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SUBSEXUAL REPRODUCTION IN *AGROPYRON*

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1. INTRODUCTION

THE grass *Agropyron scabrum* is found in both islands of New Zealand, in Norfolk Island and in eastern Australia. It is a complex of morphologically distinct populations which preserve their separate character both in nature and in cultivation. They do so because they are self-pollinated and true-breeding, and they form no natural hybrids (Connor, 1954).

A preliminary study of meiosis in this complex showed that whereas in some populations chromosome pairing was normal, in others it was less regular, and in one population there was little or no pairing at all. Four populations were therefore selected for further investigation, the results of which are described in the present paper.

2. MATERIAL AND METHODS

The parental material, derived from four areas (table 1), consisted of 30 selected plants,* which were multiplied clonally in culture and also bred. The seedling progenies totalled 664 plants.

Mitosis was studied in Feulgen squashes of root tips, following pretreatment in α-bromonaphthalene or 0·2 per cent. colchicine (3-4 hours) and fixation overnight in 2BD. For good maceration and staining, hydrolysis at 60° C. was extended to 18-24 minutes.

Meiosis in PMC was examined in temporary acetocarmine squashes, following Thomas's method (1940). Storage was satisfactory in ordinary acetic alcohol at 4-5° C.

Embryo-sacs, embryos and endosperm were traced in paraffin sections of whole buds or excised ovaries after fixing briefly in Carnoy, overnight in CrAF.

The stain was crystal-violet. T.S. and L.S. were cut at an increasing thickness of 16-36 microns.

3. PARENT PLANTS

The main details of morphology and habitat are recorded by Connor (*loc. cit.*).

All populations are normally hexaploid with 42 chromosomes (table 1). The centromeres are all submedian, and as a rule two nucleolar pairs can be recognised with certainty (text-fig. 1*a-b* ; *cf.* plate I, figs. 1-2).

In each parental group (except A, table 1) there were some cytologically abnormal plants whose abnormal character was usually expressed phenotypically. The chromosome aberrations were of two kinds.

(*a*) *Numerical*, including relative triploidy ($2n = 57, 63$; plate I, fig. 1).

(*b*) *Structural*, one chromosome showing a deficiency (text-fig. 1*a-b*).

* From the extensive field collections of Mr H. E. Connor, Botany Division, Department of Scientific and Industrial Research, Christchurch, N.Z.

This preliminary information was of immediate value. It gave some idea of the kind of chromosomal variation occurring in wild populations ; it made possible a provisional sub-grouping into chromosome classes ; and it supplied useful cytological markers. Finally,

TABLE

Versatile reproduction in

Wild population parents : 30 plants							
Group and family	No. of plants	2*n*	Male		Female		Endosperm
			Meiosis	*n*	Type of division	Presumed *n*	
North Island A	5	42 (6*x*)	21 II	21	As male		3*n*
B1	4	42	I-IV	20-22	As male		±3*n*
2	1	43	I-IV	21-23	As male		...
3	1	63	I-V	*ca.* 31	Meiosis Dyads ?, mitosis ?	63	...
South Island C1	2	42	18-21 II Rare IV	±21	Meiosis Dyads, mitosis	? 42	... ...
2	1	57	I-III	*ca.* 28	Dyads, mitosis	57	...
3	1	63	I-III Rare V	*ca.* 31	Dyads, mitosis	63	...
D1	2 normal	42					...
2	2 deficient	42	Intraplant	42 or	Dyads, mitosis	42	±6*n*
3	10 normal	42	0-8 II	±21			...
4	1 deficient	42					...

A : Wellington, N.Z. B : Foxton, N.Z.
C : Dunstan, N.Z. D : Waiau, N.Z.

it led to the suspicion that different modes of reproduction were operating in the different populations.

This expectation was then tested in two simple ways : by raising large progenies and comparing them with their respective mother-plants ; and by pollination experiments.

4. PROGENIES

The results of progeny testing are summarised in table 1. Since there were no differences between sister-families raised by isolation and by open-pollination, the two sets of data were pooled.

1

Agropyron scabrum

Progenies (isolation and open pollination) : 664 plants					Genetic mode of reproduction of parent
No. of plants	With parental chromosome no.	Aberrants			
		Chromosome no.	Presumed origin	Per cent.	
25	100 per cent.	...	...	...	Sexual
74	75·7 per cent.	n? n, $n+1$ $2n-1$, $2n+1$ or 2 $3n$, $3n+3$	(Sub-lethal) Haploid parthenogenesis Sexual Semi-sexual	6·8 4·0 8·1 5·4	Facultative apomixis
46	34·8 per cent.	n $2n$, $2n+2$ to 3	Haploid parthenogenesis Sexual	4·4 60·8	
5	0	$4{\cdot}5n$ (*ca.* 96)	Semi-sexual	100	
201	99 per cent.	$2n-1$ *	Diploid parthenogenesis	1·0	Predominant apomixis (sub-sexual)
1	(100)	...	...	...	
13	92·3 per cent.	$4{\cdot}5n$ (94)	Semi-sexual	(7·7)	
95 60 118 26	96·8 per cent. 88·3 ,, 96·6 ,, 96·1 ,,	$2n-1$,* $2n+1$ † $2n$,‡ $2n+1$, $2n+2$, $4n$ $2n-1$, $4n$ $2n+5$ §	Diploid parthenogenesis	5·0	Obligate apomixis

* one chromosome dicentric
‡ two short chromosomes
† one short chromosome
§ centric ring

A : Wellington (25 plants)

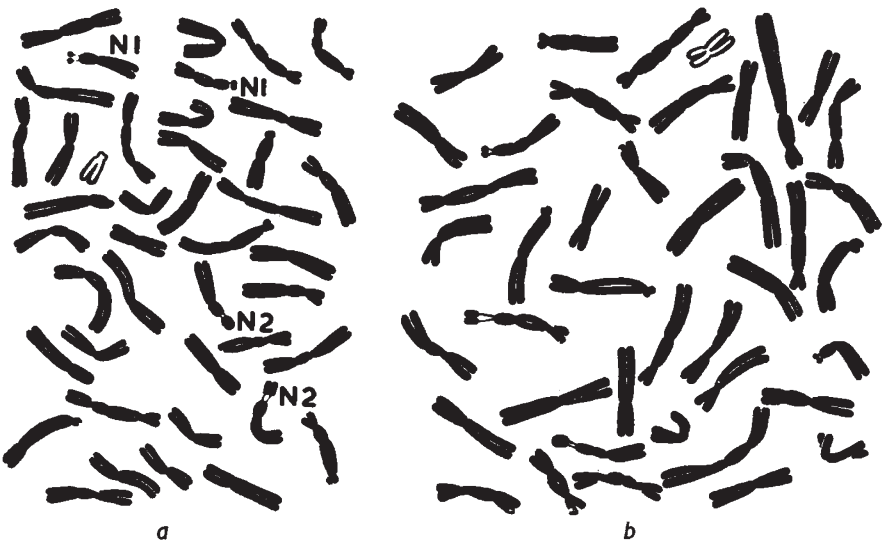
All five plants were self-fertile and gave uniform progenies without aberrations in chromosome form, number or behaviour. Further, they were successfully crossed with other species and varieties giving undoubted hybrids (table 2). Clearly this group is entirely *sexual*.

B : Foxton (125 plants)

The six parental plants were sister-plants, one generation removed from the wild type. Three other plants of the same family—two male-sterile and one "haploid" and sterile—set no seed. The next generation (ex B1 and 2) showed the same variability. "Haploids" ($3x$) and "triploids" ($9x$) were regularly produced, as well as a high proportion of unbalanced forms. Both meiosis and fertilisation were liable to fail but in an uncoordinated way with great loss of fertility.

The third chromosome class (B3) gave exclusively higher polyploids with about 96 chromosomes, presumably by fertilisation of unreduced egg-cells. In this lineage, failure of reduction was repetitive.

The group as a whole is evidently subject to non-recurrent disturbances in sexual reproduction ; in other words, it is semi-sexual or *facultatively apomictic.*



TEXT-FIG. 1.—Somatic complements ($2n = 42$) of the obligate apomicts, D2 and D4, each showing one short, deficient chromosome. ×2000.
N1 and N2 : the two pairs of nucleolar chromosomes.
(*a*) D2 ; (*b*) D4.

C : Dunstan (215 plants)

Here the identity of each parental sub-group, even when aneuploid, was preserved in the progenies. The only significant exception was in the progeny of C3 : one plant among 13 arose *semi-sexually, i.e.* by failure of meiosis but not of fertilisation. The single aberrant which appeared in the progeny of the hexaploid (C1) arose *sub-sexually, i.e.* parthenogenetically, following a suppressed meiosis in which crossing-over and segregation occurred despite the failure of reduction. It contained an isodicentric nucleolar chromosome, the remarkable properties of which have been described elsewhere (Hair, 1953).

Taken together the results indicate that the different Dunstan units are *predominantly apomictic.*

D : Waiau (299 plants)

Taking the whole progeny of the 15 parental plants we find 284 entirely like the parents and 15 which had aberrant numbers and even forms of chromosomes. Again, one had a dicentric and one had a ring, common results of inversion crossing-over. Thus the mode of reproduction was not giving stability, indeed less stability than would sexual reproduction in a polyploid. Yet the two parents with single deficient chromosomes (D_2 and D_4) had together 86 seedlings, every one of them carrying the deficient chromosome of the parent in the same heterozygous condition as the parent except one which had it in the homozygous condition, and one " tetraploid " ($12x$) which had it in double dose.

This result likewise contradicts our experience of sexual inheritance in polyploids. Both the maintenance of the parental deficiency and the introduction of new aberrations are, on the other hand, consistent with a suppression of reduction combined with a failure to suppress the irregularities of crossing-over and segregation that go with a suppressed meiosis. Obligate apomixis have been attained but subsexual recombination and irregularity remain.

The next task was to test these findings by pollination experiments.

5. POLLINATION EXPERIMENTS

(i) *Method*

Tests for apomixis were mainly confined to the supposed obligate apomicts (D), which had the advantage of being cytologically marked. Genetic markers were not available.

Plants were self-pollinated (particularly the deficiency heterozygotes), and also pollinated by various sexual species, both endemic and foreign, differing in chromosome number from the seed parents under test. Double controls were employed, as follows :

(*a*) Emasculated, isolated flowers.
(*b*) Controlled crosses between sexual species, comparable to the test crosses above.

(ii) *Results*

The emasculated controls of the predominant and obligate apomicts, a total of 1600 flowers, failed to set seed. The control crosses of sexual forms (table 2) in every case yielded the appropriate hybrids and this confirmed the normal sexual character of A populations.

Both self- and cross-pollinations of the obligate apomicts (D) gave almost entirely uniform progenies of strictly maternal character. There were only four aberrants in 106 plants, and these, as will be shown later, were probably due to maternal, subsexual irregularities in embryo-sac development.

Although the kind of pollen used had no effect upon the *character* of the progenies, it had a marked influence on the *numbers* of plants

produced. Self-pollen was more effective than cross-pollen. In cross-pollinations, the most effective male was the octoploid, next the hexaploid, and last the tetraploid. The reason for this gradation will be considered in section 9.

No seed was formed when Waiau families were pollinated by the foreign tetraploids *A. cristatum*, *A. desertorum* and *A. spicatum*, or by the decaploid *A. elongatum*. But the last two succeeded to a limited degree

TABLE 2

Results of self- and cross-pollination of sexual and apomictic forms of A. scabrum, *being the progenies of parents or seedlings in table 1 (408 seedlings)*

♀ parent	♂ parent	Flowers pollinated	Seed (total)	Progeny			Chrom. ratio : endosp./ embryo
				Maternal	Non-maternal		
					No.	Constn.	
A : Sexual :							
6x . .	Selfs	432	207	200	...	...	1·5
	4x	129	12	0	7	F_1 (5x)	1·3
	6x	270	77	0	74	F_1 (6x)	1·5
	8x	162	22	0	21	F_1 (7x)	1·7
D : Apomictic :							
6x+1 . .	Self	24	10	8	1	6x+2 def.	3
6x−1 . .	Self	20	6	6	0	...	3
6x def. .	Self	20	6	6	0	...	3
6x . .	4x	270	6	2	0	...	2·3
6x double def.	4x	44	6	1	0	...	2·3
6x . .	6x	148	11	7	0	...	2·5
6x def. .	6x	149	3	3	...	...	2·5
6x+1 def. .	6x	54	2	...	...	...	2·5
6x . .	8x	520	66	55	2	6x+1 6x−1 *	2·7
6x def. .	8x	173	16	13	1	12x	2·7
6x+1 def. .	8x	79	1	1	0	...	2·7

* One chromosome dicentric.

Def. = Deficient chromosome.

Male parents in cross-pollinations as follows :

4x : *A. enysii.* 6x : *A. scabrum ; A. kirkii.* 8x : *A. tenue.*

in stimulating endosperm development (table 6) and in more extensive trials might have resulted in mature seed.

These results permit two general conclusions :

(*a*) That obligate apomixis undoubtedly occurs in these populations.

(*b*) That seed production depends upon suitable pollination, that is to say, our populations of predominant and obligate apomicts are *pseudogamous*.

These were presumably the end-results of a gradual series of evolutionary changes, each a step in the breakdown of sexual reproduction. What these steps were is already implicit in the behaviour of the different populations described. When and how they occurred are the questions we must now endeavour to answer. For this purpose, we must examine the development of the embryo-sac, embryo and endosperm. Finally, we must determine the influence, if any, of the male gamete in the life cycle.

6. DEVELOPMENT OF THE EMBRYO-SAC (E.S.)

(i) *General*

The ovary has a single anatropous ovule, which consists of an elongated nucellus, reduced to one layer at the apex, and two double-layered integuments. The embryo-sac mother-cell (E.M.C.) is derived directly from a single hypodermal cell of the nucellus.

The E.M.C. are rarely " caught " in the act of division, and in most ovules the occurrence of meiosis (or its breakdown) must be inferred from later (or earlier) events. The most reliable criterion of a preceding meiosis (*cf.* Kiellander, 1937 ; Esau, 1946) is the presence of intact or degenerating megaspore tetrads. Their absence, on the other hand, indicates *ameiosis*. On this basis, the four reproductive groups already delimited in *Agropyron* fall conveniently into two main classes, one sexual or mainly so, the other apomictic.

(ii) *Sexual development (A and B : Table 3a)*

A plants have a normal meiosis (plate II, fig. 1) and most ovules can be classed on sight as sexual (table 3).

B1 (*6x*). Meiosis is complicated by a mixture of uni-, bi-, and multi-valent associations. Also since " triploids " (*9x*) occur in the progenies (table 1), meiosis must occasionally be suppressed.

B3 (*9x*). The original triploid plant was lost but development was examined in three triploid derivatives ex family B1. In three out of ten ovules the resting mother-cell was highly vacuolate and closely resembled the " mitotic " resting cells to be described later in the apomicts. In the remaining seven there was no evidence for a suppression of meiosis, although all progeny of the original triploid (table 1) were probably due to non-reduction. The triploids in general appear to have a capacity for apomictic reproduction which is already implicit in their own derivation from relatively diploid plants. Their apomixis, however, is facultative and constitutes a link between the sexual and predominantly apomictic populations.

The result of meiosis is a T-tetrad. The chalazal megaspore forms the E.S. which is thus monosporic (text-fig. 2*a*). It undergoes three mitoses to form an 8-nucleate E.S. Meanwhile the three sterile megaspores degenerate but their remains are visible adjacent to the E.S. up to its 4-nucleate stage. The growing sac begins to pierce

the enveloping layers of the nucellus (except at the apex) at the 2-nucleate stage.

The primary antipodals divide several times to the number of 12, 24 or even more, cells. At first regular and meristematic, the individual cells rapidly grow into irregular, vacuolate and swollen units with densely staining contents, and they ultimately form a massive tissue occupying the greater part of the chalazal region and encroaching more or less on the micropylar half of the sac. The location of this tissue in the direct path of incoming nutrients, its glandular appearance, and its later behaviour in relation to pollination and endosperm development, leave no doubt that its function is nutritive (*cf.* Brink and Cooper, 1944 ; Beaudry, 1951).

Organisation of the egg apparatus and polar nuclei follows a normal course. The polar nuclei lie in contact but do not form a true fusion-nucleus before pollination.

(iii) *Apomictic development (C and D : Table 3b)*

In the C plants only four of 191 ovules showed evidence of meiosis (table 3*b*), and these were confined to a single aberrant clone (ex C1). This rare meiosis was probably abortive : no megaspore tetrads or remains of them were seen throughout the clone or elsewhere in the three C populations jointly considered.

Ameiosis, on the other hand, affected at least 96 per cent. of the total material. Two distinct processes, the second being the more frequent, were involved : (1) Dyad Formation following a suppressed meiosis and restitution, or (2) Simple Mitosis without any trace of a meiotic prophase (*cf.* table 3*b*).

Dyad Formation. In other apomictic plants this has been attributed to two kinds of antecedent processes. The mother nucleus, which is more or less asynaptic, undergoes either :

(*a*) An abortive " pseudo-heterotypic " division, which lapses at anaphase into a single restitution nucleus, and is followed by an effective equational division (Osawa, 1913 ; Okabe, 1932 ; Bergman, 1941 ; Rosenberg, 1927) ; or

(*b*) A " pseudo-homeotypic " division in which the unpaired chromosomes, contracted as in an ordinary meiosis, pass straight into division (Gustafsson, 1934).

In *Agropyron*, the first sequence is probable, although only its later stages have been directly observed (table 3*b*). The main criterion of restitution was taken to be the constricted form of the nucleus (text-fig. 2*b* ; plate II, fig. 2).

In all observed dyads save one, the chalazal cell enlarged to form the E.S. initial, and the smaller micropylar cell degenerated (text-fig. 2*d-e*). In the odd dyad, the order was reversed (text-fig. 4*b*). This is enough to indicate between the two cells the Renner Effect. Where a 2-

TABLE 3

Summary of observations showing the sexual, mitotic, and restitutional methods of origin of the embryo-sac in different populations

(*a*) Sexuals and facultative apomicts

Group and family	2*n*	Total ovules	E.M.C.					E.S. with megaspore remnants		Estimated per cent.	
			Resting	Pro.	M I	M II	Tetrads	1-4 nuclei	8 nuclei	Meiosis	Mitosis
A : Sexual . . .	42	52	4	4	1	1	31	9	2	100	0
B : Facultative apomicts :											
B1 . . .	42	12	3	1	2	...	3	3	...	100	0
B3 (1) . . .	63-66	10	3 (2)	2	1	...	4	...	...	70	30 ?

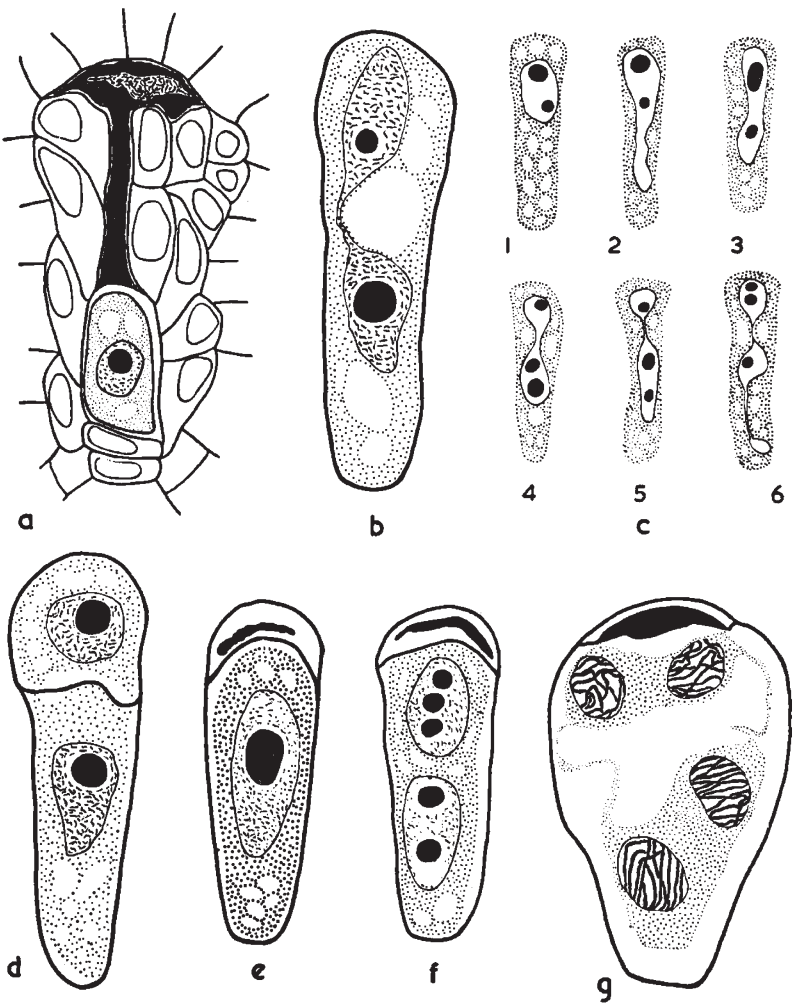
(*b*) Predominant and obligate apomicts

Group and family	2*n*	Total ovules	Mitotic E.S.			Restitution E.S.			E.M.C. (sexual)	Estimated per cent.		
			Resting vacuolate	Pro.-Ana. (3)	2-4 nuclei	Restitution	Dyads	2-4 nuclei	Diak.-AII	Meiosis	Dyads	Mitosis
Predominant apomicts :												
C1 . . .	41-42	85 (4)	15	9	12	1 ?	30	5	4	4·7	42·4	42·4
C2 . . .	57	20	3	1	14	2	...	...	...	0	10·0	90·0
C3 . . .	63	66	20	6	18	8+1 ?	12	1	...	0	33·3	66·7
C4 * . . .	94	20	15	1	4	...	...	...	...	0	0	100
Obligate apomicts :												
D1, 2 . . .	42-43	149	25	20	20	...	68	16	...	0	56·4	43·6
D4 . . .	42	11	1	5	2	...	3	...	...	0	27·3	72·7

(1) 3 plants. (2) Vacuolate. (3) 2 cells at anaphase. (4) 9 cells not classifiable.

* Presumably an obligate apomict arising in the predominant apomict population.

or 4-nucleate E.S. was associated with remnants of the abortive micropylar cell, it was classified as "restitutional" (table 3*b*), *i.e.* as originating from a previous dyad stage (text-fig. 2 *f-g* ; plate II, fig. 4).



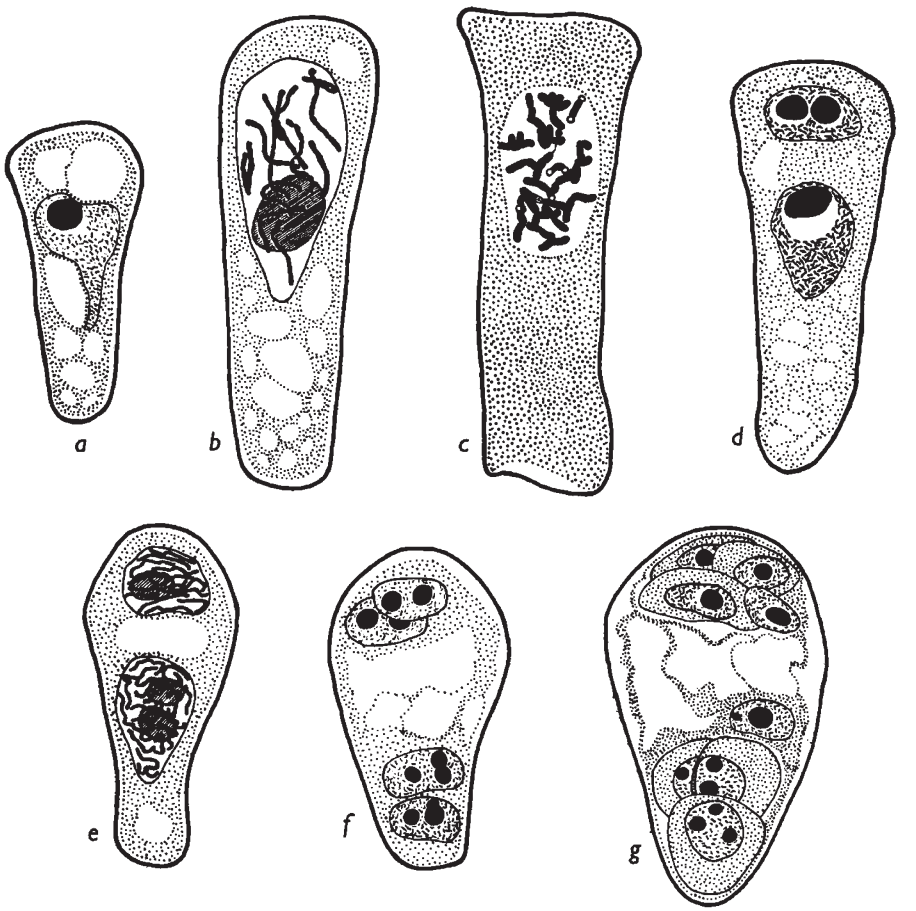
TEXT-FIG. 2.—Development of the embryo-sac. Upper end micropylar, lower one chalazal. × 750 : (*c.* × 350).

(*a*) Normal sexual development. Chalazal megaspore the definitive E.S.
(*b*) Restitution nucleus.
(*c*) 1-2 Resting nucleus in mitotic E.S. 3-6 Restitution nuclei.
(*d*) to (*g*) Successive stages following dyad formation.
(*e*) 1-nucleate.
(*f*) 2-nucleate.
(*g*) 4-nucleate E.S. with remains of abortive micropylar cell still showing.
(*a*) Facultative apomict B1 ($2n = 42$).
(*b*) Predominant apomict C2 ($2n = 57$) ; (*c*) C3 ($2n = 63$) ; (*d*) to (*e*) C1 ($2n = 41$-42).
(*f*) to (*g*) Obligate apomict D2 ($2n = 43$).

The incidence of dyads varied within and between the two populations. It was 100 per cent. in plant D2, averaged 33·3 per cent. in four triploids C3, but was nil in the high polyploid C4 of the same family.

Simple Mitosis. The evidence for a purely mitotic origin of the E.S. appears at successive stages of division in the mother-cell (table 3*b*).

In the first stage there is the *resting* E.M.C. that is to undergo mitosis. It is large, elongated and highly vacuolated, broad at the micropylar end (text-fig. 3*a* ; plate II, fig. 5). Its large nucleus tended to follow the shape of the cell. These characteristics were accentuated



TEXT-FIG. 3*a-g*.—Development of the embryo-sac by mitosis in the predominant and obligate apomicts (C1, D1, 2 and 3). Upper end of sac micropylar, lower chalazal, in all figures. ×800 to ×1100. See text.

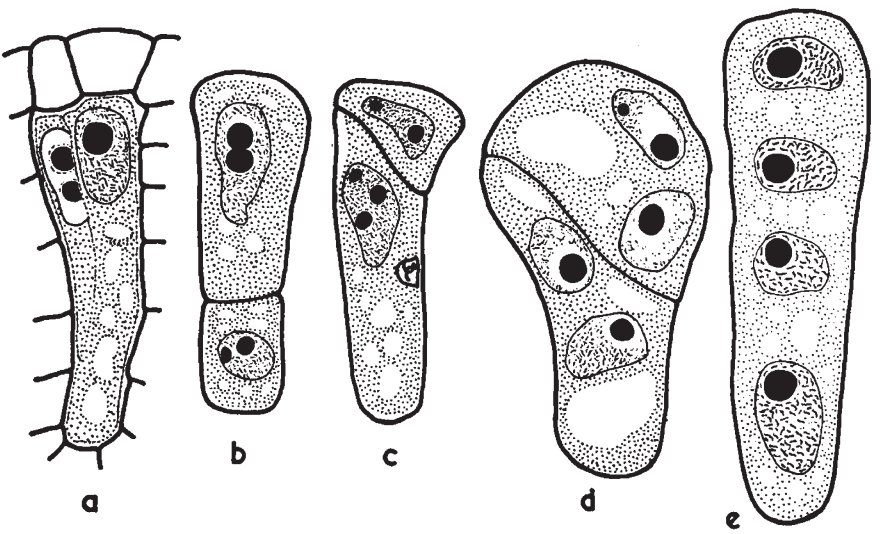
with age, and in most fixations they predominated (41·4 per cent.). The mother-cell evidently passed through a prolonged stage of growth and vacuolation before mitosis began. It finally divided by ordinary mitosis when it had already acquired the character of a uninucleate E.S. (*cf.* Stebbins, 1932 ; Keillander, *l.c.* ; Hakansson, 1943 ; Esau, *l.c.*).*

* The distinction between mitotic and restitutional nuclei was not certain at this stage. The two queried examples of restitution (table 3*b*) were of the same kind ; apart from their shape, both strongly resembled late somatic prophases (*cf.* figs. 2 and 3, plate II). Of the six resting nuclei shown in fig 2*c*, numbers 1-2 were classed as mitotic, numbers 3-6 as restitutional, but the extreme range in shape in this series indicates the possibility of misinterpretation. The difference between a supposed mitosis and the division following restitution is clear only in perfect median sections of the ovule.

The second stage is late prophase, metaphase or anaphase (text-fig. 3*b-c* ; plate II, fig. 3). The chromosomes are then only slightly more contracted than in mitosis of the nucellus. The third stage is the typical 2- or 4-nucleate-sac. It shows no trace of an earlier dyad structure (text-fig. 3*d-f*).

Summarising C and D : normal meiosis on the female side rarely occurs and never effectively. Effective development may be from dyads or by simple mitosis. One of the two dyad cells, which compete, gives the E.S. With mitosis all the products of division in the E.M.C. contribute to the E.S. : there is no formation of spores.

However established, the E.S. followed the same developmental sequence as in sexual plants, but with one important difference. Before, or by the time of anthesis, the unreduced egg had often developed precociously into a small proembryo : it had already put itself out of reach of fertilisation.



TEXT-FIG. 4.—Abnormalities in the early development of the embryo-sac in predominant and obligate apomicts.

(*a*) Two E.M.C. side by side (C2).
(*b*) Micropylar cell of dyad functional (D2).
(*c*) Dyad with oblique cell wall and one chromosome of chalazal cell in a microcyte (D1).
(*d*) Both cells of dyad developing (D1).
(*e*) 4-nucleate E.S. with linear arrangement of nuclei (C3). ×900.

(iv) *Early abnormalities*

In the C and D populations a number of abnormalities are observed prior to anthesis (table 4 and text-fig. 4). Other abnormalities originate during this period but are not seen or fully expressed until later.

7. EMBRYO AND ENDOSPERM

(i) *Sexuals and facultative apomicts (A and B)*

At anthesis the E.S. showed a well-defined resting egg and was in all respects normal (table 5). The free-nucleate endosperm was

usually initiated within 18-24 hours after pollination and in advance of divisions in the young zygote (table 6). Thus all E.S's. scored up to 48 hours after pollination had from 2-16 endosperm nuclei

TABLE 4

Abnormalities in the early embryo-sac development of predominant and obligate apomicts

Group	Example	Abnormality	Times observed	Fig. 4
D1 and 2	1	Micropylar cell of dyad, the definitive E.S.	1	*b*
,, ,,	2	Oblique cell wall in dyad	2	*c*
,, ,,	3 *	Both cells of dyad developing; wall oblique	4	*d*
,, ,,	4 †	Microcyte in chalazal cell of dyad (origin $2n-1$, etc., aberrants)	1	*c*
C1, 2	5 *	Two mother-cells side by side	3	*a*
C3	6	Linear order of nuclei in 4-nucleate E.S.	1	*e*

* Showing effects of competition between normal and supernumerary cells, the latter being usually retarded.

† Most simply explained as the result of partial restitution, giving rare hypoploid aberrants.

but only one-sixth of them showed activity in the fertilised egg. This lag was maintained at 72 hours with the endosperm at least 32-nucleate and the embryo 4-celled; and at six days with the endosperm mainly cellular and the embryo 16-celled.

TABLE 5

Variation in stages of development of the embryo-sac at the time of anthesis

Group	No. of E.S.	No. with Egg+P.N.	No. with proembryo+P.N.			Per cent. precocious
			Pro.-Telo.	2-4 cell	4-8 cell	
A : Sexual .	10	10	0	0	0	0
B : Fac. apo. .	8	8	0	0	0	0
C : Pred. apo.	20	13	1	4	2	35·0
D : Oblig. apo.	23	12	0	11	0	47·8

P.N. = polar nuclei.

The *endosperm*, mainly peripheral up to 72 hours after pollination, then began to concentrate near the proembryo and chalaza, and to form cell walls. After six days, there was little free-nucleate activity and the mass of endosperm became packed with starch.

The *antipodals*, at first greatly stimulated by pollination, declined as the endosperm expanded, the cells being compressed between the endosperm and nucellar tissue, and finally broken up and absorbed.

(ii) *Apomicts (C and D)*

At anthesis up to 50 per cent. of the embryo-sacs had an autonomous and precocious embryo of 1-8 cells (table 5, plate II, fig. 6). This confirmed apomixis and provided a quick means of assay in wild plants whose history was unknown. The same effect was shown by emasculated flowers in isolation: in both predominant and obligate apomicts, the embryo developed for as long as six days after emasculation. At this stage, one plant (C1) had a 32-cell embryo, two polar nuclei and a normal antipodal tissue.

On the other hand, no endosperm developed until 18-24 hours after pollination, and in the absence of pollen it did not develop at all. Pollination, as we saw earlier, is needed for seed production. The reason is now obvious; it is needed for the production of endosperm, in the continued absence of which early autonomous growth of the embryo ceased and the young seed starved and died. We shall see later how the pollen worked.

In apomictic plants there was little correlation between rate of development of the embryo and that of the endosperm (table 6) even long after pollination. These observations confirm the experience in *Parthenium*, and in other apomictic plants (Esau, 1946).

Cross-pollination by endemic, as well as foreign, species of *Agropyron*, though less effective than selfing or open-pollination in stimulating development, results in the same inconsistent pattern of behaviour.

(iii) *Abnormal embryo-sacs after pollination (B, C and D)*

Functional embryo-sacs differ from those of sexual plants only in the precocious development of the embryo. But a proportion of non-functional sacs among apomicts reveal a range of abnormalities (tables 6 and 7).

(a) *Failure of pollination*, or, as we shall see, failure of fertilisation of the polar nuclei, is probably a common cause of non-development.

(b) *Degeneration*: the contents of the E.S. sometimes partly or wholly degenerate; sterile sacs are found with endosperm but without egg or embryo.

(c) *Abnormal stimulation of the antipodals* was expressed in two ways: small supernumerary meristematic antipodals were sometimes found at the boundary of the normal large glandular cells, suggesting a recent burst of mitosis. Again, one, two or even more, isolated cells were actively dividing. This was more pronounced in the cross-pollination *D2* × *A. elongatum* (*10x*) than elsewhere; several cells were affected and some were clearly endopolyploid.

(d) *Correlated irregularities* in the egg apparatus, polar nuclei and antipodals are by far the most frequent. They are recorded in a

TABLE 6

Relative development of embryo and endosperm in the four populations, fixed 18 hours to 9 days after pollination

Group and family	Total E.S.	Egg		No egg +Endosp.	Proembryo	
		+P.N.	+Endosp.		+P.N.	+Endosp.
A : Sexual .	7	0	*3*	*0*	0	*4*
B : Fac. apo. .	20	0	*11*	*4*	0	*5*
C : Pred. apo.	56	44	*2*	*2*	6	*2**
D : Oblig. apo.	181	20	*23*	*22*	49	*67†*

Note : Fourteen E.S. were from cross-pollination of C1 by 4*x* and 61 from cross-pollination of D2 by 10*x*, 4*x* and 6*x*.

Pollen parents in cross-pollinations were as follows :—

4*x* : *A. enysii, spicatum* ; 8*x* : *tenue* ;
6*x* : *kirkii, scabrum* ; 10*x* : *elongatum.*

The relative developments of embryo and endosperm were the same after crossing and selfing.

* Including one of the 14 E.S. from the "cross" C1 by 4x.

† Including 13 of the 61 E.S. from the "crosses" D2 by 4x, 6x and 10x.

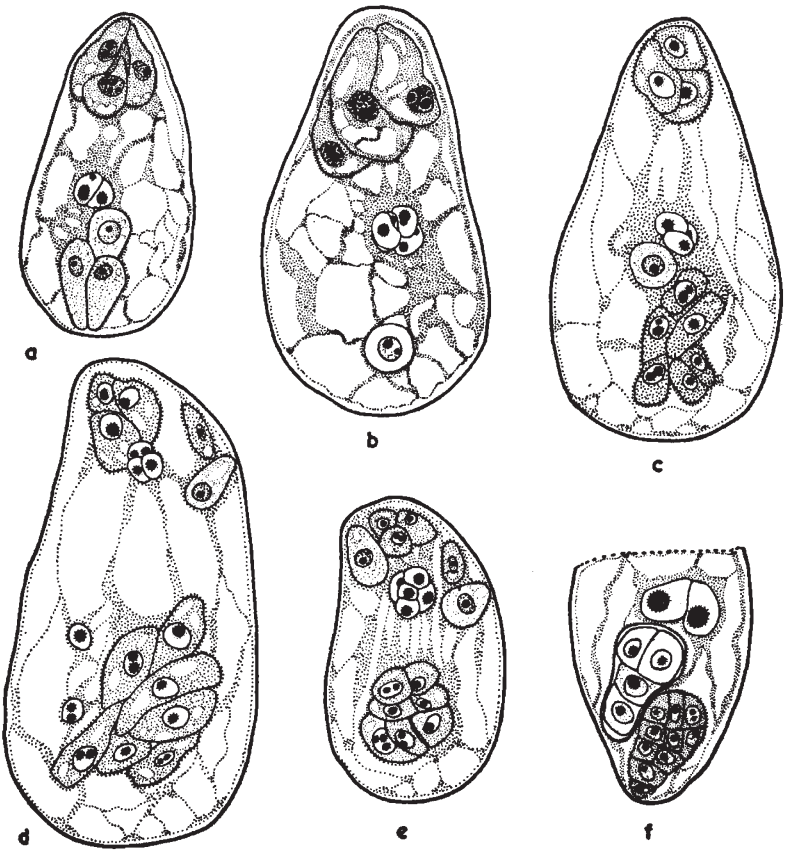
TABLE 7

Classification of mature embryo-sacs, showing the frequency of correlated abnormalities

Group and family	No. of E.S.	No. abnormal	Type or locus of abnormality				Per cent. abnormal
			Polarity	Antip.	Egg app.	P.N.	
A : Sexual .	17	0	...	...	...	...	*0*
B : Fac. apo. :							
2 . .	23	0	...	...	...	...	*0*
3 . .	8	3	3	3	2	3	*37·5*
C : Pred. apo. :							
1 . .	48	3	1	1	1	2	*6·3*
3 . .	11	1	1	...	1	...	*9·1*
4 . .	9	3	0	0	2	3	*33·3*
2 . .	31	16	11	11	8	9	*51·6*
D : Oblig. apo.:							
3 and 4 .	41	1	1	1	...	...	*2·4*
1 . .	65	7	3	3	5	5	*10·8*
2 . .	122	16	14	14	7	7	*13·1*

range of embryo-sacs fixed immediately before anthesis and at intervals up to 72 hours later. They comprise 15 per cent. of 340 E.S's.

As a rule, the different chromosome classes within each apomictic group showed the same range and types of abnormalities, and most had a high proportion of perfectly normal, though unreduced, sacs.



TEXT-FIG. 5.—Abnormal differentiation of the embryo-sacs in facultative and obligate apomicts.

(*a*) Reversed polarity with egg-like synergids. 24 hours after anthesis. D2. *Cf.* fig. 6 (3).

(*b*) Reversed polarity. No synergids ; 4 polar nuclei. 30 hours after anthesis. D2. *Cf.* fig. 6 (13).

(*c*) Central egg, two groups of antipodals at opposite poles and 3 polar nuclei. No trace of synergids. 29 hours after anthesis. D1. *Cf.* fig. 6 (12).

(*d*) Displaced egg and one synergid above ; 3 polar nuclei ; two groups of antipodals. Note the two polar-like nuclei near chalazal antipodals. 20 hours after pollination by *Agropyron elongatum* ($2n = 70$). D2. *Cf.* fig. 6 (11).

(*e*) Two eggs, one displaced, and one synergid ; 5 polar nuclei ; two groups of antipodals. At anthesis. B3.

(*f*) Two embryos side by side in the same embryo-sac. 24 hours after anthesis. D2. ×250 (rest ×150).

But three classes were exceptional : B3 ($2n = 63$), C2 ($2n = 57$), and the high polyploid C4 ($2n = 94$). C3 ($2n = 63$), on the other hand, had no more abnormalities than most of the hexaploid families. Hence an increase in chromosome number does not in itself affect the regularity of the embryo-sac. Of greater significance is the fact

that these classes stand midway between the stable extremes of normal sexuality and absolute apomixis. Their instability in all phases of development is therefore to be expected.

In most cases there is a decrease in the number of synergids, and an increase in the number of polar nuclei, eggs or antipodal groups.

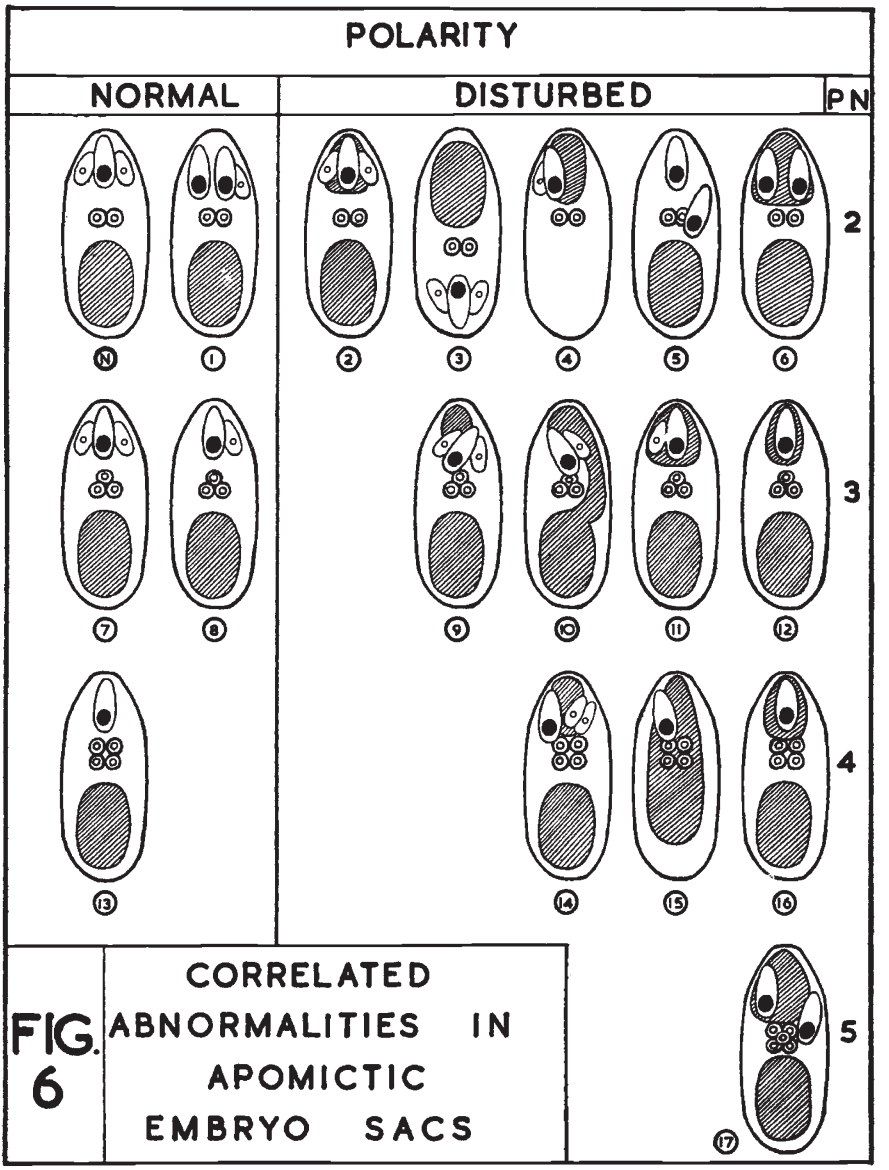


FIG. 6 CORRELATED ABNORMALITIES IN APOMICTIC EMBRYO SACS

Note: Egg nucleus solid, antipodals cross-hatched.

The variations are in position and differentiation rather than in number of nuclei (table 8). They are, moreover, the kinds of variation used in the general classification of types of angiosperm embryo-sac used by Darlington and Mather (1949, fig. 50). Since the primary nuclei

of the 8-nucleate sac are genetically identical, these variations will have no genetic effect.

Similar changes have been reported by Chiarugi and Francini (1930) in apomictic *Ochna serrulata*, and by other workers in different plants (*cf.* Maheshwari, 1950).

TABLE 8

Negative correlation of varying components of the embryo-sac

P.N.	Normal polarity Synergids			Disturbed polarity Synergids		
	2	1	0	2	1	0
(i) Predominant apomicts						
2	N	1	0	1	2	0
3	2	4	0	0	1	1
4	0	0	2	0	0	1
(ii) Obligatory apomicts						
2	N	1	0	7	0	1
3	0	3	0	1	2	1
4	0	0	2	0	0	2

Note.—Analysis of 25 per cent. of the sacs was prevented for the following reasons :—

(i) Rapid division of the primary antipodals hinders or prevents the detection of supernumerary divisions such as may occur in other components at the same time (examples 14 and 17, fig. 6).

(ii) Varied disposition of the antipodals themselves increases the difficulty by tending to obscure the normal micropylar contents of the sac.

(iii) The synergids degenerate at different times, and even in normal sacs their recognition is not always certain. Hence their absence at a particular moment, even when associated with a corresponding increase in the number of polar nuclei, eggs, or antipodal groups, cannot be taken as conclusive proof of their earlier transformation.

(e) *Disturbed polarity.* In 36 of the 50 abnormal E.S's., the polarity was disturbed in one or other of the four main ways shown in table 9. The antipodals were prominent in most of these disturbances. Thus they invaded the micropylar end and tended to displace the egg apparatus (table 9, class 2) ; or they were crowded into the micropylar end of undifferentiated sacs (class 3). When two antipodal groups were present (class 4), the ancillary, micropylar group was usually smaller than the normal, chalazal group. This anomaly was the

most frequent, and hitherto it has been reported only in *Poa alpina*,* as an exceptional event.

The abnormal ovaries of all kinds, in relation to the development of the embryo-sac (although not necessarily in relation to anthesis), were undersized. With one possible exception, the embryo-sacs were probably sterile. The exception, fixed at 20 hours after anthesis, had a small proembryo and endosperm ; here, abnormal development was confined to differential growth of the antipodals. Sterile sacs

TABLE 9

The kinds and frequencies of disturbed polarity in 50 abnormal embryo-sacs

(Three types of apomicts combined)

Class	Situation of the antipodals	Proportion abnormal
1	Polarity reversed . . .	6 per cent.
2	Differential growth . . .	16 ,,
3	Unipolar (micropylar) . .	16 ,,
4	Two distinct groups . .	34 ,,

had no endosperm, but from 2-5 polar nuclei. It is unlikely that a surfeit of polar nuclei, by itself, prevented either fusion or fertilisation. Rather it was yet another symptom of a general upset in development.

(iv) *Twin embryo*

In an obligate apomict two embryos were found side by side in one E.S. (text-fig. 5*f*). Though the possibility that one embryo arose adventitiously from the nucellus cannot be ignored, it seems more probable that it developed from an extra egg cell, the two embryos being in effect identical twins : in this case monosporic although not monozygotic. Several abnormal embryo-sacs, as we saw (text-figs. 5, 6) contained an extra egg which we supposed was a transformed synergid.

8. MEIOSIS IN P.M.C.

A : *Sexuals*

These are functionally diploid without abnormalities of meiosis (text-fig. 7*a* ; plate I, fig. 3).

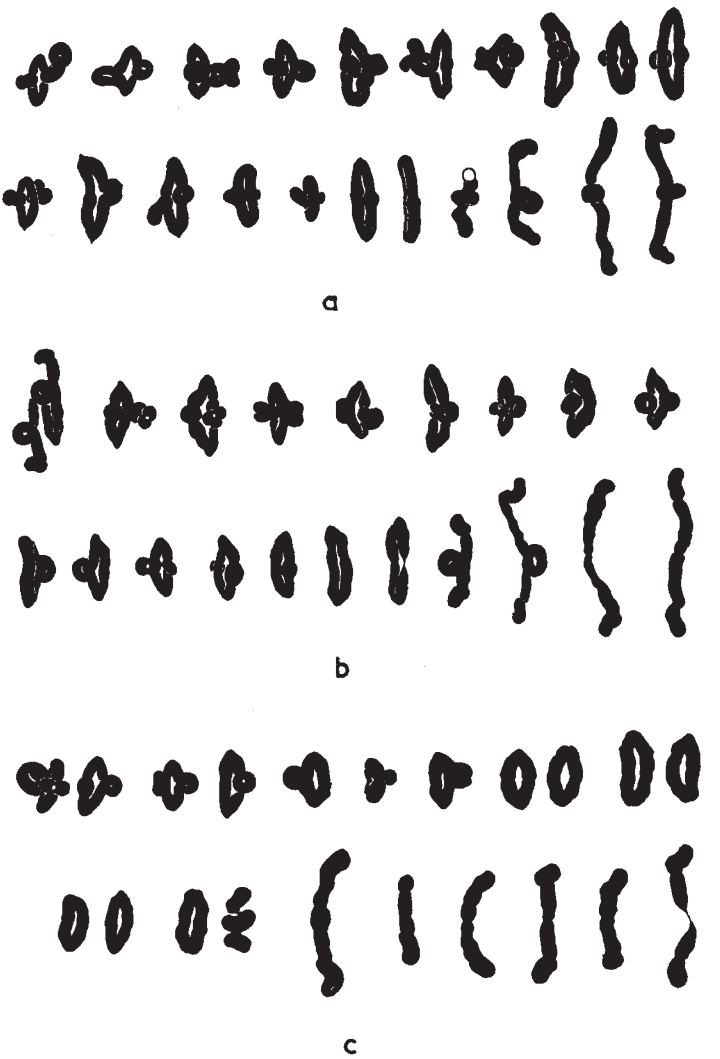
B : *Facultative apomicts*

B1 and B2 (*2n = 42, 43*). Most P.M.C. showed a trivalent or a quadrivalent, occasionally both, at M I, as well as unpaired chromosomes (table 10 ; text-fig. 7*b*). The univalents divide at either the first

* In this species, Hakansson (1943, 1944) found very strong growth of the antipodals in unfertilised flowers. Micropylar groups were regarded as the transformed cells of the egg-apparatus.

or the second division, and their irregular distribution, combined with the unequal separation in trivalents, no doubt result in a proportion of aneuploid progeny with reduced competitive power.

B3 (*2n* = *63*). A typical M I had 6 I + 6 II + 15 III and the high



TEXT-FIG. 7.—M I in P.M.C. of hexaploids : complements arranged from left to right in decreasing order of number of chiasmata per bivalent. × 1600.

(*a*) Sexual : 21II (A).
(*b*) Facultative apomict : 1IV 19II (B1).
(*c*) Predominant apomict : 21II (C1).

Note : the obligate apomict with little pairing is shown in plate I, fig. 4.

frequency of trivalents supports the view that the " triploids " arose semi-sexually, that is from failure of meiosis on one side. Quadrivalents and quinquevalents also occurred. Table 11 summarises the frequency of lagging chromosomes and of micronuclei in 373 cells.

The amount of elimination is low. Many A I laggards are presumably included in the telophase nuclei or are able to rejoin the main body of chromosomes at M II. The residue, appearing as micronuclei in the tetrads, are probably lost. But we may expect gametic numbers

TABLE 10

Chromosome associations in P.M.C. of the three kinds of apomicts

Population	2n	Mean frequency per cell of				Total cells
		I	II	III	IV	
B : Facultative :						
1 . . .	42	0·5	19·0	0·4	0·6	*19*
2 . . .	43	1·6	18·9	0·6	0·5	*26*
C : Predominant :						
1 . . .	42	1·6	20·1	0	0·05	*21*
2 . . .	57	6·3	12·6	8·5	0	*10*
3 . . .	63	5·5	7·0	14·5	0	*2*
D : Obligatory :						
1 . . .	42	37·0	2·5	0	...	*181*
1 . . .	42	35·8	3·1	0·013	...	*75*
1 . . .	41	37·9	1·5	0·007	...	*108*

of about 63/2. The chromosome numbers of all five sexual daughter-plants confirmed this expectation (table 1).

The occurrence of multivalents in plants which are numerically polyploid (6*x*) but on the whole functionally diploid, can be taken

TABLE 11

Frequencies of laggards in the "triploid" B3, showing their incorporation in the main nuclei as meiosis proceeds

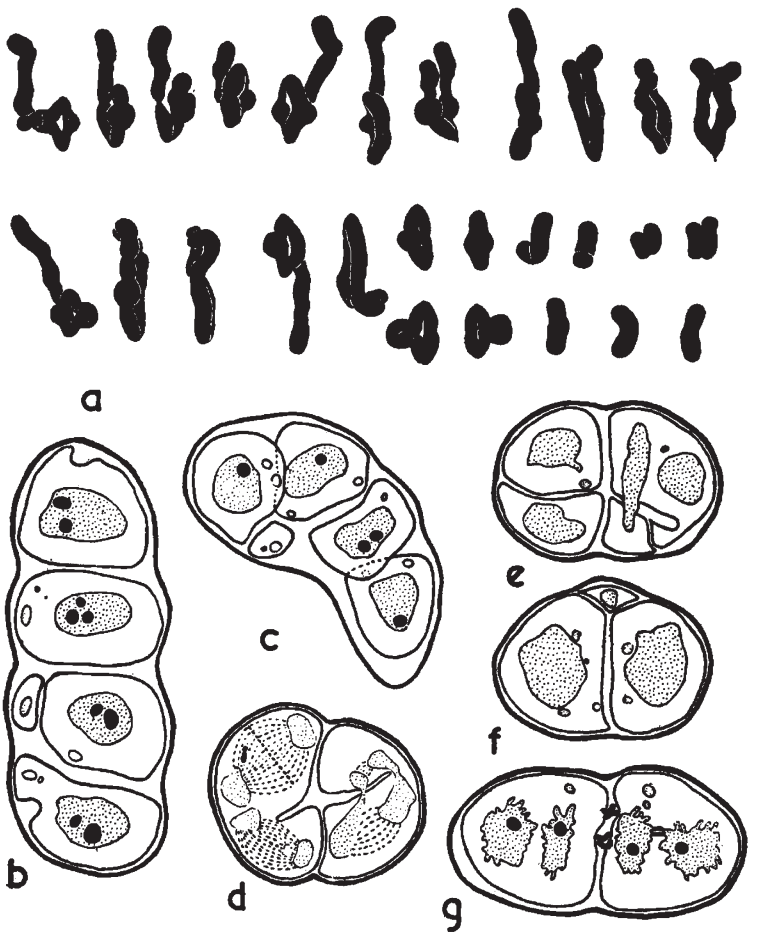
No. of cells	Nos. of laggards or micro-nuclei per cell										Mean
	0	1	2	3	4	5	6	7	8	9	
Late A I . . .	4	12	16	16	11	5	2	1	0	1	*4·7*
Interphase . . .	29	44	37	16	6	1	...	...	...	...	*3·0*
Late A II . . .	77	23	14	2	...	...	...	...	...	...	*1·2*
Tetrads . . .	33	17	4	2	...	...	...	...	...	...	*1·1*

as evidence of pairing between supposed ancestral haploid sets, or of interchange hybridity. The latter is favoured by the fact that apomictic populations will maintain or even increase whatever structural or genic hybridity may have been present in their ancestors.

C : *Predominant apomicts*

C1 (*2n* = *42*). This plant had 18-21 pairs at M I (text-fig. 7*c*) and 1-3 pairs of univalents occurred in half the P.M.C. Very rarely a single quadrivalent was found.

C2 and 3 (*2n* = *57*, *63*). The extra chromosomes were mainly found in trivalent associations (text-fig. 8*a*), confirming their presumed



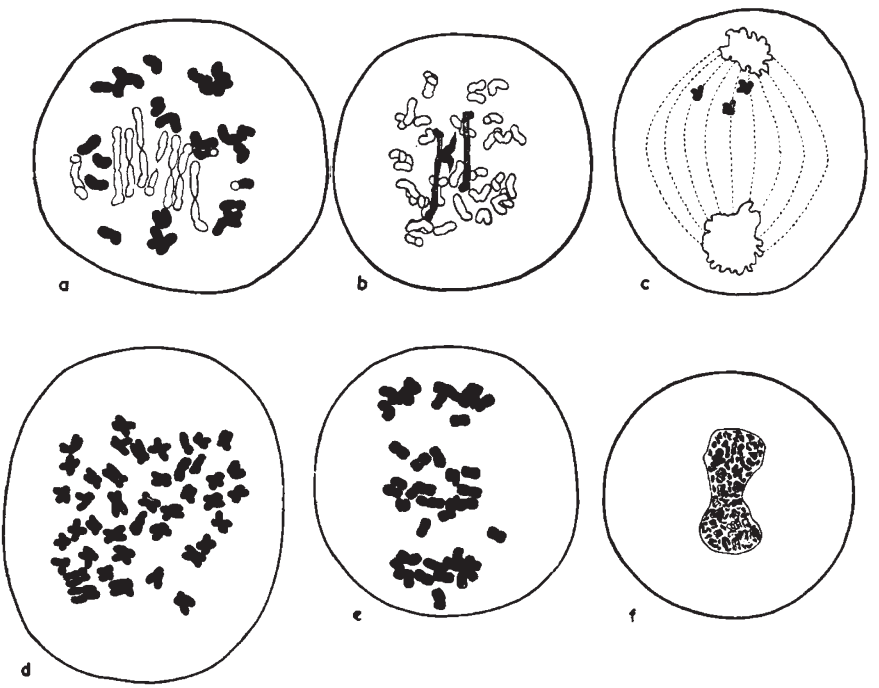
TEXT-FIG. 8.—Meiosis in the P.M.C. of the predominant apomict C3 (*2n* = 63).
(*a*) M I. 16III 4II 7 I. ×1600.
(*b*) to (*e*), (*g*) Irregular tetrads showing micronuclei, microcytes and lagging chromosomes. ×600.
(*f*) End of first division : 6 micronuclei and a microcyte. ×600.

maternal origin. The two meiotic divisions were very variable. At one extreme, the distribution of chromosomes at A I was almost regular, the probable result being functional male gametes, and, as we saw (table 1), occasional fertilisation. But at the other extreme, the range of abnormalities was endless (text-fig. 8*b-g*), and the prospect of balanced pollen grains remote.

D : *Obligate apomicts*

These plants were partly or completely asynaptic and the few bivalents formed had usually a single terminal chiasma (table 10).

Pachytene pairing was apparently complete, but it was probably incomplete in some chromosome pairs, as revealed at later prophase stages. Later association was of three kinds : by chiasmata ; by relational coiling, surviving from pachytene ; and by the parallel alignment of homologues at diakinesis without visible evidence of chiasmata or relics of relational coiling.



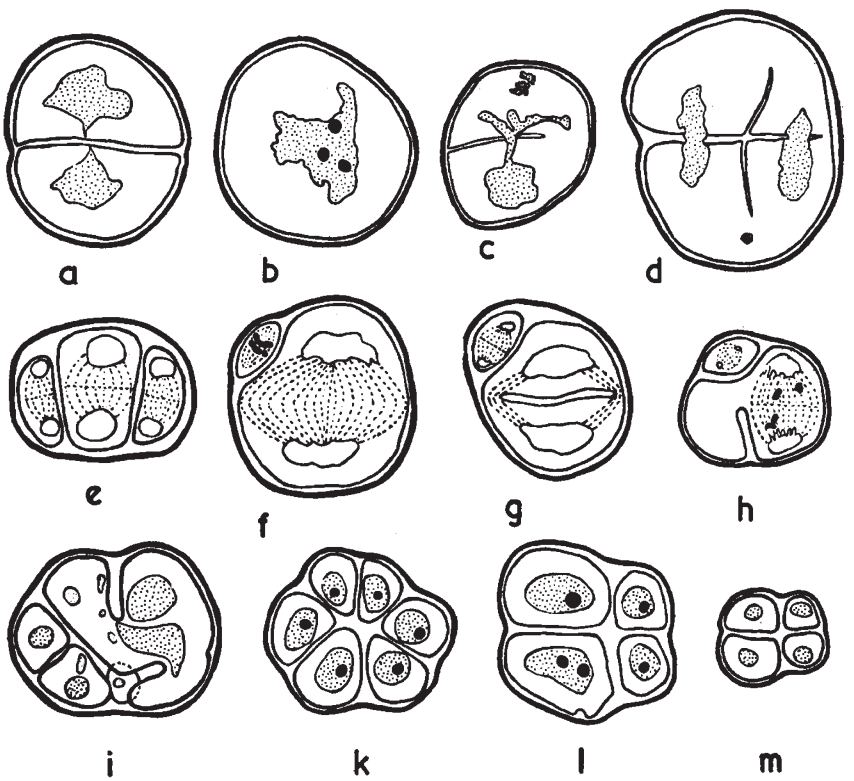
TEXT-FIG. 9.—Meiosis in P.M.C. of two obligate apomicts, D1 ($2n = 41, 42$). ×1000.
(*a*) M I showing 8II 26I.
(*b*) M I with 2II 36I and one neocentric univalent.
(*c*) Late A I with 3 lagging univalents.
(*d*) A I : 32 scattered split univalents, prior to restitution.
(*e*) A I : 42 univalents, concentrated at equator and poles.
(*f*) Restitution nucleus showing halves of split univalents still in contact.

The view that partial or complete failure of metaphase pairing is due to reduced precocity of the prophase is supported by the observations that metaphase bivalents are attenuated or semi-mitotic. This lack of spiralisation is proportional to the degree of failure of pairing (*cf.* text-fig. 9*a-b*, and plate I, fig. 4). But distinction between the obligate apomicts which are asynaptic and the groups of sexuals, facultative and predominant apomicts with nearly complete pairing under similar conditions is no doubt under genotypic control as in parallel experimental cases.

The consequences of variable asynapsis were themselves exceedingly

variable. There was, in fact, only one constant result : the unpaired chromosomes did not effectively divide at the first division (text-fig. 9*c*). The separation of half-univalents was delayed till the second division or its equivalent following restitution. This course was invariable.

The appearance of doubleness, which affected all univalents in the nucleus simultaneously, was the only reliable criterion, at least in completely asynaptic cells, of the development of the cell. Variation in the dispersal of univalents, whether they were scattered (text-fig. 9*d*)



TEXT-FIG. 10.—Meiosis in P.M.C. of two obligate apomicts, D1 ($2n = 41, 42$). ×550.

(*a*) First division successful (although a bridge has been broken).
(*b*), (*c*) First division suppressed giving restitution nuclei.
(*d*) Restitution at second division.
(*e*) Triad of cells.
(*f*) to (*h*) Division following partial restitution ; chromosome or chromosomes left out have formed micronuclei which have divided.
(*i*) to (*m*) Irregular and uneven products of a two-division meiosis.

or apparently more orderly (text-fig. 9*e*), was found equally at metaphase or at anaphase. Hence the transition from metaphase to anaphase could not be described in terms of chromosome movements.

Movement to the equator of the cell, or congression, since it was largely a movement of unpaired chromosomes, was much delayed in the obligate apomicts, reaching its extreme in cells with no bivalents at all. The remote position from the equator of many univalents

indicates that probably they had not moved at all during prometaphase. In these cells, two consequences followed :

(*a*) *Restitution* occurred because the chromosomes were so out of step in relation to the development of the spindle that they had time to split but no time to separate and move to the poles (text-fig. 9*d-e*, text-fig. 10*b-c*). All chromosomes, or nearly all, were therefore included in a common restitution nucleus (text-fig. 9*f*) and this was succeeded by a single division giving dyads and unreduced pollen grains. Individual chromosomes, or small groups, left out of the main nucleus, formed micronuclei or microcytes (text-fig. 10*c*, *f-h*).

In some anthers, restitution probably occurred at prometaphase or even earlier. The nuclei were then rounded and compact (plate I, figs. 5-6) and sometimes their contents gave the false impression that they were degenerating. These figures closely resemble the " contraction nuclei " described by Fagerlind (1947*a*, *b*) in male *Hieracium* and female *Erigeron*.

TABLE 12

The potential products of meiosis in P.M.C. : variation within one spikelet of the obligate apomict D1

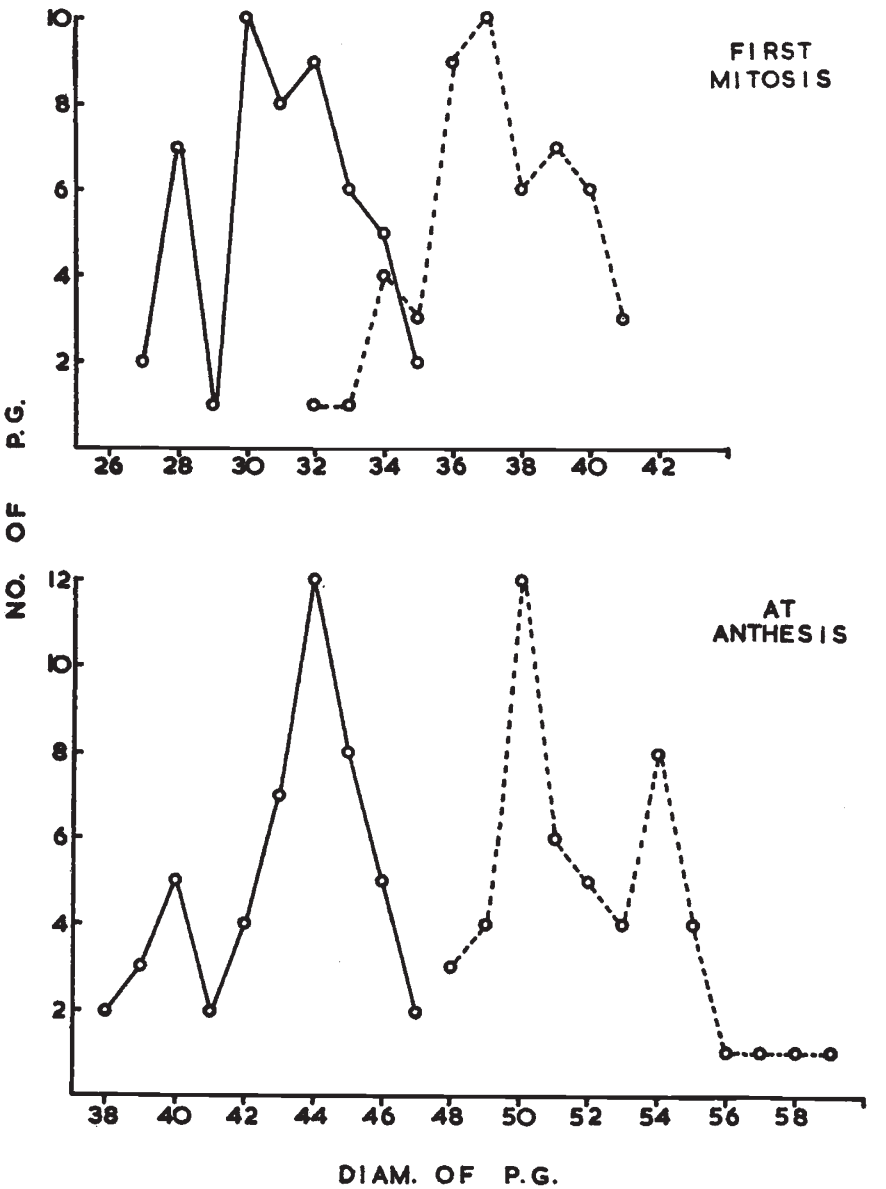
6*x* sample	Total P.M.C.	Restitution after A I			Total potential P.G.	Estimated ♂ gametes per cent.		
		Complete	Partial	None		"Diploid" 6*x*	"Hypodip" 6*x*−	"Haploid" ±3*x*
1	124	9	27	88	*424*	4·2	12·8	83·0
2-3	81	72	...	9	*180*	80·0	0	20·0

(*b*) Cell-division sometimes occurred, owing to a bipolar grouping of unsplit and static univalents which did not interfere with wall-formation. The result was sometimes a triad of cells (text-fig. 10*e*).

Neocentrics. P.M.C. with 1-2 bivalents were the more instructive since the bivalents served as reliable markers of the state of the nucleus. In one plant, a $2n-1$ aberrant arising in D1, co-orientation of the bivalents revealed an interesting abnormality (text-fig. 9*b*). One of the 37 univalents in this cell (and in eight further metaphases) was oriented like a bivalent owing to neocentric activity at its two ends (*cf.* Rhoades and Vilkomerson, 1942 ; Prakken and Müntzing, 1942, and Ostergren and Prakken, 1946).

Two-division meiosis. When 3-8 bivalents were formed, the usual result was an irregular meiosis giving tetrads and reduced but very unbalanced pollen grains. Both chromosome distribution and cell division were uneven (text-fig. 10*i-m*), and complicated by the lagging chromosomes, micronuclei and microcytes. Lagging univalents dividing at A II sometimes promoted restitution in one or both cells (text-fig. 10*d*).

Pollen grains. For present purposes, the results of meiosis in terms of the kinds of male gametes produced are of some importance. The expectations in obligate apomicts were approximately estimated by



TEXT-FIG. 11.—Graphs showing diameters in microns of reduced pollen ($3x$) from the sexual population A ($2n = 42$) and unreduced pollen ($6x$) from the obligate apomict D2 ($2n = 43$).

scoring the numbers of restitution nuclei and the recognisable products of restitution ; and also the stages, M II to tetrads, which could be related to a two-division meiosis. Three samples of cells were analysed,

one from each of the three buds of one spikelet fixed at the same time. The results were radically different (table 12).

Were gametes of these several kinds produced? This information, though not available in the same plant, was obtained from pollen grain mitoses in a closely related plant (ex D2) with 43 chromosomes. All 50 mitoses scored had 43 chromosomes or approximately so. On the other hand, no divisions at all were seen in the considerable range of smaller, usually empty and abortive grains which were distributed at random amongst the unreduced grains in any one loculus. Viable pollen grains were therefore formed. But they were predominantly, or exclusively, unreduced.

This conclusion was strengthened in another way: by measurements of known reduced (sexual) and unreduced (apomictic) pollen grains at mitosis, and at maturity. Both sets of data had the same result (text-fig. 11): in the apomict, grains were twice as large as in the sexual. We must therefore conclude that the unreduced and usually more balanced products of restitution and dyad-formation have an immediate selective advantage over the reduced and usually unbalanced segregants of an irregular meiosis, the majority of which do not survive. In a population where the pairing of chromosomes has become slightly defective it is advantageous that it should evolve towards being totally defective.

Finally, what is the function of the pollen in the obligate apomicts? One potential role is external and depends upon the ability of the pollen to function in crosses with sexual species. The second task of the pollen is internal and indispensable: it is pseudogamy. The nature of this process will now be considered.

9. PSEUDOGAMY

Hitherto the term has been used in a dual sense, to denote a stimulating effect by the pollen entering the style upon embryo and endosperm development or to indicate fertilisation of the polar fusion-nucleus. In most examples adequately investigated, the more positive interpretation is valid, and it is perhaps universally true. The crucial test is the determination of chromosome number in the endosperm, as shown so elegantly by Rutishauser (1955).

In *Agropyron*, counts were preferably made in young, free-nucleate endosperm which was multiplying rapidly. Older cellular tissue had fewer divisions and an increasing tendency for endomitosis and other irregularities. The best period was at 48-96 hours after pollination.

Counts were confined to a "selfed" obligate apomict (ex D2) with 43 chromosomes, the self-same plant in which only unreduced pollen grains were found. It had a relatively "hexaploid" endosperm with $6n = circa\ 128$. This showed, as we supposed, that "diploid" pollen is favoured by selection in this plant, and that, as we now find, its primary function is fertilisation of the polar nuclei. Following

Darlington (1952), we may distinguish the established cases of pseudogamy through triple fusion as *paramictic*.

Returning to the crossing experiments in table 2 we may recall that sexual octoploids gave better results than hexaploids or tetraploids in pollinating the obligate apomicts. Evidently this was because the apomict is accustomed to hexaploid pollen which the sexual octoploid most nearly approaches.

10. THE BREAKDOWN OF SEXUAL REPRODUCTION

Different wild populations of one and the same polymorphic species we now see in successive stages of the breakdown of sexual reproduction. This breakdown culminates in a stable system of obligate apomixis. Such a sequence has hitherto been demonstrated in *Poa alpina* (Müntzing, 1933, 1940). *Agropyron*, however, has certain entirely new features.

(*a*) All the New Zealand species of *Agropyron scabrum* are self-fertilising ; they belong to the important wheat and barley group where self-fertility is widespread and where apomixis has not previously been found.

(*b*) No natural hybrids within or between the various New Zealand species have been found.

(*c*) There is a gradual reduction in chiasma frequency which finally results in the almost complete failure of chromosome pairing found on both male and female sides in the obligate apomicts (*cf.* text-figs. 7 and 9).

The first step in the development of apomixis from sexual reproduction is the occurrence of restitution nuclei. These in a self-fertilising species presumably arise from genes leading to failure of pairing, or from " triploidy " (which in any case is likely to follow). The consequence—facultative apomixis—is therefore not due to hybridisation but to new genotypes arising from inbreeding or mutation. Types of " mutation " that would be effective in this way, such as deficiencies and dicentrics, we see later appearing in these systems, predominantly or habitually apomictic, which favour both their origin and their persistence.

The present nature, and the future trend of our facultative population (table 1), is indicated by the breeding structure of its components, as follows :

(i) Facultatively parthenogenetic " haploids " ($3x$).

(ii) Sexual " diploids " ($6x$) whose subsequent evolution may be either sexual by return to the normal diploid pool, or subsexual.

(iii) Semi-sexual " triploids " ($9x$), arising by fertilisation of restitution eggs, and immediately capable of obligate parthenogenesis though occurring in a facultative population. As in the diploids, their further evolution is subsexual.

We shall see later what the subsexual process is and how it occurs.

Two conditions, and their coincidence, are now necessary for the genesis of habitual apomixis, *viz.* :

(*a*) Regular suppression of meiosis by the segregation and selection of genetic types having this effect.

(*b*) Regular omission of fertilisation equally known to arise under genotypic control.

These conditions have largely been found in the predominant apomicts. In the " diploids ", the rare meiosis is abortive : the suppressed meiosis occasionally results in subsexual recombinants. In " triploids ", semi-sexual reproduction is rare, and in these higher chromosome classes the failure of female reduction has mainly been secured by a complete changeover from a suppressed meiosis to mitosis. We can therefore regard the segregate with 94 chromosomes (C_4) as an obligate apomict arising from its predominantly apomictic parent (C_3).

The final phase in the succession has been achieved by the obligate apomicts. Here, in all instances where the embryo-sac originates purely by mitosis, sexual reproduction, in the genetic sense, has been completely suppressed. But where the embryo-sac arises by a suppression of meiosis giving dyad formation, the restitution eggs arising in dyads show, and will always show, genetic recombination of the kind called *subsexual reproduction* (Darlington, 1937, 1939).

Subsexual recombination, as shown by Darlington, will explain " mutation " in apomicts, and the recombination of its results, both arising through crossing-over in the unreduced E.M.C. Our system of diploid parthenogenesis is therefore subject to variation by the same means as its sexual ancestors expect that the recombinations are now at a subsexual level : they no longer have to pass through a haploid sieve and therefore permit the more violent structural and numerical changes which we have actually found.

Analysis in all our apomictic groups has been simplified by the absence of apospory, revealing the crucial facts of competition between ovules whose products in the beginning are meiotic and sexual, but later subsexual and purely non-sexual. This succession is a female succession. There is also, however, a male succession. All classes of apomicts retain their male-sexuality, the opposite effect to male-sterility. This ensures the production of some viable pollen, capable of fulfilling its pseudogamous function. Good pollen still arises by reduction in the predominantly apomictic plants but by non-reduction in the habitual apomicts. Parallel genetic changes have produced parallel adaptive results on the two sides.

The morphological concomitants of the system described—a system of diploid parthenogenesis combined with subsexual recombination—have all been seen. Observations of mitosis, meiosis,

morphology, and the breeding system all agree in showing how the subsexual system works. The obligatory apomict shows how it works and how it evolves since it is the last development in the whole irreversible process.

11. SUMMARY

1. In the New Zealand grass *Agropyron scabrum* four populations A, B, C and D, all basically hexaploid, were found to differ in mode of reproduction. Representative plants and their seedlings to the number of 999 were compared in :—

(i) chromosome complements ;
(ii) behaviour at meiosis in E.M.C. and P.M.C. ;
(iii) development of embryo, endosperm and seed ;
(iv) systematic character.

These modes of study gave correlated results in the four populations as follows :

A. Completely and normally sexual : uniform in chromosome complement and in systematic character.

B. Facultatively apomictic : meiosis and fertilisation both liable to fail but in an uncoordinated way with production of " haploids ", " triploids " and unbalanced forms. Such a system may be said to be versatile, that is sexual, asexual and semi-sexual. The population is, as expected, highly variable in fertility and in systematic character.

C. Predominantly apomictic : meiosis largely suppressed on the female but not on the male side. Different chromosome types giving a limited stereotyped variation.

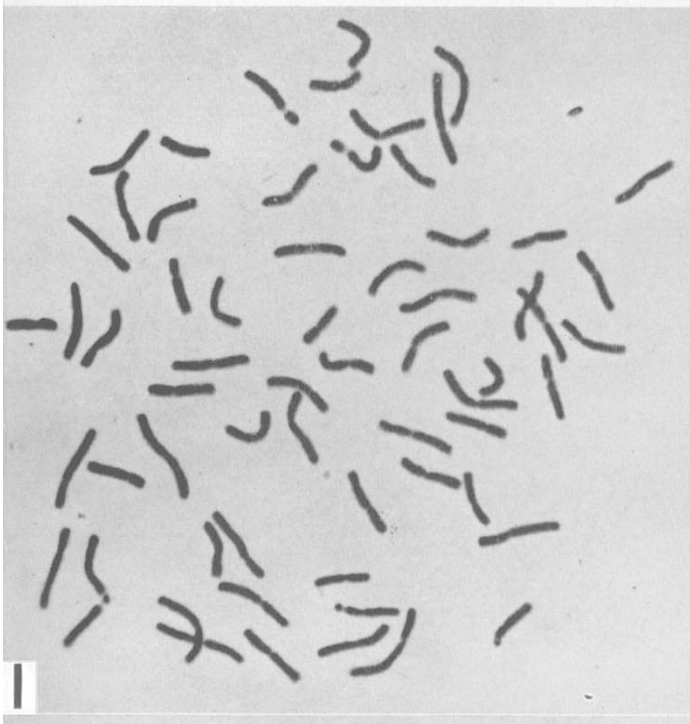
D. Obligatorily apomictic : suppression of reduction on male and female sides ; loss, gain and breakage of chromosomes in 5 per cent. of the experimental progeny but by elimination great constancy of the natural population.

2. In the three apomictic populations B, C and D :

(i) Embryo-sacs arise from mother-cells in whose meiosis reduction is suppressed but crossing-over and other chromosome changes derived from it still occur. Such reproduction is described as subsexual.

(ii) The embryo depends for its development on that of the endosperm which in turn depends on fertilisation of the fusion nucleus by a male generative nucleus. In B and C reduced pollen is chiefly effective ; in D unreduced pollen.

3. The difference between the suppression of pairing at meiosis on male and female sides in population C (and to a less extent D) shows that genotypic control, not hybridity, is responsible for the suppression of chromosome pairing in them.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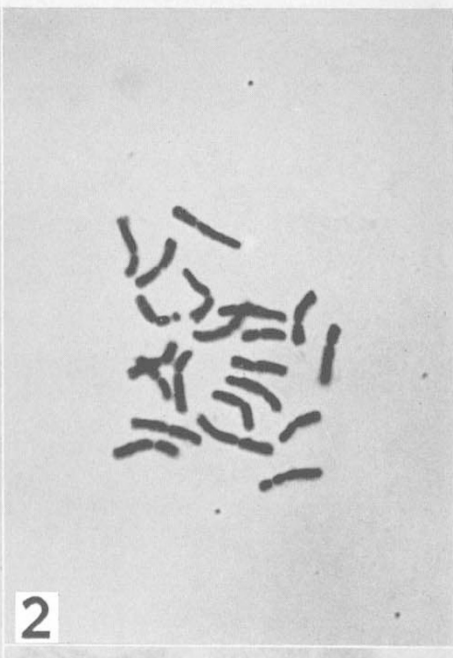
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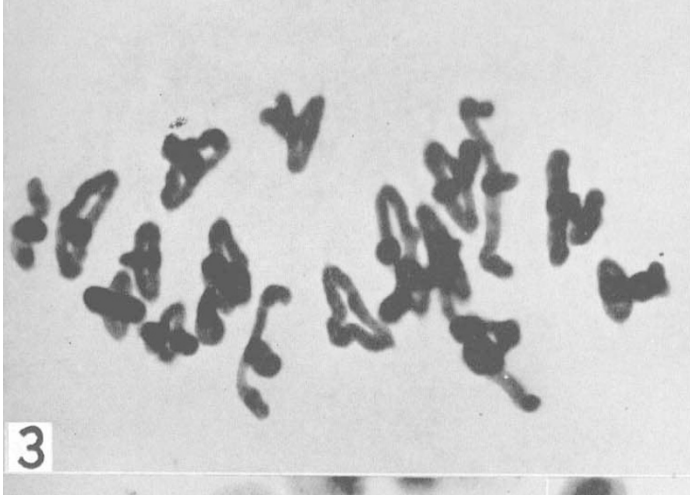
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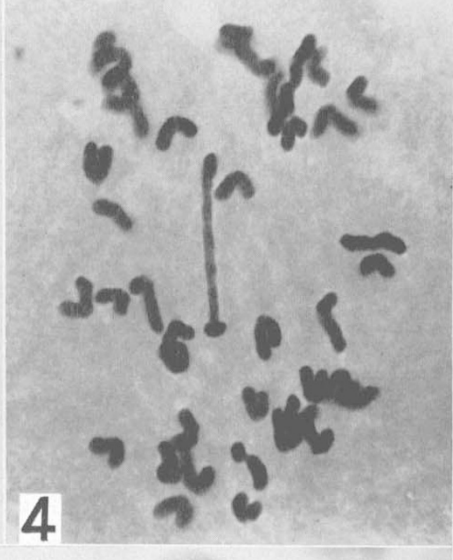
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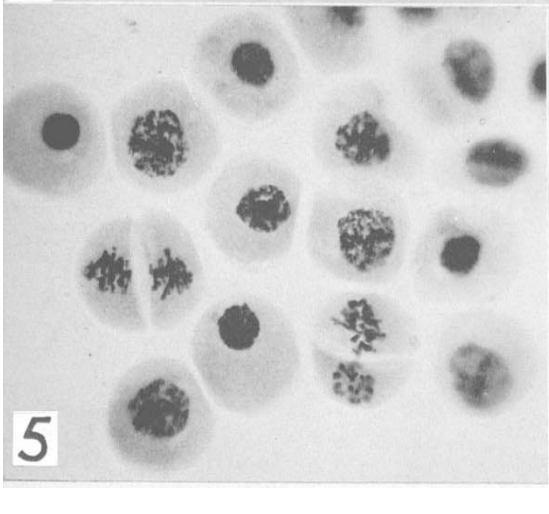
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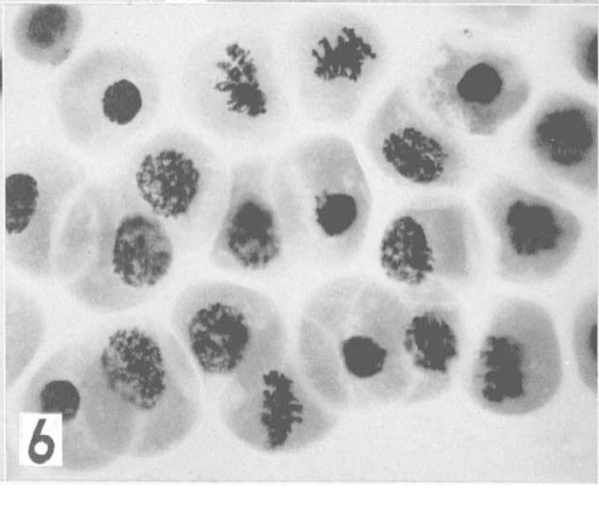
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Plate II

Early development of the embryo-sac in sexual (A) and apomictic (C, D) populations. Figs. 1-5 ×800 ; fig. 6 ×160.

FIG. 1.—Meiosis in the E.M.C., pachytene, *cf.* fig. 3 below (A).

FIGS. 3, 5.—Mitosis in the E.M.C.

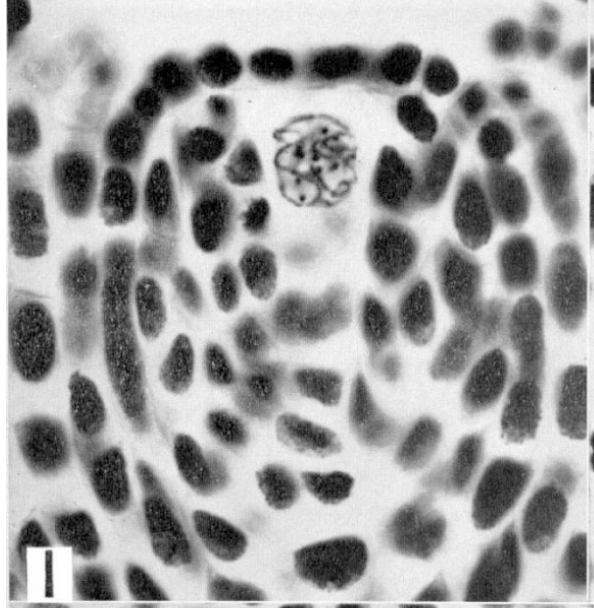
FIG. 3.—Late prophase (D_4).

FIG. 5.—Vacuolate resting stage (C_1).

FIG. 2.—Restitution in E.M.C. of C_1.

FIG. 4.—2-nucleate E.S. showing remnant of micropylar cell of dyad (C_3).

FIG. 6.—Precocious two-celled embryo at the time of anthesis (C_3).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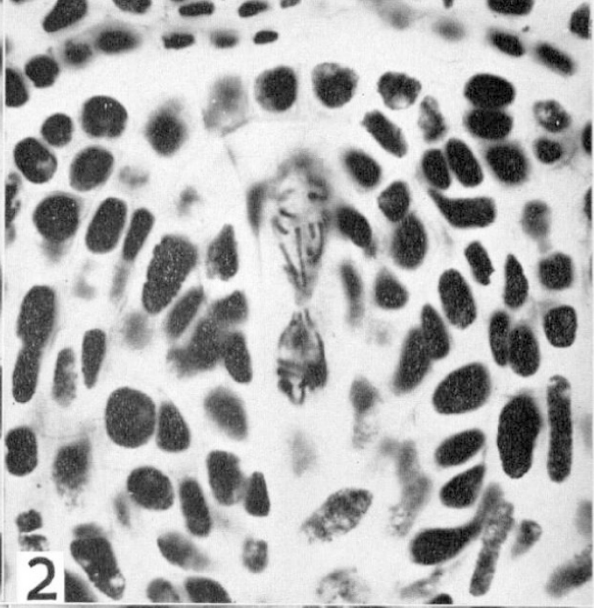
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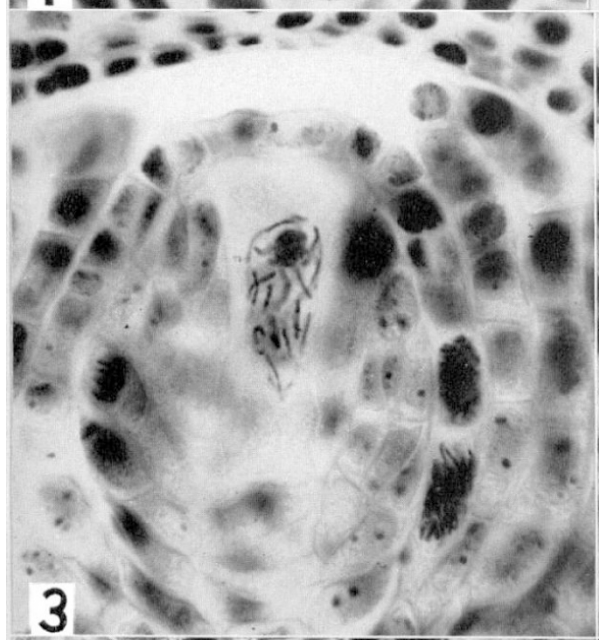
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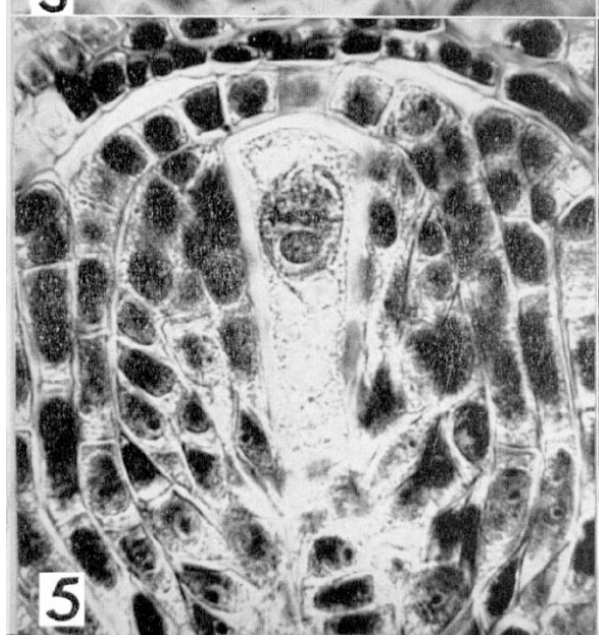
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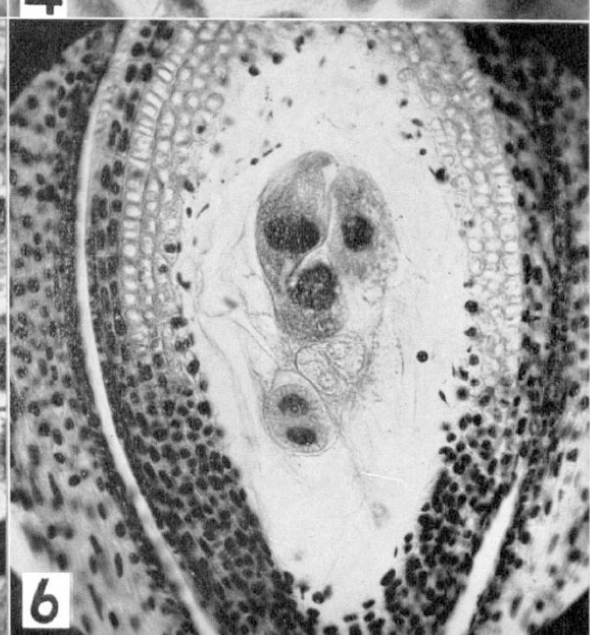
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4. The stages in the evolution of apomixis are complete in this species except for the absence of exclusively mitotic types. They appear in their simplest succession because of the strict inbreeding of all populations and the absence of the complication of apospory. Subsexual recombination provides for variation in the genotypic control of the reproductive mechanism and hence for adaptive evolution of the genetic system.

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