The Development of the Heterotypic Chromosomes in Pollen Mother-cells.

BY

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With Plates **XXVII** and **XXVIII**.

IN September, 1905, the writer (Mottier, '05) published a preliminary report on the development of the heterotypic or bivalent chromosomes in *Podophyllum*. The results of observations set forth in that communication were as follows: (1) That, in agreement with the conclusion of Farmer and Moore ('05) and others, the bivalent chromosome developed in the first or heterotypic mitosis is formed by the approximation of two chromosomes side by side, or otherwise, which were previously arranged end-to-end in the spirem, and not by the approximation of two spirems either before or during synapsis, as claimed by Grégoire and his students, Allen, Strasburger, and others. That the separation of these two chromosomes (i. e. the two segments, if the bivalent chromosome be considered as one) is a transverse division, and not a separation along the line of longitudinal fission observed in the spirem. (2) That there is no fusion of two distinct spirems as the nucleus goes into the first contraction or synapsis.

In the following communication, the writer hopes to present the results of detailed observations substantiating the above conclusions, and to show also that, in the plants considered, there is no definite or reliable evidence to support the pro-chromosome theory suggested by Rosenberg ('04), and especially emphasized by Overton ('05) for several dicotyledonous plants, and by Miyake ('05) for certain Monocotyledons. The entire mitotic history will be traced from the resting nucleus to the complete formation of the heterotype chromosomes in the pollen mother-cell of *Podophyllum peltatum*, *Lilium Martagon*, *L. candidum*, and *Tradescantia virginica*, although not in complete detail in the latter. A study of *Galtonia candicans* has also been made, and this plant was found to correspond in important details with *Tradescantia virginica*, as will be seen also from the results of other observers.

METHODS.

All material was fixed in chrom-osmic-acetic acid, after the formula published by the writer in 1897. Both the triple and iron-haematoxylin stains were used. In so far as pollen mother-cells are concerned, the triple stain of anilin safranin, gentian violet, and orange G is superior when all stages of mitosis are concerned. The iron-haematoxylin is very valuable in the differentiation of the chromatin during the prophases. In preparations stained by the iron-haematoxylin, counter-staining with eosine, orange G, gentian violet, &c., was followed for a time, but as no special advantage could be observed in the use of the counter-stain, this part of the process was finally abandoned. Preparations of almost every stage of the process described in this paper have been made in duplicate, one-half of the slides stained with the triple, the other by the iron-haematoxylin. In this way one stain serves as a control for the other.

FROM THE RESTING NUCLEUS TO SYNAPSIS.

Podophyllum. The developing anthers of *Podophyllum* were examined during the autumn and winter in order to determine the nature of the chromatin in resting nuclei in the cells of both the sporogenous and purely vegetative tissues. In stamens fixed in early October, all stages of mitosis are to be seen in the cells that are to give rise to the pollen mother-cells, as well as in purely vegetative tissue. At this time of the year, the pollen mother-cells were not differentiated, at least, in all material examined. In stamens fixed January 20, the pollen mother-cells were still undifferentiated, and about all nuclei were in the resting condition. A careful study of material collected at the dates just mentioned and in the following spring did not reveal any disposition of the chromatin into sixteen masses or lumps that might be regarded as representing the somatic chromosomes. As will be shown below for the pollen mother-cells, the chromatin of the resting nuclei exists in masses or lumps of varying sizes, and connected into a network by slender threads composed chiefly of linin. The number of chromatic masses was always greater than the number of somatic chromosomes. In fact, it is not possible to determine the exact number of these lumps, for they vary greatly in size, and three or more masses are frequently so connected by thick strands that it is not possible to know how many lumps each of such masses really represents. Frequently, in both sporogenous and purely vegetative cells of an anther, the resting nuclei will present the chromatin in rather fine granules uniformly distributed within the nuclear network. This finely granular condition of the chromatin in resting nuclei is also met with in the pollen mother-cells. In all cells showing such a distribution of the chromatin, fixation seems perfect, and it

is impossible to say whether the breaking up of the chromatin into fine granules is a normal state or due to the action of fixing fluids.

Soon after the last division in the sporogenous tissue, which gives rise to the pollen mother-cells, the nuclei pass into the resting condition, and both cell and nucleus begin to increase in size, the nucleus enlarging at first somewhat faster than the cell. The nuclear frame-work consists now of numerous chromatin masses of varying sizes connected into a network by slender threads (Pl. XXVII, Fig. 1). These threads, which consist largely of linin, vary also in thickness. The thicker ones frequently retain the safranin more strongly, thus appearing to be somewhat in the nature of chromatin, and, under such conditions, a distinction between chromatin and linin seems very difficult to determine. One or more nucleoli are also present. Fig. 1 represents a rather thick section including a tangential view of the nucleus. Fig. 2 is a median section of a nucleus taken from the same anther. This figure shows that the chromatin has a peripheral arrangement in the nucleus, a condition which is frequently observed, and that the nucleolus lies in a cavity relatively free from the nuclear network. It is seen also that the chromatin masses are larger than in Fig. 1, and that their number is greater than sixteen; hence these lumps are not pro-chromosomes. Two masses are frequently observed lying more or less closely side by side, but there is no reason to believe that such pairs are fusing. The delicate threads joining the chromatin masses may be found lying close to each other and parallel, but this does not signify that a double spirem is in process of formation. Inasmuch as the nuclei in all pollen mother-cells in an anther loculus are in almost the same stage of development, it is not very probable that Figs. 1 and 2 represent very different stages, but rather the same or very closely related stages. The greatest difference observed in the stage of development of cells in different loculi of the same anther, before the formation of the hollow spirem, was found at the time when the synaptic ball begins to loosen up. In one loculus, the synaptic mass had just begun to loosen up, as shown in Fig. 6, whilst in a neighbouring loculus the spirem had loosened to the extent of almost filling the cavity of the nucleus; but the chromatin thread had scarcely become any thicker, nor had it assumed a very regular arrangement, being much entangled in places. If there be a difference in development between Fig. 1 and Fig. 4, Fig. 4 is certainly the older, i.e. further toward synapsis. In Figs. 3 and 4, we have nuclei that are frequently met with. In Fig. 4, the chromatin is in the form of small masses of a rather uniform size, and evenly distributed in the linin network. Each mass or granule is composed of very many fine granules. The nucleoli, as in such nuclei as Fig. 1 and Fig. 2, lie within spaces comparatively free from the nuclear framework. As pointed out by the writer (Mottier, '99) in a paper on the effect of centrifugal force upon the cell, the nucleoli seem to lie within a space that

does not contain any material capable of being stained by known methods. It may frequently happen that the nucleolus or nucleoli may lie in contact with portions of the chromatin or linin threads. In Fig. 3 the chromatin is aggregated into irregular, granular shreds, giving a picture strikingly different from that of Fig. 4. These two cells were drawn from the same loculus of the anther and from the same section. In a number of preparations, nuclei of cells in about one-half of the loculus presented the structure shown in Fig. 4, while in the other half, that in Figs. 1 and 3 prevailed, with all intergrading stages between. The cytoplasm in Fig. 4 is small-meshed and finely granular, giving a rather dense and finely granular appearance. In Fig. 3, a coarser and looser reticulum is present, and in Fig. 1 the fibrillar reticulum is still looser but very sharply defined and beautiful. There can be but little doubt that these nuclei are in about the same or in closely related resting stages; for all three conditions are found in the same loculus. In many preparations of the same stage of development of the anther, about all the nuclei possessed the type of structure shown in Fig. 1. This structure is more frequently met with in the developing sporogenous tissue of young anthers and also in purely vegetative cells, yet a more finely granular state of the chromatin is likewise to be found in vegetative cells. In the preparations from which these figures were drawn, there was nothing to indicate poor fixation; the cells seemed perfectly fixed and normal in every respect. There is, of course, a possibility that the difference here manifested in the disposition of the chromatin may be due to the effect of the fixing fluid, especially the part played by the osmic acid, but as to this the writer is not prepared to speak definitely at present. As will be shown later, it is after all a matter of comparative insignificance whether the chromatin occurs in larger or smaller masses or granules in the resting nucleus.

Overton does not find favourable evidence in behalf of the pro-chromosome theory in *Podophyllum*. He states ('05, p. 130) that 'in *Podophyllum peltatum* the prochromosomes are not recognizable as such. They may be represented by groups of chromatin masses which are connected by linin threads and arranged in parallel rows during the reticulation. These chromatin masses are distributed upon linin threads running parallel to each other, and, as in *Helleborus foetidus*, there are found two distinct and independent spirems, which pass into synapsis unpaired and unfused.' Overton supports this statement in his Figs. 53 and 54 (l. c., '05). In these figures are shown parallel linin threads upon which are arranged at rather long intervals pairs of chromatin masses. The nuclei from which such figures were drawn must occur very rarely, for among a large number of preparations of *Podophyllum* no nuclei were found with parallel linin threads arranged in such diagrammatic regularity. Overton either did not find or overlooked entirely such stages as figured in my Figs. 1, 2, 3, 4. In his Fig. 5, which

is stated to occur just before synapsis, a thicker chromatin thread is shown than in his Fig. 56, which is in synapsis. If Overton's Fig. 56 correctly represents the chromatin thread in synapsis, it seems to me that his Fig. 55 is a loosening up of the synaptic mass, and hence a later stage, for I have found no such condition as his Fig. 55 before synapsis.

Cardiff ('06) claims that, previous to synapsis in *Acer platanoides*, *Salomonina biflora*, *Ginkgo biloba*, and *Botrychium obliquum*, there are formed two or more chromatin threads which arrange themselves in pairs longitudinally and move together as synapsis approaches, finally fusing in synapsis. Judging from his own experience in the study of nuclear phenomena, the writer is of the opinion that Cardiff's figures are too diagrammatic to enlist confidence in the accuracy of his interpretation.

Both cell and nucleus increase rapidly in size, so that when synapsis is complete they have about reached their maximum dimensions. In *Podophyllum* the pollen mother-cells vary considerably in size, as do also the stamens in any given flower. In thicker and longer anthers larger cells are found, but whether this is always true no effort was made to determine. It is very frequently true, however, that the cells near the middle of the anther may be very much longer than broad. Fig. 6 represents a cell which is larger than the average.

Whether the chromatin undergoes any marked change from that shown in Figs. 1-4 before contraction, as will be pointed out below for *Lilium*, cannot be stated positively. With the rapid increase in size of the cell and nucleus, the entire contents contract or ball up toward one side of the nucleus. This contraction is a rapid process, and whether the rapid growth of the nucleus is a stimulus to this contraction is an interesting question. The nucleolus may be included partly or wholly within the contracted mass, or it may lie merely in contact with it, or entirely free and at a remote side of the nucleus. As a rule, the nucleolus is included within the chromatin mass (Fig. 5). When complete synapsis is reached, the mass is tightly balled up, many nuclei showing no free portion projecting into the nuclear cavity. Frequently a few shreds of linin, including some chromatin, protrude from the mass, and sometimes a small portion of the chromatin thread may project. In some cases very fine delicate threads extend from the mass across the nuclear cavity to the nuclear membrane. There seems to be little or no regularity in the position of the synaptic mass in the nucleus as regards the upper or lower ends or the sides. Cardiff states that in the plants studied by him gravity determines the position which the mass shall take. This explanation does not hold for *Podophyllum* or *Lilium*.

In the contracted mass no definite structure can be made out. Sometimes the appearance is that of a balled-up mass of lumps or granules, and sometimes that of a chromatin thread. The nuclear membrane in the

stage of synapsis does not present the sharp contour that is seen in the resting nucleus. On the contrary, it is very delicate and appears to be composed of a network of very delicate fibrils, or as a mere cytoplasmic boundary of the nuclear cavity. The nucleus must have, however, a distinct membrane of its own, for when one observes, for example, the male nucleus in the cytoplasm of the egg or in the cavity of the embryo-sac, one can scarcely conclude otherwise. Just how long the condition of synapsis persists cannot be stated definitely. The time undoubtedly varies with the rapidity of growth and may depend upon growth conditions. The writer has observed that pollen mother-cells of *Lilium* divide rapidly on bright warm days, while in cool cloudy weather the process went on much more slowly. The synaptic period represents probably the longest pause in the mitotic process, but the exact duration and the conditions regulating the same are matters yet to be determined.

As stated above, no definite structure can be made out with certainty in the mass when synapsis is complete, but as soon as the mass begins to loosen up, as is manifested by the extension into the nuclear cavity of, at first, a few, then several loops or turns of the spirem (Fig. 6), it is clearly seen that a definite spirem is present which is split lengthwise, as evidenced by the occasional divergence of the halves, or by the presence of two rows of chromatin granules or chromomeres. However, a double row of chromomeres cannot always be seen in the free portions of the spirem that first project from the contracted mass. Up to the time of synapsis, the pollen mother-cells are polygonal in form, fitting together in complete tissue connexion, but, as soon as the synaptic mass of the nucleus begins to loosen up, the cells round off at the corners, and in a short time they will have separated from the original walls and have formed the new walls so characteristic of these cells. As stated in my earlier publication, the original, delicate walls of the cells persist for some time after the pollen mother-cells have contracted away from them. The rounding up of the pollen mother-cells seems to be brought about both by the contraction of the protoplasm of the cells and by the enlargement of the anther through growth. With the further increase in size of the loculus, the cells soon come to lie loosely in the cavity. Before describing the phenomena immediately following synapsis and the final formation of the chromosomes, the presynaptic history in *Lilium* will be given.

Lilium. Shortly after the organization of the daughter nuclei, resulting from the last somatic division in the sporogenous tissue, the chromosomes begin to reticulate into smaller masses. One or several nucleoli may be present, usually more than one. The nucleus now presents a structure consisting of irregular lumps or masses of chromatin, varying in size and held together by linin threads or strands (Fig. 15). Many of these linin strands are thicker than at a later stage and retain the safranin strongly.

Because of this fact, these strands suggest the nature of chromatin. They do not, however, hold the stain as strongly as the chromatin masses, and there can be no doubt that we have a differentiation between chromatin and linin, although what the exact difference is cannot be stated. It has been observed in a number of preparations that, at the stage represented in Fig. 15, the chromatin masses show a tendency to group themselves closely about the nucleoli, and the linin strands to radiate from the collection. This condition gives a very noticeable appearance even with low powers of the microscope. What significance, if any, such a grouping may have, the writer is not prepared to say, neither is it known to be a regular and perfectly normal phenomenon; although the preparations show no evidence of abnormal fixing. In former publications the writer expressed the opinion that the nucleolus may contribute material for the formation of chromatin. By this he does not mean, as some observers have interpreted the statement, that the nucleolus is reserve chromatin, for he has no well-defined idea of what reserve chromatin may be, or that such a substance exists.

With further development of the anther, the pollen mother-cell and its nucleus increase in size. The chromatin masses increase through growth and become more regularly distributed within the nuclear cavity, but more especially along the periphery. The nucleoli lie within the nuclear cavity, which is, as a rule, freer from the network, and their connexion with the chromatin is less intimate, or they are entirely free (Figs. 16, 17). Here, as in *Podophyllum* the chromatic masses, which are always variable in size and in shape, are connected into a reticulum by means of linin threads. These threads are sometimes slender, showing very fine granules; sometimes they are thicker and retain to some extent the chromatin stain. The same phenomena are manifested, whether the stain used was the triple or the iron-haematoxylin; but for the differentiation into chromatin and linin the triple stain seems to be superior. It may be remarked also that the number of these masses always far exceeds the somatic number of chromosomes. In preparations of this and later stages (Figs. 15, 16, 17) which are not too densely stained, it is to be seen that each chromatin mass is composed of many smaller granules (Fig. 18). Fig. 18 was taken from the same anther locus as Fig. 16. In this the stain differentiated in such a manner as to leave the linin very much paler or almost colourless. A similar condition is figured by Allen for *Lilium canadensis*. In the period of development in *L. Martagon* between the stage shown in Figs. 16 and 17 and synapsis, there seems to be undoubtedly a very remarkable and striking variation. After a slight increase in size, nuclei, presenting the structure of Fig. 17, may undergo the synaptic contraction (Figs. 22, 23). On the other hand, the chromatin masses may undergo a further reticulation into finer granules, so that the nucleus reveals a linin

thread, or network, with a single row of chromatin granules (Fig. 19). In some preparations, nuclei with the structure indicated in Fig. 17, occupy one end of the loculus, while in the other end of the same loculus the structure of Fig. 20 is present, and in the middle portion complete synapsis. In other preparations, nearly all the nuclei in the pollen mother-cells of the whole anther show the structure of Figs. 19, 20, 21, i.e. from a spirem or network with a single row of small granules to complete synapsis. In one end of such an anther there may be a few cells with the structure of Fig. 17; in others there are no such nuclei, but all show the structure as described in the preceding sentence. In none of the preparations was there anything to indicate unusual or faulty fixation.

Examining each of these conditions now in detail, it is seen that in Fig. 22, the chromatin masses are collecting into a mass at one side of the nucleus, as all conceivable stages between Figs. 17 and 23 are present in the same loculus. In Fig. 22, the linin threads are very beautifully shown, some being very delicate and staining blue or appearing colourless, whilst others are thicker and hold some of the safranin stain. Here and there two or more of these linin threads may have a parallel course, and some of the chromatin masses are paired, but how such phenomena can justify the doctrine that a double spirem is being formed is difficult to comprehend. There seems to be as strong evidence that a triple or quadruple spirem is formed, because the chromatin masses are frequently aggregated in clusters of three or more. In the second condition described above, we have a structure just preceding synapsis which differs from that shown in Fig. 22. Here it is certain that the chromatin becomes more finely reticulated, so that small and uniform granules are formed, and that these granules are being arranged into a single spirem (Figs. 19, 20). In nuclei presenting the structure of these figures, the nucleolus, or nucleolar material, is almost always flattened in one or two masses against the nuclear membrane. The chromatin thread is very frequently oriented with reference to the flattened nucleolus, as indicated in Fig. 20. This orientation gives the impression that the thread is disposed in many interlacing loops or turns, that extend out from the flattened nucleolus. There seems to be no closer connexion, however, between nucleolus and chromatin thread in this condition than in the other. Some preparations show that in the entire anther all pollen mother-cells were in some stage between that of Figs. 19 and 21. In Fig. 21 the synaptic condition is about complete. In the section from which this figure was drawn, about one-third of the nuclei presented the contracted mass, while in the rest some such condition as Figs. 19 and 20 was present. In such cells it was noticed that the flattened nucleolus was very constant and thereby conspicuous; but this was less so in the former condition, although it is frequently met with (Fig. 23). In preparations showing the structure of

Figs. 19 and 20 that were stained with the triple stain, the linin portion is colourless, while the chromatin granules remain blue. This means, of course, that in the process of differential staining the safranin is removed from the linin and chromomeres almost completely, and that the latter retain the violet stain with greater avidity. The condition of the nucleus as shown in Fig. 19 has been described by Farmer and Moore ('05, p. 508) for they state that 'the definite spirem thread can be distinguished very clearly at an early period in karyokinetic activity, certainly long before the spore mother-cells dissolve their union with each other. It forms a colourless thread, at first infiltrated with chromatin throughout, but the latter soon collects into serial beads so as to give rise to the well-known alternation of stainable (chromatin) and non-staining (linin) disks.'

Farmer and Shove ('05) figure a similar presynaptic structure in *Tradescantia virginica*. A spirem of a single row of granules has been described by Schaffner ('97, '06) as representing the presynaptic condition in *Lilium philadelphicum* and *L. tigrinum*. In fact this has been the interpretation of most cytologists in former years. Allen ('05), on the contrary, does not mention any such stage as the writer's Fig. 19, as occurring prior to synapsis.

From what has been described in preceding paragraphs, the possibility is not excluded that the chromatin may not always be in the same state of reticulation, or distribution within the linin framework, prior to the contraction into the dense mass. In Figs. 22 and 23, which do not seem in any way to represent artefacts, the chromatin exists in the form of lumps or masses, while in other cases (Figs. 19, 20, 21), there has taken place a much finer reticulation of the chromatin, so that the granules are much more numerous, of uniform size, and arranged in a single row within the linin reticulum. Whether we have in Figs. 19 and 20 a continuous thread, or thread and network combined, cannot be stated with absolute surety; but it is certain that a large part of the structure is a thread (Fig. 20) with only one row of granules, or we may say chromomeres. When we compare Fig. 18 with Fig. 19, it seems reasonable that the structure of Fig. 19 has been derived directly from that in Fig. 18. Anthers, showing the structure of Figs. 19, 20, 21, seemed to be in about the same stage of growth as those in which Figs. 22 and 23 were found, especially when one remembers that Figs. 16 and 18 were drawn from the same section. In all cases the pollen mother-cells, up to the time of synapsis, are contiguous throughout, forming a continuous tissue connexion without any intercellular spaces at the corners of the cells.

Whether the disposition of the chromatin, as described and figured for the plants in question, is due in any respect to the action of fixing fluids or not—this seems certain, namely, that, before or during synapsis, the chromatin becomes resolved into relatively small granules which do not

vary greatly in size, and which are arranged into a spirem. When such conditions as Figs. 19, 20, and 21 are found in preparations showing no evidence of poor fixation, the only conclusion that can be drawn is that the presynaptic thread consists of a single row of chromomeres. There is no evidence that two distinct spirems are present prior to the first contraction, or synapsis.

FROM SYNAPSIS TO THE LOOSE OR HOLLOW SPIREM.

Podophyllum. As stated in the foregoing, the period of synapsis represents probably the longest pause in the entire mitotic process. During the same, the cells and their nuclei may increase somewhat in size, although extensive measurements were not made to determine the amount, as this was not considered of very great importance.

The first indication of a loosening of the contracted mass is seen in the extension into the nuclear cavity of loops of the spirem, and the beginning of the rounding off of the pollen mother-cells at the corners (Fig. 6). In the section from which this figure was taken, some of the cells showed the first signs of rounding up. When the loosening of the spirem has once begun, the process seems relatively rapid, as the enlargement of the anther is also rapid, so that the pollen mother-cells are soon separated from each other, in some instances rather widely. As soon as the balled-up thread begins to loosen, some of the loops or portions of the thread which project into the nuclear cavity are seen to be double, that is, two rows of granules or chromomeres may be made out in them (Fig. 6). The double row of chromomeres is not everywhere distinguishable, for in many of such free portions of the spirem only a single row can be recognized. In exceptional cases the halves of the thread diverge for a short distance, so that it seems that the linin thread is also double, but, as a rule, the linin seems undivided. The writer wishes to emphasize the fact that neither in *Podophyllum* nor in the other plants to be described further on, are the chromomeres all of the same size, nor are they all exactly paired (Fig. 6). In a comparatively short time, the contracted mass distributes itself somewhat uniformly within the nuclear cavity, and the pollen mother-cells round off, secreting their own rather thick and characteristic cell-walls. This stage is shown in Fig. 7. In nuclei less densely stained, the two rows of chromomeres imbedded or held in the linin can be clearly observed. In exceptional cases the halves of the spirem may diverge in places, showing clearly the longitudinal fission of the spirem. In *Podophyllum*, the separation of the halves is the exception (Fig. 7). Following this stage, the spirem thickens somewhat and probably becomes a little shorter, but the contraction or shortening is very much less than has hitherto been assumed. The result is a smooth chromatin spirem, arranged in rather regular turns, and in which, as a rule, no indication of a longitudinal fission

is to be recognized with any certainty save in exceptional cases. The regularity of the hollow spirem is more pronounced in *Podophyllum* than in many other plants observed by the writer, but the regularity is not so great as in those plants in which a diagrammatic arrangement of the thread into pole and anti-pole sides has been described. The stage of the regular, hollow spirem is followed by a tendency of portions of the chromatin thread to become arranged in long loops (Fig. 8) preparatory to passing into the second contraction, for a second so-called contraction is as certain in *Podophyllum* and in *Lilium* as there is a synapsis, although, as will be shown, this contraction is different in character and duration. The important thing to be borne in mind as regards *Podophyllum* is that, in the stage of the hollow spirem, we have a smooth and rather regularly arranged chromatin thread, in which all traces of a longitudinal split are obliterated save in exceptional cases, and consequently the sister halves of this thread do not diverge, as frequently happens in *Lilium candidum*.

In *Lilium*. Turning now to *Lilium Martagon* and *L. candidum*, it will be observed that a similar sequence of events obtains here as in *Podophyllum*. Synapsis leaves the nuclear contents tightly massed or contracted into a ball, which, as a rule, lies close to the nuclear membrane at one side. In some instances the mass seemed to be more central in position. I was unable to determine any regularity in the location of the mass that might lead to the conclusion that the side against which it lay was determined by gravity. Very frequently, delicate colourless fibres or threads extend from the contracted mass to the nuclear membrane (Fig. 24). Here also the loosening of the mass is first recognized by the extension of loops or turns of the spirem (Fig. 24). As in *Podophyllum*, the chromatin thread is seen to be double. This is especially apparent in cases where the halves of the spirem diverge for a short distance (Fig. 24, at the left). The loosening continues until the hollow spirem is reached (Figs. 25, 26). The turns of the spirem, though loosely arranged, are not disposed in any marked diagrammatic regularity. There are long and regular turns intermingled with short and abrupt curves and kinks. The spirem traverses the cavity of the nucleus as well as to follow its membrane. At this stage (Figs. 25, 26), usually one large nucleolus is present, but one large and one or more smaller nucleoli may occur. In *Lilium* the chromatin spirem is very long and slender at the stage in question. In sections including an entire nucleus, it is not possible to follow the thread accurately throughout its entire course. Some parts will always be concealed by others, and for that reason I have made no attempt to represent the impossible in a camera lucida drawing. Fig. 26 represents the larger part, but not the whole nucleus. It cannot be stated, therefore, whether free ends are present, but it is the opinion of the writer that the thread is endless, although this may be, and doubtless is, a matter of no importance.

In anthers, containing the stage of Figs. 25 and 26, some of the pollen mother-cells fix and stain in such a manner that the chromatin and linin make-up of the thread is very clearly shown. For example, it is found that in one end of the anther, the cells will so differentiate in staining with either the triple or the iron-haematoxylin method, that the chromomeres are brought out very distinctly, while in the remaining part of the anther the stain is held more uniformly by the thread, and the differentiation into linin and chromomeres is not so apparent. Figs. 25 and 26 may be found in the same section of a locus. In Fig. 25, the safranin washed out of the thread almost completely, and, as a result after staining with gentian violet, the linin remained colourless or nearly so, and the chromomeres blue. In Fig. 26, which is typical of all remaining nuclei in the locus, the spirem retained the safranin more uniformly throughout, and the result was a more uniform thread, showing, however, the longitudinal fission, and the chromomeres in certain parts as a series of small lumps. As stated, the same differentiation is obtained by the use of the iron-haematoxylin stain. Whether these nuclei are to be regarded as slightly different stages, or as representing conditions in which the fixing fluid preserved the structures in such a manner as to render chromatin and linin capable of retaining the stains in a different degree, is a question. It not infrequently happens that, in a number of cells in one end of the locus, the chromatin will differentiate as shown in Fig. 25, but in all other cells of the same locus the condition of Fig. 26 prevails. However, as we know that nuclei in the same locus are frequently in different mitotic stages, the fact that some of the nuclei in the end of an anther differentiate as in Fig. 25, and others as Fig. 26, may be due to differences in the stage of mitotic development. In the cytoplasm of these cells there is also some difference in appearance. The cells showing the nuclear structure of Fig. 25 have apparently a more finely granular cytoplasm, in which the fibrillar nature is not as clearly brought out as in those cells with nuclei of the structure shown in Fig. 26. In neither case, however, was there anything that could be looked upon as bad fixing.

So far as known to the writer, the true nature of the chromatin thread immediately following synapsis can be more clearly demonstrated in *Lilium* than in any other plant. Here it is shown with sharply defined clearness that the spirem consists of what is almost universally known as linin, in which are held the granules equally well known as chromatin. The chromatin granules are arranged, for the most part, in two rows, which, the writer believes, is the result of a true longitudinal fission. Each granule is usually spoken of as a chromatin disk or chromomere. It is also evident that each chromomere is composed of a number of smaller granules. According to the writer's interpretation, each chromomere is not in the form of a disk, and he desires to emphasize again, as he stated a number of years ago, that the spirem does not consist of alternate disks

of chromatin and linin, but that the chromomeres are held in the linin. Each chromomere has a rough outline, as if eroded, which seems to indicate that each is made up of smaller granules. The chromomeres were not observed in the form of angular blocks or masses, as figured by Allen ('05). In some parts of the thread the chromomeres are seen to be regularly paired and of the same size; but in other parts they may be different in size, some being much smaller than the average, and not paired but arranged alternately. Whether this is due merely to the twisting of the spirem cannot be stated with certainty at present. Fig. 25*a* represents a portion of the spirem at the stage of development in question, as the writer has observed it.

After the stage of Fig. 26, the spirem becomes slightly thicker, and always stains as a more uniform thread in which the double nature cannot always be made out; but frequently the double character of the thread is apparent, and it is seen that the two segments are twisted about each other (Fig. 26). In preparations of this stage of mitosis, cases in which the longitudinally split spirem tends to diverge are the exception rather than the rule (Fig. 26). The rule seems to be that the halves of the spirem are so closely applied to each other as to conceal nearly, or quite, the double nature.

From the stages shown in Figs. 25 and 26, the spirem soon passes into the condition which has been described as the second contraction (Figs. 27, 28). In these figures it is apparent that the larger part of the spirem is arranged in loops which tend to radiate from the centre of the nucleus, where the thread is already somewhat closely entangled. In this stage, however, as will be mentioned in a subsequent paragraph, the sister threads do diverge considerably in many instances.

When the chromatin thread, as it emerges from the synaptic mass, is compared with the presynaptic phenomena, certain changes seem to have occurred in the interval, although it is not possible to determine even in very thin sections what actually takes place in the dense contracted mass. The writer assumes that all nuclei pass through this stage, and he has limited the term synapsis to this contraction. Judging from figures of other observers, this term seems to have been made to include the loosening up of the spirem, and thin tangential sections of the early stages of the loosening mass have been doubtless looked upon as indicating what occurs in the tightly balled-up nuclear contents. Now when we compare the chromomeres in the spirem, as it emerges from synapsis (Figs. 6, 24), with the chromatin masses, as they appear in certain cases at the beginning of the contraction (Fig. 22), it is clear that these masses of chromatin are very much larger than the chromomeres. There must take place then, in cases like Fig. 22, a further reticulation into smaller granules. In such cases there would be as good ground for the conclusion that a double

spirem is formed in the synaptic mass as for the doctrine that a single spirem is formed, which then underwent a longitudinal fission. On the other hand, when we consider the condition of the chromatin shown in Figs. 19, 20, and 21, it is clear that a single spirem, with a single row of chromatin granules, is present just prior to synapsis. There is no evidence that, in such a nucleus as Fig. 19, two spirems are present. The chromomeres in this case are smaller than those in the post-synaptic spirem, and this fact finds its most reasonable explanation in the assumption that the chromomeres of the stage of Fig. 19 unite to make the larger chromomeres which split longitudinally to form the double row in the spirem. The doctrine that the longitudinally split spirem is formed by the union side by side of two distinct spirems rests purely upon theoretical considerations, and furthermore, when we know, as will be shown in what follows, that the two members (chromosomes) forming the bivalent chromosomes are arranged end to end in the spirem, all theoretical support for the assumption of the lateral fusion of two spirems will have fallen to the ground. The explanation remaining, which is most in harmony with all known facts, therefore, is that the longitudinal fission in the heterotype mitosis is as real as in any somatic division.

THE FORMATION OF THE BIVALENT CHROMOSOMES FROM THE LOOSE OR HOLLOW SPIREM.

Podophyllum. As stated in a preceding paragraph, the longitudinally split spirem, on coming out of synapsis, thickens and shortens to some extent, as it takes on the form of a rather loose and regularly arranged chromatin thread. With this process almost every trace of the double nature disappears, so that, in the absence of any knowledge of a previous fission, it would not be possible to say that the spirem is double, since the halves are so closely applied as to be indistinguishable (Fig. 8). In cases in which the double nature can be seen, the segments are usually twisted about each other, as described by the writer in 1897. Between the stage of Fig. 8 and the cross segmentation into chromosomes, the spirem undergoes comparatively little shortening. It has been assumed by earlier observers that the spirem shortens greatly, becoming very much thicker, and that the sister threads diverge widely in many places, before the cross segmentation takes place; and such an assumption is necessary under the explanation that the halves of the bivalent chromosomes lie side by side lengthwise in the spirem. Such, however, is not the case. The spirem now tends to become arranged in the form of loops (Fig. 8). This looping leads to the second contraction, by which the loops and other parts of the spirem that are not looped contract toward the centre of the nucleus in such a manner that the loops, the parallel sides of which have become more or less closely applied and twisted about each other, tend to radiate from the more

central and entangled mass (Figs. 9, 10). During this second contraction or rearrangement the spirem becomes partly or wholly segmented into the chromosomes. In some cases the twisted loops radiate somewhat diagrammatically, but this regularity is only exceptional. Fig. 9 and 10 which are slightly different stages, show average conditions met with in the many preparations. In Fig. 9 the spirem has not completely segmented, but in Fig. 10 segmentation seems to be complete or nearly so. In this and in nearly all other cases the entangled state of the chromosomes obscures their inner ends. In a great many instances the loops and other parts of the spirem are more entangled than is shown in these figures. From Figs. 8, 9, and 10 it is seen that the two segments, which are more or less twisted about each other, represent the bivalent chromosomes, and that each bivalent chromosome is formed by the coming together or approximation of two portions that were previously arranged end to end in the spirem. In *Podophyllum* there is no marked divergence of the longitudinal halves of the spirem during rearrangement, as may occur in *Lilium*. In the majority of cases this approximation of different parts of the spirem side by side is accomplished by a looping, but the segments may come together side by side, or adhere end to end after cross segmentation, as will be seen especially in *Tradescantia*. Frequently the curved ends of the radiating loops are directed toward the nuclear membrane with the free ends at the centre, but this is not always the case, and it may not be the rule, for the free ends are often seen pointing towards the periphery (Fig. 10). The free ends of the chromosomes are almost invariably connected with the nuclear membrane by delicate fibres. It sometimes happens that the longitudinal split, which is seen in the spirem on coming out of synapsis and which disappears from sight in the hollow spirem, can be observed in each parallel part of a loop (Fig. 10). The writer understands that this longitudinal fission of the spirem persists, but from the stage of the hollow spirem to the meta- or anaphase the halves are so closely applied as to obscure the split. During the anaphase, or in the early telophase, the longitudinal fission again becomes evident, and represents the line of separation of the daughter segments in the second, or homotype mitosis. In my earlier paper this fission was regarded as a second longitudinal splitting (Mottier, '03).

Between the time of the segmentation of the spirem into chromosomes, which takes place either during or following the second contraction, and the formation of the spindle, they shorten rapidly and become very much thicker. This, and not during the hollow spirem stage, it may be added, is the period of greatest shortening and thickening of the chromosomes in all plants observed. An error made by the writer in common with almost all other observers was the assumption that the greatest shortening and thickening of the spirem took place before cross segmentation.

Soon after the stage shown in Fig. 10, the chromosomes separate and become more or less scattered within the cavity of the nucleus. The segments of the chromosomes (i. e. the two chromosomes of the bivalent structure) are variously oriented toward each other. They may still adhere as a loop, as an open or closed ring or link, as straight thick rods lying closely side by side, or attached end to end. They may or may not be twisted upon each other. These and various other dispositions of the segments toward each other may be observed in all stages from the time of complete cross segmentation of the spirem to the mature spindle (Figs. 11, 12, 13, 14). In Fig. 14 are shown a few of the various forms of chromosomes observed in the spindle. Of these, that of Fig. 14 *a* is less frequently observed. The arrangement of the two segments toward each other doubtless depends on the manner in which the spirem was looped or entangled at the time of cross segmentation. So far as the writer can see, no special significance can be attached to the various orientations of the two segments toward each other.

Now, when we take into account the development of the chromosomes of the heterotypic mitosis from the hollow spirem, but one conclusion seems possible, namely, that each chromosome appearing in the equatorial plate of the spindle is bivalent, consisting of two chromosomes that were disposed end to end in the spirem; that the two segments of the whole chromosome are not daughter segments but two somatic chromosomes. From what is known during the anaphase, it is clear that each of these segments, or somatic chromosomes, is in turn double, consisting of two daughter segments which arose by the longitudinal splitting seen immediately following synapsis. Each bivalent chromosome may, therefore, consist of four chromosomes, each member of the bivalent chromosome consisting of two daughter segments. Consequently the first mitosis is a reducing division, that is, the numerical reduction of the number to one-half is accomplished, one-half of the somatic chromosomes going to one daughter cell and the other half to the other.

Lilium. In the period of the prophase of *Lilium*, between that following synapsis and the transverse segmentation of the spirem, certain details are more complicated and may lead more readily to different interpretations. For example, in two closely related stages, it is frequently difficult to determine which is younger or older, and a careful study and comparison of the figures of the several observers seem to indicate that older stages in the prophase have been interpreted as representing earlier steps in the process. Such errors, although slight, are sometimes sufficient to throw the observer's judgement against the conclusion that all other facts seem to support.

As pointed out in a preceding paragraph, the loose spirem, following synapsis, and which is longitudinally split, soon reaches its stage of greatest

regularity (Fig. 26). It should be borne in mind that in *Lilium* there is no diagrammatic regularity in the arrangement of the spirem throughout its entire course; some of its turns follow the nuclear periphery, while others traverse the interior. In the interior of the nucleus it is not possible to follow accurately the entire thread, but it seems that there are no free ends. In this stage, as in *Podophyllum*, very frequently no definite trace of a longitudinal splitting is discernible. The stage of the loose and more regular spirem soon undergoes a rearrangement and passes into that of the second contraction, which is first indicated by a conspicuous knotting or entangling of the spirem, usually near the centre of the nuclear cavity, and by a tendency of the thread to form loops, some of which radiate from the entangled centre (Fig. 27). At this stage of development, there is a marked tendency of the sister halves of the longitudinally split spirem to diverge rather widely in longer or shorter stretches of the thread, and, in the opinion of the writer, this fact more than any other has led to a misinterpretation of the stages which follow. A careful study of the preparations showing this and closely related stages shows that, while the divergence of the sister halves is frequent at this stage, it does not seem to be the rule; for cells in which little or no divergence of the longitudinally split halves are found side by side with those in which the divarication is greatest. It does not seem probable, therefore, that the wide divergence of the halves is a rule, or that it represents a stage through which all nuclei pass. However, as will be seen, the halves come together again at a later stage and become so closely applied that no trace of the longitudinal fission can be clearly made out.

The further advance of this rearrangement, or second contraction, is manifested by a larger central entanglement of the spirem in which the nucleolus may be included (Fig. 28). A large part of the spirem is arranged in loops that tend to radiate from the centre, with the head or bend of many loops toward the nuclear membrane. All the spirem, however, is not disposed in the form of loops, but some parts may have a straight course, while others consist of short and irregular turns. It will be seen also that the free ends of some of the loops will be turned toward the periphery, and that the number of loops does not necessarily correspond to the number of chromosomes. In Fig. 28 the longitudinal fission can be seen in certain of the loops, but the halves do not diverge, while in other parts of the spirem all trace of this fission is obliterated.

The stages occurring between Fig. 27 and the complete differentiation of the chromosomes are those that offered the greatest difficulty to those who have worked with this problem, and, as stated in a preliminary communication of the writer¹, they represent those steps that were regarded as the product of poor fixation at the time of his earlier study. There is now,

¹ Bot. Gaz., loc. cit., xl: '05.

however, no very good reason to believe that these steps represent other than normal stages through which all nuclei of the pollen mother-cells of *Lilium* pass, as well as in many other plants. At the stage of Fig. 28, the double spirem is slender, having shortened and thickened very little, for the period of greatest shortening and thickening begins with, or after, the cross segmentation. The processes following the condition of the spirem shown in Fig. 28 result in that illustrated by Figs. 29, 30, 31. From these figures it is clear that adjacent and parallel portions of loops approximate side by side and in many cases twist about each other. The degree of approximation and twisting of portions of the spirem always varies in the same nucleus. Also the regularity in the arrangement of the loops and other parts of the spirem varies greatly in different nuclei. Diagrammatic regularity is the great exception. The figures commonly seen are shown in Figs. 29, 30, 31. The most regular cases were not selected for illustrations, and in the figures cited the writer has endeavoured to represent average conditions observed in the majority of nuclei. In Fig. 29, for example, the looping is much less regular; three or more smaller nucleoli are present, and a larger part of the thread is knotted and entangled in the central mass. At this stage it is not possible to say that the cross segmentation is complete, but it is certain that some of the chromosomes are differentiated (Figs. 30, 31). In the light of these facts, it is scarcely possible to conclude that parallel parts of the loops, which are more or less twisted about each other, represent the halves of the spirem brought about by the longitudinal fission. In some cases it is to be seen that each parallel part of the loop is double, such a double nature being due to the longitudinal fission (Fig. 30). When the stage in the prophase represented by Figs. 30 and 31 is reached, it is probable that cross segmentation is almost completed. Some of the chromosomes, perhaps the majority that are loops, have the bend of the loop directed toward the nuclear periphery, but others are oriented with the free ends thus situated. All of the chromosomes are not formed as loops; many appear as two rather straight or bent pieces free at both ends, which have come to lie side by side (Figs. 31, 32). Whether the approximation in these cases came about before complete cross segmentation cannot be stated. In the stage shown in Figs. 30 and 31, the chromosomes are connected with each other and with the nuclear membrane by very delicate linin fibres. It is true, of course, that the chromatin is always in communication with the nuclear membrane and cytoplasm by means of such fibres.

Following the stages of Figs. 30 and 31, the chromosomes separate from each other and tend to distribute themselves within the cavity of the nucleus. At the same time each contracts rapidly, becoming thereby shorter and thicker, and the fact that the two pieces of each chromosome are split longitudinally can no longer be definitely recognized, although

this can be seen in exceptional cases. The shortening of the chromosomes continues, and they are soon more regularly spaced within the cavity (Fig. 33). There is no great regularity in the spacing; sometimes they seem to be quite regularly disposed, but frequently two or more lie close together or in contact. Fig. 33 represents the nucleus just prior to the formation of the multipolar spindle complex, and the chromosomes have about the form that they will present in the equatorial plate apart from a further shortening. The chromosomes show clearly that some arose as loops, while others may have come about by the approximation of two pieces which may, or may not, have been entirely separated before the approximation. The segments are generally twisted about each other, although this is not universally true. It is not necessary to mention the various shapes of the chromosomes as found after their formation from the spirem and upon the nuclear spindle, since the various forms arising from the manner in which the two pieces are oriented with respect to each other have already been figured in superfluous profusion by other observers. It may be stated, however, that the number of phantastically-shaped chromosomes will depend upon the degree in which the spirem is entangled, knotted, and kinked during the second contraction and earlier. It is reasonable to believe that chromosomes coming from such nuclei as Figs. 29 and 31 will be less regular in form than those arising out of those similar to Fig. 30.

When the formation of the loops during the second contraction is duly considered, and when it is recalled that the chromatin thread shortens comparatively little in the stage of the hollow spirem, and that the sister halves of the thread, if divergent, become again closely applied,—only one explanation seems now possible to the writer, namely, that the twisted parallel parts of the loops shown in Fig. 30 result first from a looping of the spirem, followed by a more intimate approximation of the parallel sides of the loop. The somatic chromosomes are, therefore, arranged end-to-end in the spirem and not parallel side by side. There seems to be no good ground for believing that the longitudinal halves of the spirem tend always to diverge widely for longer or shorter stretches and to remain thus separated; that the divergence is followed by a sudden shortening and cross segmentation into chromosomes; and that the daughter segments fuse at one end following cross segmentation to form the loops so frequently and so abundantly present. Such a process, or one similar, must be assumed by those who believe in the doctrine that the longitudinal fission is the lateral union of two spirems in synapsis, and that the two segments of each chromosome appearing in the equatorial plate of the heterotypic spindle separate along the line of longitudinal fission.

Tradescantia. The development of the heterotype chromosomes in *Tradescantia virginica* agrees in general with that described in the pre-

ceding pages. Because of this fact only those steps will be described and illustrated which seem to the writer to be necessary to clear up certain details upon which the main difference of opinion rests.

The nucleus of the pollen mother-cell in *Tradescantia* just prior to synapsis is shown in Fig. 34. Here it is seen that the chromatin is distributed in the linin network in the form of lumps or granules of rather uniform size, which show a tendency to aggregate into masses or groups. Miyake ('05) has evidently represented a similar condition in his Fig. 135. Judging from the series of figures of Farmer and Shove ('05, Figs. 23-26), it would seem that the chromatin granules are more finely divided, unless the difference between their illustrations and that of the writer is due to a difference in magnification or in methods. Whether the chromatin becomes more finely granular before synapsis, as shown for certain cases in *Podophyllum* and *Lilium Martagon*, cannot be stated. However, there is no doubt but that in some cases, at least, the structure of Fig. 34 passes directly into synapsis, for, in some preparations, the nuclei in one loculus were found with this structure, whilst in the adjacent loculus nearly all nuclei showed almost the completely balled-up condition. Inasmuch as the nuclei in the same anther are nearly all in the same or very closely related stages, there can be little room for doubt concerning the accuracy of this statement. Farmer and Shove neither describe nor figure the tightly balled-up condition of complete synapsis. They represent the first contraction figure as a partly contracted spirem only (l. c., Figs. 27, 28, 29) without an evident nuclear membrane. In all preparations of the writer showing this stage, a well-defined boundary always exists between the nuclear cavity and cytoplasm. The pollen mother-cells still form a compact tissue, but before the contracted ball has progressed far in its loosening up the cells round off and separate. It seems that the rounding off of the cells occurs sooner, or perhaps more rapidly, than in the other plants here described.

As the spirem emerges from the contracted mass, it is seen to be double or split lengthwise, though the halves divaricate only slightly at certain places. On coming out of synapsis there is in many nuclei a very marked regularity in the loops or turns of the spirem as they emerge from the remaining entanglement, which suggests the 'bouquet stage' described for certain insects (Fig. 35). According to Farmer and Shove (l. c., Fig. 32), the chromatin thread of the hollow spirem is disposed in the form of very uniform loops, showing a marked pole and anti-pole sides. Miyake's figure (l. c., Fig. 138), however, does not show any greater regularity than in other plants.

The rearrangement of the hollow spirem, or the so-called second contraction, agrees in general with what has already been described in the preceding pages. The regularity in the formation of the loops is somewhat

less marked, and they are fewer than in *Lilium candidum* (Figs. 36, 37). The spirem segments in part, at least, during the second contraction. The loops and the parallel straight or curved pieces of the thread often show a marked tendency to radiate from the entangled remainder (Fig. 37). This occurs simultaneously in a large majority of the nuclei in a given loculus, so that the picture obtained, even with low powers, is sufficiently characteristic to attract attention at a glance. When in the stage of Figs. 36 and 37, which seems to be a regularly occurring step in this mitosis, the longitudinal fission, observable always at an earlier stage, cannot be recognized except in rare cases, and there can be no doubt but the loops and parallel pieces are due to an approximation of different parts of the spirem. This interpretation is borne out as the entangled mass of Fig. 37 is loosened sufficiently to show the individual chromosomes in their entirety. There is, however, a marked peculiarity shown in *Tradescantia*, which has been noted also by Miyake for this plant and by Strasburger for *Galtonia*. As soon as the chromosomes become more evenly distributed in the nuclear cavity, following the second contraction, some appear in the form of loops with closely applied sides; others consist of paired rods, either straight or variously bent, and still others are connected into a chain, giving the impression that the spirem had not completely segmented, or that certain segments had become attached end-to-end after cross segmentation (Figs. 38, 39). This end-to-end attachment of some of the chromosomes persists in some cases until metakinesis. In the stages of Figs. 38 and 39 and during the formation of the spindle (Fig. 40), the two members of the chromosomes may form short loops, with parallel parts more or less closely applied and either straight or twisted; or the loops may be contracted into thick rings or links, open or almost entirely closed, or the two segments may adhere endwise.

When the formation of these chromosomes is followed in detail, and when their structure is observed in the spindle during the anaphase (see Figs. 30-33, published by the writer in Bot. Gaz., xxxv, 1903), it is plainly evident that each is bivalent and that the two segments are really two chromosomes that were arranged end-to-end in the spirem. When we recall also that during the anaphase (Mottier, '03, Fig. 33) each of these segments is split lengthwise, it is highly probable that this is the fission observed in the spirem as it emerges from synapsis.

Galtonia. A thorough study of all stages of *Galtonia* was not made, but so far as observation extended the process was found to agree more closely with that of *Tradescantia* and *Podophyllum* than with *Lilium*. This fact is now apparent from the figures of Strasburger ('04) and Miyake ('05). The interpretation of the formation of the chromosomes in this plant, published by Strasburger in the Sitzungsberichte der Königlich Preussischen Akademie der Wissenschaften, is, therefore, the correct one.

The behaviour of the chromosomes from their appearance in the nuclear plate of the first mitosis to the end of the second or homotype division has been described by the writer in an earlier paper (Mottier, '03), and, as a re-examination of that part of the mitotic history has only confirmed my former observations, a repetition seems superfluous here. It is to be noted, however, that the longitudinal fission of the chromosomes during the anaphase or telophase, which was then regarded as a second longitudinal fission, is, of course, the first and only such fission in these mitoses.

GENERAL CONSIDERATIONS.

A consideration of the important facts brought out in the preceding pages seems to point with a high degree of probability to certain definite conclusions, and to indicate more or less definitely several lines of study that may lead to fruitful results. In looking over the literature of the past ten or twelve years, which deals with the mitotic process in reproductive cells among plants, one is impressed with the fact that, at different times, the attention of observers has been focused especially upon some certain phase of the karyokinetic development. As in most problems, presenting many and great difficulties, it has been possible to clear up but one point at a time. For a certain period the chief interest centred about the centrosome and the development of the spindle, with the behaviour of the chromatin taking second place. At present, however, the question of centrosome and spindle development may be regarded as pretty definitely settled, especially among the higher plants, while concerning the behaviour of the chromatin there is still much diversity of opinion among both botanists and zoologists. The study of nuclear division and the sexual process has led to the location of the hereditary substance in the nucleus, and the establishment of the doctrine of the individuality of the chromosomes, together with their periodic reduction to one-half of the number at some definite point in ontogeny. One of the most important questions pertaining to these theories was the manner in which the reduction is accomplished; and, if we now admit that this problem has found a satisfactory solution, there still remain questions suggested by those steps manifested by the nucleus in the evolution of the chromosomes themselves, as well as other equally important questions that pertain to the manner in which, at least, the more obvious hereditary characters are distributed to the respective sex cells by the individual chromosomes.

When, ten years ago, the writer established the fact that the longitudinal fission of the spirem occurred in the early prophase (i.e. just after synapsis) of the heterotypic mitosis of pollen mother-cells, it was not considered necessary, even by the leading plant cytologists, to make

critical studies of the resting nucleus. The interpretation of the structure and origin of the chromosomes was based upon the form and arrangement of these structures as they appeared just previous to, or upon, the spindle, and their subsequent behaviour during the second or homotype mitosis. The prevailing view of reduction at the time, namely, that this was accomplished during the second division, centred attention largely upon the second and upon the latter part of the first mitosis, with the very important result that the second was not the reducing division. The discovery of the Mendelian principle of the segregation of characters in hybrids stimulated cytologists to a renewed study of the first mitosis with present results.

The more recent study of the presynaptic phase has suggested new problems and new interpretations, not the least important of which is the individuality of the parental chromosomes in somatic cells, and their recognition in the resting nucleus. The writer will now endeavour to show what bearing the facts observed in the plants in question have upon some of the explanations that have been proposed.

The nucleus is almost universally recognized as being in the resting stage when the chromatin is in a finely divided state, consisting of small granules, held in a linen network of delicate threads, in which none of the well-recognized phenomena of mitosis can be detected. Of course, it is not possible, and it may not be necessary, to define the term resting nucleus with strict accuracy. In different plants, and probably under different conditions in the same organ, the degree to which the breaking up into finer granules, or the reticulation of the chromatin has taken place, varies considerably. Sometimes it is certain that just prior to synapsis the reticulation may result in a very finely divided state, in which the granules are perhaps of the same size as, or even smaller than, one-half of the paired chromomeres which appear in the spirem as it merges from synapsis (Figs. 19, 20). In other cases the granules are larger, and may be spoken of rather as lumps or masses than as granules (Figs. 22, 34). This latter condition, especially when the masses or lumps approach in number that of the somatic chromosomes, has been interpreted as indicating the nature in which the individual chromosomes are recognized in the resting nucleus, each lump being a 'prochromosome' or centre of organization of a chromosome. Thus Overton ('05) claims that in the pollen mother-cells of *Thalictrum purpurescens*, *Calycanthus floridus*, *Helleborus foetidus*, and *Campanula grandis*, the number of these masses, or prochromosomes, equals the number of somatic chromosomes. Miyake ('05) suggests a similar interpretation in regard to certain monocotyledonous species, but he does not commit himself to this proposition, stating that it is not easy to determine the number of chromatin lumps, and that in *Lilium* the number of lumps exceeds that of the somatic chromosomes. My own

results seem to show conclusively that there is no definite relation existing between the number of these lumps and the number of somatic chromosomes in *Podophyllum*, *Lilium*, *Tradescantia*, and *Galtonia*, and consequently these plants lend no support to the prochromosome theory.

In regard to the differentiation of the nuclear framework into chromatin and linin, the writer believes that there can be little doubt in this, and he differs from the view expressed by Grégoire and Wygaerts ('03) that all is chromatin. In a later publication Grégoire ('06) has modified his view in this particular, admitting that such a differentiation may exist. It is true that in resting nuclei, in which the strands of the network connecting the chromatin masses are rather thick, these strands retain more of the stain, in which case the difference in appearance between chromatin and linin seems to be small indeed; and, if the iron-haematoxylin stain is used, frequently no difference is at all apparent. On the contrary, in such nuclei as Figs. 17, 19, and 40 of the presynaptic stage, and in Figs. 7 and 25 following synapsis, good staining by both the triple and iron-haematoxylin methods shows beyond doubt that we have two substances sufficiently distinct to justify the designation chromatin and linin. By this statement the writer does not intend to convey the idea that our methods of differential staining, however perfect they may seem, demonstrate completely the difference between chromatin and linin, but to indicate that such difference really exists, leaving the ultimate nature of the two substances to be determined by future research.

This study has also shown that in the same plant the manner of the distribution of the chromatin may be different prior to the synaptic contraction (Figs. 22, 23, 19, and 20). In some cases the lumps remain relatively large, while in others a breaking-up into much finer granules obtains. In all cases, however, the thread on emerging from the balled-up mass is made up of chromomeres in which there is a general uniformity in shape and size. This shows conclusively, that, whatever else may happen within the synaptic mass, this is certain, that there does take place in cases as shown in Figs. 22 and 34 a further reticulation of the chromatin. To this phase of the subject we shall return beyond.

As repeatedly asserted in the foregoing, the chromatin spirem emerges from synapsis, showing for the most part a double row of granules or chromomeres, which I interpret as the result of a longitudinal fission of the chromomeres, and that the entire thread is double seems to be indicated by the divergence in certain instances of the sister threads (Fig. 24). That we do not see all chromomeres paired or of the same size does not argue against the doctrine of a longitudinal fission more forcibly than against the union of two different spirems; and in view of the manner in which the bivalent chromosomes are formed from the spirem, it is reasonable to conclude that, if the longitudinally split thread is not the result of fission, there

is no such fission in either somatic or spore mother-cells, and the only conclusion remaining is that the spirem is formed double under all circumstances. The writer doubts whether any cytologist is prepared at present to defend such a doctrine.

In *Podophyllum*, as the chromatin thread becomes arranged into the hollow and regular spirem, the longitudinal split, as a rule, disappears from sight. Whether the sister threads completely fuse, or become so closely applied only as to conceal the double nature, cannot be stated. The latter alternative is not improbable, especially when we remember that we have to do with two cords composed of a thick fluid substance of exactly similar consistency. The next step is the rearrangement of the spirem which results in the so-called second contraction figure, in which much of the spirem may appear as radiating loops, while the remainder is in the form of an entangled mass near the centre of the nuclear cavity. Cross segmentation now follows, and the rapid shortening of the segments begins. In the formation of the second contraction figure, and from the nature of the chromosomes as they become more or less free, it is clear that each is formed by the approximation of two parts of the spirem that were arranged end-to-end in the thread, and not by two sister segments that first separated laterally and then came together again, or united at one end to form loops, open or closed links, and so on. In *Tradescantia* the case is equally clear, though certain details are more difficult to follow than in *Podophyllum*. The same process obtains in *Lilium Martagon* and *L. candidum*, although in these species, especially in *L. candidum*, a factor enters which tends to confuse greatly the regular course of events, and which has led the writer, together with many others, into error. I refer to the tendency in many cases of the sister threads of the longitudinally split spirem to divaricate rather widely for longer or shorter intervals prior to, or during, the rearrangement of the spirem into the loops. In some cases the divergence of the halves seem to be greater when the loops were more regular, but as to this being a rule cannot be stated with certainty. It should be remembered that a wide divergence of the sister threads does not occur in all cells, and, what is of greatest importance, those which do separate, as described, come together again before cross segmentation, and become so closely applied as to conceal the double nature of the thread. This fact seems to have been entirely overlooked by Berghs ('04). When one looks at Berghs' series of figures (l. c., Figs. 1-8) it seems that he has made out a case that can scarcely be questioned. But he neither describes nor figures the stages showing the second contraction or rearrangement, as illustrated by my Figs. 28, 29, and 30. He has evidently overlooked the fact that the divaricated halves of the spirem come together again before complete cross segmentation. In his (l. c., '04, I) Figs. 2-4 he has shown only isolated portions of the spirem. In order

that his Figs. 3-5 may be convincing, it is necessary to show the relation of such pieces as Figs. 3 *a*, 3 *b*, 3 *c*, and 4 *a*, 4 *b*, and 4 *c* to a large part of the remaining thread in their respective nuclei. Berghs seems to have assumed that the sister threads divaricate, that they do not become closely applied again in the spirem, and that those which form loops and so forth unite at one end after cross segmentation. I am convinced that Overton ('05), Miyake ('05), and Allen ('05) have been led into similar errors. If there should be any question concerning this point in *Lilium*, there can be no doubt in the case of *Podophyllum*, that the bivalent chromosomes are produced by an approximation of two chromosomes arranged end-to-end in the spirem, and even as regards *Lilium* the observed facts seem to admit of no other interpretation.

That the first mitosis in the pollen mother-cells is the reducing division was first asserted by Heuser in 1884 for *Tradescantia virginica*, and ten years later by Korschelt ('95) for an animal, *Ophryotrocha puerilis*, one of the annelids. Speaking of the chromosomes when arranged in the equatorial plate of the first mitosis in the pollen mother-cell, Heuser says: 'Hier sind in der Sternform die Elemente aussergewöhnlich lang,—sie reichen fast von Pol zu Pol. Ihre eigentliche Theilung wird nicht durch Längsspaltung, sondern durch eine nochmalige Quertheilung in der Nähe des Aequators besorgt; dann erst, also nach Anlage der Tochterkerne, erfolgt die Längsspaltung der Tochterstrahlen.'

From this it is evident that Heuser regarded the first, or heterotypic mitosis, as a reducing division, and that the separation of the chromosomes was a cross segmentation and not a separation along the line of longitudinal fission. He was the first also to state that the daughter segments were split lengthwise as they passed to the poles. Heuser gave no figures of *Tradescantia* in the paper cited, and his conclusions were regarded as a mere guess, and consequently little heeded by subsequent observers. The same may be said of Korschelt's account of *Ophryotrocha*, although, judged in the light of what is now known for many animals, he seems to have made out a tolerably clear case. As regards certain worms, however, the results of Foot and Strobell ('05) seem to leave no doubt as to the correctness of this view. Even admitting that Heuser's conclusion was largely in the nature of a guess, and there have been many since 1884, it was, nevertheless, based upon certain correctly observed, though insufficient, data. To show the influence upon investigators of conclusions drawn by eminent specialists from a series of apparently correctly observed and well-organized facts, the writer takes the liberty to recall an incident that occurred when he began the study of mitosis. In 1896, while working in a foreign laboratory, he remarked to a fellow student that, if there was any such thing as a reducing division in higher plants, the same takes place during the first mitosis in the pollen mother-cells. His friend replied

with a look of amusement thus: 'Do you think Flemming has made a mistake in the salamander?' referring to that author's classic paper on the spermatogenesis in *Salamandra*. No, we did not believe that Flemming could have been wrong in the matter in question, and the most eminent cytologists at the time, including the master under whom the writer worked, could not think of a reducing division occurring except in the second or homotypic mitosis. As regards the observers who in recent years have interpreted the first mitosis as a reducing division, holding that the chromosomes separate crosswise and not along the line of the longitudinal split, it may be said without doing any injustice to the ability of these workers, that the evidence set forth by them has not been wholly convincing.

The establishment of the fact that the segments of the heterotype chromosomes (the daughter chromosomes) are split lengthwise during the anaphase, together with a more accurate knowledge of their subsequent behaviour in the reconstruction of the daughter nuclei and during the homotype mitosis, precluded all possibility of a reduction division in this mitosis in so far as the higher seed plants are concerned. Furthermore, the Mendelian principle of segregation of characters in certain hybrids seemed to demand a reducing division, and whether this principle be correct or not, it has certainly accomplished much good in stimulating a search for a reducing and a qualitative mitosis. Botanists, therefore, directed their renewed attention to the first mitosis with present results. Although Farmer and Moore were probably the first to present the most convincing series of facts in behalf of the reduction in number taking place in the first or heterotypic mitosis, yet the credit for this explanation belongs by right of priority to Heuser for the higher plants and probably to Korschelt for animals.

The belief in the individuality of the chromosomes is now almost, if not quite, universal among cytologists and students of heredity. A consideration of this doctrine in the light of the rapidly increasing data, and the very general agreement among the various observers concerning the manner in which the reduction in number of the chromosomes is accomplished, brings the investigator face to face with very important and far-reaching problems. Among these the question of the recognition of the identity of the chromosomes in the resting nucleus has received attention by recent workers, and the result has been the suggestion of the prochromosome idea. In certain plants there is a tendency of the chromatin to form lumps or masses in the resting condition, in which there is often a general uniformity in size, and when the number of such lumps approaches the number of somatic chromosomes, each mass has been looked upon by some as representing a prochromosome, or the centre of organization of a chromosome (Rosenberg, '04; Overton, '05). In the light of the facts presented in this paper, it is evident that the plants here considered lend no support to such a view. The chromatin does not always appear in the

form of definite and well-defined masses in the same plant, and when such occur their number far exceeds the number of chromosomes. When all the facts are duly considered, we must admit that there is nothing in the resting nucleus that we can look upon as representing a chromosome, for all identity of such bodies is lost. In the opinion of the writer, it is better not to associate the idea of identity with the chromosomes as these are recognized upon the mitotic figure, but this idea should be connected with smaller units. Whether these units be regarded as hypothetical or as objects capable of observation may make little difference in the construction of a theory, but in order to eliminate as much as possible the purely problematic factors, it seems to me best to select as our unit, to which we ascribe individuality, some part of the chromatin capable of objective demonstration in the resting nucleus. In so doing I have selected the term pangen of the de Vriesian terminology chiefly because this term has been used in a similar sense by others (Strasburger, '05). Differing somewhat from the meaning put into the term by Strasburger, I shall apply the term pangen to the small but distinguishable particles that compose the chromomeres. In the spirem, as it emerges from the synaptic mass (Fig. 25 *a*), the chromomeres are seen to be made up of smaller granules. These smaller granules, which I shall speak of as pangens, can be recognized as well in the resting nucleus. It may be said, of course, that each of these granules composing a chromomere can be regarded as made of still smaller bodies, and why not apply the term pangen to these smaller bodies as others have done? I have not done so because, if we apply the term to objects too small for objective demonstration, it would be just as well to apply it to molecule or atom. Furthermore, in using the word pangen to designate the small but recognizable granules composing the chromomeres, cytologists will have some definite idea of what is meant. We shall understand, therefore, that each chromomere is composed of a number of pangens, leaving the number indefinite, inasmuch as all chromomeres are not the same size, and do not appear to be made up of the same number of these granules or particles; and it may not be possible in any case to determine the number of pangens in a given chromomere. Each chromosome will then be composed of a number of chromomeres, also variable in number. The use of a long series of terms, such as pangen, pangenosome, gamosome, and zygosome, as proposed by Strasburger, seems to me to confuse rather than to elucidate, and for the sake of simplicity it is better to retain the terminology already established for the larger collections of units. Each pangen may be conceived as bearing one or more qualities, but how many and what qualities are purely matters of theory.

Turning now to the idea of hereditary qualities or characteristics, the following questions present themselves. Do the chromosomes of each

gamete possess all the characters of the species? Or does the sperm, for example, contain characters not possessed by the egg? Or does each gamete contain all the characters of the species, with some character, or characters, having a stronger potentiality in one gamete than in the other? Among plants should any greater significance be attributed to variability in the size of the chromosomes in any individual than to the variability in the number of chromosomes in different species? A perusal of the recent literature on the subject shows one that the majority of theoretical discussions concerning the transmission of hereditary characters embodies some answer to the above questions, which is either assumed in the beginning or developed in the course of the argument.

Probably the prevailing view among botanists is that, in the chromatin of each parent or in each gamete, all the qualities are represented, for upon no other assumption does it seem possible to explain the parthenogenetic development of an egg into an individual similar to one arising from fecundation. Furthermore, in the case of an homosporous fern, for example, the spores contain only one-half the number of chromosomes of the sporophyte that bears them, yet the prothallia resulting from these spores produce both male and female gametes, which upon union develop into a sporophyte similar to the parent sporophyte. From these and numerous other cases, the only conclusion that can be drawn is that the reduced number of chromosomes, that is, those of any one gamete, contain all the characters of the individual. Strasburger ('05, p. 59) seems to believe that the reduction division itself signifies the conclusion that before reduction all the primordia are present in duplicate, that is, double, while after reduction all occur single. 'Dass diese reduzierte Zahl die Anlage für alle Merkmale des gegebenen Organismus in sich fasst, geht aus dem Wesen der Reductionsteilung hervor. Vor der Reductionsteilung waren alle Anlagen doppelt vertreten, nach der Reductionsteilung sind sie einfach vorhanden.'

In order that we may comprehend the significance of this doctrine, and to be able to draw our own conclusion, it is necessary to examine the data during every step in the reducing division. In the resting stage of the microspore mother-cell of *Podophyllum* or *Lilium*, for example, we can recognize definitely, let us say, only pangens, and while we cannot follow in every detail the construction of the spirem, and hence the chromosomes, out of these pangens, we may draw certain definite conclusions from those details that admit of objective demonstration, while other interpretations must remain problematical. Prior to the contraction into the balled-up mass, the chromatin may be rather finely and evenly distributed in the nuclear framework (Fig. 19). These fine granules may be regarded as pangens. On the other hand, the pangens may be collected into larger masses, each mass being usually much larger than a chromomere (Figs. 16,

17, 18). The contracted mass loosens up as the regular spirem, in which may be recognized the series of chromomeres held in the linin matrix. These chromomeres are for the most part paired, which I think is due to the longitudinal splitting of single chromomeres. The paired chromomeres vary somewhat in size, but on the whole there is much uniformity. Some of the chromomeres are, however, not paired, at least so far as can be made out, and many of the unpaired ones are smaller than the average. Now the question is, what happens in synapsis? According to Strasburger ('05, p. 37), there takes place in the synaptic mass a rearrangement by which homologous pangens are brought together. 'Meine Ansicht ist jetzt also die, dass durch die Wechselwirkung der beiden Gamosomen ihre Pangene eine bestimmte Orientierung erfahren, so dass sie bei der darauf folgenden Streckung dieser Gamosomen eine übereinstimmende Aufeinanderfolge erhalten. Dieser vorbereitende Schritt hätte somit das Ergebniss, die homologen Pangene an der gestreckten Gamosomen einander unmittelbar zu nähern.' The bringing together of homologous pangens means that every maternal pangen is brought near to its corresponding paternal pangen. Since Strasburger regards the longitudinal fission of the spirem as being due to a union of two parental threads rather than a fission, one naturally infers that he understands that the homologous groups of pangens are brought together side by side.

We cannot, of course, determine what takes place in synapsis, but it may be possible that some kind of a rearrangement occurs. In those cases in which the larger masses of chromatin are present just prior to the contraction, it is certain that these larger masses are broken up into much smaller ones before the loosening up process begins. If any rearrangement takes place, this must happen either during the balled-up condition or earlier; for it is less probable that a rearrangement and an exchange of pangens should be brought about in the longitudinally split spirem. In view of the orientation of the parental chromosomes in the spirem, that is, end-to-end, as set forth in this paper, it seems to me that, if some sort of rearrangement or interchange takes place in synapsis, this must be different from that held by those who regard the parental chromosomes placed side by side in the spirem. To me it seems that in synapsis or earlier the pangens of like affinity, or those bearing like characters, are brought together into chromomeres. These chromomeres are then organized into chromosomes. Each chromosome is accordingly composed chiefly of homologous chromomeres, which become arranged end-to-end, and which split lengthwise to form the paired chromomeres. A longitudinal fission means an equal distribution of homologous chromosomes, and this we know is accomplished during the second or homotypic mitosis in all plants thus far thoroughly investigated.

If it is assumed that all chromosomes are not alike in every quality,

then all pangens are not alike. There is no definite evidence, as yet, among plants that any particular chromosome is entirely different from others, but there is at the same time every reason to believe that each gamete may contain all, or nearly all, of the parental qualities. It is also probable that these qualities are present in different degrees of potentiality in the different chromosomes. The theory of sex determination developed in the insects (Wilson, '06, and others) cannot be applied with equal force to plants, for no accessory chromosome, or probable sex determinant, has been observed in the plant kingdom. We may, however, reasonably attribute to the chromosomes in plants certain different qualities falling within narrow limits, and such an assumption may be based upon observed facts. It is seen that, in the spirem in which the individual chromomeres are demonstrable (Figs. 25, 25*a*), all of these are not paired, especially in the case of the smaller ones. The probabilities are that these smaller granules may become a part of one chromosome or another according to the rules of chance, or according as certain qualities of a group have a stronger affinity for each other than for others. If we are in any way justified in ascribing some individual quality or qualities to these apparently unpaired granules, then we are justified in concluding that some chromosome or chromosomes are to that extent hereditarily different from others. Otherwise, what value can a reducing division have further than that of a mass reduction? Of course, we may say also with a large degree of probability that the nuclei of the gametophyte differ from those of the sporophyte only in the amount of hereditary substance, and not a difference in the hereditary qualities of that substance.

In regard to placing any great significance in the fact that in some plants certain chromosomes are smaller and some larger than others, it seems to me that the constancy of such difference is not sufficient to warrant any definite conclusion at present, especially when the source of error in estimating the size of the chromosomes is considered, and also in the additional fact that, in many plants, one of the most conspicuous things is the difference in size of the chromosomes in similar cells. If we transfer the idea of individuality from the chromosome to the pangen, the significance of difference in size seems to be greatly diminished. In this connexion the question of the significance of the number of chromosomes in any plant arises. Why should one plant reveal only sixteen chromosomes, another twenty-four, and still others larger numbers? In *Podophyllum* and *Lilium*, which possess relatively large cells and a large amount of chromatin, we have relatively small numbers but large chromosomes. In other plants, as perhaps in the majority of Dicotyledons, there are usually smaller cells, a smaller quantity of chromatin, and a relatively large number of small chromosomes. Just what relation may exist between the size of the cell, the mass of chromatin, and the size and number of chromo-

somes in plant cells has not been ascertained, and it is apparent at the outset that accurate results will be difficult to obtain. That which is of greatest theoretical importance is the fact that the number of chromosomes in any species is constant. The number, however, whether large or small, may be due to purely mechanical causes, but whatever the determining factors may be, it is certain that these are fixed by heredity, and the difference in the size of the chromosomes in any plant may have no greater importance than the difference in the number of chromosomes in different plants. The difference in size of the chromosomes in any plant, whenever it is found to occur with a high degree of regularity and constancy, is, of course, very suggestive, and the writer does not wish to be construed as under-estimating the value of such facts, or the usefulness of theories based thereon.

In connexion with any theory of the individuality of the pangens and their distribution in reduction, there is another very important fact or series of facts to be taken into consideration. In the entire plant kingdom, with the one known exception of the genus *Pinus*, the gamete nuclei fuse in fecundation while in the resting condition, or when the chromatin in each nucleus is in the more finely divided state. It is not possible to recognize in the fusion nucleus male and female chromatin, nor is any such recognition possible in the nuclei of the sporophyte generation. This being the case, what ground is there for assuming that the male and female chromatin remain distinct throughout this generation, being merely associated in the cells? If there be any rearrangement, exchange, or pairing of homologous pangens, why should not all this take place when the nuclei fuse, rather than at the close of the cycle when the reduction in number of the chromosomes occurs? In the division of the sporophytic nuclei there is in many cases, at least, a spirem formed, and this spirem separates into double the number of chromosomes, one-half having been furnished by the egg and the other half by the sperm. In this spirem we must admit that the chromosomes are arranged end-to-end, if we believe in the individuality of the chromosomes, for each division is equational. Thus arranged, are all paternal chromosomes united end-to-end to form one continuous half of the spirem, while the other half is formed by the maternal chromosomes? Or do the parental chromosomes alternate with each other in the spirem, or merely become arranged according to the rules of chance? If the parental chromosomes are arranged end-to-end in somatic cells of the sporophyte, why should they be not so arranged in the heterotypic spirem rather than side by side, as those who maintain that the double spirem is formed by the union side by side of two spirems. In this connexion it is important to remember that the univalent chromosomes which show the longitudinal split during the anaphase of the heterotypic mitosis do not form a double spirem

in those cases in which a continuous spirem is present in the daughter nuclei (*Podophyllum*, *Lilium*), but the sister segments unite end to end in this spirem.

The doctrine of the individuality of the chromosomes requires the assumption that the reduced number contains all the qualities of the species, else how is it possible to explain the fact that the monoecious prothallium of a homosporous fern, which arises from a spore containing only one-half the sporophytic chromosomes, produces by the union of gametes a sporophyte identical with the parent sporophyte? The same applies to cases of true parthenogenesis. The fact that the eggs of such a fern do not develop into sporophytes without the union with sperms would seem to be due merely to the lack of quantity of chromatin rather than to the absence of any quality. The assumption that the chromatin of each spore contains all the necessary characters of the species seems quite essential. Yet, on the other hand, the reducing division cannot be qualitative unless some of the chromosomes contain pangens that are either not possessed by others at all, or, if possessed by all, then certain pangens must contain qualities in a greater degree of potentiality. Since any two individuals of the same species have many more qualities in common than those that are different, it follows that the chromatin would consist chiefly of pangens representing these common qualities, while the comparatively few characters of difference would be represented by a relatively small number of pangens. Moreover, the pangens representing these relatively few characters would not only be different from the rest, but their affinity for any of the numerous pangens might diminish as the qualitative difference became greater. It is also conceivable that the pangens which represent certain special or widely different qualities may have a stronger affinity for some of the more numerous pangens than for others, and in this way there could be brought about a localization of the special pangens. Consequently, in the phylogenetic development of the race a more permanent association might result between certain groups of pangens and those bearing the special qualities. In some such manner certain chromosomes might have become qualitatively different from others. The pangens representing these most markedly different qualities may be conceived as coming together in one chromosome, or as distributed among different chromosomes. The foregoing may be made clearer by an illustration. The greatest structural and functional differences between any two sexually differentiated individuals, or members of an individual, of the same species is probably that of sex; and it is possible that the pangens representing sex are those that differ most from all others. This difference may be conceived as being so great that such pangens may unite to form a distinct chromosome, or sex-differential, as seems possible in the case of certain insects. However, the pangens representing these special characteristics, whether they pertain

to sex or some other distinct quality, may be present in one, or distributed among several chromosomes. It does not necessarily follow that any given chromosome should always contain these pangens; that is to say, there is among plants no ground for the conclusion that any one chromosome contains certain qualities, and only those, throughout the history of the species.

It is worthy of note here that in *Marchantia polymorpha*, whose spores produce both male and female gametophytes, these thalli retain their unisexual character when propagated vegetatively by gemmae. Noll¹ is reported to have cultivated both thalli by means of gemmae for over thirty generations, and under varying conditions of growth, without being able to change the sexual character of either strain. In a homosporous fern, which the writer now has under investigation, the spores produce small prothallia that are strictly male, and large ones that bear archegonia. The gametophyte of this fern is reported in recent literature to be strictly dioecious, the statement being made that the large prothallia never bear antheridia. Out of a number of the larger prothallia bearing archegonia, selected at random from a pure culture, about 85 per cent. were pure females and 15 per cent. hermaphrodite. Investigation is in progress to determine the percentage of spores that produce strictly male prothallia, and also the ratio of pure females to hermaphrodite forms among the large so-called female gametophytes. At present my study has not proceeded far enough to justify any statement beyond that made in the foregoing. The commonly accepted view is that by sowing the spores thickly, male prothallia are produced in greater abundance or almost exclusively, while in the less crowded cultures affording better nutrition a larger number of spores will develop into female gametophytes. Efforts will be made in the experiments to determine more accurately, if possible, whether the sex of the prothallia is influenced or determined by conditions of growth.

If the studies under consideration bear out the conclusion obtained from an examination of a very limited quantity of material, it would seem that in the fern in question some spores are strictly male, some purely female, while others contain male and female characters with the female greatly predominating. This condition can be explained in the light of the theory advanced in this paper by assuming that some spores possess a predominance of strictly male pangens, others female, and still others pangens of both sexes with the female predominating. Neither in the spore-bearing cells of *Marchantia* nor in those of the fern in question has anything comparable to an accessory chromosome, or special sex-differential, been found, and, as in the case of the higher plants mentioned in the preceding pages, it seems not impossible that the smaller granules observed in the spirem, which are not included in any of the chromomeres of a larger and more uniform size, may represent almost exclusively male

¹ Reported by Blakeslee ('06).

or female pangens or those bearing other distinct qualities. It seems reasonable also that the sexual differentiation observed in certain moulds (Blakeslee, '06) may be explained in a similar manner.

In conclusion, I think it is not mere speculation to assert that in plants of a low degree of differentiation, the hereditary determinants or pangens are correspondingly much less specialized; but as differentiation along the lines of both vegetative and reproductive structures and functions became more and more pronounced in phylogenetic development, it is only reasonable that the pangens should undergo a corresponding differentiation. And in the higher organisms where certain sharply defined and fundamental characteristics appear, as, for example, those of sex, it follows that these important and fixed characteristics should be represented in the hereditary substance by pangens that are as distinct and as sharply defined from other pangens as the characters in question are different from all other characters of the individual. The question then to determine is whether such pangens are equally distributed among all the chromosomes, or whether any of the chromosomes are qualitatively different. To arrive at any trustworthy conclusions, it is necessary that experimental studies carried out with this end in view must go along with a cytological study of the chromatin.

SUMMARY.

The resting nucleus of the pollen mother-cell consists of a linin net in which the chromatin is held, either in the form of very fine granules of uniform size, or in larger aggregations or lumps which are composed of smaller granules. The chromatin granules or lumps are often evenly distributed within the nucleus; frequently there is a tendency toward a peripheral arrangement of the chromatin, leaving the centre freer from the granules or lumps. One or more nucleoli are present. Prior to synapsis there may be formed, in some cases in *Lilium Martagon*, a very delicate, spirem-like threadwork in which the chromatin occurs as a single row of small chromomeres; in other cases the chromatin did not seem to become so finely divided, the nuclear framework, with larger chromatin lumps, passing directly into synapsis.

Synapsis consists in the contraction of the entire nuclear threadwork into a compact and dense ball, which usually lies close to the nuclear membrane. The position of the contracted mass in the nuclear cavity does not seem to be determined by gravity. The nucleolus may or may not be included within the contracted mass. The contraction of synapsis represents the longest pause in the mitotic process. There is no union of two spirems in synapsis.

The synaptic mass on loosening up forms the hollow spirem, which is double, being due to a longitudinal splitting and not to the lateral union of two spirems. In *Podophyllum* the loose spirem is of a uniform thickness

and rather regularly arranged. In it all traces of the longitudinal fission is concealed, save in exceptional cases.

In *Lilium* the arrangement of the spirem is less regular and less easily followed. The longitudinal fission is as a rule also obscured.

The hollow or loose spirem is followed by a rearrangement of the thread which has been called the 'second contraction'. This second contraction consists in the arrangement of a large part of the spirem into loops that tend to radiate from a closely entangled central mass of the thread. The loops are formed by the proximation of the parallel portions of longer turns of the spirem. In *Lilium*, as the rearrangement into loops takes place, it frequently happens that the halves of the longitudinally split spirem tend to divaricate rather widely in places. Later, these divaricated portions come together again, and the fission is obscured. During the second contraction the cross segmentation begins. The free ends of the loops may lie near the centre with the head or bend towards the nuclear membrane, or the reverse may be true. The parallel sides of the loops are frequently closely applied and twisted about each other. Each loop represents a bivalent chromosome, each parallel part being a single chromosome. The two parallel parts, or chromosomes, were arranged tandem, or end-to-end, in the spirem. All of the bivalent chromosomes do not arise as loops; the two parts or segments may approximate in various positions. This fact accounts for the variously shaped chromosomes observed in later stages. The more entangled parts of the spirem form the curiously curved and twisted bivalent chromosomes. The rearrangement or second contraction is a regularly occurring step in the heterotype mitosis in all the plants examined.

As the bivalent chromosomes separate from the second entanglement, they tend to become distributed within the nucleus and frequently near the periphery. In *Tradescantia* and *Galtonia*, there is in many cells a marked tendency for some of the segments to adhere end-to-end, forming chain-like rows of sausage or kidney-shaped pieces. Whether this is due to incomplete cross segmentation, or to a re-adherence subsequent to cross segmentation, cannot be stated. Other chromosomes in the same nucleus may have the two parts so arranged as to form open or closed links, or these may lie side by side as two short thick pieces. In all the plants studied, the two members of each bivalent chromosome were not side by side in the spirem, representing the halves of the longitudinally split thread, but they were two different parts of the spirem that were arranged end-to-end in the chromatin thread.

As the two members of the bivalent chromosome separate in meta-kinesis or during the anaphase, each is seen to be split longitudinally, and I am convinced that this is the original longitudinal split seen in the early prophase. This longitudinal split represents the line along which the

daughter segments will separate in the second mitosis. The first or heterotypic mitosis is a reducing division; the second or homotype division is equational. To what extent the heterotype division is qualitative is an open question.

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EXPLANATION OF PLATES XXVII AND XXVIII.

Illustrating Professor Mottier's paper on *Heterotypic Chromosomes*.

All figures were drawn from sections with the aid of the Abbé camera lucida and with Zeiss apochromatic immersion 2 mm., apert. 1.40, and compensating ocular 12. Magnification $\times 1800$.

Podophyllum peltatum.

Fig. 1. Pollen mother-cell prior to synapsis. The chromatin is distributed in the linin net in the form of granules of medium size; the threadwork of the cytoplasm is very distinct.

Fig. 2. A nucleus of pollen mother-cell from the same anther. The chromatin appears in larger masses or lumps, having chiefly a peripheral position; the nucleolus lies in the centre of the cavity which is freer from the linin net.

Figs. 3 and 4. Cells just prior to synapsis, taken from the same anther loculus. In Fig. 3 the chromatin granules are collected into shreds, suggesting a spirem-like condition; in Fig. 4 the chromatin consists of small granules evenly distributed throughout the linin net. The cytoplasm is finer mesh than in Fig. 3.

Fig. 5. Complete synapsis. The entire nuclear contents are contracted into a dense mass. The cells show a complete connexion at this stage.

Fig. 6. The synaptic mass beginning to loosen up. The longitudinal fission is seen in the free parts of the spirem. At this stage the cells show the first signs of rounding off at the corners.

Fig. 7. The loose or hollow spirem. At certain places the halves of the longitudinally split spirem diverge slightly.

Fig. 8. A later stage of the hollow spirem; the section includes a large part of the nucleus. The spirem consists of a rather smooth cord of uniform thickness, in which, as a rule, no trace of the longitudinal split can be seen. In this figure the spirem has begun to arrange itself into the long loops. Between Figs. 7 and 8 we have a more regular spirem, showing fewer loops.

Fig. 9. The rearrangement or second contraction. The two limbs of each loop have approximated side by side, and some have become greatly twisted about each other. In some cases the longitudinal split can be seen in each limb of the loop. Cross segmentation has begun.

Fig. 10. Second contraction complete, as is also the cross segmentation. The bivalent chromosomes tend to radiate from the centre where their inner ends are much entangled. Either the two free ends or the head of the loops may lie toward the nuclear membrane. Preparation for the spindle formation is indicated by the presence of kinoplasmic fibres radiating from the nucleus.

Fig. 11. A later stage in formation of spindle, showing several bivalent chromosomes scattered within the spindle complex. The longitudinal split in each segment is no longer apparent.

Fig. 12. A later stage; chromosomes being arranged in nuclear plate.

Fig. 13. Mature spindle, showing forms of chromosomes commonly observed.

Fig. 14, *a, b, c, d*. Some of the usual forms of chromosomes met with.

Lilium Martagon.

Fig. 15. Nucleus of a young pollen mother-cell at the close of the last sporogenous division. The chromatin lumps composed of smaller but distinct granules show a tendency to be grouped about the several nucleoli, from which thicker and thinner strands of linin tend to radiate.

Fig. 16. A later stage in presynaptic growth. The chromatin has increased in quantity, and the nucleus has become greatly enlarged.

Fig. 17. Similar to the preceding; chromatin uniformly distributed in lumps or masses.

Fig. 18. A similar stage. The nucleus became differentiated in such a way as to show both the chromatin and the linin of the lumps.

Fig. 19. Beginning of synapsis. Here the chromatin, in the form of finely divided granules of rather uniform size, is arranged in a spirem-like net, which seems to show some orientation with reference to one of the flattened nucleoli. The nucleolus or nucleoli in such nuclei are flattened against the nuclear membrane (the sickle stage of older literature).

Fig. 20. A similar condition of the chromatin, showing a further step in the contraction.

Fig. 21. Complete synapsis of such nuclei.

Fig. 22. Nucleus undergoing synapsis in which the chromatin remained in the larger lumps.

Fig. 23. Complete synapsis of this condition.

Fig. 24. The synaptic mass loosening up. The double row of chromomeres show that the thread is longitudinally split or undergoing this process.

Fig. 25. The hollow spirem just after synapsis, showing longitudinal fission. All chromomeres do not seem to be paired. Free ends are due to cutting.

Fig. 25 *a*. A portion of the thread more enlarged.

Lilium candidum.

Fig. 26. Similar, or a little later than Fig. 25. The thread stained more uniformly, and the chromomeres are not readily seen. This figure includes more of the nucleus than Fig. 25.

Fig. 27. Hollow spirem in which the rearrangement into loops and the central entanglement have begun. At this stage the longitudinal halves of the spirem tend to divaricate for longer or shorter distances. This is not true in all nuclei.

Fig. 28. A later stage. The spirem is still quite slender, having shortened and thickened only a very little. The formation of the loops is evident, and the central entanglement about the nucleolus is more pronounced.

Figs. 29, 30, 31. Later stages. The parallel sides of loops have come closer together. The longitudinal halves of the thread that may have divaricated in an earlier stage have come together again, so that traces of this fission are becoming more difficult to observe. Cross segmentation now takes place. In Fig. 30 this may be complete; in Figs. 29 and 31 the central entanglement is too complicated to admit of a clear view. At this stage the spirem is usually as entangled as Fig. 29 and even more so. From the stage of Fig. 30, i. e. after cross segmentation, the greatest shortening and thickening of the chromatin takes place.

Fig. 32. The bivalent chromosomes are beginning to separate and to distribute themselves within the nucleus.

Fig. 33. Chromosomes distributed. The formation of the spindle begins at this stage. The loop-like character of the majority of the chromosomes is still seen. In this and earlier stages delicate linin threads extend out from the chromosomes that connect them with each other and with the nuclear membrane.

Tradescantia virginica.

Fig. 34. Presynaptic stage; chromatin in larger or smaller groups or lumps.

Fig. 35. The loosening up of the synaptic mass. The loosening spirem presents a rather regular arrangement, the so-called 'bouquet' stage. The longitudinal fission is seen in places.

Figs. 36 and 37. The second contraction figure. Cross segmentation almost complete in 37. All traces of the longitudinal splitting have disappeared from sight.

Figs. 38, 39. The chromosomes distributed in nucleus prior to formation of spindle. The segments or univalent chromosomes are frequently attached end-to-end, forming a zig-zag row of sausage-like pieces.

Fig. 40. A stage in the formation of multipolar spindle. The chromosomes are crowded together somewhat, a usual phenomenon at this stage. Some still show the end-to-end attachment. This persists in some cases even to the metakinesis (see author's figures, '03).

Mottier, ad nat. del.

MOTTIER — HETEROTYPIC CHROMOSOMES.

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MOTTIER — HETEROTYPIC CHROMOSOMES.

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Mottier, ad nat. del.

MOTTIER — HETEROTYPIC CHROMOSOMES.

Hutch. del. et imp.

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