Cytological Studies on *Oenothera*. III.

A Comparison of the Reduction Divisions of *Oenothera Lamarckiana* and *O. gigas*.¹

BY

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With Plates LXXI-LXXIII.

OF all the forms derived from *Oenothera Lamarckiana* which have appeared in the cultures of de Vries and others, *O. gigas* is to the writer by far the most interesting for the reason that we have in *gigas* a type sharply distinguished from *Lamarckiana* in a large number of particulars and clearly different in its nuclear structure, since it has twice the number of chromosomes present in the parent form. Whether others of the so-called mutants of *Lamarckiana* will show characteristic and stable differences or peculiarities of the chromosomes or chromosome count becomes a matter of importance, and we are waiting with much interest for the results of investigations upon these forms.

The writer (Davis, '11) has recently published an account of certain hybrids between *Oenothera biennis* and *O. grandiflora* that resemble *Lamarckiana* to such a degree as to justify, in his opinion, the consideration of a working hypothesis that *Oenothera Lamarckiana* arose as a hybrid between some strains of these two characteristic wild American species. The reasons, both experimental and historical, for this hypothesis will not be repeated here, especially since my studies in these directions are to be continued with other strains of these species that seem likely to give hybrids that will approach more closely to the desired end—the synthesis of a hybrid so similar to *Lamarckiana* as to be practically indistinguishable from this form by the usual taxonomic tests.

Thus, although the bearing of the possible hybrid origin of *Lamarckiana* upon de Vries' interpretation of the behaviour of this remarkable plant will not be discussed at this time, it will be apparent to the reader that if the evidence should finally indicate *Lamarckiana* to be of hybrid origin, as

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a number of writers have already suggested, many of the so-called mutants are likely to be interpreted as segregates splitting off according to Mendelian expectations. Nevertheless, whatever shall be the final judgement concerning the significance of some or perhaps most of the forms derived from *Lamarckiana*, we have in *gigas* at least a type in which profound changes have taken place in the germinal constitution through the doubling of the chromosome number characteristic of the genus *Oenothera*.

Oenothera gigas appears to be a true mutant, or sport, since it is reasonable to believe that its strongly marked peculiarities are directly associated with that change in nuclear structure which increased the number of sporophytic (somatic) chromosomes from fourteen to twenty-eight. This peculiarity of nuclear structure involves a totally different form of origin from any that is likely to be established for the other derivatives from *Lamarckiana*, and *gigas* therefore among these derivatives probably stands in a class by itself, and this is also indicated by the extreme rarity of its appearance. Whereas many of the other of de Vries' 'mutants' have appeared in cultures in sufficient numbers and proportions as to suggest a Mendelian segregation of forms from a hybrid, *gigas* on the contrary has, as far as the writer is aware, been reported as arising only seven times among the thousands of cultures of *Lamarckiana* and its derivatives made by de Vries and the later investigators who have worked with these forms.

As described in 'Die Mutationstheorie', *gigas* first appeared in 1895 among a group of thirty-two rosettes of *Lamarckiana* selected by de Vries ('09, p. 227) from a very large culture of this type in the fourth generation. It is probable that all of the *gigas* material now in cultivation is descended from this plant. On two other occasions *gigas* is believed by de Vries ('09, p. 327) to have arisen in his cultures, once in 1898 from seed of *sublinearis* (a 'mutant' of *Lamarckiana*), and once in 1899 from a cross of *lata* × *hirtella*, but neither of these plants matured. MacDougal ('07, p. 3) reports the appearance of one plant of *gigas* in his cultures of *Lamarckiana*, from seed of de Vries, at the New York Botanical Garden. Lastly, Schouten ('08; Ref., Bot. Centbl., vol. cviii, 1908, p. 246), a student of de Vries, records three plants of *gigas* in cultures from commercial seed of *Lamarckiana*. Thus *gigas* at the most has been noted only seven times, and, since apparently the cytology of de Vries' strain alone has been studied, it is by no means certain that all of the forms reported later are the same as the first example from de Vries' cultures of 1895.

The extreme rarity of *gigas* as a derivative of *Lamarckiana* seems to the writer exactly what should be expected on the supposition that this type is a true sport, or mutant. The fact of its rarity, probably based on the marked peculiarities of its nuclear organization, indicates that *gigas* is an exception, and that other derivatives from *Lamarckiana* may finally be regarded as variants of quite a different sort from the 'mutants' of de Vries'

definition. For these reasons a detailed comparison between the reduction processes of *Lamarckiana* and *gigas* is a matter of importance in any attempt to establish the fundamental differences between these two forms, and the possible manner of origin of the latter type.

The writer is pleased at this opportunity to describe the conditions in *gigas*, since this plant has been employed as one of the parents in certain crosses with wild species of *Oenothera*, and the behaviour of the chromosomes in the hybrids of the first and second generation gives promise of some interesting results which, if worked out satisfactorily, will be presented in future papers.

METHODS.

Experiments in methods of fixation have been continued since the recent study of *Oenothera biennis* (Davis, '10), where the effects of Carnoy's alcohol-acetic and chloroform-alcohol-acetic mixtures were contrasted with those of chrom-osmo-acetic acid, much to the advantage of the latter fluid. In the past summer of 1910 Bouin's fluid and Juel's fluid were given a similar trial. Anthers of *Lamarckiana*, after 4-5 hours in Bouin's fluid, were run quickly (1 hour) through grades of alcohol and washed in 80 per cent. alcohol until clear. This material exhibited a much greater degree of shrinkage than is present in anthers fixed in Flemming's fluids with the precautions outlined in my previous papers. The extent of the shrinkage, with no compensatory advantages of importance in the staining reactions, is in the writer's experience unfavourable to Bouin's fluid as a fixing agent for the anthers of *Oenothera*.

Juel's fluid (zinc chloride 2 gr., glacial acetic acid 2 c.c., 50 per cent. alcohol 100 c.c.) was tried upon the anthers of *gigas*, the material being left in the fluid 24 hours and then carried through grades of 50-85 per cent. alcohol, in which the material was thoroughly washed. The results were unsatisfactory. There was great shrinkage of the protoplasts and such changes in the structure of the cytoplasm that sharp differentiation of its structure was not possible with iron-alum haematoxylin. It was also difficult to stain the chromatin and nucleoli, which failed to exhibit the sharp outlines obtainable after fixation with Flemming's fluids.

Further experience with chrom-osmo-acetic acid has not modified the methods outlined in the previous papers (Davis, '09 and '10), but there seems to be little choice between the stronger and weaker formulae of Flemming. An essential for success with these fluids lies in such precautions as will facilitate the most rapid penetration possible, and to this end the writer knows of no better practice than that of brushing the anthers with water before immersion in the fixing fluid. The greater the attention paid to this somewhat laborious technique the less will be the amount of shrinkage

exhibited by the protoplasts, which in favourable material may be scarcely evident.

As in the previous studies, iron-alum haematoxylin was employed as the stain throughout the investigation.

OENOTHERA LAMARCKIANA.

Oenothera Lamarckiana has been made the subject of a lengthy study by Geerts ('09), which considers the development and fertilization of the embryo sac as well as the formation of the pollen. Gates's ('08) account of the reduction phenomena during pollen formation in *O. rubrinervis* considers a type so close to *Lamarckiana* as to make possible a close comparison with the latter form. There are important differences between the writer's conclusions and those of the investigators cited above in various details of the accounts of presynapsis, synapsis, and the form and behaviour of the chromosomes during the reduction mitoses. These differences become at once apparent in comparing our figures, and only certain of the most marked points of disagreement will be referred to in the text.

Presynapsis. The daughter nuclei following the last mitosis in the archesporium of the anther (Pl. LXXI, Fig. 1) exhibit clearly the fourteen sporophytic (somatic) chromosomes characteristic of the type. The chromosomes become smaller as the nucleus, increasing in size, passes into the resting condition, the development of the nuclear reticulum apparently drawing material from the chromosomes and so reducing their bulk that they can finally only be recognized in later stages as deeply staining bodies (Fig. 2). These are the chromatic bodies described by the author for *biennis* and *grandiflora*, and there can be no doubt that they are derived from the chromosomes of the preceding mitosis; they apparently correspond to the prochromosomes of Overton, Rosenberg, and others.

In the earlier presynaptic stages the chromatic bodies may frequently be counted as fourteen, but as the reticulum becomes more pronounced they cannot be distinguished from other structures, such as small nucleoli and granules which appear in the nucleus. The chromatic bodies of *Lamarckiana*, like those of *biennis* and *grandiflora*, are distributed irregularly throughout the nuclear cavity, and there is no evidence of groupings in pairs which might be interpreted as indicating the presence in the nucleus of two parallel spiremes such as have been described by Overton ('09), Lundegard ('09), and Rosenberg ('09 a). On the contrary, their position on the linin strands suggests rather an end-to-end relationship in keeping with the arrangement of the chromosomes in the spireme preceding the heterotypic mitosis.

The development of the nuclear reticulum makes it impossible to follow the chromatic bodies further, since their substance merges with the strands of the network and their form becomes lost. This is a gradual process

extending over a considerable part of the long period leading up to synapsis. It involves the gradual thickening of what are at first relatively few very delicate threads (Fig. 2) and the formation of new strands until the nucleus finally contains a close network (Figs. 3 and 4) in the meshes of which lie one or two large nucleoli, and frequently several smaller bodies of a similar nature. While on the evidence from their history it is reasonable to suppose that the chromatic bodies are chromosome centres or prochromosomes, and probably maintain their individuality in the nuclear reticulum, nevertheless such individuality is not clearly evident in the resting nucleus of *Oenothera*.

Synapsis. A contraction of the strands composing the nuclear reticulum away from the nuclear membrane (Figs. 5 and 6) indicates the approach of synapsis. This contraction, as in *biennis* and *grandiflora*, proceeds until almost all of the strands in the network are drawn together in a close mass generally at one side of the nucleus and near the nucleolus (Fig. 8), which is sometimes quite surrounded by the web of threads (Fig. 7). This contracted and confused mass of strands, the arrangement of which cannot be followed with precision, constitutes the synaptic knot, a sharply marked stage extending over a considerable period, probably of one or more days. Occasional free loops extend from the contracted mass into the nuclear cavity, and the study of these, together with such structure as is apparent in the less dense regions, makes it clear that the synaptic knot is a close association of looped threads frequently anastomosing so as to form what appears to be a tangled mass.

In such a complex association of strands it is not difficult to find threads that run closely parallel to one another for greater or less distances, or that are united at points. However, in a structure so involved and with elements so minute as are these threads, it is not safe to assume that these relations signify anything more than such a close association of strands as would naturally result from the contraction of the complexly looped and anastomosing thread or system of threads that constitute the nuclear reticulum. The evidence is entirely inadequate to support the interpretation that such parallel relations between the strands indicate the presence of two independent systems of threads (maternal and paternal spiremes), such as might be assumed to be associated with one another side by side. On the other hand it is impossible to say whether or not the coiled and twisted loops of the synaptic knot are parts of a single continuous thread or of a system of threads forming a reticulum; if the former, it must be a thread of great length.

As the process of synapsis proceeds it becomes evident that the threads thicken (compare Fig. 9 with Figs. 6 and 7), and from the later developments there is good reason for believing that this thickening is associated with a very material shortening of the thread system. The increased thickening of the strands, although clearly shown at the stages

of mid-synapsis (Fig. 9), becomes much more conspicuous in the post-synaptic stages leading to the development of the spireme. Thus with the loosening of the contracted coils of the synaptic knot there emerges a much more clearly defined system of loops than can be recognized in the complicated reticulum which entered the synaptic knot (compare Figs. 10 and 11 with Figs. 6 and 7). It is evident that the threads have become much thicker and that the thread system has grown much shorter, and the inference is natural that these developments are the result of the process of contraction so characteristic of synapsis. This history is the same as that for *biennis* and *grandiflora*.

Gates ('08) interprets the presence of parallel threads in post-synaptic stages of *rubrinervis* as indicating a splitting of the spireme, which is later closed by the union of the two threads. The writer, from his studies on *biennis* and *Lamarckiana*, cannot agree with this view, for although parallel threads may frequently be observed, there seems to him no evidence that this condition is other than such an arrangement as is to be expected when a complex system of threads has been gathered together by such a process as that of synaptic contraction. Since parallel threads may be observed in stages just previous to mid-synapsis it is but natural to expect that somewhat similar relations should be found in stages following the loosening of the synaptic knot. These threads in *Oenothera* are so delicate and their arrangement is so intricate that the establishment of such an interpretation as that of Gates is surrounded with great technical difficulties.

As in the study of *biennis*, the term 'synapsis' is reserved for this period of a first contraction of the synaptic material. A type of contraction that is frequently conspicuous at a much later period during the formation of the chromosomes (the 'second contraction') will be described in that connexion. The relation and significance of the two phenomena will be further considered in the 'Cytological Discussion'.

The Formation of the Chromosomes. Following the period of synapsis, which is of relatively long duration, there begins as described above a gradual loosening of the synaptic knot and a shortening and thickening of the thread system. Further developments now follow rapidly. The threads become more prominent (Fig. 12) and begin to take on the thickness and appearance of the spireme which is soon to be developed. During this process the complicated coiled arrangement, which because of the anastomoses exhibited the structure of a network, becomes more simple, and the threads may be followed for considerable distances in the looser convolutions. The threads remain, nevertheless, for a long time so complexly looped that their outline cannot be accurately traced throughout their entire length.

The process of shortening and thickening proceeds until an undoubted

spireme appears (Fig. 13), which is so short that the band may be followed distinctly in its convolutions throughout the nuclear cavity. The spireme is in some cases unquestionably a single thread, although the loops are frequently joined by delicate cross strands, and it is sometimes attenuated at points. In other cases it appears as though the spireme is not strictly continuous, but consists of two or more somewhat distinct portions.

Following the differentiation of the spireme (Figs. 12 and 13), a process of segmentation begins (Figs. 14 and 15) which transforms the spireme into a chain of fourteen chromosomes. This segmentation consists in a drawing apart of certain regions of the spireme as though by a process of constriction (Fig. 14), or perhaps more accurately by the condensation of the chromatin at fourteen chromosome centres on the spireme. This condensation results in a still further shortening of the spireme so that the complexities of its early looped arrangement largely disappear, and the chromosome segments may be followed for long distances as parts of a chain (Figs. 15, 16, and 17). The chain is sometimes broken as though a few segments had been displaced, which might readily result from such a looping of the spireme that parts would be thrown out of the general line of arrangement, or because portions of the original spireme were separated at points.

During the segmentation of the spireme and the condensation of its portions to form the fourteen sporophytic (somatic) chromosomes the segments are frequently found grouped at one side of the nucleus in a close mass (Figs. 18-20) that resembles superficially the chromatic contraction of synapsis. This condition is more conspicuous in *Lamarckiana* than in my material of *biennis*, and constitutes a very clearly marked stage in the reduction processes which will be described as the second contraction, since it appears to correspond closely in the time of its appearance with the 'second contraction' stage of a number of writers on different types. This stage in *Oenothera* takes place during the segmentation of the spireme or shortly after, so that the contracted material consists of chromosome segments either in process of formation (Figs. 18 and 19) or in a somewhat more advanced stage in their condensation into chromosomes (Figs. 20 and 21).

Gates ('08, pp. 11 and 12) places the stage of 'second contraction' in *rubrinervis* previous to the segmentation of the spireme, but his figures are not consistent with his account. Thus Fig. 20 exhibits a pair of chromosomes cut off from the remainder of the chromatin, which, in spite of the lack of detail shown, has the appearance of a contracted, looped, and segmented spireme, or perhaps even of a chain of chromosomes. Fig. 21, with an even greater lack of detail present in the drawing of the contracted chromatin, shows evidence of a segmenting spireme in the loop extending into the nuclear cavity. Fig. 18 may illustrate, as described, the shortened and thickened thread (spireme) entering upon the phase of second con-

traction, but there are here also indications that segmentation is about to take place.

The chromosome segments of the spireme when first formed are sometimes four to six times longer than they are broad and of slightly varying lengths even in the same nucleus, but as the process of chromatin condensation continues they become much shorter and gradually approach uniformity of size. In consequence of this process of segmentation the arrangement of the fourteen sporophytic chromosomes on the spireme is always end to end (Figs. 15-17), but as the spireme becomes older groups of chromosomes are frequently detached from the main chain. Pairs of chromosomes are thus occasionally separated which take the form of rings (Fig. 18), probably because they formed two sides of a loop in the spireme. The chromosomes of *Lamarckiana*, like those of *biennis*, are, then, not grouped regularly in pairs during the prophases of the heterotypic mitosis as was the case in material of *grandiflora* studied by the writer (Davis, '09). In that material a characteristic pairing of the chromosomes was found to result from the regular arrangement of the loops of the spireme by which adjacent chromosome segments in the chain were as a rule brought somewhat side by side, so that they remained united to form seven ring-shaped bivalent chromosomes. Such pairs or rings are not common in *Lamarckiana*, and as a rule the arrangement of the chromosomes is in the form of a single long chain or two or more shorter chains.

This method of the organization of the chromosomes in *Lamarckiana* as segments of a univalent spireme placed end to end is therefore the same as the history described by the writer for *biennis* and *grandiflora*, and in this conclusion the present paper is in full agreement with the account of Geerts for *Lamarckiana* and with that of Gates for *rubrinervis*.

The Heterotypic Mitosis. The arrangement of the chromosomes end to end in a single chain or in two or more shorter chains is frequently maintained through the prophases of the first, or heterotypic mitosis, although the chromosomes may be so closely grouped that it is difficult to follow them with precision. The cause of the intimate association of the chromosomes may readily be traced to the conditions of the second contraction from which the prophases develop, and where, as described above, the condensation of the chromosome segments appears to draw these elements together. This condensation continues throughout the prophases so that the chromosomes at the metaphase of this mitosis (Fig. 27) are very much smaller structures than the segments of the spireme from which they were derived (Figs. 15-21). As in *biennis* and *grandiflora*, the mature chromosomes are essentially similar to one another, and have usually the form of thickened V's due to the bending of the chromosome segments of the spireme; they are not rounded structures such as have been described and figured by Gates and Geerts.

Spindle formation begins, as in *biennis* and *grandiflora*, by the entrance of fibrillae into the nuclear cavity following the breaking down of the nuclear membrane. The fibrillae likewise push out into the surrounding cytoplasm and thus establish multipolar spindles (Figs. 23 and 24) similar to those described for a number of higher plants (e.g. *Equisetum*, *Larix*, *Lilium*, &c.) ; the large nucleolus disappears at the beginning of spindle formation. The chromosomes still joined end to end in one or more chains are brought by the development of the fibrillae to the centre of the spindle, where they generally lie in a rather dense group, although occasional chromosomes may be found somewhat separated from the main assemblage.

The bipolar spindle characteristic of the metaphase of the mitosis (Fig. 27) results from the rearrangement and gathering of the spindle fibres into two broad sheaves that end in granular areas which merge with the alveolar cytoplasm. The chromosomes may frequently be found still arranged in chains in the equatorial regions of the recently formed bipolar spindle (Figs. 25 and 26), but as the spindle grows older the chromosomes separate from one another, and by bending at the ends and thickening in the middle regions usually take on the form of thickened V's. There appears to be no system in the grouping of the chromosomes as they are brought to the equatorial plate just previous to the metaphase of the mitosis. Their arrangement is quite irregular, as shown in Figs. 25 and 26, and although occasional ring-shaped pairs of chromosomes may be noted which were undoubtedly derived from adjacent segments of the spireme, most of the chromosomes become so separated that it is impossible to determine with certainty what was their relation to one another on the spireme.

The V-shaped form of the chromosomes is especially evident as the two sets move away from one another towards the poles of the spindle (Fig. 28). However, during anaphase, as the chromosomes approach the poles (Fig. 29) their structure becomes complicated by a lengthwise fission of each chromosome (Fig. 30), which takes place in the plane of the page upon which the above letter (V) is printed. Thus the seven chromosomes that leave the equatorial plate of the heterotypic mitosis arrive at the poles and enter the daughter nuclei as seven split chromosomes (Pl. LXXI, Fig. 31, Pl. LXXII, Figs. 32, 33). This division is a premature fission of each chromosome in preparation for the second, or homotypic mitosis.

There was found, during the study of considerable material, only a single case that showed numerical irregularities in the distribution of the chromosomes by the heterotypic mitosis. In this example eight split chromosomes were present at one pole of a spindle (Pl. LXXI, Fig. 30), and six chromosomes were distributed between two sections of the other pole. Similar irregularities were reported by the writer (Davis, '10, p. 638) for

biennis. These cases in both *biennis* and *Lamarckiana* are of great interest, but it should be noted that they are rare, and it would be quite impossible to find sufficient material to determine whether or not fertile pollen-grains can be developed with nuclei bearing a greater or less number of chromosomes than the normal.

The group of seven split chromosomes is best observed in polar views of late anaphase, and at the time of the reconstruction of the daughter nuclei. Their history may be readily followed through the interkinesis between the heterotypic and homotypic mitoses. Following the organization of the daughter nuclei, the halves of each chromosome bend so that they separate at the ends, and thus come to have the form of two U's joined together in the middle region (Pl. LXXI, Fig. 31; Pl. LXXII, Figs. 32, 33). The seven split chromosomes become therefore seven evident pairs, the members of each pair remaining in close association until the metaphase of the homotypic mitosis. The chromosomes during the reconstruction of the daughter nuclei increase rapidly in size, and remain in this expanded state throughout the interkinesis. Nucleoli appear (Fig. 33) and sometimes the ends of the chromosomes proliferate and show a tendency to form an imperfect network. The period of interkinesis is of considerable length, with the individuality of the chromosomes generally maintained quite as clearly as is indicated in Figs. 31–33, and this history is the same in all essentials as that in *biennis* and *grandiflora* and agrees with the accounts of Gates and Geerts.

In summary of the events of the heterotypic mitosis in *Lamarckiana* it should be noted (1) that the 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 that 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 appearance of a web of fibrillae around the resting nucleus of the interkinesis together with the breaking down of its membrane indicates the approach of the homotypic mitosis. The fibrillae enter the nuclear cavity and establish a multipolar spindle (Pl. LXXII, Figs. 34 and 35), at the same time carrying the seven pairs of chromosomes towards the centre of the structure. A bipolar spindle is then developed by the rearrangement of the fibres, and the pairs of chromosomes become grouped upon an equatorial plate (Fig. 36) so that the members of the pairs will be separated and distributed by the mitosis with perfect regularity, seven chromosomes in each set. The two homotypic spindles in the

pollen mother-cell may lie side by side or at right angles to one another. The chromosomes, by condensation during the prophase, become very much smaller than in the expanded conditions of the interkinesis, and by the time that the homotypic spindle is fully developed they have returned to about the same size as when they entered the period of interkinesis. They have the form of short and slightly bent rods (Fig. 36).

During the anaphase of the homotypic mitosis (Fig. 37) and later when the chromosomes are gathered at the poles (Figs. 38-40) their form becomes more irregular, changing even more markedly after the organization of the daughter nuclei which are to enter the pollen-grains. These nuclei (Figs. 41 and 42) show at first clearly the seven chromosomes which enter into their composition, but the form of the chromosomes becomes attenuated as the nuclei enlarge. Presently a network becomes evident connecting the chromosomes, and the nuclei of the pollen-grains thus pass into typical resting conditions (Fig. 43) possessing one or more nucleoli and a delicate open reticulum in which lie deeply staining regions. These have the appearance of the chromatin bodies described for the nuclei preceding synapsis, but they are of course in the reduced number seven. The chromatic bodies become less and less evident as the nuclei increase in size, and by the time that the young pollen-grains are formed they can no longer be distinguished in the chromatic reticulum that has developed, unless some of the denser granules present may represent such chromatic centres or prochromosomes; such granules cannot, however, be counted with regularity.

This description of the homotypic mitosis in *Oenothera Lamarckiana* is in agreement in all essentials with the account of Geerts for the same form, with that of Gates for *rubrinervis*, and with the writer's accounts of *biennis* and *grandiflora*. The mitosis is clearly an equational division distributing the halves of chromosomes, the premature division of which takes place during the anaphase of the heterotypic mitosis, the halves remaining associated in seven pairs throughout the period of interkinesis.

The Partial Sterility of the Pollen in *Oenothera Lamarckiana*. De Vries noted the fact of partial sterility in *Lamarckiana* of both pollen-grains and ovules, and this subject is given considerable attention in the account of Geerts ('09, pp. 82-105), who reports that about 50 per cent. of these structures may be abortive. Geerts figures a number of pollen-tetrads and young embryo-sacs in which the nuclei give evidence of degeneration, but he does not clearly associate the phenomenon with irregularities in the distribution of the chromosomes.

Gates ('07) presents observations on the development of the pollen in the hybrid *lata* × *Lamarckiana*, showing clearly that the group of seven chromosomes which normally should enter each nucleus of the pollen-grain may be broken up with the result that extra nuclei are formed having

smaller numbers of chromosomes, and a similar case was also observed by the same author (Gates, '08) for *rubrinervis*. Both Geerts and Gates report evidence of nuclear degeneration in stages previous to the heterotypic mitosis, indicating that the causes of sterility are more deep-seated than irregularities of chromosome distribution, although the latter phenomenon is perhaps intimately associated as an effect of physiological disturbances previous to pollen formation.

The writer can only report for *Lamarckiana* the facts of certain irregularities in the distribution of the chromosomes. Thus, during the homotypic mitosis it is not uncommon to find that some of the chromosomes in a group of seven have failed to reach the poles of the spindle, and as a result form smaller supernumerary nuclei in the pollen mother-cell. Such a case is shown in Pl. LXXII, Fig. 44, where the chromosomes of three groups, a total of twenty-one, are distributed among five nuclei. Tetrads may even be formed in which large and small nuclei become associated in the same cell and pass into a resting condition, but it is not known whether such a cell can mature into a fertile pollen-grain. An irregularity in the heterotypic mitosis has also been described (Fig. 30) in which six or eight chromosomes respectively had been distributed to opposite poles, but here also we do not know whether or not fertile pollen-grains may be formed with chromosomes in a greater or less number than the normal.

An extensive field for cytological investigation is suggested by the recent paper of de Vries ('11) on double reciprocal crosses between *Oenothera biennis* and *O. muricata*. In explanation of his remarkable results de Vries postulates the development of two forms of germ cells, both male and female, carrying two different sets of hereditary tendencies. One of these two forms of gametes is eliminated from the male organ by the sterility of half of the pollen-grains, and the other form is eliminated from the female organ by the abortion of half of the ovules. As a result two groups of characters are strictly sex-limited in their inheritance, one being carried through the male and the other through the female gamete. It is too early to discuss this hypothesis from the cytological side, for the problems concerned will demand prolonged investigation. The writer's belief, based on preparations of pollen in which the entire contents of anthers were examined, is that the proportion of sterile pollen varies widely. Thus in certain races of *Oenothera biennis* from 20 to 30 per cent. of the pollen is sterile, while in other races the proportion of sterile pollen appeared to be fully 75 per cent.

It seems clear, however, that the total amount of sterility in the *Oenotheras* is much too great to be explained entirely by peculiarities of the chromosomes, or by such irregularities in their distribution as are known to occur during the reduction divisions. Thus, it is difficult to understand how the ovules of *Oenothera lutea* can be fertile and the pollen

sterile when the chromosomes are of the same germ-plasm. There are other factors at work probably of an intricate physiological nature.

For these reasons the writer is not impressed with the importance of the criticism that the partial sterility of *Lamarckiana* indicates its hybrid origin. Partial sterility in *Oenothera* and related genera, as pointed out by Geerts, is a phenomenon so common that the significance of its presence or absence may readily be unduly emphasized. So far the hybrids of the crosses between *biennis* and *grandiflora* (Davis, '11) which approach closely to *Lamarckiana* have been fertile to a high degree in the F_1 generation and then have exhibited a great range in their fertility in the F_2 , some of them being more than 50 per cent. sterile and others apparently perfectly or almost perfectly fertile. The writer will lay no emphasis on the partial sterility of *Lamarckiana* in the development of his views that *Lamarckiana* arose as a hybrid between strains of *biennis* and *grandiflora*. The behaviour of *Lamarckiana* in throwing off marked variants in such large numbers, together with the fact that *Lamarckiana* is unknown as a clear component of the American native flora, is the chief reason for his belief that this interesting plant is not, as assumed by de Vries, representative of a wild species.

OENOTHERA GIGAS.

The first announcement that the number of chromosomes in *Oenothera gigas* is double that of the parental form *Lamarckiana* appeared in a note of Miss Anne Lutz (Science, xxvi, 1907, p. 151), who reported the discovery of twenty-eight or twenty-nine chromosomes in mitotic figures of the root-tips. Gates (Science, xxvii, 1908, p. 193) shortly after confirmed this discovery in an examination of the reduction divisions in the pollen mother-cells which showed the sporophytic number of chromosomes to be twenty-eight and the reduced number to be fourteen. The present study is in agreement with the above conclusions. The material with which these two investigators have worked, and also that described in this paper, is all descended from the first plant of *gigas* which in 1895 appeared in the cultures of de Vries.

Gates ('09) has published a paper chiefly devoted to a comparison of the cell and nuclear measurements of *gigas* with those of *Lamarckiana*, to test Boveri's theory that in related forms the nuclear surface and cell volume in homologous tissues are proportionate to the number of chromosomes in the types compared. In this paper is given a short account of that period of the reduction processes from the metaphase of the heterotypic mitosis to the prophase of the homotypic. The unfortunate loss of the figures intended to illustrate this account renders impossible a close comparison between Gates's description and the conclusions of the writer,

but certain important differences in our conclusions, evident from the text, will be noted. The following account will be written with the view to comparison with the foregoing description of *Lamarckiana*.

Presynapsis. As in *Lamarckiana*, the daughter nuclei of *gigas* following the last mitosis in the archesporium (Pl. LXXII, Fig. 46) show clearly the sporophytic chromosomes, but the large number, twenty-eight, is naturally not easy to count with accuracy. As the nuclei increase in size a delicate reticulum is developed, the material of which is apparently drawn from the chromosomes since the latter decrease in size and finally can be recognized only as deeply staining granules (Figs. 47 and 48). These are the chromatic bodies and correspond to the similar structures in *Lamarckiana*.

The large number of the chromatic bodies, together with the difficulty of distinguishing them in these stages from other granules in the nucleus, makes accurate counts impossible, but there can be no doubt that the chromosomes are represented in the nuclei by deeply staining bodies upon the nuclear reticulum in such stages as are shown in Figs. 47 and 48. No evidence was discovered that the chromatic bodies are grouped in pairs and such relations to one another as appeared were rather those of an end-to-end arrangement on the strands of the network.

As the nuclear reticulum develops further (Figs. 49 and 50) the chromatic bodies become so lost in the thicker strands of the network that they can no longer be recognized. As in *Lamarckiana* the development of the reticulum is a gradual process involving the thickening of what were at first relatively few delicate threads (Figs. 47 and 48), and the development of many others until the nucleus finally becomes filled with a rather dense reticulum (Fig. 50) in which lie one or more nucleoli. In later stages and just previous to the beginning of the synaptic contraction, the network becomes more like a loosely tangled group of threads (Fig. 51), and this condition is generally more clearly shown in *gigas* than in *Lamarckiana*, *biennis*, or *grandiflora*.

Synapsis. The approach of synapsis is indicated here, as in other species of *Oenothera*, by a contraction of the nuclear reticulum away from the nuclear membrane (Fig. 52), and this contraction continues until almost all of the threads in the network are drawn together into a close mass, the synaptic knot (Figs. 53 and 54), generally at one side of the nucleus and near the nucleolus. The appearance of the synaptic knot is similar to that of *Lamarckiana* (compare Fig. 53 with Fig. 9); it consists of a contracted intricately coiled mass of threads whose arrangement cannot be followed, but from which occasional loops and strands extend into the nuclear cavity. The form of the knot may be almost spherical as in Fig. 53 or more irregular as in Fig. 54. As in *Lamarckiana*, *biennis*, and *grandiflora* the writer was unable to discover any relations between the threads other than

such a close association as would result from the contraction of a complexly looped thread or system of threads anastomosing to form a reticulum.

Following the period of mid-synapsis, when the contracted condition is most conspicuous, there begins a loosening of the synaptic knot, and it becomes at once evident that the threads have thickened and that the thread system has grown shorter (Fig. 55). Thus there emerges from synapsis a much more clearly defined system of loops than appeared in the involved tangle of threads that entered the synaptic knot (compare Fig. 55 with Fig. 53). The thickened thread system then begins to assume the appearance of the spireme, which shortly after may be clearly recognized (Fig. 56). The entire history of the formation of the spireme from the stage of the nuclear reticulum preceding synapsis seems to be a process of contraction and thickening of the threads in the reticulum, the phenomena of synapsis being an expression of this process.

The Formation of the Chromosomes. With the loosening of the synaptic knot following synapsis, as described above, it becomes at once apparent that the threads have grown markedly thicker and the thread system much shorter than at the beginning of the synaptic contraction (compare Fig. 55 with Figs. 51 and 52). Nevertheless, the thickened threads still present a very complicated coiled arrangement which, as in *Lamarckiana*, cannot be followed throughout the nucleus. The structure is, however, as far as could be determined, that of a single thread or a group of threads intricately looped and occasionally anastomosing. The spireme (Figs. 56 and 57) is developed by the continued shortening or condensation of the thickened thread system until the band may be distinctly traced throughout the greater number of its convolutions, although it is very difficult to determine whether the spireme is strictly continuous or composed of two or more portions.

There then begins the process of segmentation (Fig. 58) that transforms the spireme into a chain of twenty-eight chromosomes (Fig. 59), which because of their large number can be counted only in very favourable preparations. The process is the same as in other *Oenotheras*, and consists in the constriction of the spireme and condensation of the chromatic material at twenty-eight centres. The result of the condensation is a further shortening of the spireme, so that much of the complicated looped arrangement becomes simplified, and the segments may be followed for long distances (Fig. 59), arranged end to end like links in a chain. The chain of chromosomes is frequently broken, perhaps because the spireme is made up of somewhat distinct portions, or because certain segments become displaced or separated, as might readily take place at points in the loops.

The stage of second contraction is frequently very conspicuous in *gigas*, for the reason that so large a number of chromosomes is concerned with the phenomenon. It may appear as early as the segmentation of the spireme

(Pl. LXXII, Fig. 60), but it is most commonly noted after the process of condensation has brought the segments to a size closely approximating that of the chromosomes (Figs. 61–63, compare with Pl. LXXI, Figs. 19–21). The chromosomes are not with regularity brought together in pairs during the second contraction, although such relations are not uncommon in detached groups, and in this behaviour *gigas* agrees with *Lamarckiana* and *biennis*, in contrast to the usual formation of ring-shaped bivalent chromosomes in the writer's material of *grandiflora*.

The Heterotypic Mitosis. The prophases of the heterotypic mitosis in *gigas* give excellent stages in the development of the spindle, which is larger than that of *Lamarckiana* (compare Pl. LXXIII, Fig. 66, with Pl. LXXI, Fig. 27). The process of spindle formation begins, as in other *Oenotheras*, with the appearance of a web of fibrillae around the nucleus (Pl. LXXIII, Fig. 64), the fibrillae entering the nuclear cavity with the breaking down of the membrane. As the fibrillae also push out into the surrounding cytoplasm they establish a multipolar spindle (Fig. 65), which shortly becomes bipolar (Fig. 66) by the rearrangement of the spindle fibres.

The chromosomes may frequently be found end to end in chains during the prophases (Figs. 64 and 65), but the development of the spindle fibres finally brings the chromosomes to the centre of the spindle, where they generally lie in a crowded group until they form the equatorial plate of the heterotypic spindle. During the development of the spindle the chromosomes usually take on the form of thickened V's by bending at the ends and thickening in the middle regions, as in all other *Oenotheras* studied by the writer. They are similar in size to the chromosomes of *Lamarckiana*. There is no system apparent in the grouping of the chromosomes as they are brought to the equatorial plate. Although occasional pairs may be noted, the arrangement of the chromosomes is generally quite irregular until their alinement at the metaphase of the mitosis, when as a rule the two sets are clearly differentiated (Fig. 66).

The form of the chromosomes becomes complicated as they approach the poles of the spindle during anaphase by the lengthwise fission which is so characteristic of these forms, and which is illustrated in a number of cases shown in Fig. 67. Thus the fourteen chromosomes that pass to each pole of the heterotypic spindle generally arrive and enter the daughter nuclei as fourteen split chromosomes (Fig. 68), although the division is sometimes delayed. This division, which is a premature fission of each chromosome in preparation for the homotypic mitosis, was, however, always shown in the chromosomes of the nuclei during the interkinesis (Figs. 71 and 72). Gates ('09, p. 528) is uncertain whether the fission is always lengthwise or not sometimes transverse, but the writer has observed no evidence of departures from the manner of lengthwise division present in *Lamarckiana*, *biennis*, and *grandiflora*.

An interesting type of irregularity, present in the heterotypic mitosis, is shown in Fig. 69. In this case two pairs of chromosomes had lagged behind the main group in passing to the poles of the spindle, and were associated in a small secondary spindle within the main structure. That such stray chromosomes, as well as the main group with a chromosome content smaller than the normal, may form independent nuclei is illustrated in Fig. 70. It is, however, improbable that fertile pollen-grains can be developed under such abnormal conditions. The fact that the chromosomes in these nuclei have lost their usual form and by anastomosing appear to be developing a chromatic reticulum, indicates that the nuclei do not proceed further towards the homotypic mitosis, but probably pass into a resting condition.

The nuclei during the interkinesis between the heterotypic and homotypic mitoses show many interesting features in agreement with the conditions in *Lamarckiana* and other *Oenotheras*. This is the period when the count of the chromosomes can be most easily made, as illustrated in Figs. 71 and 72, which show fourteen pairs of chromosomes derived from the fourteen split chromosomes previously described. It is clear that the halves of the split chromosomes swing so that their ends separate and thus become a pair united only in the middle region. Although the chromosomes sometimes proliferate and show a tendency to form an imperfect network, the pairs generally remain clearly defined throughout the interkinesis. The writer has not observed cases in which the chromosome boundaries were no longer distinguishable, as reported by Gates ('09, p. 528) for certain 'semi-resting' conditions, and it may be questioned whether the latter are normal, i. e. whether such nuclei enter the homotypic mitosis and give rise to fertile pollen-grains.

The history of the heterotypic mitosis thus agrees in all essentials with that of *Lamarckiana*, some of the features being even more perfectly shown on account of the larger size of the spindles, the only complications resting with the double number of chromosomes, which are naturally more difficult to follow.

The Homotypic Mitosis. With the breaking-down of the nuclear membrane following the interkinesis (Fig. 73), the fourteen pairs of chromosomes, previously scattered through the nuclear cavity, are brought by the development of the fibrillae to the centre of the spindle, which is at first multipolar. At the equatorial plate of the final bipolar spindle (Fig. 74) these pairs of chromosomes become grouped very regularly, so that the members of the pairs are distributed in two sets of fourteen chromosomes each. The chromosomes at the metaphase of the homotypic mitosis have the form of short rods, having returned by the condensation of their material to about the same size as when they entered the period of interkinesis (compare Fig. 74 with Fig. 68).

The chromosomes become somewhat irregular in form in their passage to the poles of the spindle during anaphase (Pl. LXXIII, Fig. 75) and after the organization of the daughter nuclei that are to enter the pollen-grains (Figs. 76-78). The fourteen chromosomes may at first be easily counted in favourable preparations of the young pollen nuclei (Fig. 77), but as the nuclei enlarge the chromosomes become attenuated, and by anastomosis soon develop a chromatic network in which their outlines become lost. Deeply staining regions in the reticulum may at certain stages be recognized as chromatic bodies undoubtedly representing the chromosomes, but they cannot be positively identified in the nuclei of older pollen-grains (Fig. 79).

Thus it will be noted that the events of the homotypic mitosis in *gigas* are in agreement with those of *Lamarckiana* and the other forms of *Oenothera* that have been studied.

The size of the cells and nuclei of *gigas* as compared with that of *Lamarckiana*. It is important to compare the size of the cells and nuclei of *gigas* and *Lamarckiana* with reference to Boveri's well known conclusions from experimental studies on the eggs and larvae of sea-urchins. He was able to institute comparisons between larvae developed parthenogenetically (x chromosomes), normal larvae ($2x$ chromosomes), and giant larvae ($4x$ chromosomes) obtained by shaking eggs and thus arresting the process of mitosis after the division of the chromosomes, all of which become included in the same nucleus through the formation of a monaster. Among other conclusions, Boveri ('05) formulated a law that the size of the cells in these sea-urchin larvae is a function of the chromatin content, and that the cell volume is proportional to the number of the chromosomes.

Gates ('09) has brought together from a variety of tissues an interesting series of comparative measurements between the cells of *Lamarckiana* and *gigas*, which indicate that the cell volume throughout the *gigas* plant is uniformly greater than in *Lamarckiana*. However, the ratios between the cell volumes of the two plants in the homologous tissues studied by Gates vary greatly, ranging from a ratio of approximately 1 : 1.5 to a ratio of 1 : 3.8, instead of being in a uniform ratio of 1 : 2 as might be expected from Boveri's conclusions. The discrepancies between these results are perhaps difficult to understand, but it should be noted that the cells were measured from sections cut in paraffin (in which some distortion is inevitable), and also that no allowance was made for the size of the vacuoles, which in plant cells are large and variable, except as a rule in embryonic tissues. Thus it is extremely difficult to institute close comparisons between the size of the nuclei and plasma masses of most plant cells.

It is clear from Gates's results, nevertheless, that the cells of *gigas* are probably larger throughout the entire plant than those of *Lamarckiana*, and the greater size of many of the plant's organs (leaves, flower parts, pollen, &c.) is quite certainly correlated with this fact. An examination

of the figures accompanying this paper will illustrate the main principle. They were drawn for their cytological features alone, and without reference to comparative size, and many of the figures of nuclei do not give the outlines of the optical sections. Nevertheless, a comparison of the figures of *gigas* with corresponding figures of *Lamarckiana* will show almost uniformly the fact that the cells and nuclei in the pollen mother-cells of *gigas* are much larger than those of *Lamarckiana*. The point is brought out especially clearly in comparisons between Figs. 4 and 51, 7 and 53, 27 and 66, 32 and 66, 33 and 72, 40 and 78.

The origin and significance of the double chromosome number in *gigas*.

As might be expected, we have no direct evidence of the manner in which *gigas* obtained its double quota of chromosomes at the time of its origin from *Lamarckiana*, and since the type arises at such exceedingly rare intervals, there is little hope of obtaining such evidence unless experimental means are devised for very greatly increasing its production. There is only one observation on the *Oenotheras* that can have any possible bearing on the problem, and this is the statement of Geerts ('09, p. 52) that he found an example of the heterotypic mitosis of *Lamarckiana* showing about twenty-eight chromosomes in place of the seven pairs (i. e. fourteen sporophytic chromosomes) normally present. This interesting preparation is not figured and there are no comments upon its possible bearing on the present problem.

If material of *Lamarckiana* should be found presenting a fair proportion of abnormalities such as that described above, it might be discovered that pollen-grains and embryo sacs are occasionally formed with nuclei containing fourteen chromosomes derived from twenty-eight chromosomes appearing during the heterotypic mitosis. Chance mating of germ-cells with this double number of chromosomes would give rise to individuals agreeing with *gigas* as to the chromosome count. The presence of twenty-eight chromosomes during a heterotypic mitosis of *Lamarckiana* might come about from a somewhat earlier appearance of that premature division of the chromosomes which normally takes place in *Lamarckiana* as early as the anaphase of this mitosis. Thus the pushing forward of this premature fission of the chromosomes from the anaphase to the metaphase of the heterotypic mitosis would result in the distribution of fourteen chromosomes to each pole of the spindle. Another fission introduced before the metaphase of the homotypic mitosis would make possible a group of four nuclei at the end of the reduction divisions, each with fourteen chromosomes. Such conceivable irregularities, however unlikely they may appear at first thought, should at least be borne in mind by those who study the *Oenotheras*.

Gates ('09, p. 544) has brought forward several suggestions as to the origin of the double number of chromosomes in *gigas*, not including the possibility given above. Of these, the one that seems most probable to

Gates and to the writer supposes a doubling of chromosome count in the fertilized egg or very young embryo by the division of the chromosomes preliminary to a mitosis which failed, however, to effect their distribution. It is known that such failures to effect the normal distribution of chromosomes may result from irregularities in spindle formation, as in a monaster, by which the full set of divided chromosomes is brought together in a single nucleus which contains of course the doubled number. There is much experimental evidence on the botanical side from the studies of Gerassimow, Nĕmec, Miss Kemp, and others, and also on the zoological side, which shows that by various chemical and mechanical treatments mitoses may be interrupted at stages in their division, and two sets of divided chromosomes brought together again to form a single nucleus with twice the normal number of chromosomes.

It has long been known from the experimental studies of Gerassimow ('04), beginning as early as 1890, that the size of a cell bears a relation to the size of its nucleus. By subjecting the filaments of *Spirogyra* during cell division to low temperature, or treating them for a short time to the anaesthetic influence of ether, chloroform, or chloral hydrate, he showed that the portions of a partially divided nucleus may unite to form a single large nucleus, and that the cell increased in size. This line of investigation was later taken up by Nĕmec in a series of studies on root-tips, the results being brought together in his recent work of 1910. When growing root-tips are immersed in solutions of certain drugs, chloral hydrate being a favourite reagent, partially divided nuclei may be made to fuse, with the result that tetraploid (syndyploid) nuclei are formed having double the normal number of sporophytic chromosomes. The effect of the reagent is to disorganize the achromatic portions of the mitotic figure, or otherwise disturb their functions, so that the movement of the divided chromosomes towards the poles is arrested, and they are gathered together to form a single nucleus.

Of especial interest is Nĕmec's claim that the tetraploid number of chromosomes in some cases returns to the normal number by a heterotypic mitosis. This view is based chiefly on the peculiar appearances of the chromosomes, suggesting the diads and tetrads of the reduction division. Strasburger and others, after failing to confirm Nĕmec's observations on the 'heterotypic mitosis', have criticized his conclusions on general grounds. Miss Kemp ('11), however, has found in chloralized root-tips of the pea, an especially favourable subject, figures very similar to those of the heterotypic mitosis, but her interpretations are not those of Nĕmec. Although the chromosomes have the X, Y, and tetrad forms suggestive of the reduction division, Miss Kemp believes on good evidence that these peculiarities are due to the direct action of the drug.

The results and the interpretations involved in the investigations

mentioned above may have important bearings on the problem of the origin of such a type as *gigas*, for the origin of *gigas* was possibly concerned with the appearance of a tetraploid condition in the nuclei of *Lamarckiana*, and suggests an interesting line of experimental study upon this latter form.

It is of interest to compare *gigas* with *Lamarckiana* in view of the fact that the only cytological difference so far known concerns simply the presence in the former type of a double set of the same chromosomes which are present in the latter. *Oenothera gigas* is conspicuously larger and more robust in almost all of its parts than its parent *Lamarckiana*. The seeds and seedlings are larger; the rosettes are frequently much larger; the stems are thicker and stronger; the foliage leaves are broader; the buds are larger, as are all of the flower parts; the capsules are much thicker, although proportionately somewhat short. The height of *gigas* is, however, generally not so great as, or no greater than, that of *Lamarckiana*. The differences between the two forms thus concern the relative proportions of the organs. There are no obvious characters present in one plant that are not found in the other, and this would be expected since the inheritance is through chromosomes which are qualitatively similar.

Nevertheless, while the differences between *gigas* and *Lamarckiana* may depend chiefly on the greater vegetative vigour or luxuriance of the former type, it is clear that the expression varies in the different organs, since the results are not uniformly proportionate throughout the organization of *gigas*. That is to say, *gigas* is not a giant form of *Lamarckiana* proportionately larger in all of its parts, but it has distinct characters of its own due to different comparative relations in the form and measurements of its organs. *Gigas*, therefore, does not bear the same relation to *Lamarckiana* as the giant form of *Primula sinensis* seems to bear to the ordinary form, from which it appears to differ simply in the greater size or luxuriance of its parts. In this case as reported by Gregory ('09), the giant has the same number of chromosomes as the smaller parent form, but the cells and nuclei in the tissues studied were found to be slightly larger. These differences in size were relatively very small as compared with those between the nuclei and cells of *gigas* and *Lamarckiana*, and the two cases are evidently not of the same class. The large form of *Primula sinensis* is apparently a true giant variety of the parent type, while *gigas* is a progressive mutant, its peculiarities being clearly sufficient to separate it as a distinct species from *Lamarckiana*.

The conclusion seems unavoidable that *gigas* arose with its peculiarities fully developed as the result of profound changes in the germ-plasm of its parent *Lamarckiana*, and as far as cytological evidence goes, the doubling of the chromosome count was the most obvious cause of the changes. In this germinal modification lay the basis for the sudden appearance of a sharply marked mutant more distant from any possible parent wild species

(assuming *Lamarckiana* to be of hybrid origin) than from *Lamarckiana* itself. Furthermore, the trend of evolution in *gigas* is distinctly progressive as far as evidence is furnished by the greater size of its organs and vegetative vigour. There is apparently none of the retrogressive variation so conspicuously shown in almost all of de Vries' 'mutants' in which one or more characters of *Lamarckiana* have dropped out or are presented in reduced form in the 'mutants'. The remarkable rarity of *gigas* as a derivative from *Lamarckiana* should not, however, be forgotten in the acceptance of its being representative of a true mutant.

CYTOLOGICAL DISCUSSION.

Including the results presented in this paper, the writer has published accounts of the reduction divisions in four species of *Oenothera*, viz.: *grandiflora* (Davis, '09), *biennis* (Davis, '10), *Lamarckiana*, and *gigas*. The material of the last three species was from strains that had been bred in 'pure lines' for two or more generations; the material of *grandiflora* was from plants grown from wild seeds. In addition to these species the history of the reduction divisions of *O. muricata* has also been traced, but this history agrees so closely with the account of *biennis* that a description would be scarcely more than repetition. From these studies we are perhaps in a position to take a general survey of the conditions presented by the *Oenotheras* in relation to certain problems of cytology. It is, however, probable that important evidence on some of the chief difficulties will come from the investigation of certain *Oenothera* hybrids which will be the next step in the writer's studies, and this brief review must be, therefore, somewhat tentative in character.

The attention which in recent years has been devoted to the study of the chromosome history during interkinesis is rapidly giving important results. The work of Overton ('05, '09), Rosenberg ('04, '05, '09), and Lundegard ('09, '10) upon a variety of plants has placed the prochromosome theory in a strong position. The last statement of this view by Overton (Science, vol. xxxiii, 1911, p. 193) reports that the chromosomes following a mitosis form by alveolization independent reticula in the resting nucleus which touch one another but do not anastomose, and which during the prophase of mitosis by condensation become again transformed into chromosomes. My studies on *Oenothera* seem to favour the above view, although I have as yet been unable to trace the outlines of the chromosome reticula in the mid-period of the resting nucleus. The chromosomes may readily be followed in the process of alveolization for a considerable time following the mitosis and chromatic bodies, or prochromosomes, may be recognized at a stage during the organization of the dense chromatic network which precedes the differentiation of the spireme, when their boundaries again become lost. There is, however, a mid-period

of the resting nucleus when the chromatic material is distributed over a network so delicate as so far to defy morphological analysis, but such negative evidence in view of the minuteness of the structures in *Oenothera* has, in the writer's opinion, little or no value.

The history of the chromosomes in *Oenothera* during the interkinesis between the heterotypic and homotypic mitoses presents very important evidence in favour of the theory of the individuality of the chromosomes of which the conception of chromosome centres or prochromosomes in the resting nucleus forms a part. The writer knows of no plant types in which the chromosomes may be more easily traced through this short resting period than in *Oenothera*. The paired chromosomes characteristic of this interkinesis may be followed with absolute precision, and this stage affords perhaps the most favourable opportunity in the entire life-history of making the chromosome count. The process of expansion following the anaphase of the heterotypic mitosis increases the size of the chromosomes, but alveolization does not set in to such a degree as to obscure their outlines, although there are sometimes slight tendencies to form reticula such as are conspicuously developed in many plants (e.g. *Lilium*) at the same stage. It is possible that the period of the interkinesis is too short to allow of the complete vacuolization of the chromosomes, or perhaps their size and form make their modification more difficult. As a result the conditions usual in the mid-period of a resting nucleus have apparently been dropped out of this interkinesis in *Oenothera*; the process of alveolization never proceeds far, the chief change of chromosome form being a slight enlargement followed shortly by the condensation in preparation for the homotypic mitosis.

The most difficult periods of the reduction processes as regards both observation and interpretation are those which lead to the differentiation of the chromosomes in preparation for the first, or heterotypic, mitosis. These periods include the synaptic and presynaptic stages leading to the condition called (when present) 'diakinesis', and the peculiar phenomenon of the 'second contraction'. It has become necessary to define one's usage of the term 'synapsis' since McClung ('05) has proposed the restriction of the term to the 'fusion of simple chromosomes into multiple ones usually of bivalent value'. Chromosomal associations are the exception rather than the rule in the *Oenotheras*, an important peculiarity which will be discussed presently. McClung has proposed the term 'synizesis' for 'the condition of the nucleus in which the chromatin is found massed at one side of the vesicle, without regard to whether it is a normal phenomenon or not'. Without questioning the value of McClung's discrimination of the stages in the Orthopteran material studied by him there does not seem to the writer sufficient reason for the limitation in plant cytology of the term 'synapsis' to chromosomal fusions, since such phenomena, even

when present, are not the conspicuous stages of chromatic contraction which are so well known to botanists from their apparently universal presence in higher plants. For these reasons the writer continues to use the term 'synapsis' for that characteristic and relatively long-enduring contraction of the chromatic reticula or thread systems that appears previous to the differentiation of the spireme of the heterotypic mitosis.

There is no question in the writer's mind but that the events of synapsis are the result of a true contraction, i. e. a shortening and thickening of the strands or threads of the chromatic reticulum. Lawson ('11) has recently expressed the view that in the phase of synapsis 'there is no contraction whatever of the chromatic substance', but that the appearance of a contraction is the result of an enlargement of the nuclear cavity due to a growth which carries the nuclear membrane away from the chromatic mass. This view can fortunately be readily tested as to the facts. It will be admitted that there is frequently marked nuclear growth throughout the period of synapsis which would undoubtedly emphasize a contraction of the chromatic material, but can such growth account for the differences, in some cases very great, between the size of the nuclear cavity and its contained synaptic knot? It is a simple matter to measure the size of the nucleus and of the chromatic mass in optical section through the various stages of synapsis. Lawson holds that the chromatic mass remains essentially unchanged in its volume and presents evidence for this view in a series of figures of *Smilacina*. These figures, however, are to the writer not convincing, inasmuch as the chromatic mass of advanced synapsis (Pl. LXXI, Figs. 10-17) may be included in a circle having the diameter of the presynaptic nucleus (Figs. 1 and 2) with considerable space left unfilled. An examination of the figures of any of the species of *Oenothera* studied by the writer will show the outlines of the chromatic mass very much smaller and consequently denser during the synaptic stages than in the full-sized presynaptic nuclei, and it is his opinion that Lawson's view will not be sustained by the numerous accounts of synapsis on various material.

The relations among the strands that compose the chromatic reticulum previous to and during the synaptic contraction have so far baffled my studies. There is apparently in their arrangement no system or order of especial significance. In such a complicated and dense mass of elements (see figures) it is not difficult to find threads that run closely parallel for greater or less distances, but the writer is not willing on the evidence at hand to attach significance to these relations. It has not been possible to recognize even the elements of a simple spireme and much less conditions that might be interpreted as indicating the presence of two parallel spiremes of paternal and maternal origin. Fortunately the later history of the reduction processes clearly shows that the entire group of sporophytic (somatic) chromosomes is formed end to end (telosynapsis) by the seg-

mentation of a univalent spireme, and there is no place evident in the reduction processes of *Oenothera* for the parallel conjugation of two spiremes (parasynapsis).

This can be said respecting the results of the synaptic contraction. From the dense reticulum of the presynaptic stages there is differentiated a thread system, and during the process the threads are materially thickened and the length of the system is apparently much shortened. This history may indicate nothing more than a process of condensation and contraction of the chromatic elements of a thread system such as is found during every mitosis among higher plants, in which case synapsis may have little significance except as associated with the peculiar conditions surrounding the growth and activities of spore mother-cells. On the other hand the actual results of the synaptic contraction would certainly bring prochromosomes or other chromatic elements into as intimate a relation or proximity, short of an actual homogenous mixing of substance, as could well be imagined by the most enthusiastic advocate for transfers or mutual interactions of idioplasms. The long period in which the nucleus lies in a state of synapsis also favours the importance of the process as one of a fundamental nature. In the history of reduction in the *Oenotheras* there is apparently no phase when the chromosomes or their representatives (chromatic centres or prochromosomes) are in such intimate association and for so long a time as during mid-synapsis.

We are now ready to pass to another phase of the history of reduction that also presents serious difficulties of observation and interpretation, and which must be considered with reference to later events when the chromosomes become differentiated. This puzzling phase comprises the history from the period of mid-synapsis, characterized by the fully contracted synaptic knot, to the formation of the mature spireme which segments into the full number of sporophytic (somatic) chromosomes. With the loosening of the synaptic knot there emerges a thread system the elements of which are markedly thicker than the chromatic strands of the early and mid-phases of synapsis. These conditions are clearly shown in Figs. 15 and 16 for *grandiflora* (Davis, '09), Figs. 9 and 10, 42 and 43 for *biennis* (Davis, '10), Figs. 10 and 11 for *Lamarckiana*, and Fig. 55 for *gigas*. With the thickening of the threads there also takes place a shortening of the thread system, and there can be little doubt that the two processes are the result of the general condensation and contraction of the chromatic material throughout the period of synapsis. The next developments which lead to the differentiation of the mature spireme are more difficult to follow, for the threads take on irregularities of form and thickness which might suggest various interpretations to investigators who have reported the stages of double threads (strepsinema) in certain material. The writer, however, for reasons stated below, believes these peculiarities

to be simply features of a continued chromatic condensation and contraction which transforms the system of thickened threads into the spireme.

Grégoire ('10, pp. 325 and 387) has expressed the opinion that the 'strepsinema' stage will be found in *Oenothera* exhibiting the characteristic longitudinal division or doubling of the spireme, which is so important a feature of his views on a side-by-side association of chromosomes (parapsynapsis) leading up to diakinesis. He believes that the chromosomes will be found to arise side by side in pairs as a result of this longitudinal doubling of the spireme. Grégoire, then, suggests that the arrangement of the chromosomes in *Oenothera* in a chain is a later development due to the separation at one end of the chromosomes in each pair during diakinesis and the secondary union of the free ends of the pairs to form a chain not of $2x$ independent sporophytic chromosomes but of x pairs of chromosomes. This ingenious interpretation is intended to bring certain peculiarities of chromosome formation and arrangement in *Oenothera* into line with Grégoire's well-known theory, which he believes to be of general application throughout the animal and plant world.

The difficulties confronting Grégoire's interpretation described above seem to the writer insurmountable for the following reasons:—

First. With respect to the segmentation of the spireme, this is one of the most evident and direct processes in the entire history of reduction phenomena in *Oenothera*. It is illustrated in Pl. LXXI, Figs. 11–16 for *biennis* (Davis, '10), Figs. 13–17 for *Lamarckiana*, and Pl. LXXII, Figs. 57–59 for *gigas*. The spireme is not complexly coiled, the stages of segmentation are easily found, the form of the chromosomes is so simple that no especial difficulty is offered to the study of their arrangement, and the usual diploid (sporophytic) number, fourteen (twenty-eight in *gigas*), is so small that they may be counted with accuracy. It is perfectly clear that the spireme segments into the full set of $2x$ (diploid) chromosomes arranged end to end and not into x pairs of chromosomes arranged side by side. There is to the writer no doubt of the facts of the case or of the interpretation that these constitute the full set of sporophytic chromosomes formed end to end by the segmentation of a univalent and not a double spireme.

Second. There is no general pairing of the chromosomes in *biennis*, *Lamarckiana*, *rubrinervis*, or *gigas*. The occasional association in these species of chromosomes to form ring-shaped pairs, whether united at one or both ends, comes about through the approximation of chromosome segments forming the two sides of loops in the spireme. The exceptional conditions in certain material of *grandiflora* (Davis, '09, pp. 558 and 559), where seven ring-shaped pairs of chromosomes are formed, result from the presence of a spireme composed of complexly coiled loops which segment in such a manner that seven loops (later rings) are generally differentiated,

each composed of two chromosomes. It is to be expected that different material of *grandiflora* will show deviations from these conditions approaching more closely to those of the other species, since the differences depend on the degree of complication in the arrangement of the portions of the spireme.

It is best to approach the interpretation of the synaptic processes in *Oenothera* from the stage of the segmented spireme. We are, in the writer's opinion, on safe ground in our understanding of this condition as the segmentation of a univalent spireme into the full set of sporophytic chromosomes (diploid), and any explanation of the subtle events of synapsis must accord with this view. Should it be established that the spireme splits longitudinally before its segmentation, and that this fission is quickly closed, the most probable explanation of such a phenomenon would be the very early appearance of the division which is effected during the anaphase of the heterotypic division in preparation for the homotypic mitosis. But, as previously noted, the writer has found no evidence that such a division takes place or that there is a fusion of parallel spiremes. There is then left the interpretation of synapsis as a long-continued condensation of the chromatic elements from the stages of the presynaptic reticulum to the spireme which develops from the thickened threads that emerge from the synaptic knot, the stage of mid-synapsis (synaptic knot) being characterized by the very close association of the chromatic elements. It is possible that the above view may be modified by the study of the reduction phenomena in *Oenothera* hybrids, but the evidence so far seems in its favour.

With respect to the relations between the chromosomes in the spireme it is possible that they are alternately of paternal and maternal origin, or they may be arranged merely by the law of chance. On this point we shall probably have little or no evidence in *Oenothera* until suitable hybrids have been studied not only in the F_1 generation but also through selected forms of the F_2 and perhaps later generations. Whenever the chromosomes are associated in pairs during diakinesis it is evident that the members of a couple were adjacent chromosomes on the segmented spireme, but the phenomenon of pairing is relatively uncommon in the *Oenotheras*, and it is unsafe to lay emphasis on these conditions. Usually the chromosomes are distributed without apparent order during diakinesis, which in consequence is not so striking a stage in *Oenothera* as in those types where a regular pairing of the chromosomes obtains. The apparent lack of system in the arrangement of the chromosomes during diakinesis, however, may not be real since physiological conditions may determine a very precise distribution of the chromosomes by the heterotypic mitosis.

The phenomenon of the 'second contraction' which takes place during the segmentation of the spireme and the condensation of its portions to form the sporophytic chromosomes is frequently a clearly defined phase of the

reduction processes in *Oenothera*. Some writers have interpreted this stage as one which brings the chromosomes into close association in pairs in preparation for their distribution by the heterotypic mitosis, or even results in their temporary fusion during the period of diakinesis. The second contraction in *Oenothera* gives no support to these views, since, as pointed out, there is no general pairing of the chromosomes (certain material of *grandiflora* excepted), and little or no opportunity for close associations. The period in *Oenothera* when the chromatic elements are in their most intimate relations to one another is that of mid-synapsis, when the thickened threads are so closely tangled and massed that contact would seem almost certain, although this need not of course involve the actual fusion of idioplasms. The second contraction in *Oenothera* may then have no further significance than the assembling of the segments (sporophytic chromosomes) of a spireme so complexly looped that its parts are necessarily drawn closely together by the continuation of the general process of condensation which operates throughout the entire phase of synapsis and ends only when the mature chromosomes are ready for distribution on the equatorial plate of the heterotypic spindle. This interpretation on the evidence at hand seems to the author the most reasonable for this stage in the *Oenotheras*.

Finally we must emphasize the conclusion, also stated in the author's previous papers, that the sporophytic (somatic) chromosomes of *Oenothera* are formed end to end by the segmentation of univalent spiremes which follow synapsis. In this conclusion Gates, Geerts, and the writer are in full accord. There seems to be no possibility of interpreting the spireme as bivalent, i. e. composed of two spiremes (paternal and maternal) united side by side. Even should it be established that the spireme in earlier stages of developments shows indications of a doubling or lengthwise fission (a strepsinema stage, of which the writer has seen no clear evidence), such a fission could have no relation to the hypothesis of the conjugation of parallel spiremes, since the chromosomes in the full diploid number are formed from as many segments of the spireme and are not formed in pairs from a haploid number of spireme segments. Such a doubling of the spireme (if present) might be related to the lengthwise fission of the chromosomes which appears during the anaphase of the heterotypic mitosis in preparation for the homotypic division, but it is difficult to see any other probable interpretation of importance. Thus it appears impossible to bring the facts into accord with the hypothesis of a side-by-side pairing of the chromosomes through the association of two parallel spiremes or two parallel sets of chromosomes as held by Grégoire, Allen, Rosenberg, Overton, Stomps ('10), and their followers. The results from the studies on *biennis*, *grandiflora*, *Lamarckiana*, *rubrinervis*, and *gigas* strongly support the view of Farmer and Moore, Mottier, Digby ('10), and others of an end-to-end

relation of the chromosomes when first formed by the segmentation of a univalent spireme.

SUMMARY.

1. Chromatic bodies, probably representing chromosome centres or prochromosomes, may be recognized in the nuclei shortly after the last mitoses in the archesporium when the sporophytic chromosomes by alveolization have developed delicate nuclear reticula.

2. Shortly before synapsis the nuclear reticula become so dense in structure through the development and thickening of numerous strands that the chromatic bodies can no longer be distinguished, their substance apparently merging with that of the deeply staining networks.

3. The advent of synapsis is indicated by the slow contraction of the reticula which carries most of the strands towards the centre of the nuclei. During this process the threads become more evident in the reticula and come to lie in confused and complicated coils tightly gathered together to form the synaptic knot from which a few delicate strands and loops extend into the nuclear cavity. These threads appear at times to run closely parallel with one another, but the evidence does not justify an assumption that this relation indicates more than such a close association as would be brought about by the gathering together of a complexly looped thread or system of threads.

4. The synaptic knot, marking the period of mid-synapsis, generally lies close to the nucleolus, and is a clearly defined stage extending over a considerable period (perhaps one or more days). This is the time in the reduction processes of *Oenothera* when the chromatic elements (prochromosomes) are in their most intimate relation to one another. The results of the synaptic contraction are an evident thickening of the threads and shortening of the thread system.

5. Following the period of mid-synapsis, the chromatic material emerges from the synaptic knot by a loosening of the coils of the contracted thread system. It then becomes evident that the threads have continued to thicken and shorten although still complexly looped and coiled. From this condition is developed by further condensation the very much thicker thread that becomes the univalent spireme. Although irregularities in the form and thickness of this spireme may be observed during its development, no clear evidence has been noted that it ever becomes doubled by a lengthwise fission to give a 'strepsinema' stage.

6. A segmentation of the spireme gives rise to the full set of sporophytic (somatic) chromosomes arranged end to end. These numbers, fourteen in *Lamarckiana* and twenty-eight in *gigas*, are the diploid numbers for these species respectively. The spireme segments are always in the diploid number, which precludes the possibility of their being bivalent in

nature. The chromosomes take on their form and proportions by a shortening of the spireme segments marking the end of the long process of chromatic condensation which began in presynaptic stages.

7. The condensation of the spireme segments results in a further shortening of the spireme during which much of its complicated looped arrangement disappears. Associated with this condensation is a second contraction during which the segments of the spireme or chains of chromosomes are frequently drawn closely together in a cluster. It is doubtful whether this stage is of especial significance since the chromosomes are not regularly grouped in pairs, and it may be merely the result of the final condensation of the chromatin previous to the differentiation of the fully developed chromosomes.

8. The chromosomes separate during the prophases of the heterotypic mitosis, but, although adjacent chromosomes sometimes form rings, there is no general pairing of the chromosomes characteristic in some forms of the stage of diakinesis. The chromosomes by contracting and bending in the middle regions become V-shaped; they are all essentially similar to one another.

9. When grouped in pairs it is clear that such alternate (adjacent) chromosomes on the spireme are separated by the heterotypic mitosis, but the generally scattered arrangement makes it impossible to determine whether alternate chromosomes are always so distributed. The absence of a conspicuous stage of diakinesis with the chromosomes grouped in pairs makes the determination of this important point very difficult in *biennis*, *Lamarckiana*, and *gigas*.

10. The heterotypic mitosis is a reduction division distributing the fourteen chromosomes of *Lamarckiana* and the twenty-eight chromosomes of *gigas* in two sets of seven and fourteen chromosomes each for the respective species.

11. A lengthwise fission of each chromosome in preparation for the homotypic mitosis becomes apparent during the anaphase of the heterotypic division.

12. Thus seven split chromosomes in *Lamarckiana* and fourteen split chromosomes in *gigas* enter the nuclei of the interkinesis between the heterotypic and homotypic mitoses and may be followed as pairs of chromosomes throughout the period of the interkinesis.

13. The homotypic mitosis is an equational division distributing the members of the pairs of chromosomes present during the interkinesis.

14. Irregularities in the distribution of the chromosomes are not uncommon, and may account for a portion of the sterility of the pollen in these forms, but it seems probable that other factors of a physiological nature are operative.

15. The pollen mother-cells and their nuclei and also the pollen-

grains of *gigas* are much larger than those of *Lamarckiana*, thus supporting the view that in related types the size of the cells and nuclei are proportionate to the number of chromosomes.

16. *Oenothera gigas* is a progressive mutant, its peculiarities being clearly associated with the changes in its germ-plasm incident upon the doubling of its chromosome number.

CAMBRIDGE, MASSACHUSETTS,
March, 1911.

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EXPLANATION OF FIGURES IN PLATES LXXI-LXXIII.

Illustrating Professor Davis's paper on the Reduction Divisions of *Oenothera Lamarckiana* and *Oenothera gigas*.

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. Sections cut 6 μ thick, and stained with iron-alum haematoxylin.

PLATE LXXI.

Figs. 1-31, *Oenothera Lamarckiana*.

- Fig. 1. Telophase following a mitosis in an archesporial cell, chromosomes still evident.
 Fig. 2. Resting nucleus in a pollen mother-cell showing large nucleolus, chromatic bodies, and delicate reticulum.
 Fig. 3. The chromatic bodies are no longer easily recognized in the denser reticulum.
 Fig. 4. Nucleus filled with a dense reticulum shortly before the advent of synapsis; chromatic bodies no longer distinguishable.
 Fig. 5. The beginning of the synaptic contraction, two nucleoli.
 Figs. 6 and 7. The synaptic contraction well under way, the threads and meshes of the reticulum becoming drawn into a close mass.
 Fig. 8. Two pollen mother-cells, their nuclei in mid-synapsis.
 Fig. 9. A closely contracted synaptic knot.
 Figs. 10 and 11. The thickened threads which emerge with the loosening of the synaptic knot.
 Fig. 12. Further shortening and thickening of the threads to form the spireme.
 Figs. 13 and 14. Spireme with constrictions which show that the process of segmentation has begun.
 Figs. 15 and 16. Segmented spiremes, the chromosome segments for the most part clearly arranged end to end to form a chain.
 Fig. 17. A segmented spireme in a somewhat complicated looped arrangement.
 Fig. 18. Two chromosome segments in the form of a ring separated from the main group which is apparently in the stage of the 'second contraction'.
 Figs. 19 and 20. These illustrate the stage of 'second contraction'. The segments of the spireme are gathered in a close mass that bears a superficial resemblance to the synaptic knot.
 Fig. 21. Another form of the 'second contraction', the spireme segments more loosely associated.
 Fig. 22. Early prophase of the heterotypic mitosis. The nuclear membrane has disappeared and the nucleolus is no longer present; fourteen chromosomes could be counted, most of them still clearly arranged end to end.
 Figs. 23 and 24. Multipolar spindles; the chromosomes closely clustered as though still in the condition of the 'second contraction'.
 Figs. 25 and 26. The spindles have become bipolar. Most of the chromosomes show the end-to-end arrangement; fourteen may be counted in Fig. 25.
 Fig. 27. Metaphase of the heterotypic mitosis. The chromosomes, now bent in the form of thickened V's, show no close association in pairs.
 Fig. 28. Anaphase of the heterotypic mitosis. Seven chromosomes are present in each of the two sets which pass to the poles of the spindle.

Fig. 29. A group of seven chromosomes, still undivided, at the pole of a heterotypic spindle.

Fig. 30. An exceptional case in which eight split chromosomes, instead of the normal number seven, are clearly present at the pole of a heterotypic spindle.

Fig. 31. Early telophase of the heterotypic mitosis showing the seven split chromosomes; nucleoli have not yet appeared.

PLATE LXXII.

Figs. 32-45, *Oenothera Lamarckiana*.

Fig. 32. Pollen mother-cell following the heterotypic mitosis.

Fig. 33. Resting nucleus of the interkinesis between the heterotypic and homotypic mitoses. The seven pairs of chromosomes are shown, mostly in the form of U's joined in the bent middle region.

Figs. 34 and 35. Multipolar spindles in the prophase of the homotypic mitosis. The seven pairs of chromosomes now appear in a much more condensed form than during the interkinesis.

Fig. 36. Metaphase of the homotypic mitosis; seven pairs of chromosomes at the equatorial plate.

Fig. 37. Early anaphase of the homotypic mitosis; the chromosomes in two sets, seven in each group.

Figs. 38 and 39. Late anaphase and early telophase of the homotypic mitosis. The seven chromosomes at each pole exhibit irregularities of form.

Fig. 40. Pollen mother-cell in telophase of the homotypic mitosis; the seven chromosomes distinct in each nucleus.

Figs. 41 and 42. Telophases of the homotypic mitosis. The chromosomes are elongating and becoming irregular in form preparatory to the development of the chromatic network.

Fig. 43. Nucleus of a young pollen-grain, showing the open reticulum with certain deeply staining regions that probably represent chromosome centres or prochromosomes.

Fig. 44. Pollen mother-cell in which there was an irregular distribution of the chromosomes during the homotypic mitoses, resulting in the formation of two small extra nuclei each containing three chromosomes.

Fig. 45. Young pollen-grain with a small extra nucleus, the result of an irregularity in the homotypic mitosis similar to the sort illustrated in Fig. 44.

Figs. 46-63, *Oenothera gigas*.

Fig. 46. Telophase following a mitosis in an archesporial cell; chromosomes still evident. Compare with Fig. 1.

Figs. 47 and 48. Resting nuclei in pollen mother-cells, showing large nucleoli, chromatic bodies, and delicate reticula.

Figs. 49 and 50. Nuclei filled with dense reticula, chromatic bodies no longer distinguishable.

Fig. 51. Nucleus shortly before the advent of synapsis. Compare with Fig. 4.

Fig. 52. The synaptic contraction well under way, the threads and meshes of the reticulum becoming drawn into a close mass.

Figs. 53 and 54. Closely contracted synaptic knots. Compare with Figs. 8 and 9.

Fig. 55. The thickened threads which emerge with the loosening of the synaptic knot. Compare with Figs. 10 and 11.

Fig. 56. Further shortening and thickening of the threads to form the spireme. Compare with Fig. 12.

Fig. 57. A spireme just previous to the process of segmentation.

Fig. 58. Spireme with constrictions which show that the process of segmentation has begun. Compare with Fig. 14.

Fig. 59. A segmented spireme; the chromosome segments for the most part clearly arranged end to end in the form of a chain.

Figs. 60-62. Examples of the 'second contraction'. Segmentation has just begun in the spireme of Fig. 60. Compare with Figs. 18-20.

Fig. 63. Another form of the 'second contraction'; the spireme segments are more loosely associated. Compare with Fig. 21.

PLATE LXIII.

Figs. 64-79, *Oenothera gigas*.

Fig. 64. Early prophase of the heterotypic mitosis. Nineteen chromosomes are shown, some of them evidently paired; the remaining nine chromosomes were present in the adjacent section. The nuclear membrane has disappeared and fibrillae have entered the nuclear cavity.

Fig. 65. A multipolar spindle. Some of the chromosomes are still arranged end to end.

Fig. 66. Metaphase of the heterotypic mitosis. Fourteen chromosomes were present in each group at the equatorial plate. Compare with Fig. 27.

Fig. 67. Anaphase of the heterotypic mitosis. Fourteen chromosomes in each of the two sets which pass to the poles of the spindle, some of the chromosomes showing the fission in preparation for the homotypic mitosis. Compare with Fig. 28.

Fig. 68. Anaphase with split chromosomes at the poles of the spindle.

Fig. 69. An irregularity in the heterotypic mitosis. Two chromosomes have become separated from each of the main groups at the poles of the spindle.

Fig. 70. A condition following such an irregularity in the heterotypic mitosis as that shown in Fig. 69. The appearance of the chromatin indicates that such nuclei pass into a resting condition.

Figs. 71 and 72. Resting nuclei of the interkinesis between the heterotypic and homotypic mitoses. The fourteen pairs of chromosomes are shown, mostly in the form of U's joined in the bent middle region. Compare with Fig. 33.

Fig. 73. Early prophase of the homotypic mitosis; fourteen pairs of chromosomes. The nuclear membrane has disappeared.

Fig. 74. Metaphase of the homotypic mitosis. The chromosomes are now in a much more condensed form than during the interkinesis.

Fig. 75. Early anaphase of the homotypic mitosis. Compare with Fig. 37.

Fig. 76. Late anaphase of the homotypic mitosis.

Fig. 77. Telophase of the homotypic mitosis, the fourteen chromosomes beginning to show irregularities of form.

Fig. 78. Pollen mother-cell in telophase of the homotypic mitosis. Compare with Fig. 40.

Fig. 79. Nucleus of a young pollen-grain, showing the open reticulum.

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Davis, del.

DAVIS — REDUCTION DIVISIONS OF *OENOTHERA LAMARCKIANA* AND *O. GIGAS*.

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Huth, lith et imp

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Davis, del.

Huth, lith. et imp.

DAVIS — REDUCTION DIVISIONS OF OENOTHERA LAMARCKIANA AND O. GIGAS.

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