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A Cytological Study on *Phacellanthus tubiflorus* SIEB. et ZUCC. I.¹⁾

By

HAJIME MATSUURA

(With Plates II-IV and 6 Text-figures)

Very few contributions have been heretofore made to our knowledge on the cytology of *Orobanchaceae*. Only GATES & LATTER ('27) have dealt with the pollen development of *Lathraea clandestina* and *L. squamaria*, and CARTER ('28) has described meiosis in the EMC of *Orobanche minor*.

Members of *Orobanchaceae* are all parasitic herbs. Owing to their limited habit of living and accordingly to their limited distribution, it is not easy to obtain material for cytological work. This may be one of the reasons why the family has received so little attention from a cytological stand-point.

Phacellanthus tubiflorus SIEB. et ZUCC. with which the present paper deals is a member of the family to be found rather rarely. It represents the only species known to belong to the genus *Phacellanthus*.

The present study has been carried out since 1931. In the course of investigation, the writer has found that the species under investigation includes a series of polyploid forms (see p. 181). A detailed treatment of this subject will be given on another occasion when more data have been accumulated. In the present paper it is proposed to deal with some features noted in meiosis in a form having 35 haploid chromosomes, namely, (i) nucleolar inclusions, (ii) extrusion of nuclear substances and (iii) nuclear fusion. Some brief comment was also made of chromosome number in this species as a preliminary note.

Material and Methods

The present study is based on material collected in 1931 at Mt. Moiwa near Sapporo where some groups of the plants were found growing on the

1) Aided by a grant from the "Nippon Gakuzyutu-Sinkôkwai" (The Foundation for the Promotion of Scientific and Industrial Research of Japan).

root of *Magnolia obovata* THUNB. The first collection was made in late June of that year, and subsequent collections have been made from time to time as the plant came into bloom and set seeds.

A variety of fixing mixtures were used, of which FLEMING'S weak solution proved to give the best result. The exhaustion of air from the material was carried on in the fixing fluids by the aid of an electric air pump.

The material was sectioned in paraffin wax in thicknesses varying from 8μ to 25μ , those of 10μ being found most useful for the present study. The sections were usually stained by NEWTON'S gentian-violet method. For the material fixed in CARNOY'S solution, a modification of LA COUR'S ('31) method was found to give good but somewhat variable results. Other stainings were made with the combination of HEIDENHEIN'S iron-alum haematoxylin and safranin, and of methyl green, acid fuchsin and erythrosin. For some sections, FEULGEN'S 'Nuclearfärbung' method was applied, in order to determine the nature of the so-called 'chromatin' bodies extruded from the PMC at early prophase into the cytoplasm of an adjacent cell. The method, however, gave no satisfactory results, probably due to the inadequacy of fixing fluids used.

In order to avoid variable effects, if any, resultant from different fixations and stainings, all photos and drawings were made from sections prepared with the same treatment, that is, those fixed in FLEMING'S weak solution and stained by NEWTON'S gentian-violet method.

Observations

i) *Nucleolar Inclusions*

(Refer to Plate II)

In some species, the occurrence of crystal-like bodies in the nucleolus of the resting PMC has been reported, *e.g.*, in *Oenothera* (CLELAND '22), *Lathyrus* (LATTER '26), *Lathraea* (GATES & LATTER '27), *Oryza* (SELIM '30) etc. However very little knowledge has been gathered on their structure and behaviors at meiosis. The present material proved to be very favorable for closer observation on these bodies, as their constant occurrence within the resting nucleolus is more distinct here than in those previously described.

At the resting stage of the nucleus, the crystal-like bodies within the nucleolus were found to vary considerably in their shape, size and number. They appear to be deposited in vacuoles of the nucleolus which are present

usually as a group of unstained areas. Pl. II, Figs. 1a to 1f represent a variation of these bodies in six nucleoli, which were taken from one and the same loculus under the same microscopical field. Occasionally smaller bodies show Brown's movement. Though it was quite difficult to trace the consecutive behaviors of these so-called endonucleoli, owing to their so wide variations, none of the mother-cell of this stage revealing any definite feature on this point, the writer has very often met with cases where small bodies appear to be budded off from larger ones. Figs. 1a and b represent such a condition. Moreover, there seems to be a distinct tendency for these bodies to be fewer, the more numerous and larger the vacuoles containing no such bodies are. Compare the nucleoli, c and d, where several empty vacuoles are present, with those, e and f, containing a number of small bodies. Based on these observations and those on the nucleoli of later stages, it is most probable that the fragmentation and disintegration of the crystal-like bodies are actually and successively taking place at this stage.

These nucleolar inclusions are usually not easy to be detected, if the section is too deeply stained, being masked by the dark staining peripheral portion of the nucleolus. A close examination, however, always reveals the presence of such structure. In preparations stained with gentian-violet after NEWTON'S method, they appear frequently as yellowish brown bodies, probably due to coloration by the solution of potassium iodide-iodine. To examine the nucleolar contents more closely, however, preparations of the resting stage must be stained very faintly. In such favorably stained preparations, these crystal bodies assume their outline very sharply, showing a bright refractive character. The nucleoli represented in Fig. 1 were taken from such a preparation.

Owing to the minute size of these contents, the writer experienced much difficulty in determining their nature. However some evidence was obtained suggesting that they are probably composed of a protein reserve material. They stain yellowish brown with a solution of potassium iodide-iodine. In slides treated with MILLON'S reagent, these bodies appeared very lightish red. A concentrated solution of nitric acid gave a greenish yellow color to these bodies, and the same with an addition of ammonia gave a still deeper color, showing their outline more definitely. No conclusive reactions, however, were obtained from an attempt with a concentrated solution of nickel sulphate and also from the copper sulphate-potassium hydrate method.

The fragmentation and disintegration of these endonucleoli appear to

proceed further as the meiotic division progresses. At the synizesis stage, no large crystal-like bodies such as observed at earlier stages can be detected. Usually there are several very small vacuoles and a few well-defined 'crystal-like structures' (Fig. 5). Sometimes a large vacuole occupying the center of the nucleolus is formed, which may probably be due to the fusion of small vacuoles. At other times the vacuoles appear as colorless bright shining 'patches', a condition quite analogous to one described by LATTER ('26) in *Lathyrus odoratus*. Although on account of their extremely small size the exact determination was impossible, the writer inclines to consider that these refractive 'patches' are of a transition phase of the disintegration of 'crystal bodies'.

At the complete synizetic stage, 'nucleolar budding' frequently occurs. The extruded portions are usually very small and spherical in shape. The number of them varies considerably, two or more, however, being of common occurrence. In Fig. 5, a somewhat large extrusion from the nucleolus will be recognized. The 'nucleolar budding' in *Phacellanthus*, however, is less conspicuous than that described by DIGBY ('09) in *Galtonia candicans* and also that by LATTER ('26) in *Lathyrus odoratus*. It is not certain whether these extruded portions have some relation with the nucleolar contents. Probably they are independent in their origin from the latter, as no crystal-like bodies have been observed in them.

At more advanced stages of meiosis, *i.e.*, pachytene and diplotene, the nucleolus usually contains a single crystal-like body (Fig. 2); sometimes, several small fragments can be detected. LATTER ('26) observed the 'nucleolar body' at these stages in *Lathyrus* which is presumably "derived from the former crystal body and functions as an elaborating organ which transfers the elaborated material on to the thread with which it is in contact". In the present case, however, there was no indication of such a 'nucleolar body'. The crystal-like bodies persist yet, though there is some tendency toward a decrease in their quantity. Moreover no indication of connection was detected between these crystal bodies and the spireme.

During diakinesis and thereafter, the nucleolus shows a striking change. Firstly, the decrease of the nucleolus in size becomes more conspicuous. At mid-diakinesis, it measures 3μ in diameter, making a striking contrast with one at early diakinesis in which the average diameter was found to be about 4μ . Secondly, the nucleolar contents which have persisted as the 'crystal bodies' completely disappear at late diakinesis or the contraction stage and instead of them usually one clearly defined vacuole develops. The vacuole increase in size successively and at a stage when

the bivalents come out of the third contraction the nucleolus appears itself as just a thin film, the most of the central part of it being occupied by the vacuole. These conditions are represented in Figs. 6, 7 and 8. Finally the nucleolus disappears at a stage just prior to metaphase by the time the organization of a bipolar spindle is accomplished.

At interkinesis no nucleolar contents have been formed.

Observations on the inclusions within the nucleolus of the EMC revealed that they behave closely parallel with those in the PMC. They are found rather abundantly in the nucleoli of resting mother cells. They diminish as the meiotic developments proceed; usually only one body or two persist till early diakinesis and then the inclusions appear to be entirely disintegrated. In the EMC, the nucleolus usually disappears at mid-diakinesis. Fig. 4 represents a nucleolus of the cell at complete synizesis. Two bodies are evident within it. Note also that there is no connection of the nucleolus with the spiremes.

As will be seen from these observations, there seems to be a certain regularity in the behaviors of the crystal-like bodies within the nucleolus during the first meiotic division. It is impossible to say at present with certainty what rôle they play in the activity of the nucleolus, or whether they are merely a product consequently followed by the latter, but it seems evident in this material that no such direct relation exists between these crystalline bodies and the spireme, as claimed by LATTER ('21), GATES & LATTER ('27), etc.

ii) *Extrusion of Nuclear Substances*

(Refer to Plate III)

The extrusion of nuclear material into the cytoplasm of an adjacent mother-cell was found to be of regular occurrence in *Phacellanthus*. The phenomenon known as 'chromatin' extrusion or 'cytomixis' (GATES '11) has been heretofore repeatedly described and figured by many investigators in a number of plants. For detailed treatment of the literature concerning this subject one may properly refer to KATTERMANN ('33).¹⁾ Since in spite of numerous records, the problem of the mode of the process in connection with its causality appears not yet to have been satisfactorily explained, a detailed study has been made on the present material.

The process of extrusion in the PMC of *Phacellanthus* takes place

1) Note added to proof: cf. also KIHARA & LILIENTHAL ('34, *Japanese Jour. Genetics*, 10: 1-28).

normally while the spireme is coming out of the synizetic knot and during the inception of the pachytene stage. It may be noteworthy that at complete synizeisis such a condition has never been observed, although most of the former observers have stated that it occurs most frequently during complete synizeisis where the nucleus migrates through the cell and takes up such an eccentric position as to come in contact with the cell-wall of adjacent mother-cell. The present observation on this point seems thus to indicate that *the migration of the nucleus through the cell has no apparent relation with the process of extrusion.*

The extrusion of nuclear substances, according to the writer's observations, usually takes place simultaneously in all the PMC of these stages. The condition is quite noticeable in preparations where both early and late synizetic stages are present in the same loculus, the ejecting process usually occurring in the latter only. This synchronization of the event may offer an evidence showing that the process of extrusion can not at all be attributed to faulty fixation, for if so, all the mother-cells in the loculus would assume the same extruding condition.

As to the direction of expulsion, the writer made some careful observation. The direction was not found to be one and the same, as described by WEST & LECHMERE ('15) in *Lilium*, but also not merely haphazard, as described by most of the other observers. It was noticed in *Phacellanthus* that there is usually some regularity in the direction of the ejection, but it seems to vary concomitantly with the opening of the spireme threads from the synizetic knot. Pl. III, Figs. 9 a and b represent four nuclei in the same loculus, each of which is ejecting one pear-shaped body in the same direction, here toward the center of the loculus. Fig. 13 represents a mother-cell of a very late synizetic stage, just prior to pachytene, which is throwing off one small globular body into an adjoining cell, here too in the direction toward the center of the loculus. This mother-cell has received two larger bodies from a neighboring cell on the opposite side, the stage of which corresponds with that of the cell in Fig. 15.

Most of the former observers describe and figure cases where the extrusion takes place in the same direction as that of the contracting nuclear contents. This would lead to an impression that the event is merely an artifact induced by the fixation or treatment. These cases are in fact those the writer frequently met with. However, cases were observed also, not less frequently, where the direction of extrusion is just opposite to that of the migration of nuclear contents. These cases are clearly shown in several figures of Pl. III, especially in Figs. 11 and 12. As clearly seen

there, the process of extrusion is related with the opening of the spireme threads, but *not with the migration of chromatin materials in the nuclear cavity*. Frequently it was possible to identify small bodies which attach with the spireme in the nucleus. In Fig. 14, such a small body probably to be extruded may be recognized. A close examination reveals no material connection between such bodies and the spireme thread. Usually these so-called 'chromatin' bodies are seen at the apices of the spireme hoops which have a horse-shoe shape. It is thus quite probable that the spireme with appended 'chromatin' bodies emerges further toward the pole away from the synizetic knot and attains to the cell-wall, forming a narrow strip of nucleolar cavity along its path in the cytoplasm and finally voids the 'chromatin' bodies into the adjacent cells, through the cell-membrane probably along the plasmodesms. The existence of such plasmodesms between the pollen mother-cells has been demonstrated by several observers, such as DIGBY ('09) in *Galtonia*, GATES ('11) in *Oenothera*, WEST & LECHMERE ('15) in *Lilium*, SINOTÔ ('22) in *Iris*, etc. In the writer's preparations in which the fixation was best, these plasmic connection were also clearly visible.

The writer's observations agree with those by many of the previous authors in that extrusion into an adjacent tapetal cell is never seen. Probably this is due to the absence of plasmodesms between the PMC and the cells of the tapetum, as suggested by GATES ('11).

The extruded globules vary in size and number. But the variation is not so conspicuous as described by the former observers. Usually a nucleus of an early stage of extrusion ejects one pear-shaped globule (Fig. 9 ab), sometimes two bead-like bodies (Fig. 11), and in a few cases several of granular appearance. Evidence was also obtained showing that more than two bodies are ejected successively through the same plasmodesm or those near in position and that they accumulate together into a larger mass. Fig. 12 may represent such a condition, probably the larger one of the two extruded bodies having originated from the aggregation of two bodies, for three hoops of the spireme are recognizable. During later stages of meiosis the elimination of these bodies is usually less pronounced (Figs. 13 and 14). In the pachytene stage, however, cases of the giving off of two rather large bodies were frequently met with (Fig. 15).

The extruded material is surrounded by a clear zone. The membrane delimiting it from the cytoplasm appears to be of a similar nature to that which surrounds the nuclear cavity. The condition is quite similar with that described by GATES ('11) in *Oenothera*, WEST & LECHMERE ('15) in

Lilium and others. GATES called the clear area a 'pseudo-nucleus'. After the return of the spireme to normal position, the bodies seem to remain for a while as isolated heavily stained globules in the cytoplasm of the invaded cell (Figs. 13 and 14). These will be very quickly incorporated with the surrounding cytoplasm, no trace of them being visible afterwards. GATES describes for *Oenothera gigas* a condition of disintegration of the material assuming an appearance very similar to a spireme. Notwithstanding close examinations, nothing approaching such a condition has been observed in the writer's preparations. That the cytoplasm changes in appearance after synizesis and comes to be of denser and more fibrilous structure with numerous granules scattered throughout it in the pachytene stage, may probably have some connection with the incorporation of the extruded material into the surrounding cytoplasm.

Our present knowledge includes nothing on the exact nature of the extruded material. DIGBY ('09) called these globules 'chromatin bodies' because they readily take up 'chromatin' stains. The term has been used merely conventionally. These globules, according to the writer's observations, however, are made up of a substance entirely different from 'chromatin' partaking in the formation of the spireme. They appear first as smaller or larger roundish globules in the nuclear cavity around the synizetic knot, no connection being observable between them and the spireme threads. They are extruded into a neighboring cell by means of the stretching movement of the spireme. Very often a very fine thread passing through the cell wall appears to form a connection between the extruded bodies and the spireme threads, as described by many of the former observers. These 'connecting threads' however should be explained, in the writer's opinion, as the remaining portion of the extruded body in the plasmodesm.

DIGBY ('09) recorded nucleolar budding in *Galtonia candicans* which appears to take part in the extrusion. In *Phacellanthus*, a similar condition of the nucleolus was found to be of regular occurrence in the early synizetic stage. It was found however that the nucleolar buds soon disintegrate into smaller fragments, most of which are supposed to migrate in the nucleolar cavity and to be absorbed in the surrounding cytoplasm. However whether a part of these fragments actually takes part in the extrusion into an adjacent cell or not, it is impossible to say.

As pointed out by GATES ('11), it will be of prime importance to determine whether nuclei in which extrusion has occurred afterwards complete the meiotic processes and form normal tetrads. The data now at the

writer's disposal indicate that this does not occur in *Phacellanthus*. In this connection, attempts have been made to germinate the pollen grains. Pollen from several flowers was placed on a medium consisting of distilled water 100 cc., agar 1 g, and saccharose 12 g. The pollen was examined at the end of 24 hours and germination was found to have taken place in nearly all grains.

Discussion on Cytomixis. The extrusion of 'chromatin' bodies has been heretofore explained in several different ways. The most frequent opinion expressed is that the process is wholly or in part an artifact induced by faulty fixation or treatment. This interpretation was supported by ROSENBERG ('09 ab), NAKAO ('11), SAKAMURA ('20), YASUI ('21), SINOTÔ ('22), TISCHLER ('21), etc. KÖRNICKE ('01), however, attributed it to an abnormal physiological condition of the anther at the time of fixation or treatment. KATTERMANN ('34) is of an opinion that the phenomenon observed in his *Triticum-Secale* hybrids is based on 'eine natürliche Reaktion auf vorläufige unkontrollierbare Reize der Umwelt' and it is 'eine Abnormität in Zellgeschehen'. DIGBY ('09), GATES ('11, '12) and especially WEST & LECHMERE ('15) claim that the process represents a normal condition in the meiotic course of the PMC, although they frequently met with cases which were difficult to explain in this way.

The data presented in this paper appears to support the last interpretation. The main features of the process of extrusion in *Phacellanthus* may be profitably summarized as follows.

1) *The occurrence of the process* is synchronous and is restricted to particular stages of prophase.

2) *The quantity of the extruded bodies* appears to be nearly constant at any particular stage concerned. The size and number of them may vary, but there is a distinct tendency for them to be smaller, the more numerous they are.

3) *The mode of the process* is entirely autonomous. At an early stage of extrusion, such 'bodies' appear themselves in the nuclear cavity around the synizetic knot. They are pushed toward a pole of the cell by the elongation of the spireme, along a narrow portion of the nuclear cavity extended into the surrounding cytoplasm. Finally they are given off into the cytoplasm of an adjoining cell, passing through the plasmodesms of the cell-wall.

4) *The subsequent process* is quite normal. The spireme which took part in the process returns to a normal position, leaving the extruded bodies as isolated globules in the neighboring cell. No foreshadowing

whatsoever of unhealthiness in appearance is observable in either the parental cell or the invaded cell. The extruded bodies are quickly absorbed in the surrounding cytoplasm.

The data now at the writer's disposal seem to indicate that the extruded material has no important relation with the chromatin material which takes part in the formation of the spireme. It may be some waste products of the cells at these stages which are subjected to so sudden changes in their metabolic activity. The entire process of extrusion is a continuous one. At an early stage of synizesis, some of these products are most actively voided in the cytoplasm of the same cell (Pl. III, Fig. 10) and at a somewhat later stage, others are extruded into the cytoplasm of a neighboring cell. There appears to be no essential difference between these two processes.

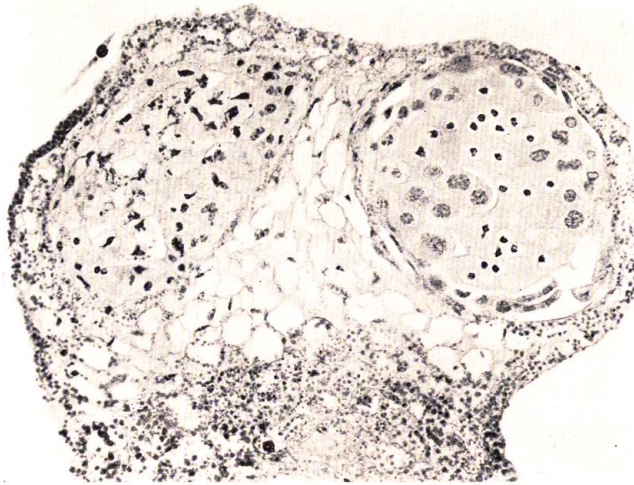
In this connection, it will be of interest to note that an analogous phenomenon was also observed at certain stages of ascus formation in *Ascomycetes* (cf. MATSUURA & GONDÔ, '35). In *Peziza subumbrina* it was found that in early stages of meiotic division I the extrusion of 'chromatin' bodies in the cytoplasm takes place by means of elongation of the spiremes, in a way similar to that described in the present paper, though no bodies to be extruded were here recognizable as isolated globules in the nuclear cavity.

From these considerations, the phenomenon may be regarded as one of the characteristic features of the prophasic stages of meiosis. SINORÔ ('21) describes many cases where the extrusion of nuclear substances occurred in cells of several other tissues such as tapetum, integument, nucellus and ovary. The writer, however, could observe no such conditions whatsoever as described by him in preparations where the fixation appears to be fairly good.

Great care must be taken on the quality of fixation in connection with these phenomena. The synizetic stage may represent the most unstable condition of the meiotic processes. The fixation at this phase may bring about very delicate effects on the cell contents. Even the most slightly bad fixation is apt to cause some serious damages, and moreover, variable penetration of the fixative will give both reliable and unreliable results in the same section. Text-fig. 1 represents one good example of this. Pollen mother-cells of the loculus on the right are quite satisfactorily fixed, while those of the other loculus, though they are perhaps of the same complete synizetic stage, are in terrible disorder. Now such a condition induced by faulty fixation is one which might be confused with the true

extrusion process. Really cases recorded so frequently as 'excessive chromatin extrusion' by many of the former observers, clearly correspond to figures shown in such damaged loculi (*cf.* figures given by SINOTÔ, DIGBY, GATES, WEST & LECHMERE, etc.). Their claim that these conditions are induced by bad fixation is correct in so far as their cases are concerned, but it must be noted that those abnormal conditions are entirely different from the case reported in the present paper.

We are not concerned here with 'blitzartige Reaktionen, die den Kern rein passiv weiterbefördern' as stated by TISCHLER ('21), but with an autonomous activity of the nucleus of PMC at particular early stages.



Text-fig. 1. A cross section of an anther, showing effect of fixation. $\times 150$.

It is rather beyond the writer's presumption at present to look for the significance of this phenomenon. It may be attributed to the economy of PMC which are supposed to be necessarily subjected to it. A possibility of certain connection of cytomixis with polyploidy of PMC should also not be excluded, as the present material seems most probably to be of a polyploid nature (*vide infra*) (*cf.* McCLINTOCK, '29). At any rate, however, the present finding that the excluded 'chromatin' bodies have no apparent connection with the substance constituting the spireme appears to indicate that 'chromatin' extrusion does not result in the loss of hereditary substances. The case described by KATTERMANN ('33) in F_1 and F_2 plants of wheat-rye hybrids reveals clearly however that the substance constituting the spireme itself is also ejected from one cell to another, result-

ing thus in the formation of multi-nuclear, hypoploid cells which are generally destined to degenerate finally, and in this respect the case appears to be entirely different from the present one.

iii) *Nuclear Fusion*

(Refer to Plate IV)

An interesting feature of the synizetic stage of PMC in the present material is the occasional occurrence of nuclear fusion. No such a phenomenon has been observed except at the synizetic stage. Cells formed by nuclear fusion necessarily have two synizetic knots in one nuclear cavity. The manner of their origin and their subsequent behaviors are represented in Pl. IV.

Very often the nuclear cavities of two neighboring cells migrate in opposite directions and the synizetic knots come to lie back to back, as represented in Fig. 17. This perhaps causes perforation in the cell-wall between them which is so thin and delicate at this stage, that the two nuclear cavities come to fuse together (Fig. 16). Usually one of the two knots migrates to the other side and conjugates with the other knot (Fig. 18). But there is never any trace of real fusion of these synizetic knots, each of which still retains its individuality.

The cell-wall which had separated the two cells before nuclear fusion took place appears to be quickly lost. This may be caused by progressive enlargement of the perforation in it. The result is the formation of giant cells, the size of which is just twice that of the normal ones. In such cells, the cytoplasm sometimes shows some trace of abnormality, being more vacuolated and more granular than in the normal cells; sometimes, however, it presents a quite normal appearance.

The subsequent behaviors of such giant cells are characterized by (1) the complete prevention of the meiotic processes, and (2) the restoration of the normal condition by the degeneration of one of the nuclear contents. Cells as represented in Figs. 19-22 are those found in a loculus in which most of the other normal mother-cells were at the diplotene stage. There are no indications of further progress in the meiotic process in such cells, each of them still retaining the synizetic stage. The apparent recovery of one of the nuclear contents is accomplished by the disintegration of one of the synizetic knots into numerous minute fragments. The secondary formation of nuclear membrane then takes place between the knots, thus completely isolating the degenerated part from the normal one. Fig. 20

represents a beginning stage in the process of degeneration. In Fig. 22 one of the nuclear contents is completely disintegrated and the nuclear cavity is divided into two by the membrane secondarily formed.

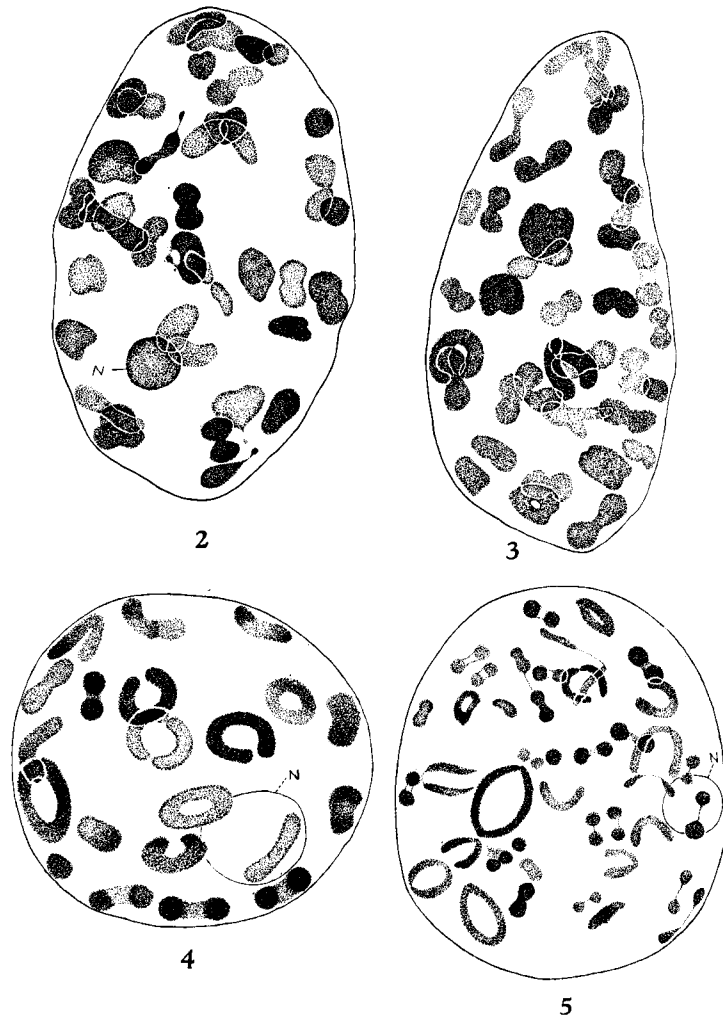
There are some indications that the degenerated part of the nucleus is subsequently absorbed by the surrounding cytoplasm, but it is not clear at present whether cells in which nuclear fusion has occurred afterwards complete the meiotic processes. There are also a few cases where both the synizetic knots show indications of degeneration at the same time. Fig. 21 probably represents such a condition.

MEDWEDEWA ('34) recorded an analogous case of nuclear fusion in early prophase of meiosis in an Italian variety of *Cannabis sativa*, but it was not clear in his instance how the fused nuclei behave later on. RUTTLE ('28) observed binucleate PMC in *Nicotiana* haploids which "resulted from the extrusion of the entire nucleus of one PMC into the cytoplasm of an adjoining PMC". Though fusion of these nuclei was not actually demonstrated, it was said that "they will doubtless degenerate". Cases recorded by KATTERMANN ('34) in *Triticum-Secale* hybrids indicate also that fusion of parts of nuclei induced by cytomixis will as a rule accompany degeneration of them.

From these and the present findings it may be inferred that the co-existence of two prophasic nuclear substances in one nuclear cavity has some fatal effects on the nuclear activity, although no definite statement is possible on the nature of these effects. Probably the present case will make a marked contrast with those of binucleate PMC as described by KARPECHENKO ('27) in *Raphanobrassica* and by SHIMOTOMAI ('31, '33) in certain species hybrids of *Chrysanthemum*, in which the fusion takes place at metaphase I through the union of the spindle fibers separately formed by each nucleus, and the resulting nuclei show no sign of degeneration.

iv) *Chromosome Number*

Phacellanthus tubiflorus appears to be a highly polymorphic species from the karyological view. The materials collected at three different places on Mt. Moiwai near Sapporo during three successive years (1931-'33) were found, to the writer's astonishment, each to represent a different chromosome situation. The material of 1931 on which the present observations described on previous pages were made, proved to have 35 haploid chromosomes. They were counted at diakinesis and metaphase I in both PMC and EMC; the diploid number, 70, was ascertained in the division of archesporial cells, and the haploid number was also counted at meta-



Text-figs. 2-5. Four nuclei at mid-diakinesis. 2 and 3, from a PMC and an EMC of the 35-chromosome type; 4, from an EMC of the 21-chromosome type; 5, from a PMC of the 42-chromosome type. Figs. 2-4, from paraffin sections, 5 from a permanent smear preparation. $\times 2200$.

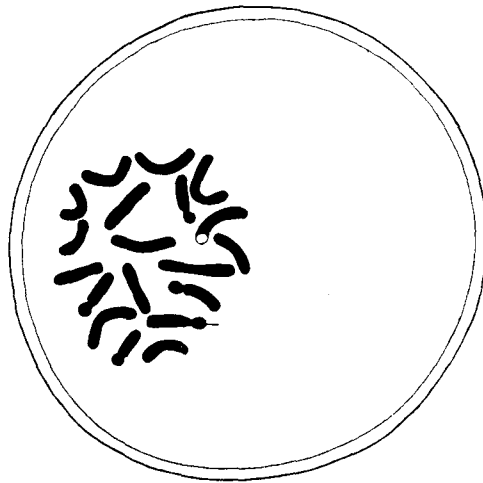
phase of the division of a megaspore nucleus. In the 1932 material, 21 bivalents were counted in diakinetive nuclei of EMC (no PMC were available). The 1933 material was found to have 42 haploid chromosomes, as counted in PMC at diakinesis and metaphase I. No marked difference in the morphology of flowering shoots was noted between these three chromo-

some types. As will be seen in Text-figs. 2-5, there is a certain great range of variation in chromosome size, especially between the 42-chromosome type and the others, but no apparent difference in the size of nuclei. Whether this difference in chromosome size is due to different treatments (for the former the permanent smear treatment was employed, TAYLOR's solution being used as the fixative, while the other two were observed from preparations of paraffin sections as described on p. 170), or whether it is of another significance, such as a possibility of relationship of this with the degree of cytomixis, it is not possible to say at present.

Numerical considerations would infer that these three chromosome numbers represent $6n$, $10n$ and $12n$, respectively, with the basic number of 7. It may be added that so far as the observations on metaphase plates in the 35- and 42-chromosome types go, the secondary association of bivalents takes place in a marked fashion. For the elucidation of the exact nature of this polymorphism however reliance must be placed upon further investigations.

We have at present five species of *Orobanchaceae* in which the chromosome number has been reported, as shown in the following list:

	n	2n	
<i>Lathraea clandestina</i>	21	—	GATES & LATTER ('27)
„ *	—	—	WITSCH (<i>ex</i> HEINRICHER, p. 129)



Text-fig. 6. Metaphase of the first vegetative division of a pollen grain in *Orobanche caerulescens*. Aceto-carmine preparation. $\times 2200$.

<i>L. squamaria</i>	21	—	GATES & LATTER ('27)
	18*	—	WITSCH (<i>ex</i> HEINRICHER, p. 129)
<i>Orobanche caerulescens</i>	20*	—	SUGIURA ('31)
	19 ¹⁾	—	MATSUURA (see Text-fig. 6)
<i>O. minor</i>	19	38	CARTER ('28)
<i>Phacellanthus tubiflorus</i>	21	—	MATSUURA
	35	70*	"
	42	—	"

* No figures have been published as yet.

It will be noted from the above list that three of the five species investigated are characterized by different chromosome numbers within the same species. If some of them were not actually based on miscountings, this heteromorphism in chromosome number must be of certain significance.

Summary

1. *Phacellanthus tubiflorus* appears to include a series of polyploid forms. Up to the present, 21, 35 and 42 haploid chromosomes have been counted in three different groups of plants. These do not accompany any marked morphological difference.

2. The nucleolus at early stages of meiosis is characterized by deposition of crystal-like bodies. Evidence suggests that they are probably composed of a protein reserve material. They are consecutively fragmented and disintegrated as the meiotic processes continue. Usually at early or late diakinesis they disappear completely. No apparent relation was observed between these crystalline bodies and the spireme (*cf.* LATTER '21).

3. The extrusion of nuclear substances takes place regularly at certain stages of synzesis and pachytene of PMC. The extrusion into the cytoplasm of the same cells is directly followed by that into the cytoplasm of a neighboring cell. These phenomena were regarded as normal and characteristic features of early stages in the meiotic course of PMC. Emphasis was placed on the essential difference of the present case from those previously reported by many authors which are to be attributed to the faulty fixation or treatment.

4. Nuclear fusion occurs frequently between two PMC at synzesis. The fused cell has two synzetic knots in one big nuclear cavity. Usually one of the two knots degenerates and the other appears to recover its

1) The chromosomes were counted at the division of pollen grains. Three metaphase figures agreed with the number indicated.

normal condition. It is not definitely clear, however, whether the apparent survival completes the meiotic processes.

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Explanation of Plates

All the photomicrographs were taken by the writer with the aid of a Leitz apparatus MA II. The different combinations of the lenses and magnifications obtained are given below:

	Objective	Eyepiece	Magnification
i) Pl. II, Figs. 1-4.	Zeiss Apo 120 (N.A. 1.30)	K. 30 ×	4000
ii) Pl. II, Figs. 5-8.	„	Homal IV	2000
iii) Pl. III, Figs. 1-15.	Leitz 1/12 Oel Immersion	„	1600
iv) Pl. IV, Figs. 16-18.	Leitz 4	K. 15 ×	330
v) Pl. IV, Figs. 19-22.	same as in (ii)		

Plate II, Figs. 1-8.

Showing the nuclear inclusions.

- Figs. 1a-f. Six nucleoli in resting nuclei of PMC., showing variation of size, form and number of the endonucleoli.
- Fig. 2. A nucleolus of a PMC at pachytene.
- Fig. 3. A nucleolus of a PMC at early diakinesis.
- Fig. 4. A nucleolus of an EMC at synizesis. The black mass of this figure is a part of the synizetic knot. Note no apparent connection of the nucleolus with the spireme.
- Fig. 5. A nucleus of a PMC at synizesis. Note: minute glöbular bodies around the knot, and vavuoles and crystalline bodies in the nucleolus.
- Fig. 6. A PMC at diakinesis. Note: a minute crystalline body in the nucleolus.
- Fig. 7. A PMC at third contraction. Note: vacuole development in the nucleolus.
- Fig. 8. A PMC at pro-metaphase. Note: more advanced stage of vacuole development in the nucleolus.

Plate III, Figs. 9-15.

Showing the extrusion of nuclear substances in PMC.

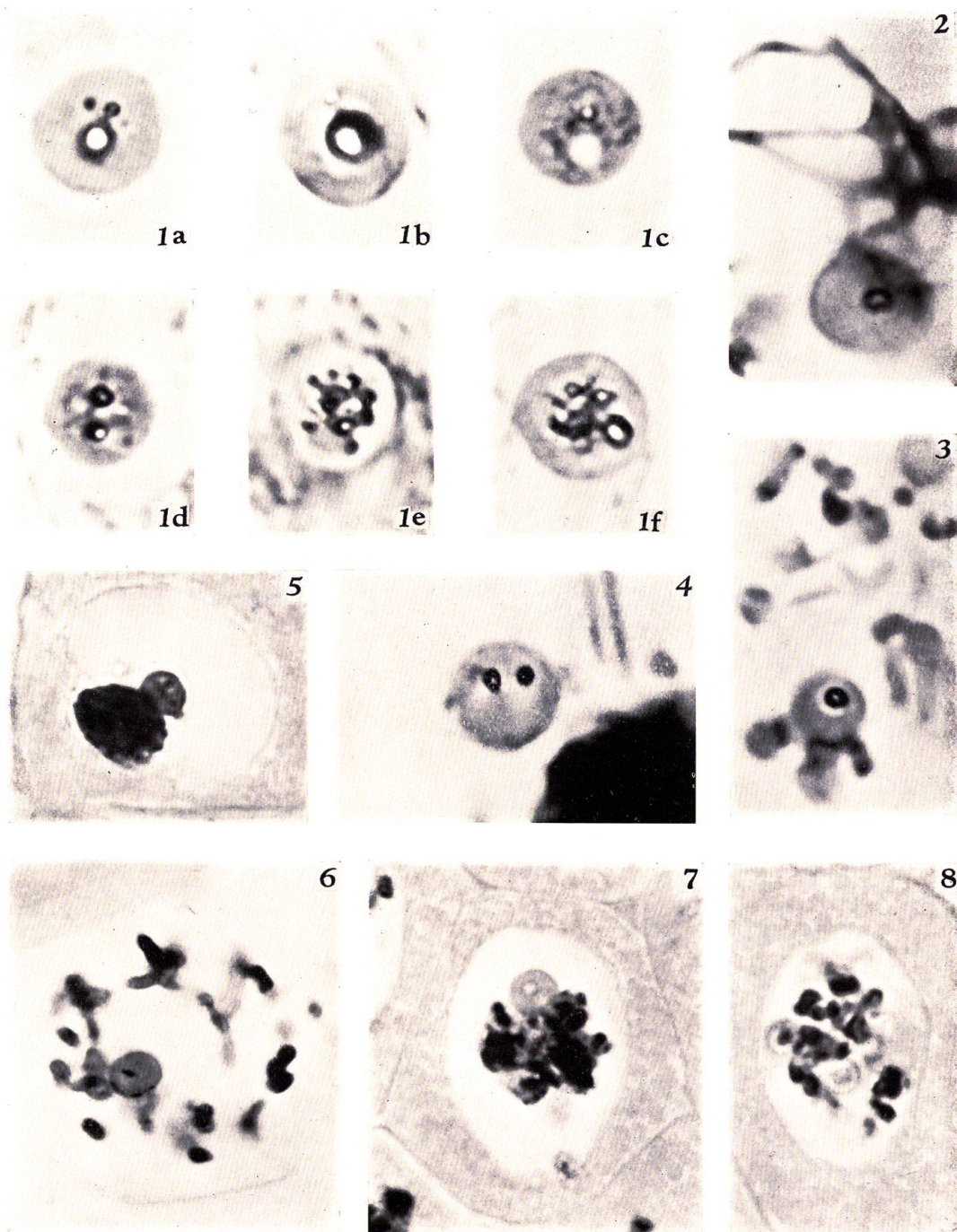
- Figs. 9 a and b. Four cells at synizesis in the same optical field (the cell in Fig. b lying just below the lowest one in Fig. a), each of which is throwing off one

- pear-shaped globule in the same direction.
- Fig. 10. A cell at a somewhat earlier stage. The extrusion of nuclear substances into the cytoplasm of the same cell is shown. Note the perinuclear zone of the cytoplasm.
- Fig. 11. A cell at synizesis extruding two bead-like bodies into two neighboring cells.
- Fig. 12. A cell throwing off two bodies into an adjacent cell, the larger one of which has originated from the aggregation of two previous smaller ones. Fig. 12a represents a portion of it. Note the relation of the extruded bodies with the spireme.
- Fig. 13. A cell at early pachytene. One small globule is ejected into an adjoining cell, and two rather large bodies have been thrown off into the cytoplasm of this cell.
- Fig. 14. A cell lying just below that in Fig. 13. Fig. 14a represents a part of the cell and shows a globule attached to the spireme.
- Fig. 15. A cell at a later stage. Two bodies are ejected into a neighboring cell. The upper part of the figure is the tapetum.

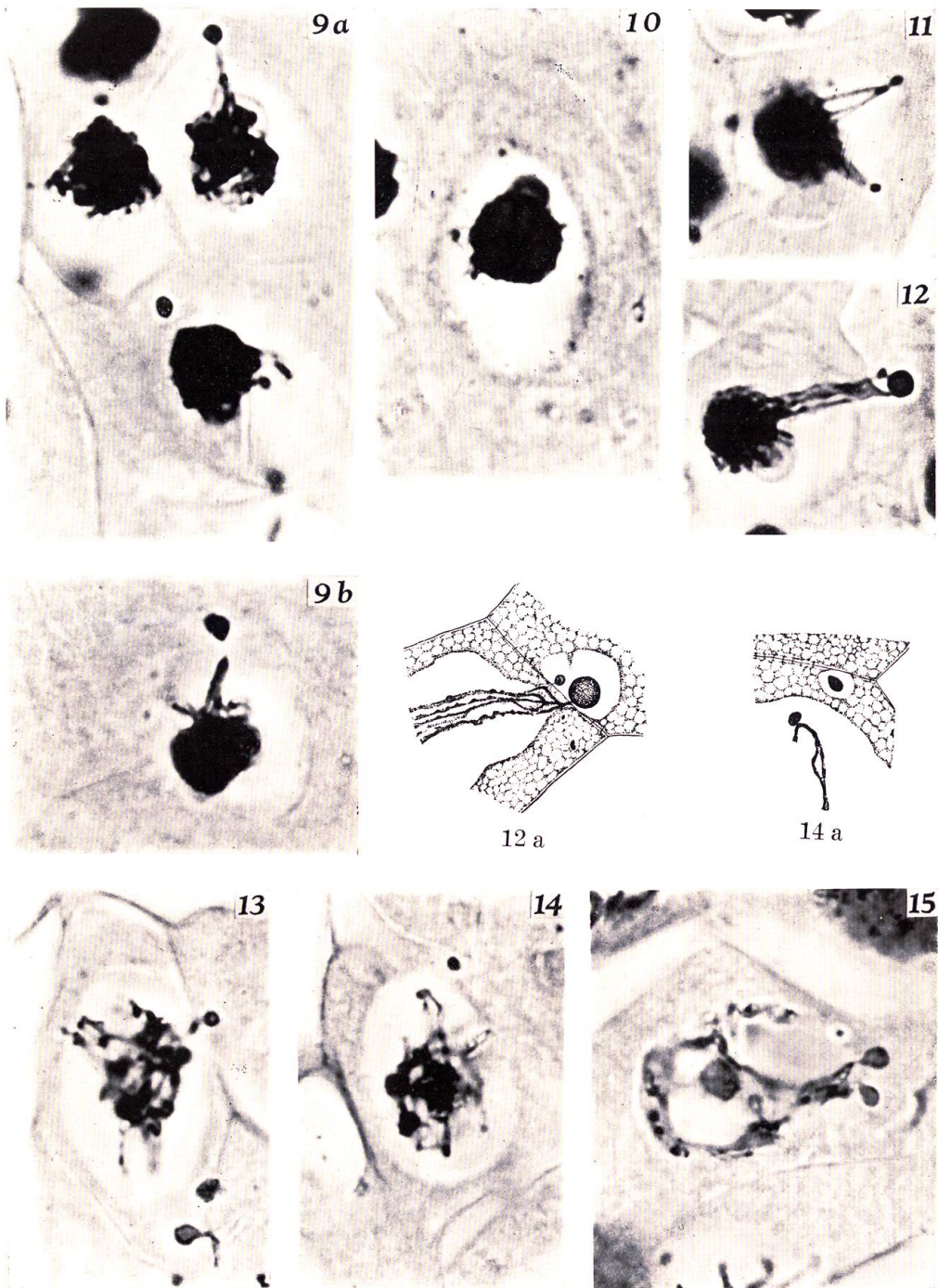
Plate IV, Figs. 16-22.

Showing nuclear fusion at the synizetic stage in PMC.

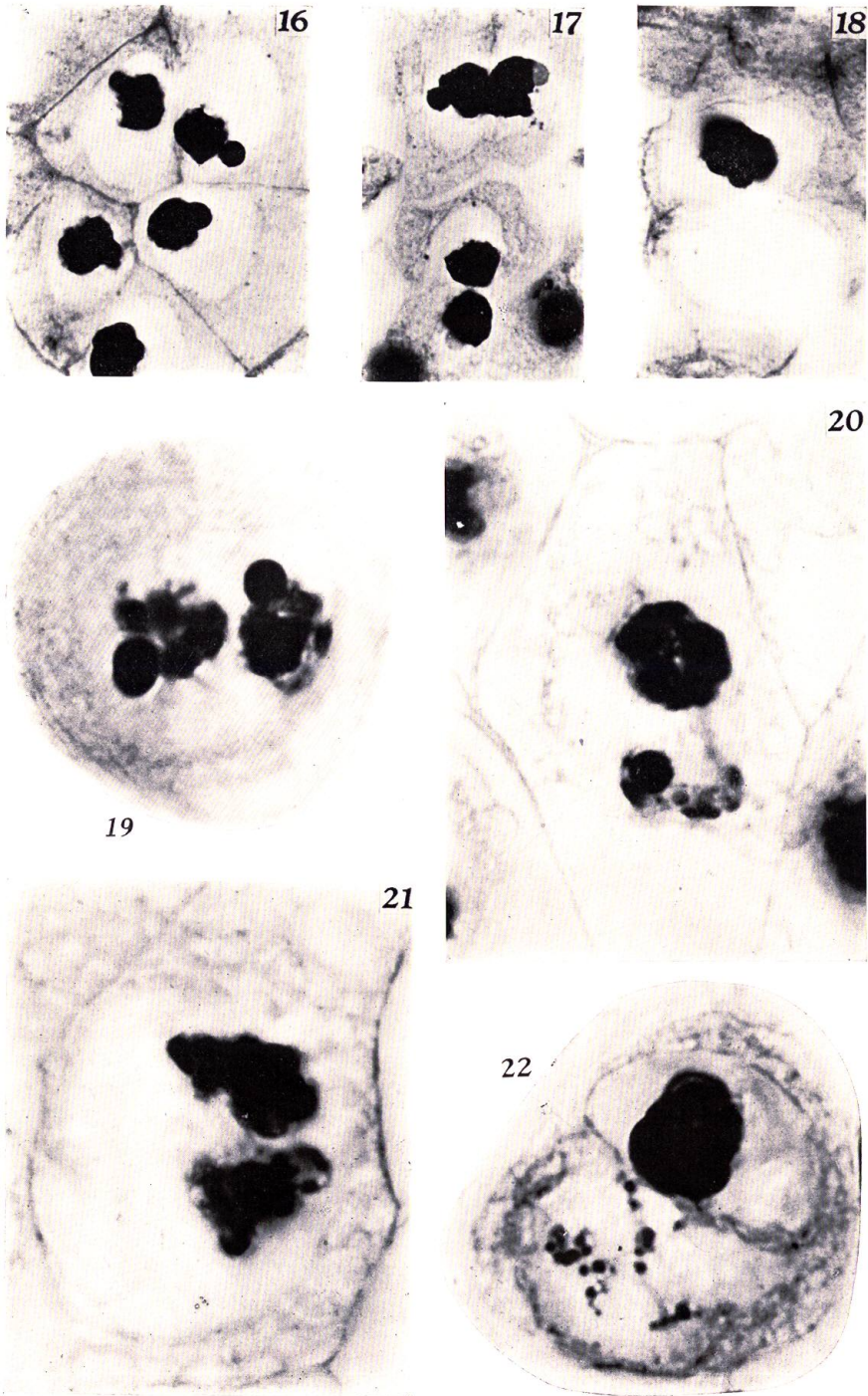
- Fig. 16. Two nuclei beginning to fuse together. Note perforation in the cell-wall between them.
- Fig. 17. Two nuclear contents lying back to back.
- Fig. 18. Accomplishment of nuclear fusion.
- Fig. 19. Two knots in one nuclear cavity.
- Fig. 20. One of the two synizetic knots is degenerating.
- Fig. 21. Both the synizetic knots show symptoms of disintegration.
- Fig. 22. Restoration of the normal condition of one of the two nuclear contents. Note fragmentation of one of them and nuclear membrane secondarily formed between them.



H. Matsuura photo.



H. Matsuura photo.



H. Matsuura photo.