Pollen Formation in *Oenothera gigas*.

BY

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With Plates LXVII-LXX.

INTRODUCTION.

IN a paper published two years ago ('09 *a*), the writer showed that the appearance of the tetraploid number of chromosomes in *Oenothera gigas* is accompanied by an increase in the size of the nuclei and cells of this mutant as compared with *O. Lamarckiana*, the parent form. This increase in size was found to vary for different tissues. The respective average volumes of the cells were approximately 2:1 in the case of the epidermal cells of the petals. This was in harmony with Boveri's law deduced from experimental cytological studies upon sea-urchin eggs, in which a doubling in the number of chromosomes was accompanied by a doubling in the volume of the cells, and in the *surface* (not the volume) of the nuclei. This numerical relationship was not found to hold, however, in *Oenothera gigas* in most of the tissues measured. Rather it was found that, in the pollen mother-cells, the *volume* of the nuclei was doubled, while the respective volumes of the mother-cells themselves were 1.5:1. In other tissues the ratios of the average cell volumes in *O. gigas* and *O. Lamarckiana* were in one case 3:1, in another tissue 3.67:1, and in still another 3.83:1. The volumes of the nuclei in the last cases have not yet been measured. Without outlining further the results of the paper mentioned, it may be stated that I concluded that the gigantic stature of *O. gigas* resulted from the larger size of its cells and nuclei, which in turn was in direct relation with the presence of the tetraploid number of chromosomes.

In complete harmony with the law for the nuclei in the pollen mother-cells of *O. gigas* and *O. Lamarckiana*, namely, that the doubling in the number of chromosomes is accompanied by a doubling in the volume of the nuclei, Tischler ('10) has recently found that in races of the Banana having respectively 8, 16, and 24 chromosomes, the volumes of the nuclei are as 1:2:3. The work of the Marchals ('09) has shown in the same manner in Mosses, that with the appearance of the tetraploid or octoploid

number of chromosomes there is a corresponding increase in the nuclear volume. As is well known, Němec ('10) has obtained similar results in his experimental production of chromosome doubling in root-tips.

It does not necessarily follow, however, that a variety with larger cells will have the tetraploid number of chromosomes. This is shown by the case of a giant race of *Primula sinensis*, in which Gregory ('09) found that, though the cells were larger, the chromosome number was unchanged. There are therefore two types of giantism in plants, one in which the tetraploid chromosome number is accompanied by a doubling in the nuclear volume and an accompanying increase, in varying proportions, in the volume of the cells; another, in which the amount of chromatin has not appreciably changed, while the cytoplasm has undergone a large increase. In the latter case there must be a marked change in the karyoplasmic relation, while in the former there is no change in the karyoplasmic ratio or, as my measurements show, a relatively small change.

A further consideration of the size relationships between *O. gigas* and *O. Lamarckiana* will not, however, be entered into in the present paper, the purpose of which is to give an account of the pollen formation in *O. gigas*. The process of chromosome reduction in *Oenothera* has already been described by me in some detail in several previous papers, including a study of *O. Lamarckiana* and several of its mutants, notably *O. rubrinervis* ('08) and *O. lata*, and the hybrid *O. lata* × *O. gigas* ('09 b); as well as a study of *O. biennis* and *O. laevifolia* ('09 c), in both of which the phenomena of reduction were found to be precisely the same as in *O. Lamarckiana* or *O. rubrinervis*.

Since diakinesis and certain other stages of the reduction process were studied with special care in a previous paper ('08), in which the method of chromosome pairing and the main events in the history of the chromosomes during meiosis were, I think, satisfactorily demonstrated, the present paper will not attempt to give another complete series showing all the events of meiosis, but attention will be concentrated upon certain stages which have been intensively studied. Of these, the series of stages or conditions which are usually grouped together under the term synapsis has received much attention, with the result that more changes in the nucleus are found to occur during this period than has previously been supposed. A type of pollen sterility which differs in certain respects from any previously known in plants will also be described, and certain variations in the phenomena of meiosis will be considered.

The material for these studies was all obtained from plants of *O. gigas* grown in connexion with my pedigree cultures, and which were derived originally through the kindness of Professor de Vries, from seeds descended through several generations from an original single mutant in his garden at Amsterdam.

The cytological methods employed need no description. For fixing solutions Bouin's fluid¹ was found satisfactory, in addition to various chrom-acetic solutions. Heidenhain's iron-alum haematoxylin was the stain most frequently employed, and a counter-stain with orange G was often useful in bringing out such structures as the cytoplasmic connexions between pollen mother-cells. The fact that the cells and nuclei are considerably larger in *O. gigas* than in the other forms has been a distinct advantage in the study of several of the stages of nuclear development. Several of these preparations were made in the laboratory of Professor Grégoire, to whom I am much indebted for laboratory facilities during my stay in Louvain.

DESCRIPTION.

Archivesporium and Synapsis.

In a longitudinal section of a very young anther a single row of archivesporial cells appears. The nuclear reticulum of these cells is at first extremely delicate, so much so that only traces of such a structure can be seen. It is always composed of finer threads than the cytoplasmic reticulum at this time. The tapetum is uninucleate and remains so until about the time of synapsis, when a simultaneous mitotic division occurs in all the tapetal nuclei. Frequently, though not always, the archivesporial cells divide to form the cells which become the pollen mother-cells. In some cases, however, these cells, by subsequent growth and without division, form the pollen mother-cells directly. Simultaneous growth of the cells, nuclei, and nucleoli of the archivesporial cells occurs, until they are enormously larger than their original size. Such a pollen mother-cell is shown in Pl. LXVII, Fig. 1. This cell is just ready for the beginning of the nuclear condition known as synapsis. Fig. 2 shows the beginning of this process, Fig. 3 a somewhat different condition occurring at this time, and Fig. 4 a slightly later stage as it sometimes occurs. As will be shown later, there is much variation in the relations between nucleus and cytoplasm at this period, but in all cases the result is a rather sudden increase in the size of the nucleus. Fig. 5 shows the typical synaptic condition after the nuclear expansion has been completed.

In another paper (Gates, '08) I have called attention to the fact that in *O. rubrinervis* there is a large amount of growth of the nucleus at the time of synapsis, without corresponding growth of the cytoplasm. With further measurements it has been shown in a recent paper (Gates, '11) that the appearance of contraction of the nuclear contents during the period of synapsis is largely, though not wholly, the result of this sudden nuclear expansion without a corresponding growth or change in position of the chromatin content of the nucleus.

¹ A note on my use of this fixing agent is to be found in Science, N. S., xxxi. 234, 1910.

A series of measurements of the pollen mother-cells and nuclei of *O. gigas* further confirms this view, and shows that while there is some growth of the pollen mother-cells during the period between the beginning and the completion of nuclear 'contraction', yet this is not in proportion to the very large increase in the size of the nucleus during this period. This result is in general agreement with Lawson's ('11) interpretation of synapsis from his study of *Smilacina*. The results of these measurements will not be presented in detail in the present connexion, but they show that, between the resting telophase of the last archesporial division and the synapsis (synizesis) condition, the pollen mother-cells and their nuclei undergo a simultaneous growth for a considerable period, during which a very large amount of increase in size takes place. In an earlier paper (Gates, '08) the same was shown to be true of the forms with fourteen chromosomes. This is followed by a rather sudden expansion of the nucleus at the beginning of 'synapsis'. The mother-cell having nearly completed its growth just before the beginning of the phenomenon of synapsis, its subsequent enlargement is relatively small, so that the ratio $\frac{\text{cell volume}}{\text{nuclear volume}}$ is conspicuously greater just before synapsis than in cells in the synaptic condition. This is clearly shown by a comparison of Figs. 1 and 5, Pl. LXVII. In Fig. 1, however, the nucleus is a trifle smaller than the average size at this time. Certain conclusions in connexion with this interpretation of synapsis have been discussed in another paper (Gates, '11) and need not be referred to here. A number of other features of exceptional interest have since developed in connexion with further study of synapsis, and these will be discussed in the present paper.

It is of course necessary to determine what pollen mother-cells are about to enter upon synapsis. One finds that in certain anthers of flowers at about this stage, some of the pollen mother-cells will be in the condition shown in Fig. 2, in which the synaptic process is just beginning. In such anthers other mother-cells do not show such 'clear spaces' adjoining their nuclear membrane, and such nuclei can safely be taken to be in the stage just preceding the beginning of synapsis.

There appears to be wide variation in the manner in which the nuclear expansion which produces the 'synaptic' condition comes about. In some cases (Fig. 2) its early stages are certainly very gradual, there being an expansion of the nuclear membrane which sets it free from the nuclear reticulum, leaving clear areas at one side of the nucleus, occupied only by karyolymph. Expansion of the nuclear membrane in this manner may continue until the nucleus has attained to nearly its definitive size. This may have been the case in the nucleus represented by Fig. 5. Sooner or later, however, in this growth period of the nucleus, the nuclear membrane is likely to become very delicate and indistinct, or it may break down

completely, leaving the nucleus in open communication with the cytoplasm. This is certainly true in fixed preparations, and is probably also true in the living cell. Fig. 3 illustrates a not uncommon condition at this time, in which there appears to have been a sudden forcible expansion or rupture of the nucleus on one side, following perhaps such a condition as shown in Fig. 2. This gives a clear area in the cell containing nothing but karyolymph or cell-sap. The main body of the nucleus is in such a case unaltered, and the amount of increase in the karyolymph can be estimated from the size of the pouch-like lateral enlargement of the nucleus. That this lateral expansion is relatively sudden is shown by the fact that the nuclear reticulum has not begun to undergo the changes which will transform it into the typical spireme condition of synapsis. Surrounding the clear area in such cases as Fig. 3, there may be a definite membrane, or it may be quite indistinct and indefinite.

Another condition very commonly found in mother-cells before the completion of synapsis is that shown by Fig. 4. Here the nuclear membrane evidently first began to enlarge, as in Fig. 2, on the left side of the nucleus. Then the membrane was ruptured, probably by the increasing pressure from within, which was causing its expansion. The result is an indefinite area on the right side of the original nucleus, which contains much karyolymph and little cytoplasm, but with no definite limiting membrane. The expansion of the nucleus in Fig. 5 probably took place in much the same way. There is thus a period during which, on one side or portion of the nucleus, the membrane partly or completely disappears. Later a definite membrane is again formed. It thus appears that the nuclear expansion, which is one of the main features of the period known as synapsis, may be gradual or relatively sudden, and that there is very commonly at this time a disappearance of the nuclear membrane on one side of the nucleus, leaving the latter and its contents in open connexion with the cytoplasm. This condition is brought about either by a rupture or by the solution of the nuclear membrane on one side of the nucleus, and there follows a period during which it is impossible to define accurately the limits of the nucleus. A portion of the membrane seems always, however, to remain intact. A little later the nuclear membrane is once more complete and perfect, the nucleus spherical, and we have the typical synapsis condition, as represented with a somewhat lower magnification in Figs. 6 and 7.

During the period when the limits of the nucleus are so poorly defined, the cytoplasm also shows a 'ragged' condition rather than a well-defined reticulum. This will be seen in comparing Figs. 3 and 5 with Figs. 1, 6, and 7. It might be concluded that these differences were due to differences in the treatment. To obviate the possibility of this conclusion, Figs. 1-7 were all drawn from the same preparation.

The two most characteristic features of this first period of synapsis

are therefore (1) the expansion of the nucleus, with frequently the partial disappearance of the nuclear membrane for a time, and (2) the rearrangement, without growth, of the structural content of the nucleus, from the reticulum to the spireme condition. This rearrangement has not begun in Pl. LXVII, Figs. 2-4, is partly completed in Fig. 5, and is quite completed in Figs. 6 and 7. It is not uncommon for threads to remain attached to the nuclear membrane, as in Figs. 4 and 5. Pl. I, Figs. 12 and 13 of a former paper (Gates, '08) show the same thing, and are also instructive (when compared with Pl. I, Figs. 6-11) in showing that expansion of the nucleus, rather than contraction of its contents, is taking place. In ordinary nuclear growth, such as occurs between successive mitoses, the reticulum also grows so that it continues to fill the nuclear cavity. But in the growth of the synaptic nucleus, the reticulum does not continue to grow, at least not to a corresponding extent, and probably not at all, and the result is that the reticulum soon occupies only a small part of the cavity of the synaptic nucleus.

Regarding the presynaptic nucleus, it will be seen from Figs. 1-4 that darker-staining portions of the threadwork frequently occur, especially near the periphery of the nucleus, but they show no features of regularity in size, shape, or number.

One feature which has been of much value in the study, particularly of the synaptic stages in *O. gigas*, is the occurrence of slightly different stages or conditions of synapsis in different anthers of the same flower, while all the mother-cells of each anther are in about the same condition. Thus in one flower the mother-cells of one anther were presynaptic, except a few which showed the beginning of the synaptic nuclear expansion. In another anther of this flower the mother-cells were all presynaptic and with numerous dark bodies lining the nuclear membrane as in Fig. 1, while other anthers showed the same condition except that the dark bodies were wholly absent. This probably indicated in this case a different physiological condition of the pollen mother-cells in these anthers rather than two successive stages during which these bodies made their appearance.

Figs. 6 and 7 show the typical synaptic condition, in which a rather dense aggregation of threads, usually with darker areas or bodies in its meshes, appears on one side of the nucleus. The threadwork appears to be more or less continuous and frequently shows a typical 'chromomeric' condition such as figured by Mottier for *Lilium* (Mottier, '09, Figs. 4 and 5). The threads are extremely delicate. This chromomeric condition of the threads will be referred to again later. The only difference between Figs. 6 and 7 is in the condition of the nucleolus. In the anther from which Fig. 6 was taken, the nucleolus was, in all the mother-cells, spherical and free in the nuclear cavity. Fig. 7 was drawn from a different flower, and in this anther the nucleoli were always flattened against the nuclear membrane as in the figure.

A study of many nuclei in the stage represented by Figs. 6 and 7 makes it evident that a certain amount of collapse together of the threadwork does take place. This is shown by the fact that the synaptic spireme not only occupies less space than the presynaptic reticulum, but has a greater density, showing that the material is more closely aggregated. That the spireme occupies less space may perhaps be partly a result of osmotic effects during the preparation of the slides. It is probable, however, that it is not to be accounted for as the result of contraction on the part of the threadwork. The threads of the presynaptic nuclear reticulum are anchored to the nuclear membrane, and this perhaps serves to keep the threadwork taut and occupying the whole of the nucleus. This is clearly shown by the fact that, when the nuclear expansion without chromatin growth begins, some of the threads frequently remain attached for some time to the membrane, and are pulled outwards with it as the pressure from within distends the membrane. Finally, when the nucleus has reached its definitive size, only a few such threads remain and the threadwork floats freely in the nuclear cavity. The expansion of the nucleus is completed before the rearrangement of its contents from the reticulum to the spireme condition takes place. It is probable that during this rearrangement a certain amount of collapse together of the threads, very few of which have any attachment to the nuclear membrane, takes place, so that the spireme, which is always placed unilaterally in the nucleus, occupies somewhat less space than did the reticulum. But while the spireme occupies usually somewhat less space than the original reticulum, yet this by no means accounts for all the empty space in the nucleus. This empty space is produced by a rather sudden expansion of the nucleus unaccompanied by any change in its reticulum. The transformation of the nuclear chromatin content from the reticulum to the spireme condition occurs apparently only after this nuclear growth is completed.

Nuclear Extrusions.

During the whole growth period of the pollen mother-cells, and the early synaptic stages (represented by Figs. 1-7), the nucleus occupies a central position in the pollen mother-cell. Then, while in the typical condition of synapsis or synizesis, the nucleus moves to one side of the cell until the nuclear membrane is in direct contact with the cell-wall. This condition has frequently been figured by previous investigators, e.g. in the papers of Overton ('09, Pl. III, Figs. 4-6) and Schaffner ('09, Pl. XII, Fig. 10), but no special significance has been attached to it. It is probable that this lateral movement of the nucleus always takes place, after its growth is completed and the transformation of its contents from a reticulum to the spireme of synizesis has occurred. This movement seems to be concerned

with a process which may prove to be of fundamental significance in connexion with the period of meiosis. This process will first be described.

After the synaptic nucleus of the pollen mother-cell has reached the side of the cell, an extrusion of material from the nucleus into the cytoplasm of the adjacent cell has been observed to take place. Pl. LXVII, Fig. 8 shows a row of pollen mother-cells in which this process is happening simultaneously in all the cells. In Figs. 9 and 10 the same process is shown under higher magnification. It will be seen that material is being extruded from the spireme of each nucleus, through an opening in the cell-wall into the cytoplasm of the adjacent pollen mother-cell. The nucleolus is always present at this time as a large spherical body, but in my observations I have never found it to take any part in this extrusion. The nucleus at this time is usually so placed that a considerable area of the nuclear membrane is in direct contact with the cell-wall, or perhaps the membrane may disappear over this area, leaving the nuclear contents in direct contact with the cell-wall and, through the openings in the wall, with the cytoplasm of the adjacent cell. The extrusion may take place through a single opening or (perhaps more frequently) through several.

In a previous paper (Gates, '08) I showed that rather large and conspicuous protoplasmic connexions occur between the pollen mother-cells in *Oenothera*. They doubtless occur in all the forms, as I have demonstrated them in several. Fig. 13 shows the connexions between two mother-cells of *O. gigas* in the condition of synizesis. It will be seen from this figure that they vary greatly in size. They are usually less numerous than in the figure, three or four being perhaps the most frequent number seen on one cell-wall. These connexions have never been observed on the walls separating the mother-cells from the tapetal cells, and apparently they only occur between mother-cells. Regarding the origin of these cytoplasmic connexions, they have not been traced to an earlier stage than the synaptic mother-cell because of the difficulty in demonstrating their presence. This requires a slight amount of plasmolysis, but not enough to rupture the connexions. When the mother-cells are very young, before the growth period begins, they are so small that no plasmolysis occurs, and the presence of connexions cannot therefore be observed in ordinarily fixed material at this stage of development. It seems most probable, however, that these connexions are formed by openings left in the cell-plate when it was originally laid down. These cytoplasmic connexions must form an important avenue of interchange between adjacent mother-cells. It is probable that during this process of extrusion the nucleus merely utilizes the connexions which happen to occupy the appropriate position.

If there are several of these openings near together, extrusion may take place through all of them. There may be a number of connexions on the

same wall, through which no extrusion takes place (Fig. 12). After extrusion has taken place, the nucleus moves back again towards the centre of the cell, and retains this position in the later stages of its development. In Fig. 12 the extrusion has evidently taken place, as shown by the dark-staining areas in the contiguous region of the adjacent cell, and the nuclei have begun to move back towards the centres of their cells. In this particular case it will be observed that two nuclei have extruded into the same mother-cell. The nuclear membrane is usually definite, sharp, and conspicuous at this time, but sometimes, as in one of the nuclei in Fig. 12, portions of it may be quite indefinite.

As already mentioned, in the cases observed (and the same process has been seen in certain anthers of several different flowers) the extrusion takes place simultaneously from all the mother-cells of a given anther, and so far as observed is always in the same direction in all the cells, indicating that the nuclear movement and extrusion are perhaps to bring about an equalization of pressure. It is very probable that the presence of these conspicuous openings or connexions between mother-cells allows of an equalization of pressure in the mother-cells from one end of an anther to the other. The movement of the nuclei and the nuclear extrusion may occur in connexion with this pressure-equalization, yet the latter seems scarcely adequate to account for it. Not infrequently the nucleus, instead of approaching the end wall of a mother-cell, moves to a side wall next the tapetum, which at this time is in direct contact with the row of mother-cells. In such cases no extrusion has ever been seen to take place, probably because there are no openings through which the extrusion could occur.

We may now trace the history of the extruded material. As shown in Figs. 8–10, extrusion of a portion of the chromatin material forming the spireme of synizesis takes place through the cytoplasmic connexions, just as a viscous fluid might, under pressure, flow through an opening or a tube, replacing the cytoplasm present. This material, which is forced through to the adjoining cell, first accumulates as a dense structureless mass or body. It is well known that, in the telophase of a nuclear division, when karyolymph begins to accumulate about the daughter group of chromosomes, a nuclear membrane is precipitated where this karyolymph comes in contact with the cytoplasm. In the same manner, an equally definite membrane is formed where the liquid accumulated about this extruded chromatin comes in contact with the cytoplasm. The result is a cavity containing chromatin and surrounded by a definite membrane. We may very well call this a pseudo-nucleus. The liquid contained in this cavity is probably the same as the karyolymph of the nucleus, and this liquid is either secreted by the extruded chromatin or at any rate produced in connexion with its activity. The chromatin in this pseudo-nucleus is at first in a compact mass, but this soon becomes looser in structure (Fig. 11, to the right), and finally (Fig. 11,

to the left) comes to appear strikingly like a threadwork or the 'spireme' of the nucleus from which it was extruded. The membrane surrounding this threadwork is at this time quite as definite as any nuclear membrane, so it is difficult to see how this differs in any particular, except its origin, from a real nucleus. It seems impossible to believe that any 'structure' persists through this series of transformations. This will be discussed later, for, without laying too much stress on this series of observations, it is obvious that they may have an important bearing on our present views of chromosome individuality and nuclear structure.

After the stage of the pseudo-nuclei shown in Pl. LXVII, Fig. 11, they become more indefinite, the membrane disappearing so that soon all that remains of the pseudo-nucleus is a densely-staining portion of the cytoplasm, as shown in Fig. 12. This becomes looser and more indefinite, taking many forms, and finally is probably entirely incorporated in the cytoplasm of the cell into which it was extruded.

This process of extrusion of chromatin from the nucleus of one pollen mother-cell into the cytoplasm of the adjacent mother-cell I call *cytomyxis*. Whether it always occurs at this stage of synapsis is not certain, but there are some observations which lead me to believe that it does not always occur. In some flowers one finds the nuclei of the mother-cells occupying positions almost or quite in contact with the cell-wall, but without any chromatin extrusion. As already stated, evidence that extrusion of chromatin has taken place remains for some time in the cytoplasm of the adjacent cell, so that in examining an anther it is easy to determine whether such extrusion has occurred. If the cytoplasm is entirely colourless (in iron-haematoxylin stain) it is evident that there has been no extrusion, no matter what the position of the nuclei. On the other hand, dark-staining cytoplasm does not always indicate that this process has taken place, for this is characteristic of degenerating cells. The position of these dark areas on the periphery and next to an adjacent mother-cell, together with their relation to the cytoplasmic connexions, serves to identify them.

It might be supposed that this process of chromatin extrusion was followed by degeneration of such mother-cells, but there are several reasons why this does not appear to be the case. In the first place, the nuclei appear perfectly normal after the extrusion has taken place, and later (Fig. 12) begin to move back towards the centre of the cell. The cytoplasm of such cells also appears wholly normal, and differs in no way from those in which no extrusion has occurred, except in having the dark areas on their periphery near certain of the cytoplasmic connexions.

At the time the movement of the nucleus to the cell-wall occurs, the spireme appears to be always on the side of the nucleus next the cell-wall, whether extrusion takes place or not. This is also true when the movement is to the wall in contact with the tapetal cells, with which there are no

observed cytoplasmic connexions. The nucleolus has never been observed to be in the 'sickle-stage' at this time, but is always spherical and floating freely in contact with the spireme. It takes no part in this extrusion.

It is probable that this lateral movement of the nucleus in the mother-cell always occurs, whether it be followed by an actual extrusion of chromatin or not. The conditions of pressure within the cells, and the presence of cytoplasmic connexions at the point of contact, probably determine whether such extrusion will occur.

It may be stated that the same process of chromatin extrusion occurs also in *O. biennis*, although Davis ('10), in his recent paper on this form, failed to observe it. In December, 1908, I showed (Gates, '09 c) that the phenomena of reduction are just the same in *O. biennis* and *O. laevifolia* as in *O. Lamarckiana* and its mutants. A recent re-examination of some of the preparations made at that time furnishes certain and conclusive evidence that the same process of chromatin extrusion occurs in *O. biennis*. Dark areas of cytoplasm had been noticed, but their significance was not understood, and it was supposed that they were connected with degeneration. They can now be clearly interpreted in the light of my observations on *O. gigas*, in which particularly favourable sections showing the extrusion were obtained. A few cases showing the actual extrusion taking place were also observed in *O. biennis*. This process, therefore, doubtless occurs in all the forms, and this is a further point showing the identity of the reduction processes in *O. biennis* and *O. Lamarckiana*.

Later Synaptic Stages.

After the nucleus moves back to the centre of the mother-cell, it retains this central position until the nuclear membrane disappears after diakinesis. The synzesis condition soon changes to one such as is to be seen in Fig. 14, in which the threadwork now occupies the whole of the nucleus once more, but the threads are beginning to show the progressive shortening and thickening which characterizes in part the succeeding stages of the nucleus. All the nuclei in one anther were in this particular condition, while other anthers of the same flower were in typical synzesis with the nucleus still central in position, and yet other anthers showed the beginning of the nuclear expansion which leads to the synzetic condition, as in Figs. 2-4. As shown by Fig. 14, the threadwork of the nucleus is fairly coarse, and the larger threads are characteristically straight and undeviating, quite unlike their usual meandering course. It is evident that a complete rearrangement of the contents of the nucleus must have taken place between this stage and the synapsis of Figs. 6 and 7. The interpretation of this stage will be referred to again later.

Pl. LXVII, Figs. 15-19 represent the same stage of the nucleus, in which a very characteristic structure is exhibited. This condition also follows soon after synizesis, and a complete rearrangement of the material from the synizetic condition has taken place. Figs. 15-17 are all sections of the nucleus at this time, representing its structure in different ways. The threads passing through the cavity of the nucleus are at this time comparatively few, as shown in Fig. 15, while the greater part of the chromatic material forms a rather delicate and very characteristic threadwork lining the nuclear membrane. Many of these threads appear in cross-section in Fig. 15. They adhere so closely to the nuclear membrane as to appear to be almost embedded in its substance. These threads are 'chromomeric' in structure throughout. They are nearly as delicate as in synizesis, and they are certainly single, not double, and contain *one* row of 'chromomeres'. This threadwork lining the nucleus frequently gives the appearance of a double nuclear membrane, i. e. one membrane within the other, but by careful focusing it can always be shown that the two apparent membranes in one focus merge into one. Figs. 18 and 19 are surface views of a portion of the nuclear membrane, to show the nature of this lining threadwork. Fig. 18 shows that free ends of the threads sometimes occur. This threadwork lines the whole of the nucleus in a remarkably uniform manner. The nuclear membrane itself is very definite at this time. As already stated, relatively few threads pass through the centre of the nucleus, and these are mostly different in structure from the threadwork lining the nuclear membrane. These central threads vary greatly in thickness, as shown by Figs. 15-17, some of them being coarse and heavy masses, while others are thin and delicate. In Fig. 17 a different representation of the chromomeric threads was attempted.

During this period the nucleolus varies in shape, frequently showing a certain amount of distortion, or being characteristically flattened as in Fig. 19.

Whether Figs. 15-19 represent a stage of development through which the nucleus always passes, or whether they represent rather a physiological condition of the nucleus which may or may not occur, is uncertain. In any case, it is a very characteristic condition, and exhibits a distribution of the chromatic material of the nucleus which is quite the reverse of that seen in synizesis, for instead of being rather closely aggregated in the synaptic ball it is in considerable part arranged as a threadwork lining the nuclear membrane. Davis ('09) has figured what is evidently the same stage in *O. grandiflora* (Pl. XLI, Fig. 13), but does not explain it.

The figures presented show how numerous are the stages or conditions of the nucleus during the period of synapsis. As I have shown elsewhere (Gates, '11), there is no reason to believe that synapsis accomplishes any special exchange of materials or influences between homologous chromo-

somes of the sporophyte. But it is well known that the synaptic period lasts for some time, and it is now evident that many changes in the condition of the nuclear contents may take place during this period.

The succeeding stages, up to the heterotypic mitosis, have not been studied in detail in *O. gigas*, and as they were investigated with special care in *O. rubrinervis* (Gates, '08), they will not be touched upon in the present paper.

Heterotypic Mitosis.

In *O. gigas*, as I have shown to be the case in the other *Oenothera* forms, the chromosomes are loosely scattered on the heterotypic spindle, so that it is frequently impossible to say which members constitute a pair. Pl. LXVIII, Figs. 20–22 represent pollen mother-cells in the metaphase of the heterotypic mitosis. In Fig. 22 the chromosomes show a rather irregular alinement in two series which have begun their journey to the poles. Usually the nucleolus disappears immediately upon the solution of the nuclear membrane after diakinesis, but occasionally it persists for a short time as a pale-staining body, as shown in Fig. 22. In connexion with a previous paper (Gates, '09 *a*) these stages were figured, but the plates were lost and I have since been unable to find again the particular cells from which they were drawn.

Fig. 23 represents an early anaphase in polar view, all the chromosomes but one being brought into view by focusing for a short distance in a vertical plane. A considerable amount of variation in the apparent size of the chromosomes is due to differences in the depth of stain. Figs. 24 and 25 are anaphases in somewhat oblique view. In both cases fourteen chromosomes can be counted in the group passing to the lower pole, while some of the chromosomes are missing from the upper pole. Fig. 26 is a later anaphase in side view.

As the chromosomes pass towards the poles of the heterotypic spindle there is much variation in their shape. Very often they appear square, oblong, triangular, or globular, with no indication of a bipartite structure. In other cases they may be somewhat elongated and with their arms bent in the form of a V. All these forms will be seen in Figs. 24 and 25. Many of the chromosomes in Fig. 26 and one of those in Fig. 24 show a sharp median constriction. In the chromosomes in Fig. 26 this appears as a definite lighter area through the median plane of the chromosome, and leads to the conclusion that this must be a transverse segmentation of the chromosome, if one can judge from their constant manner of orientation on the spindle. Other chromosomes in Fig. 26 show the 'tetrad' appearance so characteristic of the chromosomes in early interkinesis. This is due to a split (apparently longitudinal) of the more elongated chromosomes in the anaphase. There is thus wide variation in the time at which the split

which is characteristic of the heterotypic anaphase appears, and also in its direction, which apparently may be either longitudinal or transverse. The result is a very wide variation in the shape of the chromosomes as they pass to the poles of the heterotypic spindle. Davis states ('10, p. 644) that the chromosomes of the heterotypic mitosis 'in both *biennis* and *grandiflora* have the form of thickened V's and are not subglobular, as described and figured by Gates and Geerts'. With further observation he would have found that all the variations I have described occur in *O. biennis*, as well as in *O. gigas* and the other races of *Oenothera*. The split of the chromosomes for the homotypic mitosis may occur in the very early anaphase of the heterotypic, or it may be delayed so that the chromosomes retain a globular or subglobular shape with no evidence of fission even after the daughter nuclei of the heterotypic telophase have been formed. As will be seen from Pl. LXVIII, Figs. 24-26, the V-shape of the chromosomes in the heterotypic anaphase is frequently much less common than a more nearly globular shape.

When the chromosomes pass to the poles of the heterotypic spindle they at first form a very close group in contact with each other. The nuclear membrane very soon appears, as I have described elsewhere (Gates, '09 *b*, pp. 184-7), and as the karyolymph begins to increase the chromosomes continue to separate, and they remain hugging the nuclear membrane as the nucleus grows in size. They usually show clearly their bivalent character at this time, though the halves of these chromosomes are held tightly together and never appear in loose contact, yet the ends of the arms of the two halves are, usually at least, widely separated, as though they repelled each other like the strips of gold leaf in a Leyden jar. In my extensive observations of this interkinesis stage in a number of *Oenothera* forms, I have never found two chromosomes in contact at this time. They appear to be always equidistantly spaced just within the nuclear membrane, and it is probable that this manner of spacing is due to their mutual repulsion at this time.

During interkinesis there appears to be much variation in the extent to which the chromosome bivalents for the homotypic mitosis lose their compact condition and anastomose with each other. It is probable that in some cases the chromosome bivalents retain their compact condition with little change between the heterotypic telophase and the homotypic prophase. In other cases the chromosomes may become irregular in shape, with a considerable amount of anastomosis between chromosomes. Fig. 27 shows such a case in its earliest stages. In *O. gigas* this process frequently goes further, giving a series of coarse threads in which the boundaries of individual chromosomes can no longer be determined. In Fig. 27 the last of the heterotypic spindle is just disappearing. I have observed a number of times in *O. gigas* that an evanescent cell-plate may be formed

in the median plane of the heterotypic spindle. It seems to disappear with the spindle fibres, however.

Homotypic Mitosis.

Fig. 28 shows the beginning of homotypic spindle formation, the first indication being the development of a web of delicate wavy fibrils surrounding the nucleus. These become drawn out at certain points, as in Fig. 29, and then the nuclear membrane disappears. As will be seen from Fig. 28, the chromosomes in the homotypic prophase, before the disappearance of the nuclear membrane, show clearly their bivalent structure, which differs in no respect from that of the heterotypic telophase.

Just as in the anaphase and early telophase of the heterotypic mitosis the chromosomes not infrequently show no indication of a bivalent structure, so in the prophase of the homotypic mitosis this is sometimes the case. In Fig. 30 only one of the chromosomes is clearly bivalent, the rest showing little or no indication of a bivalent structure. Fig. 31 shows both the homotypic spindles in late prophase, just before the chromosomes are drawn into the equatorial plate. Nearly all of these chromosomes show their bivalent character. In this section all the chromosomes of the cell are present, there being sixteen on one spindle and twelve on the other. This means that on the heterotypic spindle in this mother-cell a 12-16 distribution of the chromosomes occurred, which is the widest departure from an equal distribution that I have found. Several cases of a 13-15 distribution in the reduction division were observed by counting the chromosomes in the early heterotypic telophase.

Fig. 32 is a side view of the homotypic metaphase, showing the chromosomes so closely massed that their boundaries are not clearly distinguishable. Fig. 33 shows all the chromosomes of a mother-cell in two rather scattered groups of fourteen each, probably in polar view, although the spindle fibres were not visible, and in this case there was no indication of the bivalent character of the chromosomes. Fig. 34 shows the homotypic spindles in side view, the chromosomes all showing their bivalent structure very clearly in this case. They are just being oriented in the equatorial plate, and several of them are still out of alignment. In Fig. 35 the chromosomes cannot all be separated from each other, and they are rather irregularly arranged on the spindles. In Fig. 36 only two of the chromosomes—one on each spindle—show their bivalent character. Their alignment is still somewhat irregular. Pl. LXIX, Fig. 37 is an early anaphase, in which the halves of the chromosomes have in most cases just separated. In this figure several of these anaphase chromosomes show a median constriction. I shall refer to this again later. In Fig. 38 the spindle to the left is in side view, showing several of the chromosomes whose halves have not yet separated, while the spindle to the right is seen in slightly oblique

polar view, and shows one of the early anaphase groups of fourteen chromosomes.

Pl. LXIX, Fig. 39 is a later anaphase of the homotypic spindle in oblique view, showing the group of fourteen chromosomes on their way to the lower pole. Only twelve of those approaching the other pole are to be seen, on account of the upper end of the spindle being obliquely cut. In that case it can be seen that the daughter chromosomes are partly looped or V-shaped, but some are long and rod-like, while others are short and rounded. There is thus also much variation in the shape of the chromosomes at this time, and this may apply to the chromosome group on a single spindle, as in Fig. 39. Pl. LXVIII, Fig. 40, shows a somewhat later homotypic anaphase in side view. The chromosomes here also show some variation in shape, but are mostly rather straight and somewhat elongated. Fig. 41 shows a homotypic spindle in anaphase, in which nearly all the daughter chromosomes are clearly constricted in the middle, and sometimes appear as though a definite transverse fission had taken place.

It has already been mentioned, in connexion with the synaptic stages, that different anthers of the same flower not infrequently show different conditions. A remarkable case of this kind has been observed in the pollen mother-cells during the homotypic mitosis. Pl. LXVIII, Figs. 32 and 35; Pl. LXIX, Fig. 37; Pl. LXVIII, Fig. 34; Pl. LXIX, Fig. 39; Pl. LXVIII, Figs. 40 and 41 are all taken from one flower. Of these, the first three are from one anther and the last four from another. A remarkable and constant difference was found between the pollen mother-cells of these two anthers. These differences (as can be observed from the figures) are (1) in the breadth of the spindles. In one anther (Pl. LXVIII, Figs. 32 and 35; Pl. LXIX, Fig. 37) these are very narrow, so much so that there is sometimes scarcely room for the chromosomes on the equatorial plate, and they project beyond the sides of the spindle, as in Pl. LXVIII, Fig. 32. These chromosomes also frequently fail to show their bivalent character at this time. In the other anther (Figs. 34, 40, and 41; Pl. LXIX, Fig. 39) the spindles are remarkably broad, and it would appear (although this is not certain) that the number of spindle fibres must be greater. The chromosomes usually appear as clear bivalents on the equatorial plate. This difference in the spindles was very striking and was found to be constant between different anthers. (2) While the walls of the mother-cells in anther no. 1 were smooth and even, as they ordinarily are, yet in anthers having the broad spindles already described the walls were always very irregular, showing extensive growths or proliferations subsequent to the separation of the mother-cells which takes place usually soon after synapsis. The character of these growths of the cell-wall can be judged from Pl. LXVIII, Figs. 28 and 34; Pl. LXIX, Figs. 38 and 39. This difference between pollen mother-cells is therefore not merely a matter of variation, but the broad homotypic spindles and

proliferated mother-cell walls were a constant feature of certain anthers in this flower, while certain other anthers showed with equal constancy very narrow spindles and smooth regular mother-cell walls. The cause or meaning of this difference is not evident. In other flowers the most usual width of the homotypic spindle is between these two extremes, as shown by Pl. LXVIII, Fig. 36, and Pl. LXIX, Fig. 38.

Pl. LXVIII, Fig. 42, shows an early telophase of the homotypic mitosis. Many of the chromosomes have the median constriction which appears to be characteristic of them at this time, not only in the homotypic telophase but in somatic telophases as well, as I have shown for *O. Lamarckiana* in a previous paper (Gates, '07, pp. 18–20). Figs. 43 and 44 are early homotypic telophases drawn with higher magnification, and nearly all the chromosomes show clearly a bivalent structure. Since the late anaphase stages of the heterotypic mitosis in *O. gigas* sometimes show a clear transverse constriction (Fig. 26), and since a similar constriction frequently appears in the early telophase of the homotypic mitosis, it appears probable that this may, in both cases, bear a relation to the succeeding karyokinetic division. It seems further probable that, in *O. gigas* at least, there is no significant difference between a longitudinal and a transverse fission of the chromosomes.

Fig. 45 is a slightly later stage in the homotypic telophase. The nucleus has grown and a nucleolus has appeared, while the chromosomes are beginning the process of 'stringing out' to form an anastomosis of coarse threads. In Pl. LXIX, Fig. 46, this process in the nuclei has gone somewhat further, the chromosomes having elongated considerably to form thick, somewhat ragged threads. This figure also shows the condition of the fibrillae in the cytoplasm just previous to the beginning of cell-plate formation. In comparing Pl. LXVIII, Figs. 42–45, and Pl. LXIX, Fig. 46, with those of the heterotypic telophase, it will be seen that while there has been no change in the size of the cell, the chromosomes, although the same in number, are only half the size of the bivalents in the heterotypic telophase, and the volume of the nuclei is correspondingly smaller. In this case the *amount* of chromatin evidently determines the size of the nucleus, for the number of bodies is the same in each case, and the size of the individual bodies is the differential factor.

The Pollen-tetrads and Pollen-grains.

Figs. 47 and 48 show the tetrad of young pollen-grains still within the mother-cell wall. After the tetrahedral walls come in, dividing the contents of the mother-cell into four pyramids with spherical base, whose apices meet at a central point, the walls begin to separate at the apex and the contents of each cell form a flattened disc, spherical in surface view, discoid in side view. This withdrawal of the cells leaves them arranged so

as to occupy nearly the whole periphery of the mother-cell, but with a large cavity in its centre. It would seem that this change in the shape of the young pollen-cells must be from forces inherent in the cells themselves, with which the mother-cell can have little concern. The discoid shape of the young pollen-grains, and afterwards of the older ones, is clearly self-determined.

Miss Lutz ('09) was the first to make the interesting observation that in *Oenothera gigas* the pollen-grains have characteristically four lobes or interstitial bodies, while all the other species of *Oenothera*, including *O. Lamarckiana* and its other mutants, are known to have usually three. These four interstitial bodies in the *O. gigas* pollen-grain are symmetrically placed at the corners of a square (Pl. LXIX, Fig. 49), showing that the four are as characteristic of *O. gigas* as three, placed at the corners of a triangle, are of *O. Lamarckiana* and its other mutants. Sometimes, however, one finds five interstitial bodies symmetrically placed, as in Fig. 50. At the age of the pollen-grain represented by Figs. 49 and 50, the cytoplasm still fills the whole cell, the nucleus is centrally placed, spherical, and with a definite membrane. Its reticulum is rather coarse, and two or three nucleoli are usually present.

Figs. 51 and 52 show a much later stage, in which the pollen-grain has undergone a great deal of growth, and the interstitial bodies give a more or less marked quadrangular appearance to the grain. The nucleus usually appears amoeboid in shape at this time, with very little chromatic content, and the cytoplasm is in coarse strands occupying only a portion of the cell. Beer ('06) has studied carefully various features in connexion with the growth and development of the triangular pollen-grains in *O. longiflora* and *O. biennis*.

In *O. gigas*, pollen-grains having more than four lobes are relatively common. Occasionally the lobes may then all be symmetrically placed, as in Fig. 50, but much more frequently the four original lobes are placed symmetrically, and the extra lobes are intercalated between these in various positions, frequently in a plane above or below the original ones. This is shown in Figs. 53 and 54, the first of which has four lobes rather regularly placed, and a smaller fifth lobe which is below the plane of the others. Fig. 54 has seven lobes, the three somewhat irregular and smaller additional lobes being all on the left side of the figure. It is therefore clear that in *O. gigas* the pollen-grain is constructed on a basis of four, just as in the other species it is on the basis of three, although grains with more than three lobes do occur. The reasons for this rather remarkable change are not clear, although one is tempted to speculate on the different size relationships of the cells and nuclei in the young pollen-grains, as a possible basis for this alteration. The doubling of the chromosome number, with the attendant doubling in the volume of the nuclei, but a different ratio of

increase in the cytoplasm, gives a new ratio for $\frac{\text{nuclear surface}}{\text{cell surface}}$, and this may conceivably have been the factor determining the appearance of a quartet instead of a triad of interstitial bodies.

This completes the account of the normal development. Many cytological features which were described in previous papers have not been repeated here, only the distinctly new features being emphasized.

Sterility in *O. gigas*.

I wish now to describe an interesting type of sterility in *O. gigas*, which differs in some respects from anything previously described in plants. Its peculiarity consists essentially in this: The cytological phenomena of reduction go forward in the normal manner, but the pollen mother-cells retain their archesporial appearance, their walls remaining in contact with each other and with the tapetum to form a compact tissue, so that they can never be set free or discharge pollen, even though the reduction processes are normally completed. This appears to be due primarily to a failure of the surrounding tissues of the anther to grow and form a cavity which allows sufficient space for the mother-cells to round off during synapsis and float freely in the cavity of the anther. Other features of this process will appear in connexion with the description. In all, four flowers, all from the same plant, were found to show this condition in various stages.

Fig. 55 is from a longitudinal section, showing some of the pollen mother-cells and their surrounding tapetal cells as they appear under a low power. Though the tapetal cells are binucleate and the mother-cells are in interkinesis, there is no tendency for the mother-cell walls to break apart or separate from the tapetum. The walls of the mother-cells frequently become much thickened and cutinized at this time. Fig. 56 is from another anther of the same flower, and shows a portion of a row of mother-cells. In the two long central cells of the row there has been an attempt at wall-formation after the heterotypic mitosis, which resulted only in an incomplete segmentation of the cytoplasm. The four cells so formed are each undergoing the second reduction division. There is frequently a more or less abortive attempt to form a wall after the heterotypic mitosis.

Figs. 57 and 58 are drawn with a little higher power. They each show two pollen mother-cells from a row, and all are in the heterotypic telophase. In all there has been a partially abortive attempt to form a wall separating the interkinetic nuclei, and in two cases this has resulted in a complete constriction of the cytoplasm. The lower mother-cell in Fig. 58, particularly, shows evidence of irregularity in the chromosome distribution during the heterotypic mitosis. The reduction processes are not entirely normal, and some of the evidences of irregularities will be seen in later figures. Pl. LXIX, Figs. 59–61, Pl. LXX, Fig. 62, represent stages of the homotypic prophase

under a high power. The chromosomes in these figures appear considerably larger than in the figures previously described. This is not due, however, to their containing more chromatin, but to the fact that many of the chromosomes are less dense and compact, and therefore stain less deeply. But although not alveolated, they occupy a larger space, and therefore of course, although they stain less deeply, appear to contain more chromatin. On account of this difference in compactness, the chromosomes differ greatly in apparent size, and in Pl. LXIX, Figs. 59–61, Pl. LXX, Fig. 62, while most of the chromosomes are in evident pairs, it is not possible in every case to say which are pairs and which single chromosomes.

Figs. 63 and 64 are anaphases of the heterotypic mitosis, showing the polyhedral character of the cells. Figs. 65–67 are later stages, showing the heterotypic telophase. All are from the same flower. In Figs. 65 and 66 the heterotypic spindle is in different stages of its disappearance, and a definite cell-plate is laid down. In Fig. 65 the interkinetic chromosomes are very compact and show no indication of bivalence, while in Fig. 66, which is a later telophase, the daughter nuclei have grown to larger size, the chromosomes are less compact, and most of them show clearly a bivalent structure.

In this telophase the chromosomes frequently undergo a sort of swelling or distension without vacuolation. They thus occupy a larger area, as already mentioned, but are less deep staining and less compact, though without any open spaces. Then stringing out at the corners occurs, and then the vacuolation process may begin.

Even the smaller nuclei in Fig. 66 afford evidence that their size depends upon the amount of chromatin they contain. In all the Figs. 65–67 there is evidence of irregularities in the chromosome distribution. It will be seen also that these mother-cells are polygonal, and still in contact with other cells on all sides.

One of the most striking features of these sterile anthers is the enormous variation in the size of the mother-cells. In normal development the pollen mother-cells, even when still in the archesporial condition, are approximately equal in size. In these sterile anthers, one mother-cell may have many times the volume of an adjacent mother-cell of the same anther.

Fig. 68 shows a mother-cell in the prophase of the homotypic mitosis. There was an incomplete attempt at segmentation (?) of the cytoplasm after the first division. These prophase chromosomes nearly all show their bivalent structure. Fig. 69 represents a homotypic telophase. The daughter nucleus in the higher focus on the left, being uncut, shows fourteen chromosomes. Here also there has been an incomplete attempt at a segmentation of the cytoplasm. Fig. 70 represents a condition sometimes occurring, in which a complete cell-wall is formed following the heterotypic mitosis. The two daughter cells are each in the telophase of another (the homotypic) mitosis,

and cell-plates are again being formed on these spindles. In Fig. 71 the same stage is to be seen, but no wall was formed after the first division. It is, of course, often difficult to determine just what stage is represented in a given mitosis, for the appearances are very different from those of ordinary pollen formation, and frequently can only be identified by the number and condition of the chromosomes.

Plate LXIX, Figs. 72–75 are homotypic telophase groups more highly magnified. Fig. 72 shows the chromosomes when they have just reached the pole of the homotypic spindle. Figs. 73–75 are slightly later, after the nuclear membrane has been formed. These nuclei are uncut and show all the chromosomes, fourteen in the first two cases and fifteen in the third. Thus one chromosome occasionally finds its way to the wrong pole, as I have shown in other forms of *Oenothera*, and such irregularities occur also in normal development in *O. gigas*, as pointed out previously in this paper.

In Plate LXX, Fig. 76, an evident cleavage of the cytoplasm of the mother-cell followed the heterotypic mitosis, and one of the daughter nuclei has divided again, this being followed by the beginning of a cleavage of the cytoplasm. Fig. 77 is a mother-cell in which there have been evident irregularities, and scattered spindle fibres appear in the cell. Fig. 78 represents two mother-cells in which there have been abortive attempts at cell-wall formation following division of the nucleus. In Fig. 79, in the upper mother-cell, no walls were formed and nuclei of varying size lie in the cytoplasm, while in the lower mother-cell degeneration of the nuclei is evidently taking place. The relationships of the cells, as well as other features, make it evident that these all represent mother-cells. The remarkable variation in the size of these mother-cells has already been mentioned, and is very well shown by comparing, e. g., Figs. 79 and 80, which are drawn with the same magnification. The latter tiny cell has undergone division, but several extra nuclei have been formed in the cytoplasm. In Fig. 81 a better attempt was made at completing the tetrad formation in the mother-cell, but three small nuclei were formed.

Figs. 82–84 show other more or less complete attempts to produce tetrads. In Fig. 84 apparently only one of the nuclei has undergone the homotypic division. Fig. 85 shows a characteristic condition which sometimes occurs after the formation of the tetrad nuclei. The mother-cell becomes scanty in cytoplasm, and the nuclei move together and form a group in the centre of the cell.

Of course, in all the mother-cells here described the tetrad of cells formed can never function as pollen-grains. All the mother-cells remain closely surrounded by the tapetal cells at their sides and by other mother-cells at their ends, and there is no tendency for them to become free and separate. As already stated, this appears to be due to the failure of the surrounding cells of the anther to continue their

normal growth. It is evident that this type of sterility is not due primarily to any failure of the cytological processes concerned with reduction, for these are followed through in many cases without irregularities. The tapetal cells, after the karyokinetic division of their nuclei which makes them all binucleate, do not undergo the further nuclear divisions and other changes which are characteristic of these cells in ordinary cases, but their later alterations seem to be suspended. Yet they seem well equipped for performing their glandular function, and the mother-cells are apparently not at all lacking in nourishment. This condition, in which the pollen mother-cells retain their archesporial condition and remain in contact although their nuclei undergo the normal reduction processes with few irregularities, has been found in four flowers of *O. gigas*, all taken from one plant. In one of these nearly all the anthers were in different stages of reduction, a condition already mentioned as being of much value in connexion with the interpretation of synapsis stages. In this flower, in anther no. 1 the pollen-tetrads had been more or less successfully formed, as in Pl. LXX, Fig. 83, but the walls of the mother-cells were very thick and cutinized. They still formed a compact tissue, and evidently could never round off and separate to form pollen-grains or free pollen-tetrads. The mother-cells and also the tapetal cells of this anther have very little contents and are evidently lacking in nourishment. In anther no. 2 of this flower the mother-cells are in interkinesis (Plate LXX, Fig. 65). In some cases a wall has been formed between the daughter nuclei, in others there is only a segmentation of the cytoplasm or an evanescent cell-plate. Anther no. 3 is in much the same stage as anther no. 2, some of the cells being in the homotypic prophase. In anther no. 4 the two reduction divisions had been completed, as in Plate LXX, Fig. 80, and small extra nuclei in the cytoplasm indicated frequent irregularities of the spindles. In anther no. 5 the mother-cells had undergone the reduction division, followed by segmentation of the cytoplasm, and the daughter nuclei had passed into the resting condition. In no. 6 the mother-cells were mostly in the early homotypic telophase, and a segmentation of the cytoplasm had followed the reduction division. In no. 7 the mother-cells were chiefly in interkinesis, showing the chromosome bivalents of this time. Anther no. 8 was in a stage just following synapsis, as illustrated by Plate LXVII, Figs. 15-19. In all the anthers except the first the tapetal nuclei are binucleate and well filled with cytoplasm, in the condition they normally exhibit about the time of synapsis. The other three flowers which showed this type of sterility were more uniform in the stage of development of the mother-cells throughout each flower.

Somatic Mitoses.

No figures of the somatic mitoses in *O. gigas* will be presented in the present paper, but the fact that they are much larger than in the forms with

fourteen chromosomes is a distinct advantage in the study of mitosis in nuclei, which are so small in comparison with other seed plants. Two points have been definitely determined from my study of these somatic divisions in *O. gigas*. (1) In the prophase the spireme segments into a single chain of twenty-eight chromosomes, all of which were arranged end to end in a single series. The spireme is usually wound regularly just within the periphery of the nucleus. (2) Soon after this segmentation, while still in the prophase condition, the chromosomes show, in many cases at least, a clear longitudinal split. Thus the split in the chromosomes occurs long before the equatorial plate stage.

DISCUSSION.

Several special features in connexion with the data in this paper call for further discussion. Phenomena of chromatin extrusion, evidently very similar to those described here for *Oenothera*, have been described by Miss Digby ('09) in the pollen mother-cells of *Galtonia*. The chief difference exhibited by these processes in the two cases is that in *Galtonia* the nucleolus as well as the spireme extrudes bodies, while in *Oenothera* the nucleolus apparently takes no part in the process. In *Oenothera* the extrusion takes place, usually at least, through cytoplasmic connexions already present between mother-cells, the viscous chromatic material extruding through these pores and forming a solid mass in the other cell.

This may have an important bearing on our present theories of nuclear structure, for the exuded or extruded material quickly forms a pseudo-nucleus by the accumulation of karyolymph and the precipitation of a membrane where this comes in contact with the cytoplasm. Furthermore, the extruded chromatin, though it at first forms a body as solid as a nucleolus, soon spreads out and forms a structure closely resembling a reticulum or spireme. It seems probable from these observations that far too much objective morphological significance has been attached to various reticulum and spireme stages of the nucleus. It is evident that the greatest care must be exercised in interpreting spireme and reticulum stages as structures having a definite morphological meaning. Rather would it seem that these appearances depend upon the condition of physical aggregation of the chromatin material. For example, the spireme condition may be assumed as the result of the electrical charges held by the chromatin molecules. While cytological observation is of course very important as indicating different physiological conditions and stages of development in the nucleus, yet the chromosomes are not necessarily to be regarded as a congeries of definite bodies or particles which persist and multiply individually from stage to stage. This is an important modification of the ordinary method of cytological interpretation, for it assumes that we already see with our higher

powers the smallest nuclear elements which exist as distinct elements of organic structure. Many of the so-called structural features of the nucleus are such as might be assumed by any other colloidal material as well as chromatin, under similar physical conditions of aggregation.

Therefore it seems probable that our present microscope powers already disclose all of 'structure' that is to be found in the nucleus, and the search for structure within structure down to and beyond the limits of our present powers of magnification is probably a vain one. Chamberlain ('09) has found that in the enormous nuclei in the reproductive cells of Cycads, no more 'structure within structure' is to be seen than in the very much smaller nuclei of other plants. The appearance is rather that of a colloidal material loosely aggregated. It is not always sufficiently kept in mind that the 'structures' studied in the nucleus represent the coagulation and precipitation figures resulting from certain conditions of distribution of the viscous, colloidal chromatin materials in the living nucleus, although these different distributions are doubtless in themselves of the highest physiological significance.

This point of view does not imply that the chromosomes are chemically alike, and there is much evidence that they are not. Further, in the absence of definite observational evidence of self-propagating differential elements within the chromosome, it seems best at present to formulate hypotheses without making the assumption that such exist.

It is evident that this process of chromatin extrusion may also have an important bearing on our views of chromosome individuality, but it does not seem best to discuss this matter further until the significance of the process is better understood. Since this process, similar in the main essentials, has now been observed in two widely separated genera of Angiosperms, belonging respectively to Monocotyledons and Dicotyledons, it will probably be found to be of general occurrence. I have used the term cytomyxis to indicate that the process is probably one of equalization between different mother-cells. It is probable that actual extrusion of chromatin does not always occur, but that synapsis *may* be passed through without it, although the movement of the nucleus to the side of the cell probably always does take place. This process may have an important significance from the standpoint of heredity and the life cycle. A somewhat similar process has recently been described by Carruthers ('11) in the Ascomycete *Helvella*, although here the extrusion is from the nucleus into the cytoplasm of the same cell, and not into another cell.

The type of sterility here described is an interesting one. The plant which showed flowers in this condition also showed normal pollen development in other flowers, and its pollen was used successfully in making crosses. De Vries mentions (*Mutationstheorie*, ii, p. 59) that one plant of *O. gigas* in his cultures in 1899 was wholly sterile after repeated artificial

pollination. This was presumably due to a defect of the ovules, but it is not stated whether the pollen was good. In 1909 I observed one plant of *Oenothera* which was wholly sterile in its anthers, but produced plenty of large seed capsules, and therefore must have borne normal ovules and was presumably pollinated from adjacent plants. Regarding this plant, I need only say that it was one of a culture derived from near Liverpool, England, and resembling *O. grandiflora*, Ait., in many of its characters. Material was collected for a cytological study, but has not yet been examined, so it cannot be stated what was the nature of the sterility in this case. The plant differed in no other particular from other plants in the same culture and it bloomed abundantly, but the anthers in every flower examined at different times were dry and empty.

The observations on this type of sterility indicate the correctness of Tischler's ('08) conclusion, from his study of sterile hybrids, that the sterility does not depend upon any form of chromatin repulsion. It does not seem possible to define the general cause of sterility more definitely than 'lack of nutrition'.

In my previous paper on *Oenothera gigas* ('09 a), dealing with the size relationships of cells and nuclei, the point in the life cycle of *O. Lamarckiana* at which this mutant originated was discussed, and the evidence which is brought together in that paper pointed to the probability that the tetraploid chromosome number originated in the early divisions of the fertilized egg, through the failure of a nucleus to complete its division after the fission of the chromosomes had taken place. This gives two sets of fourteen chromosomes which are identical two by two. Strasburger ('10) agrees with this view as to the manner of origin of the *O. gigas* mutant, but Stomps ('10) thinks it more probable that the tetraploid number of chromosomes originated through the union of two germ cells in each of which reduction had failed to take place, which would be in accord with de Vries' theory regarding the origin of a mutation. As I have pointed out elsewhere (Gates, '09 a, p. 544), there are several reasons why this manner of origin is less probable than the one I have suggested. If Stomps' theory were correct, we should have a mutant occurring with twenty-one chromosomes, and it would be much more frequent in occurrence than in *O. gigas*. Such a mutant has never yet been found, and all the other mutants which are known have fourteen chromosomes, as in *O. Lamarckiana*. In the paper referred to (Gates, '09 a, p. 545) I made a list of cases of related species one of which has the diploid and the other the tetraploid number of chromosomes, the inference being that, as in the case of *O. gigas*, the tetraploid species had originated from a related diploid species. Although a number of such cases are now on record, there is not, so far as I am aware, a single case of a species whose sporophyte has the triploid number of chromosomes. But several such hybrids are known, notably

among plants *Drosera rotundifolia* $\times$ *D. longifolia* (thirty chromosomes), and *Oenothera lata* $\times$ *O. gigas* (twenty-one chromosomes). The former hybrid has, I believe, only been observed in the wild condition where both parent species occur in the same locality, so that it is probably a hybrid, although there is no experimental proof of its hybrid origin. Its characters agree with this supposition, and it has never been suggested that this form might be a mutant instead of a hybrid, nor would the suggestion have any probability. Stomps ('10, p. 59) cites an observation of Geerts ('09, p. 52), who found a single case of a megaspore mother-cell in *O. Lamarckiana* having twenty-eight instead of fourteen chromosomes. Stomps makes the assumption that such a mother-cell would undergo reduction, and that fertilization with a normal male cell would occur, giving a form having twenty-one chromosomes. It is probable that the tetraploid number of chromosomes originated in this cell through its failure to complete a mitosis. It does not follow that such a megaspore mother-cell would undergo reduction to fourteen chromosomes followed by embryo-sac formation and fertilization of the egg. From what we know of the close relation between the occurrence of the tetraploid condition and apogamy, it is at least equally probable that this tetraploid megaspore mother-cell would develop apogamously, without undergoing reduction or fertilization, and would therefore give rise to a form having twenty-eight chromosomes. Indeed, this may have been the manner of origin of *O. gigas*, instead of the failure to complete a mitosis in the fertilized egg as I previously suggested. A decision as to whether this suspended mitosis occurred in the megaspore mother-cell or in the fertilized egg or young embryo will only be possible with further observations, but either of these hypotheses seems at present more probable, and more in accord with related facts, than an origin from the union of two unreduced germ cells.

It further seems probable, though not certain, that whenever in the life cycle the duplication in the number of chromosomes occurred in *O. gigas*, other simultaneous changes took place in the germ-plasm. My previous paper ('09 a) on this subject was an attempt to explain as far as possible the peculiar characters of *O. gigas* on the basis of these changes in the dimensions of the cells. It is probable that such an explanation is inadequate to account for *all* the new characters of *O. gigas*, and that the appearance of the tetraploid number of chromosomes was accompanied by other changes. The further application of this method of analysis to *O. gigas* and other forms having a tetraploid number of chromosomes will aid in determining the nature and extent of any such change. It is to be hoped that an analysis of other cases from this point of view will be attempted.

SUMMARY.

In this study of pollen development in *Oenothera gigas*, several special features of synapsis are emphasized. At the beginning of synapsis there is a rather sudden increase in the volume of the nucleus, there being in some cases a distension of the nuclear membrane and in other cases a rupture of the membrane and accumulation of karyolymph in the cytoplasm. The nuclear reticulum finally floats freely in the karyolymph, and then the rearrangement of the chromatin material from the reticulum to the spireme condition takes place.

Following this there is, at least in some flowers but probably not in all, an extrusion of chromatin from the nucleus of one mother-cell through cytoplasmic connexions, into the cytoplasm of an adjacent mother-cell. For this process I have suggested the name *cytomyxis*. Probably the nucleus always moves to one side of the cell at this time, but this may or may not be followed by extrusion of chromatin.

The extruded chromatin accumulates in a mass after passing through the cell wall. A clear liquid appears around these masses, and a membrane delimits the clear area from the cytoplasm, forming what I have called a pseudo-nucleus.

Later, these masses loosen up, and acquire an appearance very similar to a spireme. The membrane afterwards disappears and the extruded chromatin finally appears to be incorporated with the surrounding cytoplasm.

It is evident that this process may have an important bearing on current conceptions of the life cycle and heredity, and on the theory of chromosome individuality. It is not improbable that various features of the finer morphology of the nucleus depend upon the physical condition of aggregation of the nuclear contents, rather than upon autonomous self-propagating 'structures' as such.

After the chromatin extrusion has taken place, the nucleus moves back to the centre of the cell and undergoes various other transformations during the remainder of the period known as synapsis. The most conspicuous of these is one in which the larger part of the chromatin content of the nucleus is in the form of intertwining chromomeric threads which line the nuclear membrane.

The chromosomes are loosely arranged in the equatorial plate of the heterotypic spindle. As they pass to the poles they frequently undergo a split, which in some cases gives evidence of being transverse, in others longitudinal.

In one flower examined, two of the anthers exhibited constant differences in their mother-cells. In one anther all the mother-cells had smooth walls and the (homotypic) spindles were very narrow. In the other anther the

cell-walls all showed outgrowths or proliferations, and the spindles were constantly very much wider.

There is much variation in the shape of the daughter chromosomes of the heterotypic mitosis, and in the time when the fission of the chromosomes for the homotypic mitosis occurs. In the anaphase or telophase of the homotypic mitosis the chromosomes usually show a median constriction.

A type of pollen sterility was studied in certain flowers, in which the reduction processes are normal or nearly so but the surrounding tissues fail to grow, remaining in contact with the mother-cells, which therefore retain their polyhedral shape. In some cases the four nuclei resulting from reduction move together to form a group in the centre of the mother-cell. In other cases a wall may be formed after the heterotypic mitosis, or the cytoplasm of the mother-cell may undergo cleavage to form a tetrad. The usual irregularities observed in hybrids also sometimes occur.

It is considered most probable that the mutant *O. gigas*, with the tetraploid number of chromosomes, originated through a suspended mitosis, either in the fertilized egg or in the megaspore mother-cell.

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EXPLANATION OF PLATES LXVII-LXX.

Illustrating Dr. Gates's paper on Pollen formation in *Oenothera gigas*.

(The lenses used were a Leitz $\frac{1}{12}$ inch oil immersion, ap. 1.40 with compensating oculars, the figures being reduced one-fourth in reproduction. This gives magnifications which allow comparison of the size of the cells in *O. gigas* with those of other *Oenotheras* figured in my previous papers.)

PLATE LXVII.

Fig. 1. Archesporial pollen mother-cell just previous to the beginning of synapsis. Many dark bodies appear near the periphery of the nucleus. × 2,850.

Fig. 2. Nucleus showing the beginning of synapsis. The nuclear membrane is gradually expanding and being pushed away from the reticulum. × 2,850.

Fig. 3. Mother-cell in the beginning of synapsis. Apparently a rather explosive rupture has taken place on one side of the nucleus, producing a cavity in the cytoplasm filled only with karyolymph. The cytoplasmic reticulum presents a rather ragged appearance. No change has yet taken place in the nuclear reticulum. × 2,850.

Fig. 4. A slightly later condition, in which the distension of the nuclear membrane has been followed by its rupture at one place, producing an ill-defined area in the cytoplasm on the right side filled with cell-sap. On the left side a few nuclear threads remain attached to the membrane. × 2,850.

Fig. 5. Synapsis is nearly completed. A single nucleolus is enclosed in the meshes of the delicate threadwork, which is nearly transformed into a spireme. The nuclear membrane is very indistinct on one side and the cytoplasmic reticulum is 'ragged'. × 2,850.

Fig. 6. Pollen mother-cell in synapsis (synizesis). The nuclear threads are extremely attenuated. The nuclear membrane is intact and the cytoplasmic reticulum is not ragged. × 1,875.

Fig. 7. Same synaptic condition as Fig. 6. Darker areas appear on the nuclear threadwork, which is quite transformed into the spireme condition. The nucleolus is attached to the nuclear membrane. × 1,875.

Fig. 8. Row of pollen mother-cells, showing the extrusion of chromatin from the nucleus of one mother-cell into the cytoplasm of the next, to form pseudo-nuclei. × 600.

Fig. 9. Three pollen mother-cells, showing how the extrusion takes place. $\times 1,275$.

Fig. 10. The same more highly magnified. $\times 1,875$.

Fig. 11. Section of pollen mother-cell, showing the two pseudo-nuclei formed by extrusion from the adjacent cell. In the one on the right the chromatic material is just beginning to break up, and on the left this process has gone further, producing an appearance resembling a spireme. In the next section the nucleus of this cell and its cytoplasmic connexions are seen. $\times 1,875$.

Fig. 12. Chromatin has been extruded from the nuclei to form dark masses in the cytoplasm of the adjoining cell. The nuclei are moving back towards the centre of the cell. $\times 1,875$.

Fig. 13. Showing numerous cytoplasmic connexions between pollen mother-cells. $\times 1,875$.

Fig. 14. A later stage in which the nucleus occupies a central position. The nuclear threadwork has been completely rearranged since synapsis, the threads now occupying the whole cavity, and being characteristically straight instead of meandering. $\times 2,850$.

Fig. 15. A synaptic stage in which most of the threads line the nuclear membrane and are seen here in cross-section. A few threads, varying greatly in thickness, pass through the cavity of the nucleus. $\times 2,850$.

Fig. 16. Same stage as Fig. 15. $\times 2,850$.

Fig. 17. Same stage, differently represented. $\times 2,850$.

Fig. 18. Section of nucleus at this time, showing only the 'chromomeric' threadwork lining the nuclear membrane. Some free ends of threads appear. $\times 2,850$.

Fig. 19. Same as Fig. 18, showing also the nucleolus and several other bodies attached to the nuclear membrane. $\times 2,850$.

PLATE LXVIII.

Fig. 20. Pollen mother-cell in heterotypic metaphase. Twenty-six chromosomes are visible, rather irregularly scattered on the spindle, several apparent pairs. $\times 2,100$.

Fig. 21. Heterotypic spindle in metaphase, showing twenty chromosomes. $\times 2,100$.

Fig. 22. Heterotypic spindle in early anaphase, showing nineteen chromosomes and a persistent nucleolus. $\times 2,100$.

Fig. 23. Heterotypic spindle, early anaphase in polar view. Twenty-seven chromosomes visible. $\times 2,100$.

Fig. 24. Heterotypic spindle, late anaphase, slightly oblique cut. All fourteen chromosomes present at lower pole, and twelve at upper pole of spindle. $\times 2,100$.

Fig. 25. Oblique cut, later anaphase, 14 + 11 chromosomes visible. $\times 2,100$.

In Figs. 22, 24, and 25 the anaphase chromosomes vary in shape, being mostly sub-globular, rod-shaped, or V-shaped, but very few show indications of their bivalent character.

Fig. 26. Late heterotypic anaphase. Six of the chromosomes show a clear median transverse constriction or segmentation, while several others have the characteristic appearance of 'tetrads'. It appears that both transverse and longitudinal divisions of the chromosomes may occur at this time. $\times 2,100$.

Fig. 27. A late stage of interkinesis. The remnants of the heterotypic spindle still remain in the cytoplasm. The chromosomes do not always anastomose to this extent during interkinesis. $\times 2,100$.

Fig. 28. Homotypic prophase, showing beginning of spindle formation and chromosome bivalents. The mother-cell wall has characteristic outgrowths. See text, p. 924. $\times 2,100$.

Fig. 29. Slightly later stage than Fig. 28, showing fourteen bivalent chromosomes on the multipolar spindle. $\times 2,100$.

Fig. 30. Slightly later stage of homotypic spindle. The spindle fibres are being rearranged and attached to the fourteen chromosomes, only one of which in this case shows its bivalent character. $\times 2,100$.

Fig. 31. Homotypic prophase. The chromosomes are all present, sixteen on one spindle and twelve on the other. They vary much in size and shape, but most show their bivalent character. This unequal distribution must have occurred in the heterotypic mitosis. $\times 2,100$.

Fig. 32. Homotypic metaphase. In all the mother-cells of this anther the spindles were very narrow, so that there was scarcely room for the chromosomes. $\times 2,100$.

Fig. 33. Homotypic metaphase in polar view, showing the two scattered groups of fourteen chromosomes. $\times 2,100$.

Fig. 34. Homotypic metaphase. In this anther all the mother-cells had proliferated cell-walls and very broad spindles. The chromosomes are all clearly bipartite. $\times 2,100$.

Fig. 35. Another homotypic metaphase, showing narrow spindles and smooth mother-cell wall. $\times 2100$.

Fig. 36. Homotypic metaphase, in which only two chromosomes, one on each spindle, show their bivalent character. Thirteen chromosomes visible in left-hand spindle, and a nucleolus persists in the cytoplasm. $\times 2,100$.

Fig. 40. Homotypic spindle in late anaphase. Spindle cut, but 12 + 13 chromosomes present. $\times 2,100$.

Fig. 41. Section of another homotypic spindle in anaphase, in which nearly all the chromosomes show a clear median constriction. There are also several rods and one V. $\times 2,100$.

Fig. 42. Homotypic telophase, showing many of the daughter chromosomes transversely constricted. $\times 2,100$.

Figs. 43 and 44. Somewhat later homotypic telophase nuclei, showing the bivalent appearance of many of the chromosomes. $\times 2,850$.

Fig. 45. Later homotypic telophase, in which the nucleus has grown larger, a nucleolus has appeared, and the chromosomes have begun to lose their compact structure. $\times 2,850$.

PLATE LXIX.

Fig. 37. Early homotypic anaphase. Spindles very narrow. Several of the daughter chromosomes show a median constriction. $\times 2,100$.

Fig. 38. Homotypic mitosis. Spindles broad and cell-wall proliferated. The spindle on the left shows several of the chromosomes whose halves have not yet separated. The spindle on the right is an early anaphase, showing in oblique view one of the groups of fourteen daughter chromosomes. $\times 2,100$.

Fig. 39. Later homotypic anaphase, slightly oblique cut, showing 14 + 12 chromosomes on the spindle. Chromosomes partly looped and partly rod-shaped or nearly globular. Cell-wall proliferated. $\times 2,100$.

Fig. 46. Late homotypic telophase. The chromosomes are elongating and 'stringing out' to pass into the resting condition. Cell-plates not yet formed. Cell-wall proliferated. $\times 2,100$.

Figs. 47 and 48. Tetrads of young pollen cells within the mother cell-wall. $\times 525$.

Fig. 49. Young pollen grains of *O. gigas* with four interstitial bodies. $\times 1,275$.

Fig. 50. Similar grain, with five interstitial bodies symmetrically placed. $\times 1,275$.

Figs. 51 and 52. Typical four-lobed pollen grains of *O. gigas*. Nucleus amoeboid in appearance, cytoplasm in strands. $\times 1,275$.

Figs. 53 and 54. Five-lobed and seven-lobed grains, with the extra lobes asymmetrically placed. $\times 1,275$.

Fig. 55. Longitudinal section, showing a row of pollen mother-cells in interkinesis, and the surrounding binucleate tapetal cells. The mother-cell walls have remained in direct contact with the tapetal cells. $\times 600$.

Fig. 56. A row of mother-cells still in contact with each other, and polygonal, although undergoing the reduction divisions. In the two middle mother-cells a cleavage of the cytoplasm follows the heterotypic mitosis. $\times 900$.

Figs. 57 and 58. Each figure shows two mother-cells in interkinesis. In two of the cells a cleavage of the cytoplasm has appeared between the daughter nuclei, and in one a small extra nucleus appears in the cytoplasm. $\times 1,125$.

Figs. 59–61. Homotypic prophase nuclei before the disappearance of the nuclear membrane. Most of the chromosomes are clearly in pairs, and they vary greatly in size owing to differences in their compactness. $\times 2,550$.

Fig. 72. Early homotypic telophase group of chromosomes. $\times 2,850$.

Fig. 73. A later stage of the homotypic telophase, after the formation of the nuclear membrane. Fourteen chromosomes. $\times 2,850$.

Fig. 74. Slightly later stage, nucleus uncut, showing fourteen chromosomes and a nucleolus. $\times 2,850$.

Fig. 75. Same as Fig. 73. Showing fifteen chromosomes and a nucleolus. In Figs. 72–75 the chromosomes show no sign of a median constriction as in normal development. $\times 2,850$.

PLATE LXX.

The figures in this plate are all from flowers showing abnormal development, in that the pollen mother-cells retain their polyhedral shape.

Fig. 62. Homotypic prophase, showing spindle formation and the polygonal character of the mother-cell. $\times 2,550$.

Figs. 63 and 64. Heterotypic anaphase, showing the polyhedral shape of the cells and the compact, univalent structure of the chromosomes. $\times 1,875$.

Figs. 65 and 66. Heterotypic telophase, showing an attempt at cell-wall formation. In Fig. 66 small extra nuclei appear in the cytoplasm. $\times 1,875$.

Fig. 67. Heterotypic telophase with extra nuclei in the cytoplasm. $\times 1,875$.

Fig. 68. Homotypic prophase, showing bivalent chromosomes. The heterotypic mitosis in this mother-cell was followed by segmentation of the cytoplasm. $\times 1,875$.

Fig. 69. Homotypic telophase. One nucleus uncut shows fourteen chromosomes. A cleavage of the cytoplasm appears in the line which was the equatorial plane of the heterotypic spindle. $\times 1,875$.

Fig. 70. Homotypic telophase. A complete cell-wall has been formed, following the heterotypic division. $\times 1,125$.

Fig. 71. Same stage as Fig. 70, but no wall formed after the heterotypic mitosis. $\times 1,125$.

Fig. 76. Late homotypic telophase. Segmentation of the cytoplasm followed the heterotypic mitosis, and a cleavage of the cytoplasm is appearing between the two daughter nuclei on the left. $\times 1,125$.

Fig. 77. This mother-cell shows evident irregularities, with extra nuclei, a cleavage of the cytoplasm, and scattered spindle fibres. $\times 1,125$.

Fig. 78. Two mother-cells with abortive attempts at wall formation following division of the nucleus. $\times 1,125$.

Fig. 79. Two mother-cells after irregular reduction divisions. In the upper cell small extra nuclei lie in the cytoplasm, while the nuclei of the lower cell are degenerating. $\times 1,125$.

Fig. 80. A very small mother-cell (cf. Fig. 79), showing irregularities in the chromatin distribution. $\times 1,125$.

Fig. 81. A completed tetrad, but three small nuclei formed in the cytoplasm. $\times 1,125$.

Figs. 82-84. More or less complete attempts to form pollen tetrads. Walls are formed within the mother-cell, although the latter remains in contact with adjacent cells. In Fig. 84 apparently only one daughter nucleus has undergone the second mitosis. Mother-cell walls very thick. $\times 1,125$.

Fig. 85. A condition sometimes found after the completion of tetrad formation. The four nuclei move together to the centre of the mother-cell, which contains little cytoplasm. In this case no walls were formed within the mother-cell. $\times 1,125$.

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