Cytological Studies on *Oenothera*. I.

Pollen Development of *Oenothera grandiflora*.

BY

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With Plates XLI and XLII.

THE interest which attaches to the *Oenotheras* on account of their intimate relation to the mutation theory of De Vries has already led to their cytological study by three investigators. These authors have, however, approached the problems either through *Oenothera Lamarckiana* itself or through some of its derivatives. They have consequently dealt not with old or well-established wild species, but with forms of recent or uncertain origin, for the relation of *Oenothera Lamarckiana* to the American flora is certainly problematical. The present study will treat of a characteristic native species which is perhaps as true to its type as any American form. Further investigation may show that there are certain advantages in approaching the cytological study of the *Oenotheras* through species of established position.

Oenothera grandiflora, Ait., is probably one of the most favourable of the American types for cytological research on the pollen mother-cells, since the remarkably large and showy flowers have anthers which are about twice the size of those of *O. biennis* and almost as large and robust as the anthers of *O. gigas*. The plants which furnished the material for this investigation were grown from seed collected by S. M. Tracy at Dixie Landing, near Tensaw, Alabama, September 3, 1907. The seedlings were started during the winter in the hot-houses of the Harvard botanic garden and planted at Woods Hole, Massachusetts, in the spring of 1908. They developed during the summer into sturdy plants more than two metres high, which flowered profusely in September.

I am greatly indebted to the Bureau of Fisheries for the hospitality of its laboratory at Woods Hole during several months, and to Miss Sarah B. Fay for garden facilities.

METHODS.

It is difficult to fix the anthers of *Oenothera* satisfactorily, and great care and patience are necessary in such a manipulation as will assist the killing fluid to wet quickly the surface of the anther, otherwise the

penetration from the tips is so slow that fixation is generally imperfect. A fixing fluid of the following formula was chiefly employed: 1 per cent. chromic acid 25 cc., 10 per cent. glacial acetic acid 20 cc., 1 per cent. osmic acid 10 cc., water 45 cc. The material was left in this solution 2-4 hours and then transferred for about 20 hours to a similar solution without osmic acid. The good effects of the osmic acid were in this way obtained without excessive blackening, and this may be readily removed with a very short immersion of the sections in a dilute alcoholic (70 per cent.) solution of hydrogen peroxide. A stronger chrom-acetic fixing fluid with osmic acid omitted also gave good results: 1 per cent. chromic acid 40 cc., 10 per cent. glacial acetic acid 40 cc., water 20 cc. Iron-alum haematoxylin proved to be the most satisfactory stain for the nuclei, which are relatively small.

THE VEGETATIVE MITOSES IN THE ANTHER.

It is important, before considering the events of synapsis and the two mitoses (heterotypic and homotypic) in the pollen mother-cell, to describe the characteristics of the vegetative divisions of the sporophyte. These may be studied in the root tips, as Miss Lutz has already done for a number of forms of *Oenothera*. There are, however, obvious advantages, because of the close relationships of the nuclei concerned, in selecting sporophytic mitoses close to or immediately preceding the reduction divisions of pollen formation.

The archesporium in the anthers of *Oenothera* is differentiated at a very early period as a single row of cells running lengthwise in the middle region of each of the four portions that become pollen chambers. As the anther grows there are cell-divisions in the archesporium both crosswise and lengthwise, and thus the single row of archesporial cells becomes transformed into a somewhat irregular double row throughout most of its length. There are also numerous nuclear divisions in the cells surrounding the archesporium (which cells form in the mature anther a well-defined tapetal tissue) and in other regions of the anther as well. Consequently the anther, as well as other parts of the developing flower, presents excellent subjects for the study of vegetative sporophytic mitoses.

This section of the paper will be concerned chiefly with the final mitoses in the archesporium which convert the primary single row of cells into the double row that become the pollen mother-cells. These mitoses appear in no way different from those in the developing tapetum and in other parts of the anther, but are more easily studied because the archesporial cells are somewhat larger.

The resting nuclei in the archesporium and throughout meristematic regions of the developing flower are similar in structure. Each contains, as a rule, a single large nucleolus, usually accompanied by

one or more small nucleoli, and a variable number of deeply staining chromatic bodies of different forms and sizes (Pl. XLI, Figs. 1 and 8). The chromatic bodies are distributed rather uniformly around the periphery, closely pressed against the nuclear membrane, and frequently traces of a very open, delicate, connecting network may be observed.

The nucleoli vary in size and in numbers. Instead of one large structure there are occasionally two or three nucleoli of approximately equal size. The smaller nucleoli may be so nearly the size of the chromatic bodies as to be scarcely distinguishable from the latter except by their more spherical form and generally more central position in the nucleus. Small nucleoli frequently lie beside the largest, and stages indicating that they are absorbed by the latter are not uncommon, as has been reported by Gates ('08) for *O. rubrinervis*.

The chromatic bodies are of especial interest in view of Rosenberg's ('04) conclusion that structures, apparently similar to these, in the resting nuclei of *Capsella* and some other forms, represent the chromosomes. It is frequently possible to make counts of these bodies that approximate the sporophytic number of chromosomes, which is fourteen, but one finds other nuclei in which the number of evident bodies is less, and also nuclei with more numerous and smaller granules. The investigations of Overton ('05, '09), which appear to have established the presence in the nucleus of prochromosomes representing the chromosomes of mitosis, make it probable that similar conditions are present in *Oenothera*, but I have not as yet been able to follow the history of the chromatic bodies with sufficient exactness to be certain that they correspond to prochromosomes.

The first indication of approaching mitosis is the appearance of a network distributed throughout the interior of the nucleus (Fig. 2), but denser in the peripheral regions. As it develops, the chromatic bodies decrease in size, apparently contributing their substance to the reticulum, and finally they can no longer be differentiated. The smaller nucleoli are then more easily recognized, since there are no longer chromatic bodies with which they may be confused.

Certain strands of the chromatic network gradually thicken and become sharply defined as a coiled and looped thread, portions of which in early stages of development are frequently united to form a very open network (Fig. 3). This thread becomes the spirem which, when fully developed, is clearly continuous for a great length, although portions of the thread in places may appear to fuse at points of crossing. It is, of course, possible that all points of union during the development of the spirem, as described above, are merely those of adherence through the medium of accessory substance, and that the true chromatic spirem is at all times a single thread.

The spirem at an early stage of development is composed of a single

row of granules resembling a string of beads (Fig. 4). These granules are apparently the chromomeres of various authors. They later become no longer recognizable as the spirem thickens and grows shorter. The fully developed spirem is a deeply staining thread wound around the periphery of the nucleus, and at this stage generally appears continuous, although the structure is so small as to make the determination of the latter point very difficult.

Fourteen chromosomes are formed by the cross-segmentation of the spirem. These may sometimes be counted within the nucleus before the formation of the spindle (Fig. 5). They have the form of long rods variously bent, as would be expected of segments derived from a coiled spirem thread.

The count of the chromosomes is, however, most readily made from polar views of the equatorial plate (Fig. 6), where they lie in approximately the same plane. The chromosomes at this time are usually V or U-shaped, but the arms may be bent at various angles. The daughter-chromosomes following the metaphase of the mitosis have a more regular form, which is clearly shown in stages of anaphase (Fig. 7). They lie with the points of the V's directed to the poles of the spindle, and in appearance are quite indistinguishable from one another. The nucleoli disappear during the development of the spindle, which is too small to be a satisfactory subject for study.

Immediately following the organization of the daughter-nuclei the chromatic material takes the form of a loose reticulum, in the meshes of which the new nucleoli appear. As the nuclei enlarge, the strands of the reticulum become thinner throughout most of the structure, and the chromatin becomes distributed around the periphery in the form of deeply staining bodies of various forms and sizes (Fig. 8). These are the chromatic bodies so characteristic of the resting nuclei in meristematic regions of the flower.

The nuclei shown in Fig. 8 are in young pollen mother-cells of the fully differentiated archesporium which, as described before, generally consists of two rows of cells, with, however, frequent irregularities of placement probably due to the adjustment of the cells to mutual pressure. There follows now a long period of growth, during which the nuclei double their diameter and the pollen mother-cells become three or four times larger in diameter. An excellent idea of the extent of these changes may be obtained by comparing Fig. 8 with Figs. 36-39, all drawn under the same magnification (2,000 diameters), the latter illustrating mature pollen mother-cells.

SYNAPSIS.

The events of synapsis and the formation of the bivalent chromosomes are of all phases in the process of pollen formation very much the most difficult to understand. The changes of structure are subtle and hard to interpret, and the correct seriation of stages requires the greatest care. There is, moreover, in the nature of the synaptic processes, which are chiefly concerned with coiled threads, a degree of variation in the form and arrangement of the structures concerned that is frequently very puzzling. Great assistance, however, is afforded by the fact that at no time are all of the anthers of an *Oenothera* flower at exactly the same period of development, and the differences are sufficiently great to be very helpful in determining which of a series of synaptic stages are older and which younger. Indeed, there are at certain periods of synapsis marked differences between the stages of development in the four pollen chambers of the same anther, and even in different portions of the same pollen chamber. As is always true, as far as the writer is aware, the period of synapsis is very much longer than that of any other phase in the history of pollen and spore formation.

The structure of the resting nucleus following the last mitosis in the archesporium is shown in Fig. 8, where it will be seen that the chromatic material is distributed as a number of deeply staining bodies around the periphery. The nuclei remain for some time in this condition. The first indication of approaching synapsis is the appearance of extremely delicate strands connecting these bodies (Fig. 9), and forming a very open network. The strands then rapidly thicken and become more numerous (Fig. 10) until finally the nucleus is filled with a relatively close reticulum (Fig. 11). During its development the chromatic bodies become smaller, apparently contributing their substance to the strands composing the network, and the nucleoli are left as the only large structures lying freely within the nucleus. The smaller nucleoli appear more conspicuous because there are no longer chromatic bodies with which they may be confused.

The advent of synapsis is marked by the beginning of a general slow contraction of the reticulum away from the nuclear membrane, a contraction which carries most of the strands towards the centre of the nucleus (Fig. 12). During this process of contraction there is a marked change in the structure of the reticulum, which results in the differentiation of very numerous threads which are coiled in an intricate manner. These are at first united to one another at many points, but as synapsis proceeds the thread structure becomes more and more evident until it becomes clear that a spirem is to be developed.

The synaptic contraction generally draws inward with it all of the smaller nucleoli which, together with the coiled and crowded threads, form a ball or knot so dense and compact that it is impossible to differentiate

clearly all of its parts. Sometimes, however, an occasional small nucleolus will be left free in the nuclear cavity (Fig. 13). There are usually at the edge of the synaptic knot a number of threads in the form of loops that extend often to the periphery of the nucleus (Fig. 14), but more frequently lie at some distance from the nuclear membrane (Fig. 15). It is clear that the process of contraction is a gradual one, and that some of the threads are drawn in more slowly than others and consequently remain free from the bulk of the contracted structure. Even in advanced stages of synapsis there are frequently present some extremely delicate threads, which later disappear, lying close to the nuclear membrane (Figs. 13, 15, and 19).

Differential staining of the chromatic threads, especially of such free loops as are favourable for study, shows them at certain stages to be composed of denser, deeply staining regions arranged like a string of beads (Fig. 14). These are apparently the oft-described chromomeres comparable to similar structures on the early spirem of the vegetative nucleus (Fig. 4).

The total length of the thread system in the early stages of synapsis is certainly very much greater than that of the early spirem of the vegetative nucleus, and apparently even greater than might be indicated by the proportionate increase in the size of the nucleus (compare Figs. 3 and 4 with Figs. 13-15). It is impossible to say whether the coiled and twisted elements of the synaptic knot are organically parts of a continuous spirem. They are certainly united to one another at many points, but this union may be merely an adherence which does not signify the branching of a true chromatic thread.

As would be expected of such complex structures as the various stages of the contracted synaptic knot it is not difficult to find threads that run closely parallel with one another for considerable distances. Furthermore, as stated above, the threads are certainly united at points (Figs. 14, 15, 16, and 17), but they form such various angles and lie in such complicated coils that a clear understanding of their intimate relations to one another was impossible with the technique employed. Further study employing methods of fixation suggested by Overton ('09) may give clearer information on the conditions. The writer has, however, so far seen no evidence that these parallel threads ever fuse for considerable lengths, or that there is present in the nucleus two independent spirems (maternal and paternal) which might be assumed to unite with one another side by side. The further history of the threads in relation to the bivalent chromosomes also gives no support to such an interpretation. Neither has the writer observed any stages that would establish the splitting of a spirem, such as might indicate a premature fission related to the lengthwise division of the chromosomes in the homotypic mitosis.

The contraction of the chromatic material during synapsis is apparently caused by the gradual shortening and thickening of the threads which later

become the spirem. This process may be readily followed and is illustrated by Figs. 12-20, which show that the threads of the older stages are more than twice as thick as those of the younger, and that the total length of the threads (so far as they may be traced) becomes very much less as synapsis proceeds.

The forms of the fully contracted synaptic knot are exceedingly varied. In the most extreme cases the threads are drawn tightly together into an irregular lumpy mass (Figs. 21 and 23). More often, however, there are several well-defined loops extending into the nuclear cavity from the dense contracted centre of the synaptic knot, as illustrated in Figs. 19, 20, and 23. The number of loops is not fixed, and they are distributed irregularly and are of various lengths. The loops that extend to the periphery of the nucleus are attached to the nuclear membrane, which is frequently drawn inward at such points probably because of the pull exerted by the contracted threads. Even when the contracted material is in the form of a dense mass (Figs. 21 and 22) it is always possible to distinguish definite lobes that in earlier stages were undoubtedly loops, but are now tightly drawn against the synaptic knot.

The mid-phase of synapsis, or the period of greatest contraction, is illustrated by Figs. 21, 22, and 23, which are fairly typical of various conditions. It should be noted that the nuclear cavity contains only the chromatic mass, the single large nucleolus (rarely two large nucleoli), occasionally smaller nucleoli (Fig. 21), and perhaps remnants of the extremely delicate threads (Fig. 23) which are present close to the periphery in earlier stages of synapsis (Figs. 13, 15, and 19). The chromatic mass may lie near the centre of the nucleus (Fig. 22), but it is usually somewhat pressed against the large flattened nucleolus and consequently at one side. The latter situation suggests a polar organization of the nucleus that is frequently emphasized by the radiating arrangement of the loops from the chromatic mass as a centre (Figs. 20 and 23). The chromatic loops are very important structures, for some of the ring-shaped bivalent chromosomes arise directly from them.

THE FORMATION OF THE BIVALENT CHROMOSOMES.

As stated before, the structure of the synaptic knot when fully contracted is very difficult of analysis. The chromatin frequently has the appearance of a lumpy mass (Figs. 21 and 22), the surface being broken by irregular globular projections, some of which were formerly the loops that extended into the nuclear cavity. In other cases these loops may remain clearly defined, although much shorter than in earlier stages of synapsis (Fig. 23). Differential staining clearly indicates that the contracted synaptic chromatin has regions of greater density, some of which

are loops of the now very much shortened and thickened spirem, and there seems no reason to doubt that a structural organization is present no matter how compact and homogeneous the synaptic knot may appear.

The chromatic material emerges from synapsis by a general loosening up of the elements which compose the contracted knot, and then for the first time it becomes evident that the chromatin has taken the form of a group of rings. Later the rings may be easily counted, the number being seven, which is half the number of chromosomes present in the vegetative or sporophytic mitoses. These become the seven bivalent chromosomes characteristic of the heterotypic mitosis.

The origin of certain of the rings from chromatin loops which extend from the synaptic knot appears very clear in such preparations as have a synaptic knot of the looser type, such as is illustrated in Figs. 20 and 23. In these cases each loop is transformed directly into a chromatic ring, and frequently three or four of the seven rings composing the group arise from such loops (Figs. 24, 25, and 26). The remainder of the rings in such nuclei are derived from the contracted portion of the synaptic chromatin, where the shortened and thickened coils of the spirem are so closely pressed together that the elements cannot be distinguished. Rings that are formed from loops are generally at first exceptionally large (Figs. 25, 26, and 29), but they rapidly grow smaller by evident condensation as the nucleus prepares for the heterotypic mitosis.

It was impossible with the technique employed to trace with exactness the development of the rings from synaptic knots of the dense much-contracted type, such as are shown in Figs. 21 and 22. These rings are organized when the chromatin is in the contracted state, and appear fully formed with the loosening up of the synaptic knot as that structure emerges from synapsis (Fig. 27). Such rings are readily distinguished from those formed from the free loops because of their small size and more regular and compact form (Figs. 27 and 28). They are at first closely grouped around the nucleolus (Fig. 27), but later become more generally distributed in the nuclear cavity (Fig. 28), illustrating the phase termed diakinesis. Frequently two or three of the rings become separated early from the others, and consequently lie freely in the nuclear cavity, while the remainder are still so closely massed in a group that their outlines cannot be clearly followed. Such free rings are very conspicuous and may be readily studied (Fig. 28).

It is certain that the fully developed rings are generally completely closed. It is exceptional to find a ring open on one side, but such are sometimes present. The rings are, however, not uniform in density, but are generally thinner at one or two points. These conditions become much more evident as a nucleus approaches metaphase of the heterotypic

mitosis, when two much thickened halves of a ring (each a sporophytic chromosome of this bivalent structure) become clearly defined, connected at two points by very delicate strands (Pl. XLII, Fig. 37).

When groups of chromosomes are viewed in favourable positions, two, three, or more rings may sometimes be seen actually linked together, thus forming short chains or clusters of rings. The appearance of these linked chromosomes is shown in Figs. 29 and 30, and there seems to be no doubt of the condition, since careful focussing enables one to follow the parts of the links above and below one another.

An explanation of the groups of linked rings seems to require their origin from an involved tangle of loops, united at certain points, which finally segment in such a manner that the fused loops (now rings) are linked together. Such an arrangement may be constructed from a piece of string (representing a spirem), loops of which are passed through one another and then tied together by thread; finally, if the string be cut at the proper points the loops will be found to separate as linked rings.

The groups of linked rings remain together until the metaphase of the heterotypic mitosis, when they become separated as the halves of the rings (sporophytic chromosomes) are drawn apart. Such complicated arrangements of the rings on the equatorial plate are probably sometimes responsible for a confused cluster of chromosomes that is difficult to interpret, as illustrated by Fig. 35.

In concluding the account of synapsis and of the formation of the bivalent chromosomes, certain striking features should be noted that are of importance in relation to the hypotheses that have been offered in discussions of the structure and origin of heterotypic chromosomes:—

(1) The spirem at no period lies freely in the nucleus in such a form that it may be followed for any considerable portion of its length. It is from the beginning an involved coiled structure, the loops of which adhere to one another at points especially in contracted portions of the synaptic knot.

(2) The spirem does not segment into fourteen chromosomes, lying freely in the nuclear cavity or later becoming associated in pairs.

(3) The chromosomes appear at the outset as seven pairs (bivalent chromosomes) which have the form of generally closed rings, and remain so united in pairs until metaphase of the heterotypic mitosis.

(4) The origin of some of the rings (bivalent chromosomes) from loops of the spirem extending freely into the nuclear cavity indicates that the halves (sporophytic chromosomes) are segments of the spirem arranged end to end, which are brought together in ring-shaped pairs by the coiled arrangement of the spirem.

(5) The occasional presence of linked rings indicates that loops of the spirem sometimes become interlaced in such a manner that after the

segmentation of the spirem the loops (now rings) are joined together like links in a chain.

(6) It seems impossible in this form to divide the phases of synapsis into periods of a first and second contraction as has been done in some other studies. The changes of structure during synapsis are so gradual as to make sharp divisions between the periods very difficult to define. Furthermore, the degree of variation in the form of the chromatic material at the same phase of synapsis makes it unsafe to assume that looser types of synaptic contraction indicate periods of expansion to be followed later by further contractions.

THE HETEROTYPIC MITOSIS.

The prophases of spindle formation of the first, or heterotypic, mitosis in the pollen mother-cell appear shortly after the seven chromatic rings, or bivalent chromosomes, emerge from the synaptic knot and are distributed through the nuclear cavity presenting the stage called diakinesis (Figs. 28 and 31). The rings by this time have grown smaller, that is, their substance has become more condensed, as may be seen by comparing Figs. 31, 32, and 33 with Figs. 25, 26, and 29. The halves of the rings soon become more sharply defined and gradually take on the bent or V-shaped forms, characteristic of the sporophytic chromosomes with which they are identical. These half-rings, or sporophytic chromosomes, however, remain connected in pairs so that the rings are still intact. It is clear that the process of condensation and differentiation continues through the stages of prophase up to the period of metaphase when the two sets of sporophytic chromosomes are ready to be distributed by this, the heterotypic mitosis.

The process of spindle formation in its main features follows a history similar to that described for the heterotypic mitosis in a number of higher plants (e. g. *Equisetum*, *Larix*, *Lilium*, &c.). The nucleus becomes surrounded by a web of fibrillae which enter the cavity of the nucleus with the breaking down of its membrane (Figs. 31, 32, and 33). The large nucleolus at this time stains faintly and soon disappears. The developing fibrillae gradually fill the nuclear cavity, and at the same time push their way out into the cytoplasm in several directions, giving the well-known multipolar stages of spindle formation. Sections of prophases showing three or four poles are common (Figs. 35 and 36).

The chromatic rings are carried towards the centre of the developing mass of fibrillae, where at first they frequently lie in a group so closely massed that their arrangement can be made out only with difficulty (Fig. 35). However, one or more of the rings are generally apart from the main group and their forms may be clearly seen (Figs. 34 and 36).

The spindle at metaphase of mitosis (Plate XLII, Figs. 37 and 38) is a rather broad bipolar structure developed from the multipolar conditions

of prophase by the gathering of the fibrillae into two opposite sheaves. No centres were observed at the poles of the bipolar spindle, the fibrillae ending in a granular area which merged into the surrounding alveolar cytoplasm.

The chromosomes become generally arranged in a very symmetrical manner to form the equatorial plate at the metaphase of mitosis. The condition illustrated by Fig. 37 is thoroughly typical. It will be seen that the sporophytic chromosomes are still joined together in pairs forming the rings, but they are now mostly V-shaped instead of semi-circular, the points of the V's being directed towards the spindle poles. The sporophytic chromosomes may be readily counted at this stage of mitosis, and the number is fourteen, grouped of course in seven pairs derived from the seven rings characteristic of the prophases. The only observed irregularities of this arrangement (in well cut sections) were instances where one or more rings lagged behind the others in taking their positions in the equatorial plate and in completing the separation of the chromosomes comprising such pairs (Fig. 38).

The V-shaped form of the chromosomes becomes even more evident during anaphase, as the two sets of chromosomes move away from one another towards the poles of the spindle (Fig. 39). Sometimes the arms of the V's are brought so closely together that the line of contact might be mistaken for a line of premature fission in preparation for the second or homotypic mitosis, but this is not the case.

The fission of the chromosomes in preparation for the succeeding homotypic mitosis takes place in the latter part of anaphase of the heterotypic division when the chromosomes are congregated at the poles of the spindle (Fig. 40). The split is lengthwise of each V-shaped chromosome in the plane of the page on which the above letter (V) is printed. However, at this time the arms of the V's generally separate somewhat, so that most of the chromosomes are not so sharply bent as in earlier stages of this mitosis. This premature fission, peculiar to late anaphase, is most readily observed when the chromosome group at a pole is cut obliquely (Fig. 41) so that the chromosomes are viewed at various angles.

There is a well-defined period of rest between the heterotypic and homotypic mitoses in the pollen mother-cell that is especially interesting for the history of the chromatin, which may be very easily followed in this form. The seven split chromosomes that gather at the poles of the heterotypic spindle are almost always arranged so that six of them lie in a circle around the seventh (Fig. 42). At this time the line of fission is clearly marked, and the chromosomes look like seven pairs of rods when the group is viewed from above the pole of the heterotypic spindle, as shown in Fig. 42. In reality, however, the chromosomes are not rods but are V or U-shaped, the bent ends pointing downward or away from the pole of the spindle.

There then develops around each group of chromosomes at the pole of

a heterotypic spindle a vacuole-like region, the boundary of which becomes the membrane of the daughter-nucleus. The seven split chromosomes, which were at first massed closely together, separate as the daughter-nucleus gradually increases in size, and become distributed rather symmetrically around its interior just under the nuclear membrane. A change in the form of the split chromosomes becomes then at once apparent. The ends of one chromosome of the pair swing away from the ends of the other until they lie in approximately the same plane, when the structure becomes that of two U's joined together at the bent middle regions. This condition is illustrated in Figs. 44 and 45, which also show the development of the nucleoli that are conspicuous in the full-sized resting nucleus.

A careful study of stages makes it perfectly clear that the halves of the split chromosomes are not crossed, even though the form of the double structure is sometimes like that of an X, but that they lie side by side joined at the bent middle regions. This striking association of chromosomes is very characteristic of these resting nuclei. There is sometimes a tendency on the part of the free ends of the chromosomes to extend and to branch, and even to become united with one another, thus forming a loose and imperfect network (Fig. 43). However, I have never seen a resting nucleus in which the outlines of one or more chromosomes could not be accurately followed. Fig. 45 shows a pair of nuclei that are fully at rest; in the lower one of the two the seven split chromosomes are entirely distinct from one another and may be counted, and in the upper one (cut by the knife) four of the split chromosomes may be readily distinguished.

There can be no doubt that in this type the chromosomes maintain their individuality in the resting nucleus between the heterotypic and homotypic mitoses. This conclusion is further supported by the structure of the chromosomes as they appear in the early stages of the homotypic mitosis to be described below. The point is one of considerable importance because of the views held by some plant cytologists that the chromosomes may entirely lose their individuality in the resting nucleus.

In conclusion, it is evident that the heterotypic mitosis distributes the fourteen sporophytic chromosomes in two groups of seven each, and consequently is a reduction-division. These sporophytic chromosomes are grouped in pairs as ring-shaped bivalent chromosomes which appear after synapsis, and are characteristic of diakinesis and the prophases of spindle formation. The sporophytic chromosomes are V-shaped at the metaphase of mitosis and appear essentially similar to one another. There seems to be as great uniformity in their shape and size as is common for the heterotypic mitoses of plants.

THE HOMOTYPIC MITOSIS.

The spindle of the homotypic mitosis is formed as in the heterotypic division from a mesh of fibrillae that develop around the resting nucleus and enter the nuclear cavity with the dissolution of its bounding membrane (Fig. 46). The prophases likewise frequently show multipolar spindles (Figs. 47 and 48) that become bipolar at the metaphase of mitosis. The two spindles may lie parallel in the pollen mother-cell or at right angles to one another.

The chromosome group emerges from the resting nucleus at the beginning of the homotypic mitosis in very much the same form as it entered that structure at the end of the heterotypic. There are the same seven split chromosomes, the halves of which are bent outward so that they are joined to one another only in the bent middle region. This peculiar chromosome group presents an interesting contrast to the seven rings characteristic of the heterotypic mitosis (compare Figs. 46-48 with Figs. 34 and 36).

The seven split chromosomes are gathered in the usual manner at the equatorial plate, where they are arranged so that the halves separate into two sets at the metaphase of mitosis (Figs. 49 and 50). A comparison of this stage with the metaphase of the heterotypic mitosis (Fig. 37) shows that the daughter chromosomes are not so large as those of the latter mitosis and have the form of short and sometimes slightly bent rods rather than of V's. One or both ends of the chromosomes may become somewhat enlarged, giving them an irregular appearance that is frequently quite pronounced during anaphase of this mitosis (Fig. 51).

Each daughter-nucleus following the homotypic mitosis thus receives one set of the halves of the seven split chromosomes. These halves may be readily counted in favourable preparations of anaphase (Fig. 52), and especially when the chromosome group at the pole of the spindle is viewed from above (Fig. 53). The arrangement of the chromosomes in late anaphase and early telophase is similar to that in the heterotypic mitosis (i. e. six chromosomes are grouped in a circle around the seventh), but in this group there is not present of course any trace of a premature fission in preparation for a succeeding mitosis.

As the four daughter-nuclei following the homotypic mitosis increase in size, the chromosomes which enter into their construction begin to elongate and finally to branch. Although they may be distinguished in the younger nuclei, such as are shown in Fig. 54, their forms become irregular in the older (Fig. 55), where a network is finally developed. The nucleus of the young pollen-grain (Fig. 56) generally has several medium-sized nucleoli, and the chromatin is distributed in the form of small deeply staining bodies connected with one another by a delicate open reticulum.

It is clear that the homotypic mitosis is an equational division distributing the halves of the seven sporophytic chromosomes that are separated from a similar set by the heterotypic mitosis. These seven sporophytic chromosomes become divided lengthwise during anaphase of the heterotypic mitosis, and the halves remain closely associated to form seven split chromosomes, which may be followed through the period of interkinesis between the heterotypic and homotypic mitoses.

CYTOLOGICAL DISCUSSION.

As stated before, three investigators have within recent months published the results of cytological studies on *Oenothera Lamarckiana* or certain of its derivatives.

Miss Anne M. Lutz has made extensive chromosome counts chiefly from studies of root tips, and reports some interesting variations from the numbers typical of the genus, which all authors agree are fourteen chromosomes for the sporophyte and seven for the gametophyte. The most remarkable exception is found in *O. gigas* which presents twenty-eight chromosomes in the sporophyte. It is to be hoped that her papers, largely in the nature of preliminary announcements,¹ will soon be followed by detailed accounts with figures.

Geerts has published preliminary notes on a lengthy study of *O. Lamarckiana*,² which include an investigation of the embryo sac as well as of pollen formation. A full account of his conclusions, to be accompanied by figures may be expected in the *Recueil des Travaux Botaniques Néerlandais*. The absence of figures in his preliminary papers makes a close comparison of his results and those of the present paper difficult, and I shall postpone for the present their consideration.

Gates has studied the processes of pollen formation in *O. Lamarckiana* and several of its derivatives, and reported the results in a number of notes³ and in two illustrated papers (Gates, '07 and '08). The last of the two illustrated papers (Gates, '08) gives his cytological conclusions based chiefly on an investigation of *O. rubrinervis*. I shall not, however, discuss these in relation to the present study of *O. grandiflora* until I have had an opportunity myself to study *O. Lamarckiana* or some of its relatives. The results of Gates's investigation of *O. rubrinervis*, as presented in his last paper, differ from those here recorded in a number of important particulars, the chief of which are as follows:—

1. Following synopsis, Gates describes and figures a thick spirem which may be traced continuously for considerable lengths in the nuclear

¹ Science, 1907, p. 151; 1908, p. 335; 1909, p. 363.

² Ber. deut. bot. Gesellsch., 1907, p. 191; 1908, p. 608.

³ Science, 1907, p. 259; 1908, p. 193 and p. 335; 1909, p. 269.

cavity and which segments crosswise to form a chain of fourteen chromosomes.

2. Pairs of chromosomes may be precociously cut off from the spirem, the members of the pairs bending around to form loops but never becoming united to form rings.

3. The chromosome segments are at first several times longer than broad, but by a process of condensation become nearly globular or pear-shaped.

4. The chromosomes are at first arranged irregularly on the heterotypic spindle, many of them frequently remaining separate and unpaired.

5. Such a distribution allows irregularities of distribution in the heterotypic mitosis, so that both (unpaired) chromosomes belonging to one pair will occasionally enter the same daughter-nucleus.

It seems to the writer best to defer a detailed discussion of the cytological problems touched by the present study until further investigations have been made on this interesting genus, but a few important considerations may be briefly outlined.

It is clear for *Oenothera grandiflora* that the sporophytic chromosomes become associated in ring-shaped pairs, which first appear as the chromatin emerges from synapsis, and are characteristic of the period termed diakinesis and of the prophases leading to the heterotypic mitosis. These ring-shaped bivalent chromosomes are assembled in a very orderly manner at the equatorial plate of the heterotypic mitosis, where they remain intact as rings until the separation of their halves into two numerically equivalent sets of sporophytic chromosomes. There is little or no opportunity for irregularities in the distribution of the numbers when the normal processes of the mitosis follow such a history.

The V-shaped sporophytic chromosomes of the heterotypic mitosis are in general similar to one another in form and size. There is apparently no more variation in these respects than is shown in the heterotypic mitosis of plants in general. The condition as illustrated in Fig. 37 is the normal one for this form, and variations from this as shown in Fig. 38 indicate only that certain rings (bivalent chromosomes) occasionally lag behind the others in completing the separation of the sporophytic chromosomes.

The chromosomes may readily be followed between the telophases of the heterotypic and the prophases of the homotypic mitoses, maintaining their individuality with very little change of form throughout this period of interkinesis. This history has also been noted by Gates and Geerts in *O. Lamarckiana* and *O. rubrinervis*. It adds further evidence against the views of Mottier ('03 and '07), Lewis ('08), and others that chromosomes lose their individuality in the resting nucleus between the heterotypic and homotypic mitoses.

The interpretation of synaptic and presynaptic conditions presents far

greater difficulties than the post-synaptic events considered above. Not only must one deal with more intricate structure but also with conditions where the necessities of the best possible technique are far more exacting than in the later stages of the reduction processes. The last contribution of Overton ('09) offers such important evidence on the structure and arrangement of the chromosomes during presynaptic phases in the history of the pollen mother-cells that the writer prefers to defer an expression of opinion concerning the conditions in *Oenothera* at these periods until further studies have been concluded.

Overton ('09), from studies on *Thalictrum*, *Calycanthus*, and *Richardia*, believes that the chromosomes are represented in the vegetative (somatic) nuclei by prochromosomes arranged in parallel pairs separated by linin intervals indicating the presence of parental sets of chromosomes associated together to form heterogeneous spirems. The synaptic contraction is a gradual process during which parental spirems become more closely associated but still remain distinct, and the chromosomes of each pair are in their most intimate relation during post-synaptic stages. Each pair becomes a bivalent chromosome composed of two sporophytic chromosomes, the bivalent structures being characteristic of diakinesis. No folding process to form these bivalent chromosomes seems possible. The sporophytic chromosomes of the bivalent diakinetik pairs undergo lengthwise divisions, thus forming apparent tetrads. The heterotypic mitosis distributes entire sporophytic chromosomes, which remain distinct during the following interkinesis. The homotypic mitosis separates the split halves of the sporophytic chromosomes, and these may be followed in the pollen-grain as prochromosomes arranged in a single row.

It will be noted at once that Overton's conclusions offer strong support to the theory of chromosome reduction held by Grégoire ('04, '07), Allen ('05), Rosenberg ('05), and certain later writers. This group believe that two sets of sporophytic chromosomes of maternal and paternal origin are represented in the nucleus by two distinct spirems or sets of threads which become closely associated in synapsis, so that by a parallel arrangement of the spirems the chromosomes of one set are paired with those of the other to form the bivalent chromosomes of the heterotypic mitosis. Another group of investigators, represented by Farmer and Moore ('05), Mottier ('07), Strasburger ('04), and others, hold that the sporophytic chromosomes are arranged end to end on a single chromatic thread and that the bivalent chromosomes of the heterotypic mitosis are formed from loops of this single spirem that include a pair of sporophytic chromosomes which thus come to lie side by side.

The writer, on theoretical grounds, has been very strongly inclined towards the views of the first-named group represented by Allen, Grégoire, Overton, and Rosenberg. It must, however, be pointed out that the present

study of *Oenothera grandiflora* presents conditions that the writer cannot bring into harmony with their conclusions. Our present information for this type strongly indicates a pairing of the sporophytic chromosomes according to the method held by Farmer and Moore. The writer is disinclined to believe that so important a process in the life-history of plants as that of chromosome reduction is likely to be effected by two methods so diverse as those held by the opposing groups of investigators mentioned above, but there is at present a conflict of such apparently good evidence that one may well hesitate before taking a positive position. The conditions in *Oenothera grandiflora* that seem to require the explanation of chromosome association advanced by Farmer and Moore may be summarized as follows:—

1. The arrangement of the chromatic material during synapsis is characterized by the presence of several loops extending freely into the nuclear cavity from the synaptic knot as a centre (Figs. 19–23).

2. These free loops thicken as synapsis proceeds, and it seems clear that some of the ring-shaped bivalent chromosomes are formed directly from them.

3. The fact that the ring-shaped bivalent chromosomes are sometimes linked together is difficult to understand except on the theory that they have become so associated by the segmentation of a much-coiled spirem the loops of which were interlaced.

SUMMARY.

1. The vegetative (somatic) nucleus contains chromatic bodies that frequently approximate the number of the sporophytic chromosomes, which is fourteen. It seems probable that they are prochromosomes. These bodies in the resting nucleus are connected by a delicate open network which becomes much more conspicuous and denser before the development of the spirem of the vegetative mitoses.

2. The spirem arises from this reticulum which later disappears, after which the spirem may be followed for great lengths as a single thread. Fourteen chromosomes in the form of long rods variously bent are formed by the cross-segmentation of the spirem.

3. Following the vegetative mitoses, the chromosomes become distributed through the nuclear cavity of the resting nuclei, where they take on the form of chromatic bodies.

4. Previous to synapsis the nucleus becomes filled with a close reticulum during the formation of which the chromatic bodies lose their rounded form and could not be followed.

5. Synapsis consists of a general slow contraction of the reticulum away from the nuclear membrane, a contraction that carries most of the

strands towards the centre of the nucleus. During the process of contraction numerous threads are differentiated from the reticulum which become coiled in a very intricate manner.

6. The synaptic contraction draws the coils of threads into a dense knot close to the large nucleolus, which generally lies at one side of the nucleus. Loops of the threads extend into the nuclear cavity from the synaptic knot as a centre. The threads gradually thicken as synapsis proceeds, and the length of the thread system is very much shortened.

7. When fully contracted, the synaptic knot consists of much-thickened threads (constituting the spirem) which are drawn so tightly together that their arrangement cannot be traced. The loops extending from the contracted mass are at this stage thick and very conspicuous.

8. The contracted material emerges from synapsis by a general loosening up of the elements of the contracted knot. It then becomes at once apparent that the chromatin is in the form of a group of seven rings, some of which are derived from the loops referred to in paragraph 7. The rings are generally closed, but consist of semicircular halves, which are the fourteen chromosomes. The rings are therefore bivalent chromosomes, very characteristic structures of diakinesis and the prophases of the heterotypic mitosis.

9. The seven ring-shaped bivalent chromosomes become much condensed during the prophases of the heterotypic mitosis. They then become arranged at metaphase to form the equatorial plate, after which the halves separate as two sets of V-shaped sporophytic chromosomes (seven in each set) that pass to the poles of the spindle. The heterotypic mitosis is therefore a reduction division. A lengthwise fission of each chromosome which takes place during anaphase is the premature division of these elements in preparation for the succeeding homotypic mitosis.

10. The sporophytic chromosomes of the heterotypic mitosis are V-shaped and appear essentially similar to one another. There seems to be as great uniformity in their shape and size as is common for the heterotypic mitoses of plants.

11. A resting nucleus is organized between the heterotypic and homotypic mitoses, and throughout this period of interkinesis the seven split chromosomes may be readily followed.

12. The homotypic mitosis distributes the halves of the split chromosomes into two sets (seven in each), and is consequently an equational division.

WOODS HOLE, MASSACHUSETTS.

April, 1909.

LITERATURE CITED.

- ALLEN, C. E. ('05): Nuclear division in the pollen mother-cells of *Lilium canadense*. Ann. Bot., xix, 1905, p. 189.
- FARMER, J. B., and MOORE, J. E. S. ('05): On the meiotic phase (reduction division) in animals and plants. Quart. Journ. Micr. Sci., xlviii, 1905, p. 489.
- GATES, R. R. ('07): Pollen development in hybrids of *Oenothera lata* x *O. Lamarckiana*, and its relation to mutation. Bot. Gaz., xliii, 1907, p. 81.
- ('08): A study of reduction in *Oenothera rubrinervis*. Bot. Gaz., xlvi, 1908, p. 1.
- GRÉGOIRE, V. ('04): La réduction numérique des chromosomes et les cinèses de maturation. La Cellule, xxi, 1904, p. 297.
- ('07): La formation des gémis hétérotypiques dans les végétaux. La Cellule, xxiv, 1907, p. 369.
- LEWIS, I. M. ('08): The behaviour of the chromosomes in *Pinus* and *Thuja*. Ann. Bot., xxii, 1908, p. 529.
- MOTTIER, D. M. ('03): The behaviour of the chromosomes in the spore mother-cells of higher plants and embryo sac mother-cells. Bot. Gaz., xxxv, 1903, p. 250.
- ('07): The development of the heterotypic chromosomes in pollen mother-cells. Ann. Bot., xxi, 1907, p. 309.
- OVERTON, J. B. ('05): Ueber Reduktionsteilung in den Pollenmutterzellen einiger Dikotylen. Jahrb. wiss. Bot., xlii, p. 1905, 121.
- ('09): On the organization of the nuclei in the pollen mother-cells of certain plants, with especial reference to the permanence of the chromosomes. Ann. Bot., xxiii, 1909, p. 19.
- ROSENBERG, O. ('04): Ueber die Individualität der Chromosomen im Pflanzenreich. Flora, xciii, 1904, p. 251.
- ('05): Zur Kenntnis der Reduktionsteilung in Pflanzen. Bot. Not., part i, 1905, pp. 1-24.
- STRASBURGER, E. ('04): Ueber Reduktionsteilung. Sitzber. Kön.-Preuss. Akad. Wiss. Berlin, xviii, 1904, p. 587.

EXPLANATION OF FIGURES IN PLATES XLI AND XLII.

Illustrating Prof. Davis's paper on pollen development of *Oenothera grandiflora*.

All figures were sketched with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 mm. (aper. 1.30) in combination with the compensating ocular No. 12, giving a magnification of 2,000 diameters. Figures 1-10 from sections 4 μ thick; 11-18, 21, 28, 30, 32-42, 44, 46, 52-56 from sections 5 μ thick; 22, 24-27, 43, 45, 47-51 from sections 7 μ thick; 19, 20, 23, 29, and 31 from sections 10 μ thick. Sections stained with iron-alum haematoxylin.

PLATE XLI.

Oenothera grandiflora.

Fig. 1. Adjacent sections from nucleus in young archesporium, showing chromatic bodies and large nucleolus.

Fig. 2. Nucleus of archesporium, reticulum formed preparatory to the development of the spirem.

Fig. 3. Nucleus of archesporium, early stages of the spirem showing the threads united at points.

Fig. 4. Nucleus of archesporium spirem with granules (chromomeres?) arranged in a single row.

Fig. 5. Segmented spirem in archesporial nucleus resulting in fourteen sporophytic chromosomes.

Fig. 6. Metaphase of mitosis in archesporial cell viewed from the pole of the spindle, fourteen chromosomes.

Fig. 7. Anaphase of mitosis in archesporial cell, showing V-shaped chromosomes.

Fig. 8. Pollen mother-cells after the final mitosis in the archesporium, nuclei with chromatic bodies and large nucleoli.

Fig. 9. Nucleus before synapsis, chromatic bodies connected by a delicate reticulum.

Fig. 10. Later stage, the reticulum more prominent in preparation for synapsis.

Fig. 11. Nucleus just before the beginning of synapsis, a close reticulum present.

Fig. 12. Early stage of synapsis, system of threads developing from the reticulum shown in Fig. 11.

Fig. 13. Synaptic contraction well under way. Most of the threads are in closely twisted coils near the large nucleolus, certain very delicate threads lie close to the nuclear membrane.

Fig. 14. Synapsis, the loops extending from the synaptic knot consist of rows of granules (chromomeres?).

Fig. 15. Synapsis, coiled and looped threads around the large nucleolus, very delicate threads close to the nuclear membrane.

Figs. 16-18. Synapsis, the threads thickening.

Fig. 19. Synaptic contraction almost complete, showing the dense knot, the loops extending into the nuclear cavity, and some delicate threads lying close to the nuclear membrane.

Fig. 20. Synaptic contraction probably complete, thickened loops extending from the synaptic knot.

Figs. 21 and 22. Complete synaptic contraction of the dense type, the loops represented by protrusions from the synaptic knot.

Fig. 23. Contracted synaptic mass ready to loosen up, the loops and prominent protrusions probably now organized into ring-shaped bivalent chromosomes.

Fig. 24. A stage in the loosening of the synaptic knot. The loop-like structures are chromatic rings.

Figs. 25 and 26. The ring-shaped chromosomes shortly after their emergence from the synaptic knot. The rings are large at this stage.

Fig. 27. Approaching the phase of diakinesis. Most of the rings are still clustered around the large nucleolus.

Fig. 28. Diakinesis, the rings now much more condensed than when first organized (Figs. 24-26).

Fig. 29. A group of rings, certain of which are linked together—a stage in the emergence of the rings from the synaptic knot similar to Figs. 24-26.

Fig. 30. A group of linked rings.

Fig. 31. Early prophase of the heterotypic spindle, large nucleolus still present. The nuclear membrane has disappeared, chromatic rings distributed in the nuclear cavity, which is surrounded by a web of fibrillae.

Fig. 32. Multipolar spindle, chromatic rings gathered in the centre of the nuclear cavity.

Fig. 33. Certain chromatic rings show clearly their bivalent nature, being composed of two thickened semicircular halves.

Fig. 34. The seven bivalent ring-shaped chromosomes gathering at the equatorial plate.

Fig. 35. Some of the chromosome rings densely clustered at the equatorial plate as if they might be linked together.

Fig. 36. Pollen mother-cell in prophase of the heterotypic mitosis, showing the relation of the multipolar spindle to the surrounding alveolar cytoplasm. Seven bivalent chromosomes are gathered in the centre of the spindle.

PLATE XLII.

Oenothera grandiflora.

Fig. 37. Pollen mother-cell at metaphase of the heterotypic mitosis, the two sets of sporophytic chromosomes still united in pairs forming rings. The similarity of the chromosomes in form and size is clearly shown.

Fig. 38. Another metaphase illustrating the fact that certain rings may lag behind the others in completing the separation of their halves (sporophytic chromosomes).

Fig. 39. Early anaphase of the heterotypic mitosis, showing the V-shaped sporophytic chromosomes, all of similar form and size.

Fig. 40. Late anaphase, chromosomes gathering at the poles of the spindle.

Fig. 41. A group of chromosomes in late anaphase of the heterotypic mitosis (cut obliquely), showing certain of the chromosomes split lengthwise.

Fig. 42. Group of seven split chromosomes gathered at the pole of the heterotypic spindle viewed from above.

Fig. 43. Telophase of the heterotypic mitosis, the halves of the split chromosomes bending away from one another, showing a tendency to branch and to form an open network, small nucleoli appearing.

Fig. 44. The resting nucleus of the interkinesis between the heterotypic and homotypic mitoses. Seven split chromosomes are present, the halves in the form of U's joined together in the bent middle region.

Fig. 45. Pollen mother-cell containing resting nuclei in the interkinesis following the heterotypic mitosis, seven split chromosomes evident in the lower nucleus of the two.

Fig. 46. Prophase of spindle formation of the homotypic mitosis, the seven split chromosomes distributed throughout the nuclear cavity surrounded by a web of fibrillae.

Figs. 47 and 48. Multipolar stages in the formation of the spindle, the split chromosomes being gathered at the equatorial plate.

Fig. 49. Metaphase of the homotypic mitosis, split chromosomes at the equatorial plate.

Fig. 50. The separation of the halves of the split chromosomes.

Fig. 51. Anaphase of the homotypic mitosis, showing certain irregularities in the forms of the chromosomes.

Fig. 52. Anaphase, the two groups of chromosomes, seven in each.

Fig. 53. Late anaphase of the homotypic mitosis, the upper group of seven chromosomes viewed from the pole of the spindle.

Fig. 54. Early telophase following the homotypic mitosis, the chromosomes becoming elongated and irregular in outline.

Fig. 55. Pollen mother-cell shortly before the formation of the pollen tetrad, chromosomes of irregular form connected by a very open reticulum.

Fig. 56. Nucleus of young pollen-grain, chromatic bodies present in an open reticulum.

DAVIS — POLLEN DEVELOPMENT OF OENOTHERA GRANDIFLORA

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Davis, Bradley M. 1909. "Cytological studies on Oenothera. I. Pollen development of Oenothera grandiflora." *Annals of botany* 23, 551–571.
<https://doi.org/10.1093/oxfordjournals.aob.a089238>.

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