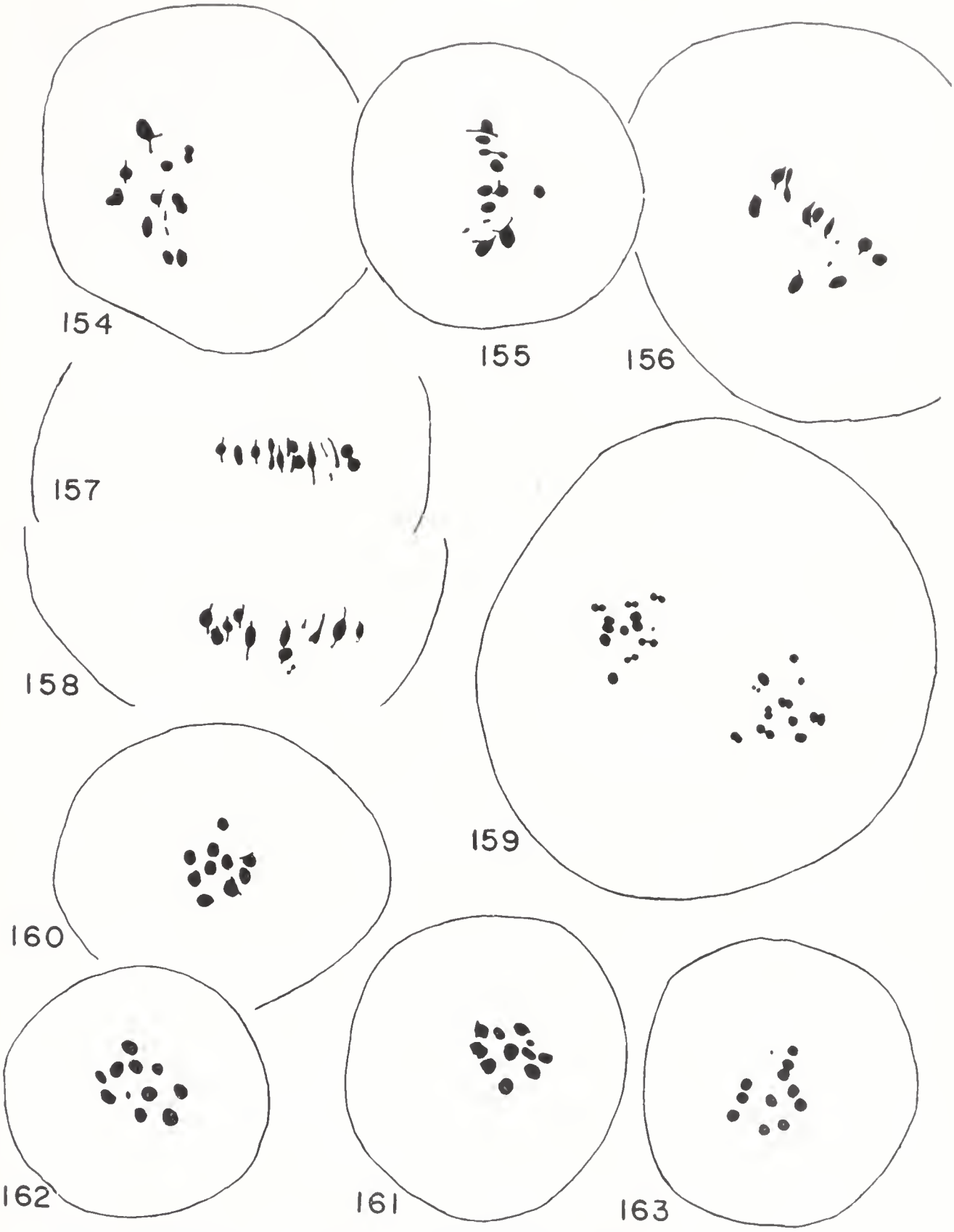

PLATE XVII. FIGS. 154–159. *Orthotrichum tenellum* ($n = 11$). FIGS. 154–158. Various stages of first meiotic metaphase, from early to median, to show behavior of minute pair that dissociates precociously. FIG. 159. Metaphase of second division in polar view. FIGS. 160–163. *Orthotrichum cylindrocarpum* ($n = 11$), polar view of first meiotic metaphases. All figures $\times 2160$.

ing to the section *Rupestria* that were investigated had the chromosome number, $n = 6$: *Orthotrichum affine, O. bolanderi, O. lyellii, O. rupestre* and its var. *globosum*, and *O. texanum*. In this connection, it should be noted that *O. speciosum*, belonging to the same section, has been reported to have the same chromosome number, from Finnish material (Vaarama 1950b). Such uniformity of chromosome number would seem to indicate natural relationships for the species grouped in this section. In two populations of *O. rivulare*, in the well-marked section *Rivularia*, we found the chromosome number, $n = 11$, which is also present in *O. cylindrocarpum* and *O. tenellum*, in the section *Tenella*, and in the Japanese species, *O. consobrinum* (Yano 1951). Vaarama (1953b) reports the number, $n = 13$, for *O. stramineum*, of the section *Straminea*, which would indicate a still different series of chromosome numbers in a further section of the genus.

Neckeraceae

Porothamnium bigelovii (Sull.) Fleisch. (figs. 164–166): $n = 12$ (11?). The only question concerning the chromosome number of this species arose from the appearance of polar views of metaphase plates of the first meiotic division; one pair of chromosomes appeared to be recently separated, but still adjacent (fig. 166). Furthermore, in pre-metaphase stages, the chromosome number, $n = 11$, was occasionally encountered, lending support to the idea that one of the chromosome pairs perhaps disjoins prematurely. This species possesses unusually small sporocytes, a fact all the more remarkable when the large size and handsome appearance of the moss is taken into consideration. The material investigated was found on a steep, rocky bank of a cold stream in the Big Basin State Park, Santa Cruz County (LEA 30, March 18, 1953).

Summary. Neckeraceae is a large, wide-ranging, essentially tropical and subtropical family that consists of about twenty genera. The chromosome number just indicated for *Porothamnium bigelovii* is of importance in being the first reported for the genus. It is to be hoped that the much debated taxonomic problems involved in the interrelationships of *Porothamnium, Porotrichum,* and *Thamnium,* which have led to much nomenclatural confusion, may perhaps find some clarification through continued chromosomal studies. Yano (1951) has reported the chromosome number, $n = 11$, in two species of *Thamnium*.

Thuidiaceae

Claopodium whippleanum (Sull.) Ren. & Card. (figs. 167–169): $n = 11$. The chromosomes of this very abundant and almost weedy Californian species fall into widely differing size classes at the first meiotic metaphase. Furthermore, the members of one small bivalent tend to disjoin prema-

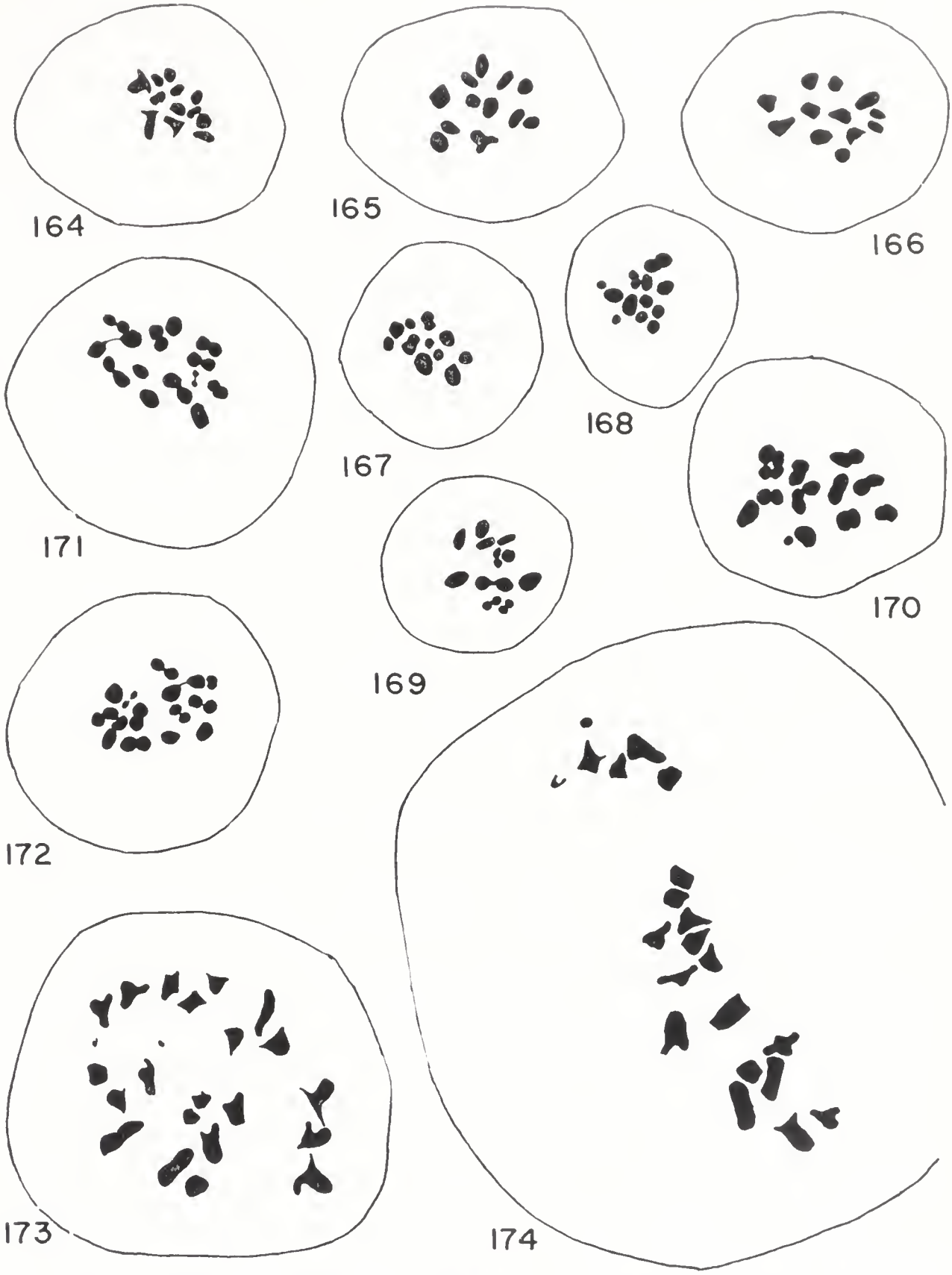

PLATE XVIII. FIGS. 164–166. *Porothamnium bigelovii* ($n = 12$), polar view of first meiotic metaphases, from early to median. FIGS. 167–169. *Claopodium whippleanum* ($n = 11$), polar view of first meiotic metaphases. FIGS. 170–172. *Amblystegium juratzkanum* ($n = 13$), polar view of first meiotic metaphase. FIGS. 173 and 174. *Hygroamblystegium irriguum* ($n = 20$). FIG. 173. Late diakinesis-early metaphase stage. FIG. 174. Side view of first meiotic metaphase. All figures × 2160.

turely, but since this process was observed in all its stages, it did not confuse the chromosome count unduly. In view of the few chromosome counts reported previously in this family, for several very distantly related Japanese species, no cytotaxonomic conclusions can yet be drawn. Our material came from a population growing on soil over large boulders in the deep shaded canyon of a stream, Big Basin State Park, Santa Cruz County (VSB 37, March 18, 1953).

Summary. The Thuidiaceae, a medium sized family containing some 15 genera, is widely distributed geographically. With the exception of *Thuidium,* with more than 150 described species, the genera are rather small. The only chromosome number previously reported for members of the genus is $n = 10$, for *Thuidium japonicum* (Shimotomai & Koyama 1932), *T. viridiforme*, *T. micropteris,* and *T. uliginosum* (Yano 1951).

Amblystegiaceae

Amblystegium juratzkanum Schimp. (figs. 170–172): $n = 13$. With the exception of an extremely small pair, the chromosome number of this species agrees very well with that of *A. serpens,* as reported by Marchal (1912) and by Wettstein (1924). The bivalent chromosomes fall into several size classes, and were observed to tend toward premature dissociation. The material was collected from a population growing on moist stones in a pool at the Japanese Garden, in Golden Gate Park, San Francisco (VSB 33, March 19, 1953).

Hygroamblystegium irriguum (Wils.) Loeske (figs. 173–174): $n = 20$. Marchal (1912) reported the chromosome number of this very common and widely distributed aquatic moss to be $n = 12$, on the basis of European material, of course. We find that the Californian population studied possesses a very different chromosome complement, of which one bivalent is extremely small, and divides prematurely. The occurrence of chromosome races in so widely distributed and variable a species is not surprising, and American specimens vary from the European type to such an extent that we may really be dealing with two distinct species. The population investigated here grows at and below water level on rocks in a pool at the Japanese Garden, in Golden Gate Park, San Francisco (LEA 32, March 19, 1953).

Summary. The dozen genera comprising the Amblystegiaceae are all relatively small, but of very wide geographical distribution, usually in moist to very wet habitats. Members of this family may attain a very great abundance in bogs, swamps, and pools, and may even become the dominant vegetation there. *Amblystegium serpens* was used as experimental material both by the Marchals (1911, 1912) and by Wettstein (1924), and through apospory, a series of polyploid chromosome races was produced,

with the chromosome numbers, $n = 24$ ($2n$) and $n = 48$ ($4n$). Vaarama has reported for this same species the chromosome number, $n = 22$, from populations from Finland (1950b) and from California (1953b). Marchal (1912) reported chromosome numbers for species in two other genera, *Hygroamblystegium irriguum* ($n = 12$; as *Amblystegium*) and *Leptodictyum riparium* ($n = 24$; as *Amblystegium*). Moutschen (1952) has recently confirmed Marchal's count for *Amblystegium riparium*, and has induced autopolyploidy in this species by means of colchicine. Heitz (1928) has given estimates of the chromosome number of *Calliergonella cuspidata* as $n =$ "(9)10"; of *Calliergon richardsonii* as $n = 20$; and of *Campylium stellatum* as $n =$ "6–8." Vaarama (1950b, 1953b) has reported the chromosome numbers of three species of *Drepanocladus: D. fluitans*, $n = 22$; *D. exannulatus* and *D. uncinatus*, $n = 12$. This family, like most aquatic and semiaquatic groups of plants, is considered to be an unusually difficult one by botanists because of the great variability of the species under differing environmental conditions with resulting taxonomic confusion.

Brachytheciaceae

Homalothecium nevadense (Lesq.) Ren. & Card. (figs. 175–180): $n = 12$. The chromosomes of this species are distinguished not only by the two very small pairs, but also by the fact that several of the normal bivalent chromosomes seem quite constantly to absorb the stain to a lesser degree than others. Consequently, division figures rather characteristically presented an odd appearance of deeply and lightly stained chromosomes. This phenomenon was seen in several capsules, thus removing the chance that it might represent the idiosyncracy of some individual sporophyte. Furthermore, the chromosomes appeared to be somewhat diffuse and not as sharply delimited as in the other mosses studied. Whether this behavior was caused by the methods used in fixing and staining, or whether by some condition inherent in the species, we are unable to say, since similar phenomena were not observed elsewhere. Vaarama (1950b) remarks that the matrix of the chromosomes of *Brachythecium* appears to be less firm than usual, so that the bivalents tend to stick together very commonly. The material was collected from a population that abounds on huge, mist-swept boulders at the foot of Bridal Veil Falls in Yosemite National Park, Mariposa County (WCS 69, April 20, 1953).

Summary. With more than 20 genera, the Brachytheciaceae occur in all parts of the world, although most commonly and abundantly in the more temperate climates. Containing over 200 species, *Brachythecium* is by far the largest genus, although *Rhynchostegium* contains more than 100 species. Chromosome numbers for six species of *Brachythecium* have been reported, as follows: *B. albicans*, $n = 9$, *B. populeum*, $n = 9$, 10, *B. salebro-*

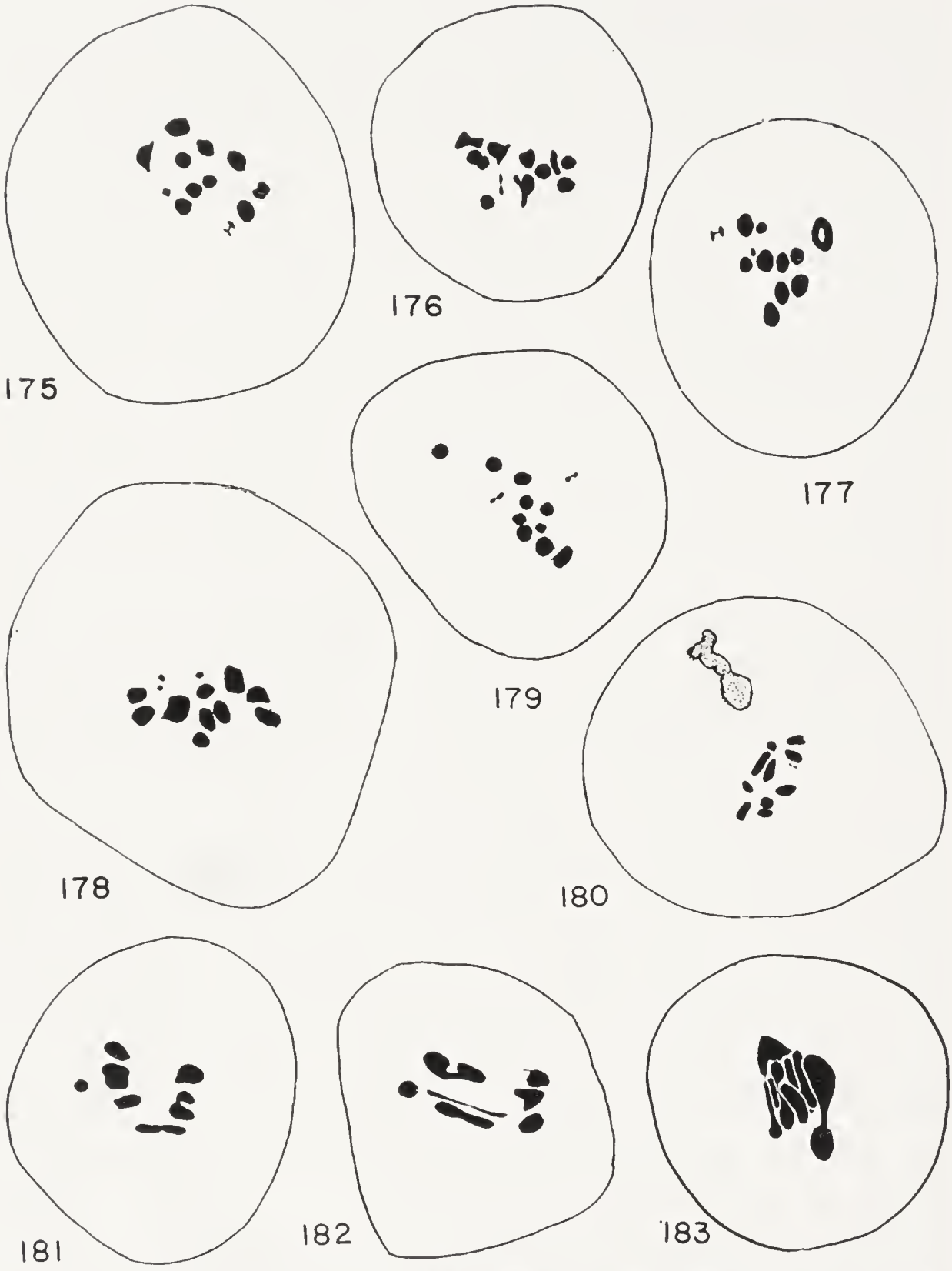

PLATE XIX. Figs. 175–180. *Homalothecium nevadense* ($n = 12$). Figs. 175–179. Various stages of first meiotic metaphase, from very early to median, to show behavior of minute pairs of "accessory" chromosomes. Fig. 180. Polar view of second metaphase, showing minute chromosome dividing; one chromosome plate in side view. Figs. 181–183. *Atrichum undulatum* (California race, $n = 7$). Figs. 181 and 182. Metaphase stages of first meiotic division. Fig. 183. Anaphase of first division, showing heteromorphic chromosomes. All figures $\times 2160$.

sum, $n = 13$, *B. starkei* ssp. *curtum*, $n = 20$, *B. relutinum*, $n = 11$ (Vaarama 1950b), and *B. rutabulum*, $n = 11$ (Sinoir 1952). Heitz (1928) has estimated the chromosome number of *Eurhynchium rusciforme* as $n = 6–8$; of *E. schleicheri* as $n = 8(9)$; and of *Scleropodium purum* as $n = 9–11$. As already noted, this family seems to be characterized cytologically by the difficulties it presents in the fixing and staining of chromosomes.

Polytrichaceae

Atrichum undulatum (Hedw.) Beauv. (figs. 181–183): $n = 7$. The moss reported upon here is a very common one in the Coast Ranges of California, where it has rather generally been called *A. undulatum* (Koch 1950), although Lesquereux (1868) reported what is undoubtedly the same thing, from "Banks, Santa Cruz Mts., alt. 2,200 ft.," as *A. angustatum* (Brid.) Br. & Sch. However, it should be pointed out that this species is neither *A. undulatum* nor *A. angustatum*, as it differs from the former in being clearly dioecious, in its chromosome number (Lowry 1948, Sinoir 1952), and in the very low and inconspicuous lamellae on the upper surface of the leaf; and from the latter in the broad, strongly undulate leaves, with a relatively narrow costa. Very young plants offer a remarkable resemblance to *A. crispum*, in the short, broad, obtuse leaves. Older leaves develop conspicuous undulations as well as spines on the dorsal surface, which preclude any possibility that it may be *A. crispum*. Some possibility exists that this species may be identical with one already described from the Orient or even from tropical America. Since a final identification of this species is certain to be a time-consuming and laborious task, we have decided to continue using the name, *Atrichum undulatum*, because of its previous application to our material, but with the understanding that some other name will eventually have to be applied to it.

The chromosomes are clearly in 7 pairs, with one larger and obviously dimorphic bivalent. Our material was collected in the Coast Range west of Stanford University, on a soil bank along Alpine Road near its junction with Page Mill Road, at an altitude of over 1000 feet (WCS 44, March 31, 1953). An interesting feature of this collection was the production, in late March, of occasional young sporophytes, accompanying numerous older, deoperculate sporophytes. The long drought in early winter of 1953 and its termination through the resumption of winter rains apparently enabled a new crop of sporophytes to develop, quite out of season.

Summary. Of the more than a dozen genera of Polytrichaceae, a family of large and conspicuous mosses, only three have been investigated cytologically. The chromosome numbers of seven species of *Polytrichum*, nine species of *Pogonatum* and three species of *Atrichum* have now been reported, and distinct cytotaxonomic significance appears in the fact that

TABLE I. *Chromosome numbers in fifty-five species of Californian Musci (Names in italics represent first reports)*

Name and source of material	Reference Numbers	Chromosome Number	Figures
Fissidentaceae			
Fissidens limbatus Sull.			
Stanford University, Santa Clara Co.	LEA 1	$n = 5$	1–4
Fissidens pauperculus Howe			
Muir Woods, Marin Co.	LEA 53	$n = 12$	5–7
Ditrichaceae			
Ceratodon purpureus (Hedw.) Brid.			
Big Basin State Park, Santa Cruz Co.	WCS 43	$n = 13$	8–10
Yosemite National Park, Mariposa Co.	VSB 55	$n = 13$	
Ceratodon purpureus fo. *xanthopus* (Sull.) Britt.			
East of Tomales, Marin Co.	WCS 57	$n = 13$	11
Ditrichum schimperi (Lesq.) Paris			
Muir Woods, Marin Co.	LEA 59	$n = 26$	12–13
Pleuridium bolanderi C. Müll.			
Hedgpeth Ranch, Sonoma Co.	WCS 12	$n = 26$	14–16
Dicranaceae			
Anisothecium varium (Hedw.) Mitt.			
Stanford University, Santa Clara Co.	LEA 2	$n = 14$	17–19
Dicranoweisia cirrata (Hedw.) Lindb.			
Big Basin State Park, Santa Cruz Co.	WCS 31	$n = 11$	20–22
Encalyptaceae			
Encalypta vulgaris Hedw. var. *mutica* Brid.			
Mt. Hamilton, Santa Clara Co.	WCS 39	$n = 13$	23–25
Pottiaceae			
Aloina ambigua (BSG.) Limpr.			
Stanford University, Santa Clara Co.	LEA 6	$n = 24$	26–28
Barbula brachyphylla Sull.			
Mt. Hamilton, Santa Clara Co.	WCS 45	$n = 12$	29–30
Barbula convoluta Hedw.			
Searsville Lake, San Mateo Co.	WCS 36	$n = 11$	31–32
Barbula vinealis Brid.			
Stevens Creek, Santa Clara Co.	WCS 8	$n = 14$	
San Mateo County Park	VSB 19	$n = 14$	33–34
Desmatodon hendersonii (Ren. & Card.) Williams			
Alum Rock State Park, Santa Clara Co.	WCS 51	$n = 13$	35–37
Phascum cuspidatum Hedw. var. *americanum* R. & C.			
Hedgpeth Ranch, Sonoma Co.	WCS 13	$n = 29–30$	41–43
Mt. Hamilton, Santa Clara Co.	WCS 41	$n = 26$	38–40
Pottia davalianum (Smith) Steere			
Stanford University, Santa Clara Co.	LEA 7	$n = 30$	44–45
Timmiella vancouveriensis Broth.			
Muir Woods, Marin Co.	WCS 58	$n = 14$	46–48
Tortula bolanderi (Lesq.) Howe			
Searsville Lake, San Mateo Co.	VSB 38	$n = 13$	49–50
Tortula laevipila (Brid.) Schwaegr.			
Alpine Road, San Mateo Co.	VSB 47	$n = 12$	51–52
Tortula muralis Hedw.			
Stanford University, Santa Clara Co.	WCS 5	$n = 48$	53–54
Tortula princeps De Not.			
Mt. Hamilton, Santa Clara Co.	VSB 52	$n = 12$	55–57
Stevens Creek, Santa Clara Co.	WCS 5a	$n = 24 + 1$	58–59
Hedgpeth Ranch, Sonoma Co.	WCS 15	$n = 36 + 2$	60–61
Tortula subulata Hedw.			
Yosemite National Park, Mariposa Co.	WCS 71	$n = 49$	62–63

TABLE 1 (continued)

Name and source of material	Reference Numbers	Chromosome Number	Figures
Weissia viridula Hedw.			
Searsville Lake, San Mateo Co.	VSB 26	$n = 13$	64
Mt. Tamalpais, Marin Co.	WCS 75	$n = 13$	65–66
Grimmiaceae			
Grimmia alpestris (Web. & Mohr) Nees			
Yosemite National Park, Mariposa Co.	WCS 72	$n = 13$	67–70
Grimmia apocarpa Hedw.			
Mt. Hamilton, Santa Clara Co.	VSB 40	$n = 13$	71–73
Yosemite National Park, Mariposa Co.	WCS 70	$n = 13$	74–75
Grimmia pulvinata (Hedw.) Smith			
Stevens Creek, Santa Clara Co.	WCS 9	$n = 13$	
Mt. Diablo, Contra Costa Co.	LEA 28	$n = 13$	76–77
Grimmia trichophylla Grev.			
Mt. Diablo, Contra Costa Co.	VSB 29	$n = 13$	78–79
Rhacomitrium depressum Lesq.			
Yosemite National Park, Mariposa Co.	WCS 67	$n = 14$	80–82
Funariaceae			
Funaria hygrometrica Hedw.			
Hedgpeth Ranch, Sonoma Co.	WCS 11	$n = 28$	83–84
Funaria muhlenbergii Turn. var. *patula* BSG.			
Alum Rock State Park, Santa Clara Co.	VSB 50	$n = 28$	85
Bryaceae			
Bryum argenteum Hedw. var. *lanatum* BSG.			
Hedgpeth Ranch, Sonoma Co.	WCS 4	$n = 12$	86–88
Bryum capillare Hedw.			
Stanford University, Santa Clara Co.	LEA 3	$n = 10+$	89–91
Bryum pseudotriquetrum (Hedw.) Schwaegr.			
East of Tomales, Marin Co.	LEA 61	$n = 11$	92–95
Epipterygium tozeri (Grev.) Lindb.			
Stanford University, Santa Clara Co.	WCS 17	$n = 11$	96–99
Alpine Road, San Mateo Co.	WCS 48	$n = 11$	
Orthodontium gracile Schwaegr.			
La Honda Road, San Mateo Co.	LEA 22	$n = 12$	104–107
Pohlia longibracteata Broth.			
San Mateo County Park	VSB 18	$n = 12$	100–103
Mniaceae			
Leucolepis menziesii (Hook.) Steere			
San Mateo County Park	LEA 21	$n = 5$	108–111
Aulacomniaceae			
Aulacomnium androgynum Schwaegr.			
Big Basin State Park, Santa Cruz Co.	VSB 42	$n = 12$	112–114
Bartramiaceae			
Anacolia menziesii (Turn.) Paris			
West of Saratoga, Santa Clara Co.	WCS 34	$n = 8$	115–118
Anacolia menziesii var. *baueri* (Hpe.) Paris			
Mt. Diablo, Contra Costa Co.	VSB 27	$n = 7$	119–121
Hedwigiaceae			
Hedwigia ciliata (Hedw.) Brid.			
Mt. Hamilton, Santa Clara Co.	LEA 46	$n = 11$	122–124
Pseudobraunia californica (Sull.) Broth.			
Hedgpeth Ranch, Sonoma Co.	WCS 20	$n = 11$	125–126
Orthotrichaceae			
Orthotrichum affine Brid.			
Yosemite National Park, Mariposa Co.	WCS 64	$n = 6$	127–129
Orthotrichum holanderi Sull.			
Searsville Lake, San Mateo Co.	LEA 23	$n = 6$	130–133
Mt. Diablo, Contra Costa Co.	LEA 24	$n = 6$	

TABLE I (continued)

Name and source of material	Reference Numbers	Chromosome Number	Figures
Orthotrichum cylindrocarpum Lesq.			
South of Olema, Marin Co.	WCS 74	$n = 11$	160–163
Orthotrichum lyellii Hook. & Tayl.			
Mt. Diablo, Contra Costa Co.	WCS 25	$n = 6$	134–136
Yosemite National Park, Mariposa Co.	VSB 54	$n = 6$	
Orthotrichum rivulare Turn.			
Stevens Creek, Santa Clara Co.	LEA 10	$n = 11$	146–151
Ukiah, Mendocino Co.	WCS 66	$n = 11$	152–153
Orthotrichum rupestre Schleich.			
Yosemite National Park, Mariposa Co.	LEA 68	$n = 6$	137–139
Orthotrichum rupestre var. globosum (Lesq.) Grout			
Yosemite National Park, Mariposa Co.	WCS 65	$n = 6$	140–142
Orthotrichum tenellum Bruch			
Mt. Tamalpais, Marin Co.	WCS 60	$n = 11$	154–159
Orthotrichum texanum Sull.			
Yosemite National Park, Mariposa Co.	VSB 56	$n = 6$	143–145
Neckeraceae			
Porothamnium bigelovii (Sull.) Fleisch.			
Big Basin State Park, Santa Cruz Co.	LEA 30	$n = 12(11)$	164–166
Thuidiaceae			
Claopodium whippleanum (Sull.) Ren. & Card.			
Big Basin State Park, Santa Cruz Co.	VSB 37	$n = 11$	167–169
Amblystegiaceae			
Amblystegium juratzkanum Schimp.			
Golden Gate Park, San Francisco Co.	VSB 33	$n = 13$	170–172
Hygroamblystegium irriguum (Wils.) Loeske			
Golden Gate Park, San Francisco Co.	LEA 32	$n = 20$	173–174
Brachytheciaceae			
Homalothecium nevadense (Lesq.) Ren & Card.			
Yosemite National Park, Mariposa Co.	WCS 69	$n = 12$	175–180
Polytrichaceae			
Atrichum undulatum (Hedw.) Beauv.			
Alpine Road, San Mateo Co.	WCS 44	$n = 7$	181–183

with very few exceptions the species have the chromosome number, $n = 7$, or some multiple of it (Lowry 1948, Sinoir 1952). The genus *Atrichum* is of special interest as it demonstrates a well-developed polyploid series. *Atrichum angustatum* has the chromosome number, $n = 7$ (Lowry 1948, Tatuno 1951), whereas *A. undulatum* is apparently a diploid or triploid form, with the chromosome number, $n = 14$ or $n = 21$ (Lowry 1948, Kurita 1937). It is of interest to observe the close correlation of dioecism with haploid species (in terms of the gametophyte), and of monoecism with diploid ones, as clearly pointed out in the genus *Mnium* by Heitz (1942) and by Lowry (1948).

Discussion. Chromosome numbers. Even including chromosome numbers for over 50 species of mosses reported in the present paper, the total number of species for which definite chromosome counts have been published still remains considerably below 200, out of about 20,000 described species of mosses. In view of this very small proportion of species whose

chromosome numbers are known compared with those that have not received cytological investigation, it would be premature to make any large-scale generalizations at this time. However, some very general conclusions can be reached, even if the details may be considerably changed as studies on chromosome behavior in mosses continue. Compared with pteridophytes (Manton 1950), the chromosome numbers in bryophytes are relatively small,

TABLE II. *Frequency of occurrence of chromosome numbers among moss species*

Chromosome Number	Number of Species	Chromosome Number	Number of Species
5	3	20	5
6	23	21	2
7	26	24	4
8	7	25	1
9	4	26	5
10	12	28	7
11	21	30	2
12	25	36	1
13	17	48	1
14	14	49	1
15	2	52	1
16	3	60	1
18	2	66	1

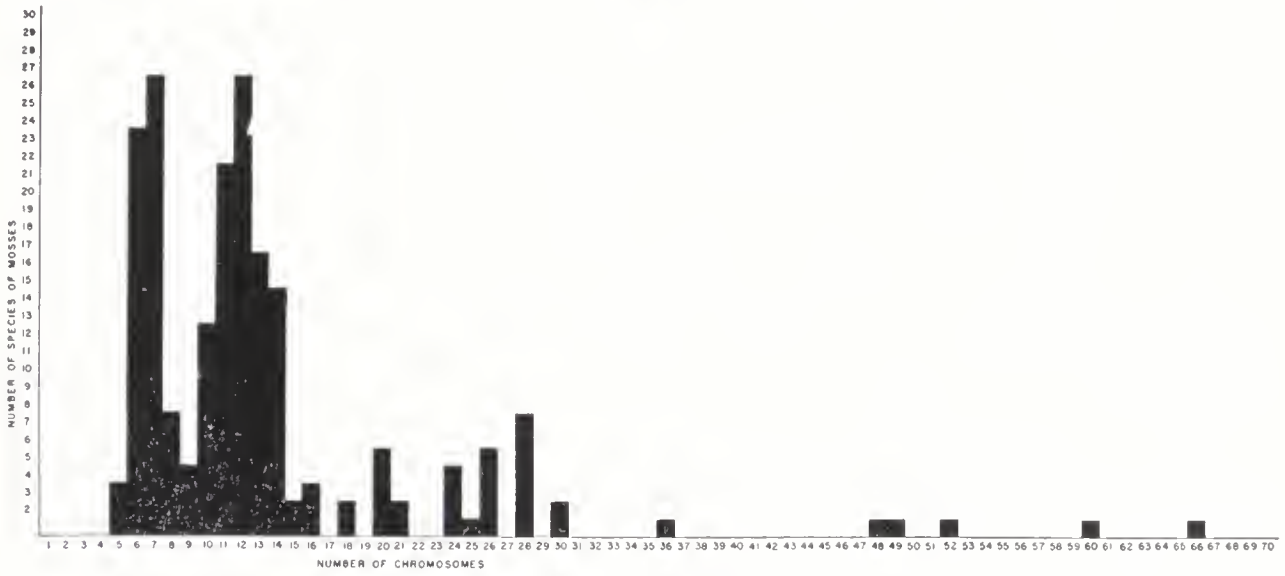

TABLE III. *Graphical representation of chromosome number frequency in mosses, based on all published reports of definite numbers (through 1953), as well as the present investigation.*

and agree surprisingly well with the basic numbers found in flowering plants. The lowest chromosome number yet found in mosses is $n = 5$, reported previously for only two species, *Mnium* (*Leucolepis*) *menziesii* (Lowry 1949) and for *Hylocomium* (*Pleurozium*) *schreberi* (Vaarama 1950b). The present investigation substantiates the count for *Leucolepis menziesii* and adds *Fissidens limbatus* to the list of those with five pairs.

The highest numbers of chromosomes yet known in mosses have been reported by Vaarama (1953b), for *Tortula muralis,* where $n = 60$ and $n = 66$, and for *Phascum cuspidatum,* where $n = 52$. In the course of the present investigation, high chromosome numbers were found in *Tortula muralis* ($n = 48$) and in *T. subulata* ($n = 49$). All these species with high chromosome numbers belong to the same family, the Pottiaceae, in which lower chromosome numbers are also found. The Mniaceae seem to display consistently the lowest numbers of chromosomes for a whole family, with the basic numbers, $n = 6$, $n = 7$, and $n = 8$, and their multiples, except for *Leucolepis menziesii,* already mentioned, in which $n = 5$. A summary of the chromosome numbers so far definitely reported for mosses, excluding estimates, and including the present investigation, shows several interesting features (Table II). An interesting feature of this table is the unexpeetedly elear polymodality of chromosome numbers in mosses, a condition that will probably be emphasized by future work, rather than reduced. The numbers, $n = 6$ and $n = 7$, oceur in almost equal frequeney, in 23 and 26 species, as do the numbers $n = 11$ and $n = 12$, in 21 and 25 species. Smaller modes are found at $n = 20$, with five species, $n = 25$, with four species, and $n = 26$ and $n = 28$, with five and seven species, respectively. Because of the unexpected clearness of the modes in this preliminary analysis, it seems highly possible that each one represents a level of ploidy. Consequently, although many species and genera would seem to have the basic number of $n = 10$, $n = 11$, $n = 12$, $n = 13$, or $n = 14$, these may be already at a tetraploid level (in terms of the sporophyte). Since the autoploid condition presents certain advantages to the moss gametophyte, as discussed later, this condition has probably been stabilized for so long in some species or groups that now it does amount to the basic number. On the other hand, further investigation may still result in the discovery of a true haploid number in related species or genera.

Before chromosome counts in species of mosses can be accepted finally, much more work needs to be done. Within species, inconsistencies already have been discovered among the chromosome numbers of different populations in different areas. There is no question that chromosome raees exist in nature, as shown for *Timmia cucullata* by Lowry (1953), for *Tortula ruralis* and *Orthotrichum tenellum* by Vaarama (1953a, b) and for *Atrichum undulatum* by Heitz (1926, 1928). Further examples can be cited from the present investigation, as *Phascum cuspidatum* and *Tortula princeps.* The identification of Californian moss populations with European speeies may occasionally result in considerable straining of speeifie concepts, as suggested by the fact that several of the California mosses have different ehromosome numbers than the European species under whose names they pass, as *Phascum cuspidatum* ($n = 26$ or 29–30 in California,

$n = 52$ in Finland), *Weissia viridula* ($n = 13$ in California, $n = 14$ in Finland), *Grimmia apocarpa* ($n = 13$ in California, in two populations; $n = 12$ in Finland), *Funaria muhlenbergii* ($n = 28$ in California, $n = 26$ in Europe), *Bryum argenteum* ($n = 12$ in California, $n = 10$ in Europe), *Bryum pseudotriquetrum* ($n = 11$ in California, $n = 10$ in Europe), *Mnium pseudopunctatum* ($n = 14$ in Michigan, $n = 13$ in Europe), *Hedwigia ciliata* ($n = 11$ in California, $n = 22$ in Finland), and *Hygroamblystegium irriguum* ($n = 20$ in California, $n = 12$ in Europe). As a consequence of the foregoing considerations, a chromosome number reported from a single area or a single population, although in itself a significant datum, cannot be accepted finally as the chromosome number of the species throughout its whole geographic range.

Chromosome studies of the somatic tissues of the gametophyte of mosses present the advantages, as shown by Lowry (1948), that morphological distinctions are generally much more obvious than in the more contracted meiotic chromosomes, and that the presumably haploid chromosome number is encountered there, free of the complications often found at meiosis. An additional point of interest, apt to be overlooked, is that the gametophyte population is usually clonal. With very few exceptions the sexual plants are long-lived, and they are generally provided abundantly with some means for vegetative reproduction, as from the continued growth of the original protonemata, from the repeated growth and branching of the plants themselves, from regeneration of almost any cell of the stem or leaf, and from specialized cellular structures of various types, as gemmae and propagula, that result in the rapid propagation of the green plants. In some species, perhaps because of the success of vegetative propagation, sporophytes are rarely or never produced. The sporophytes, however, can not be members of clones, strictly speaking, since each one results from a separate fertilization, and so is homologous to a whole seed plant. However, because of the large expanses of a single gametophytic clone, especially if it is monoecious, the parentage of the sporophytes is probably very uniform, although no studies on this problem in nature have been undertaken. Nevertheless, since each sporophyte can have a somewhat different genotype, if accidents of fertilization so dictate, considerable variation in genetic stability can be expected. The discovery by Vaarama (1953a) of two chromosome races in the two sporophytes of *Orthotrichum tenellum* he studied and our finding of three chromosome races in three populations of *Tortula princeps* might indicate a considerable degree of hybridization with a possible impact on chromosome numbers and speciation.

Polyploidy. It should be recalled here that the first polyploid series known in plants was created artificially in mosses through apospory. The discovery by Pringsheim (1878) that the young sporophytes of several

species of mosses would regenerate gametophytic tissues was so far ahead of his time that its importance was not then properly appreciated. With the realization of the cytological differentiation of sporophyte and gametophyte and of the alternation of a haploid sexual generation with a diploid asexual generation by Strasburger (1894), the significance of apospory was finally understood. The brilliantly conceived and executed researches of the Marchals resulted in the experimental production of a tetraploid race ("var. *bivalens*") of *Bryum capillare* (Marchals 1911) and of tetraploid and octaploid ("var. *bivalens*" and "var. *quadrivalens*") races of *Amblystegium serpens* (Marchals 1911, 1912). Wettstein and others, in more modern investigations (1923–1924), succeeded in producing autoploid and amphiploid races in several mosses, including *Funaria hygrometrica* ($n = 14$, $n = 28$, $n = 56$), *Physcomitrium piriforme* ($n = 18$, $n = 36$, $n = 72$), *Bryum caespiticium* ($n = 10$, $n = 20$), and *Amblystegium serpens* ($n = 12$, $n = 24$). Heitz (1945) was able to produce polyploidy in *Aulacomnium androgynum* through the action of colchicine, although the chromosome numbers were not established definitely. Moutschen (1952) obtained similar results in *Amblystegium riparium* with colchicine. The discovery of polyploids in natural populations was much delayed, however, largely because of the technical problems already outlined. The first instance of polyploidy in mosses, in nature, seems to be represented by the report by Heitz (1926) of a diploid (in terms of gametophyte) race of *Atrichum undulatum* with the chromosome number of "14–16," and his subsequent report (1928) of a triploid race with the number "(20)21(22)." Later (1942), Heitz elaborated on this thesis to some extent, and pointed out the existence in nature of paired species, one diploid and the other tetraploid, as *Mnium spinosum* and *M. spinulosum*, *Mnium orthorhynchum* and *M. marginatum*, *Mnium punctatum* and *M. pseudopunctatum*. He further showed an interesting relationship between such paired species, that the haploid species are heterothallic and that the diploid species are homothallic, indicating a strong possibility that the diploid species arose through sporophytic regeneration or apospory. Of course, there is equal possibility that such races have arisen through unreduced spores, and occasional giant spores are seen in mosses. Vaarama (1949) has reported the occurrence of polyploid areas in the archesporium of *Rhacomitrium ramulosum* and of *Grimmia muhlenbeckii*, which could give rise to diploid spores. It is clear that diploid sectors or chimeras of the gametophytic moss plant would still retain the sex of the haploid parent tissue, and would be genetically unable to produce a monoecious plant. This fact lends some strength to Heitz's hypothesis that natural apospory has been effective in producing diploid races or species of mosses. Since these paired diploid species have not yet been reproduced in the laboratory through the experimental in-

duction of apospory, we still do not know whether in nature they arose long ago and have undergone further evolution to reach their clear-cut isolation as species, or whether they have been produced repeatedly from time to time, up to the present. The latter possibility presents a very striking and unique means of polyphyletic speciation, by which all populations comprising a species might not be related, except in terms of the parent species. Lowry (1948) has confirmed most of Heitz's findings, and has reported other cases of paired species in *Mnium*, of the same type, as well as haploid and diploid races (in terms of the gametophyte) within *Mnium cuspidatum*. As already indicated, we have found that a California population in *Heduigia ciliata* has the chromosome number, $n = 11$, without doubt, whereas the same species has been reported from Finland as having the number, $n = 22$ (Vaarama 1950b). Furthermore, we have found diploid ($n = 12$), tetraploid ($n = 24 + 1$) and hexaploid ($n = 36 + 2$) races of *Tortula* princeps, out of the three races studied, raising some interesting speculations on what further investigations of this handsome species will bring to light. Other examples of natural polyploidy, although at a somewhat different level, can be cited. Lowry (1953) has demonstrated that a population of *Timmia cucullata* in southern Michigan has the chromosome number, $n = 16$, whereas a population from Wisconsin, studied in considerable detail by Scheuber (1932), is reported to have the chromosome number, $n = 12$. Another example of this type is given by *Tortula muralis*. In two populations from Berkeley, Vaarama (1953b) found chromosome numbers of $n = 60$ and $n = 66$, whereas a population at Stanford University has the number, $n = 48$. Again, the future of investigations on this species would seem very promising, for obvious statistical reasons.

The surprisingly large proportion of species that are apparently tetraploid or of higher ploidy, shown by Table II and its extension in graph form, is reflected by cytological behavior. Multiple associations of chromosomes at meiosis were observed occasionally to regularly in several species, notably *Timmiella vancouveriensis* (fig. 48) and *Bryum capillare* (fig. 90). Quadripartite chromosomes were observed from time to time at the first anaphase, and a clear case of multiple association at the second division was seen in *Bryum argenteum* var. *lanatum* (fig. 88), but not well understood. One of the most common evidences of polyploidy in species with apparent basic numbers of 10, 11, 12, 13 and 14, is the obvious duplication of chromosomes, and their tendency to remain in proximity, even into the second division. The prevalence of tetraploid species in which the basic number appears to be an odd one, especially $n = 11$ or $n = 13$, seems to be explained by the usual presence of one or more larger "pairs" which give evidence of being multivalent structures. *Ceratodon purpureus* offers an excellent example, in which the large sex chromosome (Heitz 1932, Ja-

chimsky 1935) is accompanied by 12 autosomes of about the same size. The rather clear secondary pairing of autosomes (figs. 8, 9) would seem to indicate that the present chromosome number, $n = 13$, may well have been derived from an original number, $n = 14$, or from the hybridization and subsequent doubling at some remote past time of two species with the chromosome number, $n = 7$. In highly polyploid members of the Pottiaceae, many of the chromosomes at the first metaphase, in polar view, appear to be in rows or in chains, probably reflecting the mutual attraction of duplicated chromosomes (figs. 53, 54 and 61).

The original, truly haploid, chromosome number of mosses is of eourse impossible to determine at present, at least without comprehensive genetical work. However, there seems to be some evidence for the view that the most frequent basic numbers so far encountered, $n = 6$ and $n = 7$ (Table II) may themselves represent chromosome duplication in whole or in part. Many of the excellent figures given by Lowry (1948), of somatic chromosomes from the gametophyte of different species of *Mnium* with the basic number, $n = 6$, show more than a suggestion of such duplication, not only in shape and size, but also in orientation. Lowry's table of chromosome length (1948) does not offer much positive evidence for such a view, however, since in only one species did he find two chromosomes of the same length. An apparent duplication of chromosomes is also seen occasionally at meiosis in species with low basic numbers, as in *Orthotrichum rupestre* (fig. 137). If the gametophyte of mosses is genuinely haploid, then the genes cannot occur in allelomorphic pairs, but would have been segregated at meiosis.

Aneuploidy. Most reports of aneuploidy in mosses seem to represent races derived from chromosome fragmentation, as shown most graphieally in *Orthotrichum tenellum* by Vaarama (1953a). A few aneuploid races may possibly be indicated by reports of varying chromosome numbers in the same species by different authors, although the chance of error or misinterpretation of chromosome counts cannot be overlooked, especially in early investigations, before the development of adequate techniques. For example, earlier workers reported the chromosome number, $n = 6$, in *Polytrichum commune* (Woodburn 1915), *Polytrichum juniperinum* (Allen 1912), and *Polytrichum piliferum* (Vandendries 1912), whereas recent investigations (Vaarama 1950b) show the chromosome number of these three species to be $n = 7$. In these examples, we are undoubtedly dealing with the results of refinement of technique rather than aneuploid raees. Other disagreements in published chromosome counts, where the European population differs from the American one, have already been listed. These differences may be due as much to overextension of speeifie or varietal limits as to true intraspecific aneuploidy. However, an outstanding example of

true aneuploidy was discovered in the course of the present investigation, in *Phascum cuspidatum* var. *americanum*, in which a race from Mt. Hamilton, Santa Clara County, has the chromosome number, $n = 26$ (figs. 38–40), whereas a race from Sonoma County has the number, $n = 29$ and 30 (figs. 41–43). These races have been isolated geographically for long periods of time, since they occur in separate mountain ranges, within the system of the Coast Range. The chromosomes differ not only in number, but in the presence of two pairs of very minute chromosomes in the Sonoma County race. It seems safe to predict that further examples of aneuploidy as clear as this one will be discovered as chromosome studies on mosses continue.

Chromosome fragmentation may result in races of mosses with different chromosome complements, as beautifully demonstrated by Vaarama's somewhat fortuitous discovery that, of two capsules of *Orthotrichum tenellum* from the same population, one had nine bivalents whereas the other had ten. After an analysis of the bivalents, he offers the following explanation "it seems most probable that the increased bivalent number of sporogone 2 is due to fragmentation of the long A-bivalent present in sporogone 1. The fragmentation has obviously taken place through the submedian constriction present in the A-bivalent, and the two new bivalents formed correspond to the arms of the long chromosome. It may also be stated as likely that the A-bivalent is a fusion product of the two bivalents mentioned present in sporogone 2." A similar situation was discovered in *Weissia viridula,* in which the California population contains thirteen bivalents, whereas the European one is reported to have fourteen bivalents (Vaarama 1950b). Vaarama figures these fourteen bivalents as more or less the same size (his fig. 2a, b), whereas all the sporocytes seen, of the Californian race, contain twelve bivalents of approximately the same size and one very large one (figs. 64–66). It is clear that the fragmentation of this large chromosome would result in a 14-bivalent race—or that the more or less permanent association of two bivalents in the European race would result in the condition seen in the Californian race. A study of somatic chromosomes in these races, both of *Orthotrichum* and of *Weissia,* would be very rewarding, as providing material more suitable for quantitative studies. Lowry (1948) has demonstrated clearly that chromosome fragmentation has taken place in a Michigan race of *Mnium pseudopunctatum,* in which $n = 14$. In this species he found two very minute somatic chromosomes averaging .075 μ in length and two much longer chromosomes, averaging 2.7 μ long, with terminal attachment regions. It would seem clear from this evidence that the minute chromosome represents the short arm and the longer chromosome with terminal attachment represents the longer arm of a J-shaped chromosome with a subterminal attachment, a normal type in *Mnium*. The population studied by Lowry is of especial interest since in

Europe this species is reported to have the chromosome number, $n = 13$ (Heitz 1942, Vaarama 1950b), which would indicate that only one of the J-shaped chromosomes of the pair had fragmented. The discovery by Vaarama (1950b) of races within *Brachythecium populeum* with chromosome numbers of $n = 9$ and $n = 10$ may also be related to chromosome fragmentation.

Accessory chromosomes. Accessory chromosomes or accessory isochromosomes were first reported in Bryophyta by Vaarama, in two species of mosses, *Grimmia muehlenbeckii* (1949) and *Dicranum majus* (1950a). Later, he reported this type of chromosome in *Ulota curvifolia* (1950b) and in *Orthotrichum tenellum* (1953a, b). In the present investigation, very small chromosomes and chromosome pairs, of the kind that Vaarama has called accessory chromosomes, were found regularly in 16 species of California mosses, as follows, *Anisothecium varium* (figs. 17–19), *Aloina ambigua* (figs. 26–28), *Desmatodon hendersonii* (figs. 35–37), *Phascum cuspidatum* var. *americanum* (the Sonoma County race, figs. 41–43), *Tortula princeps* (figs. 58–61), *Tortula subulata* (figs. 62–63), *Bryum capillare* (figs. 89–91), *B. pseudotriquetrum* (figs. 92–95), *Orthodontium gracile* (figs. 104–107), *Anacolia menziesii* (figs. 115–118), *Orthotrichum rivulare* (figs. 146–153), *O. cylindrocarpum* (figs. 160–163), *O. tenellum* (figs. 154–159), *Amblystegium juratzkanum* (figs. 170–172), *Hygroamblystegium irriguum* (figs. 173–174), and *Homalothecium nevadense* (figs. 175–180). Very minute supernumerary chromatic bodies were observed very rarely in other species that did not seem typically to have accessory chromosomes, but, of course, they could have been overlooked. Such bodies were seen, for example, in one sporocyte of *Bryum argenteum* var. *lanatum* (fig. 87), and in *Orthotrichum bolanderi* in only two of the four anaphase groups following the second meiotic division (fig. 133), in a single sporocyte. There is some possibility that these are the result of chromosome fragmentation in single sporocytes. In still other species, the range of the size of bivalents decreases in a regular scale to the point at which the smallest pairs fall within the size range of the so-called accessory chromosomes, as in *Fissidens pauperculus* (figs. 5–7), *Timmiella vancouveriensis* (fig. 47) and *Tortula bolanderi* (fig. 50), as well as in many others of the obviously polyploid members of the Pottiaceae, *Rhacomitrium depressum* (fig. 82), and *Aulacomnium androgynum* (figs. 112–114).

With the exception of *Bryum capillare*, in which five very minute chromosomes are grouped closely together just before the first metaphase (fig. 89) and are then apparently distributed unequally during anaphase (fig. 91), the so-called accessory chromosomes of the remaining 15 species behaved with some to very great regularity. Except for their very small size and their prevalent tendency to dissociate either before or after the first

meiotic metaphase, they seem to behave reasonably normally as bivalents, and to divide regularly in the second division (fig. 159), so that each spore will contain a minute chromosome representing the original accessory chromosome. As the normal bivalents in mosses seem to demonstrate a considerable amount of precocious dissociation, and as they may also be very small, thus providing a point at which normal bivalents and accessory chromosomes find common ground, we decided to include all chromatic bodies that behaved regularly in the chromosome count. Because of the large number of species which were observed to have accessory chromosomes—more than 25 per cent of the Californian species studied—considerable attention was given to their behavior, as a result of which we find ourselves in some disagreement with Vaarama, especially in his analysis of *Orthotrichum tenellum* (1953a). Vaarama reported the presence of two accessory chromosomes in "sporogone 2," which had 10 normal bivalents, and four accessory chromosomes in "sporogone 1," which had only nine normal bivalents. Although we have not rediscovered the anomalous race represented in Vaarama's "sporogone 1," we have investigated many capsules from a population with 10 bivalent chromosomes, and with what Vaarama has reported as two accessory chromosomes. However, even though some of our evidence may appear to be circumstantial, it is our opinion that these accessories really represent one bivalent structure. Although this tiny bivalent obviously dissociates very early, the orientation of the two parts at the first metaphase certainly conforms to the usual behavior of bivalent chromosomes (figs. 154–156), and their habit of dividing prematurely during the first anaphase (figs. 157–158) definitely parallels the not uncommon behavior of univalent chromosomes. Furthermore, in *Orthotrichum cylindrocarpum*, which is so closely related to *O. tenellum* that it has been considered to be only a variety of it by Koch (1950), the accessory chromosomes appear as a single minute bivalent (figs. 160–163). In *O. rivulare,* which is not particularly closely related to *O. tenellum*, but which has the same chromosome number, the accessory bivalent is obviously double at diakinesis (fig. 146) and at early metaphase (figs. 147–149). At metaphase, its parts disjoin to produce much the same condition as seen in *O. tenellum* (fig. 150). The very similar chromosomal behavior of these three species, except for the exceptionally precocious disjunction of the so-called accessory chromosomes of *O. tenellum*, has led us to the conclusion that in all of them, $n = 11$. Exactly parallel situations may be found in other genera and families, and after a survey of so many species, one can not help but be impressed by the regularity in behavior, rather than the lack thereof, of the accessory chromosomes. A study of the somatic chromosome complements of species with accessory chromosomes at meiosis would now seem to be an urgent necessity. A point of considerable interest

conversely, is that all the species in which accessory chromosomes have been found so far are probably polyploid (Tables II and III). It is also of some significance that accessory chromosomes have not yet been shown to occur in species of mosses with such basic chromosome numbers as $n = 5$, $n = 6$, or $n = 7$. Nevertheless, accessory chromosomes may eventually be discovered to occur at the diploid level in mosses, even if with considerably smaller frequency than in polyploids. This possibility is clearly demonstrated by Cleland's (1951) recent report of the occurrence of "extra, diminutive chromosomes" in two wild races of *Oenothera hookeri* in California. His stimulating discussion of the possible methods by which such chromosomes might arise is certainly applicable to the situation in mosses.

Sex chromosomes. Sex chromosomes were first discovered in plants in the hepatic, *Sphaerocarpos* (Allen 1917), on a presumptive basis, at first, but later they were demonstrated to be the sex-determining mechanisms through long-continued and fruitful genetic experiments (Allen 1945). However, in many other dioecious bryophytes, larger and usually dimorphic chromosome pairs have been called sex chromosomes without further proof, and have been so accepted in the literature. It should be pointed out that any such report of sex chromosomes, without genetical proof, can be only an assumption or an hypothesis. In mosses, sex chromosomes have been reported in perhaps a dozen species by Heitz (1932), Shimotomai and Koyama (1932), Shimotomai and Kimura (1936), Jachimsky (1935), Vaarama (1950a), and Tatuno (1951). In the course of the present investigation, dimorphic chromosomes were observed distinctly in *Ceratodon purpureus* (figs. 8–11), *Weissia viridula* (figs. 64–66), *Leucolepis menziesii* (figs. 109–111) and in the Californian race of *Atrichum undulatum* (fig. 183). In many other species, dimorphic chromosomes were observed at certain stages of the first meiotic division, but not so constantly or as conspicuously as in those just listed. A detailed investigation of heterochromosomes in Californian mosses should be rewarding, because of the large number of dioecious species, and the many indications of interesting phenomena seen in our preliminary observations. The otherwise excellent work of Ernst-Schwarzenbach on sexual dimorphism and heterospory in *Macromitrium* (1944) leaves untouched this aspect of the chromosomal situation which would appear to be a central part of the whole problem. The sexual dimorphism in the gametophytes of some mosses is remarkable in that the male plant is reduced to an almost microscopic, bud-like structure that is epiphytic on the large female plant, recalling the nannandrous condition of some species of *Oedogonium*. The only species of this sort that has been studied cytologically is *Dicranum majus* (Vaarama 1950a), in which a complicated chromosomal situation was discovered, including a large bivalent assumed to be the site of the sex-determining genes.

Meiotic features. Unlike the situation reported in previous papers (Vaarama 1949, 1950b), we found prophase stages of meiosis very abundantly in the Californian populations, including well-marked stages of diakinesis. In California, the sporophytes of most mosses mature in late winter and early spring, when the cold nights, especially at higher altitude, must cause the suspension of meiosis. In the present investigation, it was observed that the sporocytes of mosses brought in from the field early in the day were often in late prophase or tetrad stages. Exposure to light and warmth, however, induced the young sporocytes to resume meiosis. In view of the many chromosomal aberrations noted at the first and second meiotic divisions, a careful study of prophase behavior is urgently needed, especially of pachytene stages.

Premature disjunction and division. One of the serious problems in counting the chromosomes of mosses is the precocious dissociation of bivalents. As already outlined under the discussion of several highly polyploid members of the Pottiaceae, the counting of chromosomes in some species must amount to an estimate rather than an exact count, because of the high proportion of univalent chromosomes at the first division. Occasional cells are seen in which all the bivalents have dissociated before the first metaphase, as in *Fissidens limbatus* (figs. 3 and 4). Fortunately, the univalents can be identified with some certainty because of their smaller size (compare figs. 1 and 2 with 3 and 4). If it were not for the smaller size of the chromosomes where double the usual number is present, one could interpret these as polyploid cells. At the anaphase of the first division, the univalent chromosomes may divide prematurely in preparation for the second division, to such an extent that accurate counts might be jeopardized were it not for the proximity and the orientation of the chromosomes. Vaarama (1950b) reported behavior of this sort in *Weissia viridula*, and we have observed the same phenomenon in several species, most notably in *Pseudobraunia californica* (fig. 126). In occasional figures of some species, the first anaphase chromosomes clearly show a quadripartite nature, either as the result of long established polyploidy or in preparation for the first somatic division of the spore, a point that was not resolved. The double nature of the chromosomes at the first anaphase also appears in the metaphase of the second division (fig. 159).

Delayed disjunction. The lagging of chromosomes was observed in many sporocytes, and has been well documented by Vaarama (1949) in members of the Grimmiaceae, as well as in other mosses (1950b). Nevertheless, in observations on thousands of tetrads during the course of this investigation, not one was seen with more or less than four spores, except those in one capsule of *Weissia viridula*, where giant spores and diads were found. It may be that spores containing other than the usual complement abort

or disintegrate after the separation of the four members of each tetrad. The techniques evolved for estimating chromosomal aberrations in higher plants by counting the proportion of fertile to sterile pollen should eventually prove to have some application to mosses.

Satellites. Although undoubtedly present in somatic chromosomes of many species, satellites were seen at meiosis in a single species, *Leucolepis menziesii* (fig. 111), which appears to be the first moss for which these chromosomal features can be reported.

Chiasmata. It was observed that each species has its own fairly constant proportion of bivalents with interstitial and terminalized chiasmata. The constant occurrence in many species of nearly equal numbers of bivalents of the two types would seem to indicate that external factors are not responsible for the inhibition of terminalization, or conversely, were not responsible for conditions aiding the terminalization of chiasmata. Consequently, it is concluded that the nature of chiasmata in the bivalents of mosses, at least in the species investigated, is determined genetically rather than environmentally. Diakinesis stages were found to be especially helpful in interpreting chiasmata, as seen in *Dicranoweisia cirrata* (fig. 20) and *Orthotrichum lyellii* (fig. 134).

Sporocyte size. The spore mother cells varied greatly in size among the species investigated, from 10 μ in diameter in *Fissidens* (figs. 1–7) to more than 40 μ in *Pottia* (fig. 44) and *Pseudobraunia* (fig. 126). No correlation could be seen between the size of sporocytes and other factors, as annual habit, size of plants, dryness or moistness of habitat, number of chromosomes, or total amount of chromatin present. Small sporocytes may have very large numbers of small chromosomes, as in *Tortula muralis* (fig. 53), or a few large chromosomes as in *Atrichum* (figs. 181–183) and *Leucolepis* (figs. 108–111). Conversely, large sporocytes may have a small number of chromosomes, as in *Hedwigia* (figs. 122–124) and *Pseudobraunia* (figs. 125 and 126), or a much larger one, as in *Funaria* (fig. 85).

General conclusions. Investigations on the chromosome behavior of mosses are still too few for any final and detailed conclusion. Nevertheless, some generalizations are possible. For example, in most families in which several representatives have been studied, it becomes clear that the chromosome numbers within the family tend to be related to each other in terms of a basic number or within a rather narrow range of variation. Within genera, likewise, a marked correspondence in the basic chromosome number usually appears, in the few cases where several species in the same genus have been studied. Conversely, the conspicuous deviation from the normal chromosome number in the genus *Mnium* found in *Leucolepis menziesii* establishes a cytological basis for its recognition as a valid segregate genus, as suggested by Lowry (1948). Further studies on chromosome numbers

and behavior in large and taxonomically difficult genera will be especially helpful in interpreting the position of anomalous members of such genera, in establishing the limits of sections and subgenera, and perhaps in estimating the degree of hybridity and genetic stability in the species. However, a great deal of detailed cytological research is necessary before the results of chromosomal studies will be really useful to taxonomists at the specific and subspecific levels. Lowry's detailed investigation of the somatic chromosomes of American populations of several species of *Mnium* represents the first serious cytotaxonomic study of mosses, and establishes the value and utility of such work. Persson (1949) has made some penetrating and thoughtful comments on the lack of morphological and anatomical features of cryptogamic plants, as compared with the phanerogams, that creates many difficulties in distinguishing between vicarious races and species. He even suggests the somewhat metaphysical possibility that different species might appear identical, simply for want of distinguishing features. This question is an appropriate one here, in view of the current assumption, already mentioned, that many European and American moss populations belong to the same species, and the very general application of European names and concepts to American species. However, it has been shown in the present investigation, and to some extent in earlier ones, that the chromosome complement of some of the American populations differs from that of the supposedly identical European species. Thus, the chromosome numbers, morphology, and behavior may furnish useful new criteria for the better understanding of an old problem.

Too few genera and families of mosses have been studied cytologically to enable us to see any clear relationship between chromosomes, either in number or morphology, and phylogeny. The fossil record of bryophytes is almost non-existent, because of the small size and delicate structure of the plants (Steere 1946), so that all phylogenetic arrangements are almost purely speculative, with serious conflicts among them. The high chromosome numbers and numerous polyploid series found in the Pottiaceae represent an advanced feature, in terms of evolutionary specialization. It is still too early to correlate chromosome behavior with annual and perennial habit, as data on annual species is nearly lacking.

From time to time the question arises whether the species of cryptogams are coordinate with or equivalent to the species of higher plants, a problem that has deserved serious consideration by botanists. The cytological behavior of bryophytes, both normal and anomalous, appears to parallel that of phanerogams very closely, as is shown especially well by the existence of polyploid and aneuploid races, the multiple association of chromosomes during the first and second meiotic divisions, the precocious disjunction of bivalent chromosomes and of chromatids, and the presence of so-

called accessory chromosomes, and of sex chromosomes. Finally, it seems safe to conclude that species of mosses, and of bryophytes in general, in spite of their relatively simple morphology, present the same kind and degree of complexity as those of higher plants, and that speciation within natural populations of mosses is governed by the same patterns of cytological behavior.

Summary

1. The chromosome behavior at meiosis of 73 populations of Californian mosses, mostly from the San Francisco Bay region, has been studied.

2. Chromosome numbers were determined for 55 species, representing 39 species, 16 genera, and 2 families whose cytology had not been investigated previously.

3. Previous reports on chromosome numbers of mosses are integrated with the present results, where appropriate, and a general discussion of the significance of chromosome numbers within families is given.

4. Since the chromosome studies were made on temporary mounts, through the use of aceto-orcein and the squash method, voucher specimens have been preserved, with detailed locality data, so that any of the populations may be found again if further investigation is desirable.

5. In the species investigated, a range of chromosome numbers from $n = 5$ to $n = 49$ was found.

6. A graph of the frequency of species with different chromosome numbers is clearly polymodal, with the highest frequencies at $n = 6–7$, $n = 11–12$, $n = 20$, $n = 24$, and $n = 28$, suggesting a high incidence of polyploidy.

7. Polyploidy was encountered within several species, notably in *Tortula princeps,* in which three different populations were found to represent three different stages of polyploidy, $n = 12$, $n = 24+$, $n = 36+$.

8. Aneuploidy was discovered in several species, but was most conspicuous in *Phascum cuspidatum* var. *americanum,* in which two races presented the chromosome numbers, $n = 26$ and $n = 29–30$.

9. So-called accessory chromosomes were found in more than 25 per cent of the species studied. As they differ from normal bivalent chromosomes primarily in their small size and generally precocious disjunction, they were included in the chromosome counts. These minute chromosomes were found only in species that appear to be polyploid.

10. Larger, heteromorphic bivalent chromosomes, assumed to be sex chromosomes, were seen in several species.

11. Multiple association of chromosomes was found in the first meiotic division of several species whose chromosome number indicates their intrinsic polyploidy. Other cytological evidence for polyploidy was the precocious division of first anaphase chromosomes into two or rarely four parts.

12. Precocious disjunction of bivalents was not infrequent, but very rarely involved all bivalent chromosomes.

13. Species of mosses are concluded to be completely comparable to those of higher plants. Speciation in populations of mosses appears to be governed by the same chromosomal behavior patterns, and perhaps at an even higher rate.

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