Cytological Studies on *Oenothera*. II.

The Reduction Divisions of *Oenothera biennis*.¹

BY

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With Plates LII and LIII.

THE following account of the reduction divisions of *Oenothera biennis*, L., is presented as a part of the writer's plan to study certain characteristic native species of *Oenothera* as a basis for comparison with *O. Lamarckiana* and some of its derivatives, and as preliminary to the investigation of certain hybrids which may readily be obtained. The first paper of this series, 'Pollen Development of *Oenothera grandiflora*' (Davis '09), gives an account of the reduction divisions in the pollen mother-cells of one of the larger American species, which exhibits some interesting peculiarities that are more easily understood after comparison with the conditions in *O. biennis*. Both of these American wild species give opportunity for a comparison of the reduction processes with those of *O. Lamarckiana* and *O. rubrinervis* studied by Geerts and Gates.

As is well known, *Oenothera biennis* is a species which exhibits a considerable range of variation in the size of its flowers, character of its foliage, and habit of growth, so that it is frequently possible to distinguish forms from different regions of America, and it is probable that races or strains may be readily differentiated by pure cultures. The fact that the flowers are self-pollinated before the opening of the bud would undoubtedly greatly assist in holding any differentiated races reasonably true to their types. The strain which furnished the material for this investigation was derived from a rosette transplanted from waste ground at Woods Hole to the author's garden, in 1908. The rosette was selected with some care as an interesting type to hybridize with *O. grandiflora* with certain ends in view. The cross was made during that season and some of the hybrids of the first generation have been briefly described in a paper, 'Notes on the Behavior of Certain Hybrids of *Oenothera* in the First Generation' (Amer. Nat., vol. xliv, p. 108, 1910).

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Should certain of these hybrids or others of later seasons prove of sufficient interest in the second generations this Woods Hole strain of *biennis*, which was constant in cultures of 1909, will be described in some detail. For present purposes, however, it will be sufficient to state that the strain is representative of the green-stemmed (lacking red dots), broader-leaved types of *biennis* with medium-sized flowers. The main stem grows to a height of about 1 m., and long side branches arise from near the base of the plant. The leaves on the lower portions of the stem are elliptical, the larger about 14 cm. long and 4 cm. broad. The inflorescence is characteristic of *biennis* in having bracts about $\frac{1}{3}$ as long as the flowers. The petals are about 1.3 cm. long.

METHODS.

Considerable attention was given during the summer of 1909 to the problems of fixation, especially to test the effects of Carnoy's alcohol-acetic and chloroform-alcohol-acetic mixtures which Overton ('09) found so satisfactory for certain types. The chloroform-alcohol-acetic mixture caused such shrinkage, even when used for so short a time as ten minutes, that it was worthless for the stages of synapsis and later. The alcohol-acetic mixture was less violent in its action, but nevertheless proved for the same reason to be unsatisfactory for older cells where there is a large amount of cell-sap in cytoplasmic vacuoles. These two fluids of Carnoy have, however, a value for *Oenothera* in the study of presynaptic stages, where the cells are relatively small and the cytoplasm dense, because of the sharp staining quality of the chromatin with haematoxylin.

The most satisfactory fixation was obtained with chrom-osmo-acetic fluids when due precautions were taken to facilitate rapid penetration. To attain this necessary end the anthers, freed from floral envelopes but still attached in a group to the ovary, should be thoroughly brushed with water before immersion in the fixing fluid. Satisfactory results were also obtained when such groups of anthers were dipped for a few seconds in Carnoy's alcohol-acetic mixture before immersion in the chrom-osmo-acetic acid; in this procedure the former fluid appears to prepare the surface of the anthers so that the latter fluid is able to penetrate more quickly. Experience has shown that the formula known as strong Flemming (1 per cent. chromic acid 75 c.c., 2 per cent. osmic acid 20 c.c., glacial acetic acid 5 c.c.) gives somewhat better results than the weaker fluids used in the study of *O. grandiflora* (Davis '09, p. 552). There is another advantage in employing rather strong fluids since material appears to cut better after their use, perhaps because the acids may free the tissue from raphides.

As in the previous study of *Oenothera grandiflora* iron-alum haematoxylin proved to be the most satisfactory stain.

THE REDUCTION DIVISIONS IN THE POLLEN MOTHER-CELL.

It seems best for the sake of clearness to describe and figure separately the events of reduction in the anther and in the ovule. Although, as would be expected, the cytological features of the processes are essentially the same for both organs, there would necessarily be some confusion in an attempt to present and describe two groups of figures side by side in parallel series. The account of the reduction divisions in the ovule will be made very brief, but the series of stages will be presented in some fullness for comparison with the figures that illustrate the events within the pollen mother-cell.

Presynapsis. The structure of the resting nucleus of the pollen mother-cell following the last mitosis in the archesporium of the anther is shown in Plate LII, Fig. 1. As in the case of *Oenothera grandiflora* (Davis '09) there is present besides the large nucleolus a number of deeply staining bodies generally distributed around the periphery of the nucleus, and connected with one another by very delicate strands. The nature and significance of these bodies are of importance in relation to current views that chromosomes are represented in the resting nuclei by chromatic centres or prochromosomes. The bodies as observed in *O. biennis* stain deeply with haematoxylin, and hold their colour tenaciously after the manner of chromatin. They may frequently be counted as fourteen, which is the number of the sporophytic (somatic) chromosomes, or in numbers somewhat under fourteen. Counts of deeply staining bodies in excess of fourteen may also be made in the nuclei, but small granules possibly nucleolar in character are practically indistinguishable from these bodies in the nuclei of *Oenothera*. The writer is inclined to believe that these chromatic bodies are the prochromosomes of Overton ('05, '09), but it should be noted that there is no evidence that they are arranged side by side in pairs on a system of threads that might be interpreted as representing two parallel spiremes. The chromatic bodies of *O. biennis* are distributed irregularly throughout the nuclear cavity, but wherever two lie close together (Figs. 1, 2) the arrangement appears to be end to end upon a delicate strand that runs in the direction of their longer axes. This is possibly a point of significance since the chromosomes differentiated after synapsis are clearly segments of a single spireme and are consequently joined end to end.

The nuclei remain for a long time in the resting condition described above. With the approach of synapsis the delicate strands connecting the chromatic bodies thicken and become much more numerous, forming a clearly defined network. Stages in this process are indicated in Fig. 3, showing a nucleus in a cell adjacent to that illustrated in Fig. 2, and also in Fig. 4, which shows two nuclei separated from one another by a single cell. The nucleus finally becomes quite filled with a dense reticulum (Fig. 5), at

which stage the chromatic bodies can be distinguished only with difficulty, if at all, since they lie in the meshes of the deeply staining strands of the network. A single large nucleolus is present and frequently one or more smaller nucleoli. It is important to note that this linin network develops as a reticulum from its earliest inception; there is at this time no indication of a continuous spireme, much less two parallel spiremes such as have recently been described for a number of forms by Overton ('09), Lundegardh ('09), and Rosenberg ('09 a).

The fate of the chromatic bodies in *Oenothera* as the nucleus approaches synapsis is a problem of great interest, but at present all that can be said is that their substance becomes apparently merged with that of the reticulum, from which they cannot finally be differentiated. It is, however, possible that in this reticulum there are regions, corresponding to the chromatic bodies, which are quite distinct from the linin substance of the network that in earlier stages (Figs. 1-4) is easily distinguished from the chromatic bodies. Such a chromatic framework, if present, may be responsible for the spireme which appears during synapsis.

Synapsis. The approach of synapsis is indicated by the contraction of the reticulum away from the nuclear membrane (Fig. 6), and this contraction proceeds until almost all of the threads and meshes in the network are drawn into a close mass (Fig. 7) that lies generally near the large nucleolus. While the contraction is in progress the reticulum takes on the character of a tangle or ball of threads, constituting the synaptic knot, from which a few loops and very delicate strands usually extend into the nuclear cavity (Figs. 7-10). It is impossible to follow the individual threads in the denser portions of the knot, but the free loops at the edge give an opportunity for more detailed study.

Although it is frequently possible to find threads that run closely parallel to one another for some distance the writer is unable to present any evidence that this condition is other than such a close association as would be brought about by the gathering together of a complexly looped thread or system of threads into a tight mass. The loops appear to be quite independent, as though they were parts of a much tangled system rather than portions of a split spireme, or of two independent systems of threads (maternal and paternal spiremes), which might be assumed to be associated with one another side by side. It is, however, impossible to say whether or not the coiled and twisted loops of the synaptic knot are parts of a single continuous thread; if so, it must be a thread of great length.

The seriation of such a group of stages, as is shown in Figs. 7-10, is rendered somewhat easier by the fact that the pollen mother-cells separate from one another during the process of synapsis, each one rounding off and coming to lie freely in the pollen chamber. Fig. 7 is undoubtedly the earliest stage of this group of figures, because the pollen mother-cells were still

angular and united, while Figs. 8–10 are from sections in which the cells had rounded off and separated.

As synapsis proceeds it becomes very clear that the threads thicken (compare Figs. 9 and 10 with Figs. 7 and 8), and there is good evidence that the system becomes shorter. Thus the threads are very much more prominent in the later stages of synapsis (Figs. 8–10) than at the beginning of the contraction (Figs. 6 and 7), and in the later stages the structure begins to take on the appearance of a much-looped and coiled spireme. This history of the differentiation of the threads during synapsis is the same as that for *O. grandiflora* (Davis '09).

It will be noted that the term synapsis is reserved for this period of the first contraction of the chromatic material. A type of contraction that frequently takes place much later, during the formation of the chromosomes, will be described in that connexion.

The Formation of the Chromosomes. The period of synapsis, described above, is of relatively long duration, as is shown by the fact that in an inflorescence one to three buds may be found in which all, or almost all, of the nuclei in the pollen mother-cells are in this stage. The succeeding developments, leading to the development of the thick spireme from which the chromosomes are formed, take place more quickly. The chromatic material emerges from the contracted condition of the synaptic knot by a loosening of the coils, with the result that a thickened and very much shorter thread appears, which is evidently a spireme. Such a stage is shown in Fig. 11, which is of especial interest in comparison with Fig. 10, since the two nuclei were from opposite ends of the same pollen chamber. The spireme even in this thickened and much shortened condition (Figs. 11–13) is generally complexly looped and coiled, but the greater part of the thread system can be traced generally without great difficulty, which is impossible in the stages of synapsis.

Shortly after the emergence of the thickened spireme from the synaptic knot there appear indications of a process of segmentation that is to transform the spireme into a chain of fourteen chromosomes. Early stages of this process are shown in Figs. 12 and 13, where the segments are present as long, variously bent rods with thin regions between them, as though separating by constriction. The contraction which has been going on in the spireme continues in these segments until their long diameter is not more than two or three times their width, and by this process of condensation the spireme becomes so much shortened that the complexities of the looped arrangement largely disappear, and it is frequently possible to follow the chain of fourteen chromosome segments from end to end as a single segmented spireme (Fig. 14). Later the spireme may become broken into groups of chromosome segments (Figs. 15 and 16), each group showing the segments arranged end to end; finally during the prophase of the heterotypic

mitosis the segments as fully developed chromosomes separate from one another.

During the process of condensation and shortening of the chromosome segments in the spireme there is frequently found near the nucleolus a massing of the chromatic material (Figs. 17 and 18) which bears a superficial resemblance to the synaptic knot (Figs. 8-10). This is apparently the condition termed the 'second contraction' and considered by some authors as of considerable significance, since it is supposed to bring the chromosomes into close association as pairs in preparation for the stage of diakinesis. The material, however, offered little support to such an explanation, because the chromosomes exhibit only slight tendencies to group in pairs, and it is doubtful if the stage itself is a regular occurrence. It is hard to see for the species under consideration any significance in this 'second contraction' further than an assemblage of the chromosome segments of a spireme so complexly looped that its parts are necessarily drawn close together during the condensation which transforms the fourteen segments into chromosomes.

It is interesting to note the differences in the formation of the chromosomes between this material of *Oenothera biennis* and that of *O. grandiflora*. The spireme of *grandiflora* emerges from the synaptic knot as a much more complexly coiled thread (Davis '09, Figs. 16-18) than that of *biennis*, and the subsequent condensation and shortening of the spireme draw the chromatic material into a closely contracted mass, from which several thick loops extend into the nuclear cavity (Davis '09, Figs. 19-23). These latter conditions might be considered representative stages of a 'second contraction', but in *grandiflora* the changes of structure are so gradual and the variation of form is so great that it is very difficult to distinguish such a phase. Finally the thickened and contracted spireme of *O. grandiflora* breaks up into seven rings, some of the loops being transformed directly into these structures. The later history shows that each ring is bivalent in character, since it separates into semicircular halves (sporophytic chromosomes) during the heterotypic mitosis. There are then fourteen sporophytic chromosomes as in *biennis*, and these are likewise segments of a spireme arranged end to end, but the involved coils of the spireme bring the segments together in loops in such a manner that the fourteen chromosomes become grouped in pairs to form seven rings (bivalent chromosomes). It would be hasty to conclude that the close association of the chromosomes in pairs, characteristic of the material of *grandiflora* studied by the writer, involved any greater affinity between the segments of the spireme than in *biennis*. The striking differences between the two species in the final grouping of the chromosomes are perhaps simply due to the peculiarity of a more complicated looped spireme in the case of *grandiflora*. Furthermore, it will not be surprising if the study of

other strains of the variable *O. grandiflora* showed departures from the conditions in the material described by the writer, and that following synapsis types of chromosome organization will be found which resemble more closely those of *O. biennis*.

The important point to emphasize in this connexion is that in both *grandiflora* and *biennis* the chromosomes are organized from segments of a spireme arranged end to end, and that the history of the reduction divisions in this respect is the same as that described by Gates ('08) for *rubrinervis* and Geerts ('09) for *Lamarckiana*.

The Heterotypic Mitosis. The condensation of the chromatin, which takes place during the organization of the spireme and formation of the chromosomes, continues throughout the prophases of the first, or heterotypic, division. As a result the chromosomes at metaphase of this mitosis (Figs. 22 and 23) are very much smaller structures than the segments of the spireme (Figs. 13 and 14) from which they are derived. The mature chromosomes are essentially similar, generally with the form of thickened V's due to the bending of the spireme segments, and are not rounded structures such as have been described and figured by Gates and Geerts.

The process of spindle formation is the same as that of *grandiflora*, and similar to that described for a number of higher plants (e. g. *Equisetum*, *Larix*, *Lilium*, &c.). Fibrillae enter the nuclear cavity with the breaking down of the nuclear membrane, and also push out into the cytoplasm in various directions and thus establish a multipolar spindle (Figs. 19 and 20); the large nucleolus disappears at an early stage in the process. The group of chromosomes is brought by the development of the fibres to the centre of the spindle where the chromosomes lie in irregular clusters, in which, however, some may frequently be found still united end to end in short chains of two to five chromosomes.

The multipolar condition of prophase gradually changes to a typical bipolar spindle characteristic of metaphase (Figs. 21-23) by a rearrangement and gathering of the spindle fibres into two broad sheaves which end in granular areas that merge with the alveolar cytoplasm. During this development the chromosomes separate from one another and usually take on the form of thickened V's by the bending of the ends and the thickening of the chromatin in the middle regions. The arrangement of the chromosomes as they are brought to the equatorial plate during metaphase is quite irregular. Although occasional pairs are found sufficiently closely associated to indicate that they were adjacent segments of the spireme, most of the chromosomes are so separated and scattered (Figs. 21 and 22) that it is impossible to determine with certainty what was their relation to one another in the spireme.

In this respect these conditions in *biennis* are in sharp contrast to those in the material of *grandiflora*, studied by the writer, where the arrangement

of the chromosomes at the equatorial plate was very symmetrical because they remained joined in pairs in the form of seven rings throughout the pro-phases of the heterotypic mitosis (Davis '09, Figs. 31-36). The separated and scattered arrangement of the chromosomes in *biennis* allows far greater opportunity for numerical irregularities in their distribution during the heterotypic mitosis than is possible when they are closely associated in pairs.

As the two sets of chromosomes (seven in each set) move away from one another towards the poles of the spindle the V-shaped form is especially evident (Fig. 23), but during anaphase of the mitosis their structure becomes complicated by a lengthwise fission of each chromosome in the plane of the page upon which the above letter (V) is printed. The seven chromosomes which leave the equatorial plate for each pole of the heterotypic spindle arrive as seven split chromosomes (Fig. 24), this division being a premature fission of each chromosome in preparation for the second, or homotypic, mitosis. The group of seven split chromosomes is best observed in polar views of late anaphase (Fig. 25), and at the time of the reconstruction of the daughter nuclei (Fig. 26).

During the examination of a large amount of material in the stages described above (Figs. 25 and 26) two cases were observed that showed numerical irregularities in the distribution of the chromosomes by the heterotypic mitosis. In both examples eight split chromosomes were found at the poles of the heterotypic spindle where the normal number is seven. Fig. 27 illustrates this exceptional condition, and in this instance five chromosomes were present at the opposite pole of the spindle; six would be the number expected, and it is quite probable that a chromosome had been carried away from the group by the microtome knife. The rarity of these exceptions would make it very difficult to determine whether or not such irregularities might continue throughout the homotypic mitosis and give rise to functional pollen grains with smaller and larger chromosome content than the normal.

The history of the seven split chromosomes is readily followed through the interkinesis between the heterotypic and homotypic mitoses. After the daughter nuclei are organized the halves of each chromosome separate somewhat (Fig. 28), and the ends swing apart until the halves lie in approximately the same plane and resemble somewhat two U's joined together at the middle region (Figs. 29 and 30). It then becomes evident that there are seven pairs of chromosomes in each nucleus, a condition which is, as a rule, most clearly shown in polar views of the structure. The chromosomes increase markedly in size, and remain in this expanded condition throughout the period of interkinesis. The free ends of the chromosomes sometimes exhibit a tendency to branch and become united to form a loose and imperfect network, but generally most of the chromosome pairs of

a nucleus are as clearly defined as in Figs. 29 and 30. The daughter nuclei remain for some time in this resting condition with the seven pairs of chromosomes distributed around the periphery and with one or more nucleoli present. It is clear that the chromosomes of *biennis* maintain their individuality throughout the interkinesis, and their behaviour in this respect is in complete accord with the history during the same phase in *grandiflora* (Davis), *Lamarckiana* (Geerts), and *rubrinervis* (Gates).

The chief conclusions from this study of the heterotypic mitosis of *Oenothera biennis* are (1) that fourteen V-shaped sporophytic (somatic) chromosomes are distributed in two groups of seven each, so that the mitosis is a reduction division; (2) that the chromosomes are assembled irregularly at the equatorial plate, and there is no uniform grouping of the structures in pairs, although occasional pairs may be found; (3) that the chromosomes are essentially similar to one another in form and size; and (4) that during anaphase there is a fission of the chromosomes, so that seven split chromosomes enter each daughter nucleus and are evident as seven pairs during the period of interkinesis.

The Homotypic Mitosis. The approach of the homotypic mitosis is indicated, as in the heterotypic division, by the appearance of a delicate web of fibrillae around the resting nucleus of the interkinesis (Fig. 30). With the breaking down of the membrane the fibrillae enter the nuclear cavity (Pl. LIII, Fig. 54, prophase of homotypic mitosis in the ovule) and establish a multipolar spindle which quickly changes into a bipolar structure. The seven pairs of chromosomes are carried towards the centre of the developing spindle (Figs. 31 and 32), where they form an irregular cluster. During these changes the chromosomes by condensation become much smaller than in the expanded condition of the interkinesis, and by the time the homotypic spindle is fully developed (Pl. LIII, Fig. 33) they have returned to about the same size as when they entered the period of interkinesis. The pairs of chromosomes are arranged quite regularly on the equatorial plate of the homotypic spindle, so that as a result the members of each pair are separated by the mitosis. The two spindles may lie side by side or at right angles to one another (Fig. 33).

The form of the chromosomes at metaphase of the homotypic mitosis is that of short and sometimes slightly bent rods, but during anaphase (Fig. 34) one or both ends generally enlarge somewhat, so that the outline becomes irregular. The irregularities of form grow more pronounced after the organization of the daughter nuclei (Fig. 35), in which a chromatic network is finally formed by the elongation, branching, and anastomosis of the chromosomes. The nucleus of the young pollen grain thus passes into a typical resting condition (Fig. 36) possessing one or more nucleoli and a delicate open reticulum in which lie thickened deeply staining regions. The latter are frequently so definite in outline that it seems probable they

are chromatin centres or prochromosomes, but their form is exceedingly variable, and one cannot make constant counts.

The study of the nuclei in older pollen grains and of the mitosis which differentiates the generative from the tube nucleus presents certain technical difficulties that make the investigation of this phase of the gametophyte somewhat difficult, and the writer has not yet taken it up in detail.

The history of the homotypic mitosis in *Oenothera biennis* is identical in all essentials with that of *grandiflora*, *Lamarckiana*, and *rubrinervis*. The mitosis is clearly an equational division distributing the halves of chromosomes whose premature division takes place during the anaphase of the heterotypic mitosis, the halves remaining associated as seven pairs throughout the period of interkinesis.

THE REDUCTION DIVISIONS IN THE OVULE.

In some respects the ovule furnishes better material than the anther for studies on the reduction divisions, since when the outer walls of the ovaries are carefully cut away it is frequently possible to obtain a better and more uniform fixation than in the case of the anther. It is, however, at times far more difficult to seriate stages. The study of Geerts ('09) on the ovule of *Oenothera Lamarckiana* gives an opportunity for some comparison of that form with *biennis*.

The reduction divisions in the ovule of *Oenothera* occur just previous to the differentiation of the embryo sac which arises from one of a group of four cells homologous with a pollen tetrad. The embryo sac therefore develops from the homologue of a megaspore and not from the homologue of a megaspore mother-cell as in many types (e. g. *Lilium*, &c.). The four cells of the group (tetrad) are arranged in a line and arise, through a heterotypic mitosis followed by two homotypic divisions, from a single large megaspore mother-cell which is differentiated in the nucellus of the very young ovule at the time of the development of the integuments. Geerts ('09) reports for *Lamarckiana* that the embryo sac always develops from the upper cell of the row of four (tetrad), the other three cells breaking down. This is not true for the present form of *biennis*, in which the embryo sac appears to develop rather more frequently from the lowest cell of the row (i. e. farthest away from the micropylar end), although often arising from the upper cell.

Geerts ('09, Pl. XXII) has pointed out in tabular form that the reduction divisions in the ovule of *Lamarckiana* take place at a stage of the flower's development long after the homologous mitoses in the pollen mother-cell, and the same history is true of *biennis*. Indeed, the pollen tetrads are fully formed before the rudiments of the ovules have begun to develop their integuments.

We shall now outline briefly, and for the sake of comparison with the more detailed account of the pollen mother-cell, the history of synapsis and of the reduction divisions in the ovule.

Presynapsis. The resting nuclei of the megaspore mother-cells (Fig. 37) show the presence of chromatic bodies similar to those described for the nucleus of the pollen mother-cell (Figs. 1-4), but here also it is frequently possible to make counts above and below the number fourteen, irregularities for which various explanations might be suggested. The writer is, however, inclined to believe that certain of these structures are prochromosomes. The chromatic bodies are distributed upon a very delicate linin reticulum, and wherever two lie close together (Fig. 37) their arrangement appears to be end to end upon a strand of the network.

On the approach of synapsis the delicate reticulum is gradually replaced by a dense network of deeply staining threads (Figs. 38 and 39) which fill the nucleus (compare with Figs. 4 and 5), and at this stage the chromatic bodies cannot be distinguished with certainty.

Synapsis. A contraction of the dense reticulum (Fig. 40) marks the advent of synapsis when the meshes of the network become drawn together into a close mass, the synaptic knot (Fig. 41), generally near to the nucleolus. After remaining for a considerable time in this contracted condition the synaptic knot appears to loosen, when it is evident that the threads of the system have become much thicker (Figs. 42 and 43) and begin to resemble a much looped spireme. This history of synapsis is illustrated for the nucleus of the pollen mother-cell by Figs. 6-10.

The Formation of the Chromosomes. Following the loosening of the coils of the synaptic knot there is a rapid development of an evident spireme by the contraction of the thickened threads. The spireme (Fig. 44) is very much shorter and thicker than the system of threads that emerges from synapsis, and is not so complexly looped. A segmentation of the spireme then transforms this structure into a chain of fourteen chromosomes (Figs. 45-47, compare with Figs. 12-17). Occasionally the segmented spireme or the group of chromosome segments derived from the spireme is found so contracted (Fig. 48, compare with Fig. 18) as to suggest conditions of synapsis, but this stage, which some authors would term a 'second contraction', does not appear to be uniformly present, and is not, in the opinion of the writer, of especial significance.

The Heterotypic Mitosis. The fourteen chromosomes, which result from the segmentation of the spireme, appear at the equatorial plate of the heterotypic spindle in the form of rod-shaped structures (Fig. 49), some of which even at this late stage of mitosis are frequently found still arranged end to end in short chains. By continued condensation the chromosomes are further shortened, and later take on the form of V's, a condition which is most evident at metaphase (Fig. 50), and also when the two sets of

chromosomes, seven in each group, separate to pass to the respective poles of the spindle (Fig. 51). The chromosomes are not closely or uniformly associated in pairs either during their organization or in their arrangement at the equatorial plate.

The chromosomes divide lengthwise as they pass from the equatorial plate to the poles of the heterotypic spindle, so that each daughter nucleus following the heterotypic mitosis receives seven split chromosomes (Fig. 52). The seven pairs of chromosomes maintain their individuality throughout the period of interkinesis (Fig. 53), during which they increase in size.

Figs. 49–53, outlining the history of the heterotypic mitosis and the period of interkinesis in the ovule, may be compared with Figs. 19–30, which illustrate in greater detail the same events in the pollen mother-cell.

The Homotypic Mitosis. The seven pairs of chromosomes present during the interkinesis become by condensation much smaller during the prophase of the homotypic mitosis (Fig. 54), and have the form of short rods by the time that they are arranged on the equatorial plate (Fig. 55, compare with Fig. 33). The members of each pair are distributed by this mitosis into two sets, seven chromosomes in each, the chromosomes assuming a more irregular outline during anaphase (Fig. 56, compare with Fig. 34). With the organization of the daughter nuclei and the development of cell plates across the two homotypic spindles, a row of four cells is organized homologous with the pollen tetrad. The nuclei of these cells pass into a resting condition (Fig. 57, compare with Fig. 36), the chromatin forming a reticulum upon which lie chromatic bodies, variable in number, some of which are probably prochromosomes.

The chief stages in the development of the embryo sac were traced, and the main features of Geerts' account were confirmed. There are only two mitoses, and these result in a group of two synergids and an egg nucleus at the micropylar end of the sac with a single polar nucleus below, antipodals therefore being absent. It is very difficult to obtain good fixation of stages in the development of the embryo sac and during fertilization and in the early segmentation of the fertilized egg, and the writer is not yet ready to take up the cytological features of this phase in the life-history of *Oenothera*.

As is well known the anthers of *Oenothera biennis* discharge their pollen in the bud, so that the base of the stigma is well covered when the flower opens. Sections of the present material showed that the pollen tubes reach the embryo sacs even before the opening of the buds. With such conditions it can readily be understood that ordinarily the production of seeds by *Oenothera biennis* through cross pollination is unlikely. Cross pollination of this form in nature would appear to be probable only when its own pollen is insufficient to provide for the number of ovules matured, a possibility that seems remote.

VEGETATIVE MITOSIS IN THE OVULE.

The nucellus of the developing ovule presents a tissue especially favourable for the study of the vegetative mitoses which proved to be in all essentials similar to those described in the anther of *O. grandiflora* (Davis '09). The resting nucleus contains a single large nucleolus, frequently accompanied by one or more smaller nucleoli, and a very delicate reticulum in which lie small deeply staining granules of varying sizes (Fig. 58). Some of the granules are sufficiently large to suggest chromatic bodies or prochromosomes, but the writer, after many attempts to establish such relationships, cannot report satisfactory results. The granules are so variable both as to their size and number that uniform counts or groupings could not be made, yet it seems probable that some of the structures are prochromosomes. It is, however, unfortunate for the prochromosome hypothesis that these structures are so difficult of demonstration in the resting nuclei of vegetative tissues, since, if they really stand for permanent structures (chromosomes) of the cell, it should be possible to follow them with a fair degree of accuracy.

The thickening of threads in the network (Fig. 59) indicates the approach of mitosis. These threads have at first a somewhat beaded appearance due to irregular thickenings or to associations of granules, but later the structure becomes more uniform (Fig. 60). Finally there is developed a much-coiled spireme (Fig. 61), which cannot, however, be traced as a single continuous thread since there are occasional points of union of the thread system.

The spireme breaks up into fourteen chromosome segments which have at first the appearance of long, variously bent rods (Figs. 62 and 63), but which by condensation become much shorter and somewhat thickened. The chromosomes, at metaphase of mitosis, may be readily counted in favourable polar views of the equatorial plate (Fig. 64). The form of the mature chromosome is that of a more or less bent rod.

During anaphase of mitosis the V-shaped daughter chromosomes are gathered into two groups with the points of the V's directed towards the poles of the spindle (Fig. 65). Following the reconstruction of the daughter nuclei (Fig. 66) the chromosomes may for a short time be recognized as bands or threads more numerous on the sides of the nuclei furthest from the cell plate, but their outlines are soon lost in the chromatic reticulum which is shortly developed. The reticulum is at first rather coarse and open, but presently becomes very delicate as the nucleus passes into the resting condition shown in Fig. 58. The large nucleolus which disappears during the mitosis is quickly formed again at an early stage in the reconstruction of the daughter nuclei.

CYTOLOGICAL DISCUSSION.

The foregoing description of the reduction divisions in *Oenothera biennis* agrees with the account of Gates ('08) for *O. rubrinervis*, and Geerts ('09) for *O. Lamarckiana*, in the following chief particulars:—

1. The fourteen sporophytic (somatic) chromosomes that enter the heterotypic mitosis are formed by the segmenting of the spireme which follows synapsis, and are consequently arranged end to end.

2. Although some of these fourteen chromosomes may be grouped in pairs at the equatorial plate of the heterotypic spindle, they are frequently so scattered as to be quite independent of one another. Under these conditions it seems impossible to distinguish two sets of chromosomes (such as might be regarded as of paternal and maternal origin), and there is possible a degree of irregularity in their distribution which is not generally present in the heterotypic mitosis, and was not present in the material of *O. grandiflora* studied by the writer (Davis '09). The heterotypic mitosis is a reduction division.

3. The chromosomes (after the lengthwise splitting in the anaphase of the heterotypic mitosis) may be followed through the interkinesis as seven pairs of chromosomes in each resting nucleus.

4. The members of the seven pairs of chromosomes, present during the interkinesis, are distributed by the homotypic mitosis, which is therefore an equation division.

The periods of the reduction divisions outlined above, that is from the segmentation of the spireme which follows synapsis to the conclusion of the homotypic mitosis, are the least difficult of all the phases to study. Nevertheless this account of *Oenothera biennis* is not in agreement with the descriptions and figures of Gates and Geerts in a number of important particulars. The chief of these differences is in the structure of the chromosomes of the heterotypic mitosis, which in both *biennis* and *grandiflora* have the form of thickened V's and are not subglobular as described and figured by Gates and Geerts. This divergence of results at a very characteristic stage of the reduction processes is but an example of a number of important differences between the writer's accounts and those of the above authors at various phases of the periods under discussion. However, it does not seem best to discuss these different results in detail, since the reader can readily compare our various conclusions as shown by the figures.

The phases of synapsis and presynapsis are very difficult of study, and there are better reasons to expect different accounts and interpretations on the part of investigators than in the stages following the appearance of the spireme as described in the above paragraph. Geerts gives very little information on the periods of synapsis and presynapsis in *Oenothera Lamarckiana*. Gates ('08, Fig. 17) figures for *O. rubrinervis* a close associa-

tion of threads following synapsis, interpreted by him as a splitting of the spireme, which is later closed by the union of the two threads. While it is not difficult in these stages and earlier to find threads that run closely parallel with one another, the structures are so minute, and the coiled arrangements of the threads so intricate, that the writer is not convinced that these relations signify more than such an association as would naturally result from the contraction of a very complicated thread system.

The events of the synaptic contraction and of the presynaptic phases are probably of great significance, but the research so far upon *Oenothera* does not give a satisfactory explanation. All that can be said is, that from a dense reticulum, which develops during the presynaptic stages, there is differentiated a complicated thread system that during the synaptic contraction lies in intricate coils, and that from this thread system, following synapsis, is developed the very much shorter and thicker spireme. It is evident that during synapsis the threads become shorter and thicker, but it is not clear whether or not the synaptic contraction is merely the result of this process or has some deeper significance. Although the threads are reasonably distinct during synapsis, their relation to one another is obscured by the presence of substances either entangled in the thread system or forming with the threads a reticulum.

It seems to be established for *Oenothera* that the chromosomes of the heterotypic mitosis are not formed side by side through the parallel association of two spiremes, but are developed from a single spireme, which by segmentation forms a chain of fourteen chromosomes arranged end to end. This fact makes it unnecessary to assume that the synaptic contraction has for its purpose the bringing together of two distinct spiremes (of maternal and paternal origin), and it is at least possible that the peculiarities of synapsis may have no deeper significance than the contraction and condensation of the chromatin on a very long and complicated system of threads. It is not easy to understand how the chromosomes of the chain could be arranged with such precision that they are alternately of maternal and paternal origin, which would be the logical arrangement if it be assumed that the chromosomes are distributed to give pure germ cells. The ring-shaped pairs of chromosomes characteristic of the heterotypic mitosis of *Oenothera grandiflora* evidently result from the close association of adjacent segments in the loops of the spireme, and similar pairs when present in other species of *Oenothera* have probably the same method of origin.

It is clear that the evidence from this study, in agreement with the conclusions of Gates and Geerts, supports the theory of the end-to-end arrangement, following synapsis, of the full set of sporophytic (somatic) chromosomes through the segmentation of a single spireme, as held by Farmer and Moore ('05), Mottier ('07, '09), Strasburger ('04), and their followers. The fact that many of the chromosomes in *Oenothera biennis*,

rubrinervis, and *Lamarckiana* are not closely associated in pairs does not affect the main point, which is that they are arranged end to end in a single spireme during the prophases of the heterotypic mitosis. The very clear association of the chromosomes in pairs shown in the material of *O. grandiflora* studied by the writer is explained by the more complicated looped structure of the spireme in this form, which brings adjacent chromosome segments into such intimate relations that they remain together as ring-shaped pairs (bivalent chromosomes). The writer can see no possibility of explaining the reduction phenomena of *Oenothera* by the theory of a side-by-side pairing of chromosomes through the parallel association of two spiremes as held by Grégoire ('04, '07), Allen ('05), Rosenberg ('05, '09 a), Overton ('05, '09), and others.

With respect to the attractive theory of Rosenberg ('04, '05) and Overton ('05, '09) that chromosomes are represented in the resting nuclei by chromatic centres or prochromosomes, this study offers only indirect evidence. Chromatic bodies may be readily distinguished in resting nuclei, and it seems probable that some of these are prochromosomes, but the counts are too variable to establish their identity with the chromosomes. Furthermore, the chromatic bodies could not be traced to the chromosomes differentiated in the prophases of mitosis since their outlines became lost in the chromatic network which precedes the differentiation of the spireme. The fact that the chromosomes may be followed through the interkinesis between the heterotypic and homotypic mitoses offers strong support to the theory of the individuality of the chromosomes, but it must be borne in mind that these two mitoses (heterotypic and homotypic) are closely bound together as parts of a common process (that of chromosome reduction), the phylogenetic relations of which to the events of vegetative mitosis are not understood. The writer hoped that material fixed in the fluids of Carnoy, as employed by Overton, might give better results for the study of the chromatic bodies in resting nuclei and in the early stages of mitosis and synapsis, but such material proved to be no more favourable than that killed in Flemming's fluids.

SUMMARY.

1. The resting nuclei of the pollen mother-cell and megaspore mother-cell contain chromatic bodies (variable as to number), some of which are probably chromosome centres, or prochromosomes. These structures lie in a very delicate reticulum accompanied by one or more nucleoli.

2. Shortly before synapsis the nucleus becomes filled with a close reticulum, and the chromatic bodies become lost in the deeply staining strands of this dense network.

3. The advent of synapsis is marked by a slow contraction of the reticulum away from the nuclear membrane, carrying most of the strands

towards the centre of the nucleus. During the process of contraction numerous threads are differentiated from the reticulum and lie in complicated coils. Although it is frequently possible to find threads that run closely parallel with one another, the writer is unable to present any evidence that this condition is other than such an association as would be brought about by the gathering together of a complexly looped thread, or system of threads, in a tight mass.

4. The synaptic contraction draws the coils of threads into a dense knot close to the nucleolus, which generally lies at one side of the nucleus. A few loops and delicate strands 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 becomes materially shortened.

5. The chromatic material emerges from synapsis by a loosening of the coils of the contracted thread system, when it becomes evident that the threads are very much shorter and thicker, although still complexly looped and coiled. From this condition is developed by further contraction a very much thicker thread which becomes the spireme.

6. The spireme by segmentation gives rise to the fourteen sporophytic (somatic) chromosomes, which are therefore arranged end to end. These chromosome segments continue the process of contraction, which has been going on in the spireme, until their long diameter is not more than two or three times their width, and the spireme becomes so much shortened that the complexities of the looped arrangement largely disappear, and it is possible to follow the chain of fourteen chromosome segments for the entire length of the spireme.

7. The condensation and shortening of the spireme frequently draws the chromosome segments into a close group, giving the condition described as a 'second contraction'. It is, however, doubtful if this stage is a regular occurrence or of especial significance.

8. The chromosome segments of the spireme break apart during the prophase of the heterotypic mitosis, and by further contraction and bending in the middle region become the fourteen V-shaped chromosomes characteristic of this mitosis. The chromosomes are all essentially similar to one another.

9. Although not infrequently found in pairs the chromosomes have generally a scattered distribution at the equatorial plate, which may give an opportunity for numerical irregularities in their distribution by the heterotypic mitosis.

10. The fourteen sporophytic (somatic) chromosomes are distributed as two sets by the heterotypic mitosis, which is therefore a reduction division.

11. A lengthwise fission of each chromosome, in preparation for the homotypic division, becomes apparent during the anaphase of the hetero-

typic division, so that seven split chromosomes enter the resting nucleus of the interkinesis between these two mitoses.

12. The seven split chromosomes may be readily followed as seven pairs throughout the period of interkinesis.

13. The homotypic mitosis is an equational division distributing the members of each pair of chromosomes, seven to each daughter nucleus.

14. The reduction divisions in the ovule result in a row of four cells homologous with the pollen tetrad. The embryo sac appears to develop rather more frequently from the lowest cell of this row (i. e. the cell farthest away from the micropylar end), although often arising from the upper cell.

15. The vegetative mitoses in the nucellus of the ovule are in all essentials similar to those described for the anther of *Oenothera grandiflora* (Davis '09).

CAMBRIDGE, MASSACHUSETTS,
April, 1910.

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EXPLANATION OF FIGURES IN PLATES LII AND LIII.

Illustrating Prof. Davis's paper on the reduction divisions of *Oenothera biennis*.

All figures were sketched with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 mm. (num. aper. 1.30) in combination with the compensating ocular No. 12, giving a magnification of 2,000 diameters. Figure 1 from section 4 μ thick; Figures 2-11, 24, 25, 27-36, and 42, from sections 5 μ thick; 12-23, 26, 37-41, and 43-66, from sections 6 μ thick. Sections stained with iron-alum haematoxylin.

PLATE LII.

Oenothera biennis.

Figs. 1-32. Illustrating reduction phenomena in the pollen mother-cell.

Fig. 1. Resting nucleus in pollen mother-cell, showing a large and a small nucleolus, chromatic bodies, and delicate reticulum.

Fig. 2. Similar to Fig. 1, but with a denser reticulum.

Fig. 3. Nucleus neighbouring to that shown in Fig. 2, the strands of the reticulum becoming thicker.

Fig. 4. Two nuclei separated by a single cell: *a*, with a delicate reticulum; *b*, with a much coarser network.

Fig. 5. Nucleus filled with a dense reticulum shortly before the advent of synapsis, chromatic bodies no longer distinguishable.

Fig. 6. The beginning of the synaptic contraction.

Fig. 7. Synaptic contraction well under way, the threads and meshes of the reticulum drawn into a close mass.

Fig. 8. A closely contracted synaptic knot.

Fig. 9. A synaptic knot with loops and threads extending into the nuclear cavity.

Figs. 10 and 11. Nuclei from opposite ends of the same pollen chamber. Fig. 10, from lower end, the threads beginning to shorten and thicken to form the spireme. Fig. 11, from upper end, the spireme which emerges from the synaptic contraction following the shortening and thickening of the thread system.

Fig. 12. A spireme with constrictions which show that the process of segmentation has begun.

Fig. 13. A segmented spireme.

Fig. 14. The segments of the spireme by condensation of the chromatin have become much shorter and thicker.

Figs. 15 and 16. The segments of the spireme are more nearly the size of the chromosomes present at metaphase of the heterotypic mitosis (compare with Figs. 20-23); their end-to-end arrangement is still clearly evident.

Figs. 17 and 18. The chromosome segments in about the same stage as shown in Figs. 15 and 16, but gathered in a close mass which bears a superficial resemblance to a synaptic knot. This is apparently the stage termed by some authors a 'second contraction'.

Fig. 19. Prophase of the heterotypic mitosis. Most of the fourteen chromosomes in the centre of the multipolar spindle are united end to end, nucleolus no longer present.

Fig. 20. Spindle taking on a bipolar form, many of the chromosomes still arranged end to end.

Fig. 21. Bipolar spindle, the fourteen chromosomes gathered in an irregular group, certain of them apparently associated in pairs.

Fig. 22. Metaphase of the heterotypic mitosis. The chromosomes, now bent in the form of thickened V's, show no very close association in pairs.

Fig. 23. Anaphase of the heterotypic mitosis. Seven chromosomes are in each of the two sets which pass to the poles of the spindle.

Fig. 24. Telophase of the heterotypic mitosis. The seven chromosomes at each pole of the spindle are split lengthwise.

Fig. 25. A group of seven split chromosomes gathered at the pole of the heterotypic spindle, viewed from above.

Fig. 26. A group of seven split chromosomes just before the organization of the daughter nuclei following the heterotypic mitosis.

Fig. 27. An irregular distribution of chromosomes by the heterotypic mitosis. Eight chromosomes instead of seven have been brought to the pole of the spindle; five chromosomes (one missing) were found at the opposite pole.

Figs. 28 and 29. Resting nuclei of the interkinesis between the heterotypic and homotypic mitoses. Seven pairs of chromosomes are present in each nucleus, mostly in the form of U's joined together in the bent middle region.

Fig. 30. Nucleus of the interkinesis surrounded by a web of fibrillae preparatory to the organization of the homotypic spindle.

Figs. 31 and 32. The seven pairs of chromosomes being gathered at the equatorial plate shortly before the metaphase of the homotypic mitosis.

PLATE LIII.

Oenothera biennis.

Figs. 33-36. Illustrating reduction phenomena in the pollen mother-cell.

Fig. 33. Metaphase of the homotypic mitosis; seven pairs of chromosomes at the equatorial plate. The two spindles lie at right angles in the pollen mother-cell, so that the equatorial plate of one spindle is viewed from the pole.

Fig. 34. Anaphase of the homotypic mitosis; the chromosomes of the two sets, seven in each group, show irregularities of form.

Fig. 35. Telophase of the homotypic mitosis; the seven chromosomes in each nucleus still distinct.

Fig. 36. Nucleus of a young pollen grain, showing the open reticulum upon which lie deeply staining chromatic bodies (prochromosomes?).

Figs. 37-57. Illustrating reduction phenomena in the ovule.

Fig. 37. Resting nucleus in megaspore mother-cell, showing large and small nucleolus, chromatic bodies, and delicate reticulum.

Fig. 38. Development of a much coarser reticulum from the stage shown above, chromatic bodies no longer distinguishable.

Fig. 39. Nucleus filled with a dense reticulum just before the advent of synapsis.

Fig. 40. The synaptic contraction under way.

Fig. 41. A synaptic knot.

Figs. 42 and 43. The thickened threads which emerge with the loosening of the synaptic knot.

Fig. 44. Further shortening and thickening of the threads to form the spireme.

Fig. 45. A segmented spireme.

Figs. 46 and 47. The chromosome segments, for the most part still arranged end to end.

Fig. 48. Chromosome segments gathered in a close group bearing a superficial resemblance to a synaptic knot, apparently the stage termed by some authors a 'second contraction'.

Fig. 49. Bipolar spindle, the fourteen chromosomes gathered in an irregular group.

Figs. 50 and 51. Metaphase of the heterotypic mitosis; the chromosomes bent mostly in the form of thickened V's.

Fig. 52. Telophase of the heterotypic mitosis; the chromosomes at the poles of the spindle are split lengthwise.

Fig. 53. Resting nucleus of the interkinesis between the heterotypic and homotypic mitoses. Seven pairs of chromosomes are present, mostly in the form of U's joined together in the bent middle region.

Fig. 54. Prophases of the homotypic mitosis in companion cells, showing multipolar spindles and the seven pairs of chromosomes.

Fig. 55. Metaphase of the homotypic mitosis.

Fig. 56. Anaphase of the homotypic mitosis, showing the two sets of daughter chromosomes, seven in each set, that result from the separation of the members of the pairs.

Figs. 57. Nucleus of megaspore, showing reticulum and deeply staining chromatic bodies (prochromosomes?).

Figs. 58-66. Illustrating the vegetative mitoses in the cells of the nucellus.

Fig. 58. Resting nucleus, showing delicate reticulum, minute chromatic bodies, and large nucleolus.

Figs. 59 and 60. The thickening of the strands of the reticulum preparatory to the formation of the spireme.

Fig. 61. The spireme giving evidence of segmentation to form the chromosomes.

Figs. 62 and 63. Chromosome segments of the spireme.

Fig. 64. The group of fourteen chromosomes at the equatorial plate viewed from above.

Fig. 65. Anaphase of a vegetative mitosis; the daughter chromosomes gathered at the poles of the spindle in the form of attenuated V's.

Fig. 66. Daughter nuclei; some of the chromosomes still recognizable as bands or threads, more numerous on the side of the nucleus farthest from the equatorial plate.

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B.M. Davis del.

DAVIS — REDUCTION DIVISIONS OF *OENOTHERA BIENNIS*.

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B.M.Davis, del.

DAVIS — REDUCTION DIVISIONS OF OENOTHERA BIENNIS

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