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THE REDUCTION DIVISION IN THE MICROSPOROCYTES OF *OENOTHERA BIENNIS*.*

BLANCHE MCAVOY.

While making a study of the reduction division in *Fuchsia* (8) it became necessary to review the literature on the *Oenotheras*. Finding that Geertz (7), Gates (3, 4, 5 and 6), and Davis (1 and 2), did not entirely agree among themselves and finding also that my study of *Fuchsia* (8) did not agree in all respects with that of any of the investigations on the evening primrose, I also became interested in the problem presented by the reduction division of *Oenothera*.

Geertz (7) describes the threads occurring in the early stages of *Oenothera lamarckiana* as being irregular in thickness and containing small discs of chromatin. He calls the contraction stage synapsis and speaks of loops extending out from the contracted knot. He says the fully formed chromosomes are found immediately after the contraction and that the bivalent chromosomes are produced by a pairing of univalent chromosomes, but he does not find a conjugation of two threads during the contraction. He also observes a longitudinal splitting of the chromosomes just after the transverse split occurs.

Gates has made various studies of the *Oenotheras* namely *O. rubrinervis* (4), *O. lata* x *O. gigas* (6), *O. lata* x *O. lamarckiana* (3), and *O. gigas* (5). In his paper on *O. rubrinervis* (4) he insists that the contraction stage is not an artifact but a natural stage leading to synapsis. After the contraction the chromatin material arranges itself in threads which shorten, contract and finally constrict so as to show fourteen univalent chromosomes. These break apart in pairs, each pair fusing together to form a bivalent chromosome. His second paper (6) is a study of the continuity of chromosomes. He claims that there are two methods

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of chromosome formation, one involving a side to side pairing, the other an end to end. He finds a continuous spirem and twelve chromosomes but makes no mention as to how the chromosomes are formed. In *O. gigas* (5) he notes an irregularity in the way homologous chromosomes seem to pair.

Davis first studied the reduction division in *O. grandiflora* (1). In the early sporocyte he describes chromatin material around the periphery connected by delicate strands. These strands thicken by what seems to be a process of absorption of the chromatin bodies and fill the nucleus with a close reticulum. He calls the synzytic contraction synapsis. At the end of the contraction stage the spirem has assumed the shape of seven bivalent chromosomes some of which, he says are linked together. These rings are later pulled apart on the spindle.

In his second *Oenothera* paper on *O. biennis* (2) he calls the dark staining masses found around the periphery of the nucleus prochromosomes. He finds no evidence that they are arranged in pairs, but says whenever there are two together they lie end to end. Later on he finds a spirem out of which is constricted a chain of fourteen chromosomes. He speaks of a longitudinal split which appeared before the heterotypic chromosomes reach the poles.

The buds of *Oenothera biennis* which were used as material for this study were collected west of Cincinnati during the summer of 1912. They were killed in Schaffner's weaker chromacetic acid and run up through the grades of alcohols to absolute. The imbedding was done from chloroform. Sections were cut 10 microns thick and stained. Both Delafield's and Heidenhain's haemotoxylin were used, the Heidenhain's giving the better results. The iron was used for four hours and the stain over night.

In the very young sporocytes (Fig. 1) there is a reticulum on which can be seen an indefinite number of chromatin masses or granules. A little later (Figs. 2 and 3) this chromatin material collects in seven little masses which represent the protochromosomes. In some of the sporocytes these protochromosomes appear double. Their double nature is more easily studied in the preparation than reproduced on paper for the two parts of a single protochromosome can often be seen best by focusing. The masses are so large that on first sight they might almost be taken for the bivalent chromosomes except for the small size of the young sporocyte and the condition of the tapetum. The tapetum in the younger stages has but one nucleus to each cell while in the later phases each tapetal cell has two nuclei. In passing from the younger to the older stages the tapetum retreats from the sporocyte as the sporocyte increases in size and rounds up. The nucleolus is quite distinct and need never be confused

with the chromatin masses since there is a difference in the way the two stain. The protochromosomes are connected by delicate strands.

Figures 4, 5 and 6 show the protochromosomes in various stages of transformation, while their chromatin is apparently being distributed in the form of granules on the spirem. In Fig. 4 there are still six good sized masses although part of the chromatin has already been distributed. Fig. 5 shows four large masses and two small ones with a spirem forming in the cavity. By the time the sporocyte is as far advanced as the one shown in Fig. 7 the spirem is complete and the protochromosomes are entirely gone. All this time the sporocytes are gradually growing larger.

Somewhat later the chromatin material becomes loosened from the nuclear wall and collapses in a mass in the nuclear cavity, but the synizetic knot is never so close as in some species. Figures 8, 9 and 10 show synizesis in different stages. In figure 10 most of the spirem can be plainly seen. The granules along it are easily made out and the whole spirem is looped and twisted. The nucleolus is not confused with chromatin material on account of the differentiation of the stain. The nuclear cavity is enlarged and frequently the cytoplasm is contracted away from the cell wall. The spirem after the synizesis is granular and looped, and can be traced for some distance. (Fig. 11.)

Figure 12 shows a continuous spirem. In the preparation the spirem could be traced throughout its complete distance without a break. In the drawing the nucleolus seems to cover the spirem and obscure its continuity, but in the preparation, by focusing, the spirem could be seen to be complete throughout its entire length. The spirem is distinctly granular and is thrown into loops three of which can not be mistaken and four more can be made out without much difficulty. Figure 13 shows loops while figures 14 and 16 show seven definite loops. In figure 14 one loop is filled up with stain. In the next figure (Fig. 15) five definite loops show and two masses, one smaller than the other. Figure 16 is probably the best figure to show that the spirem is continuous and is thrown into seven definite loops. Two of them have a double twist. The spirem is granular and lies between the nucleolus and the nuclear wall. In figure 13, 14 and 15 the loops are crossed in the center and beneath the nucleolus and so the continuity of the spirem can not be observed. The looping of the thread shows plainly also in figures 17 and 18, but the continuity of the thread can not be seen plainly on account of the nucleolus. The spirem is granular. In these two sporocytes (Figs. 17 and 18) the nuclear wall seems to be disappearing although in most cases the nuclear wall does not go until the chromosomes are formed.

Gates (4) in his paper on *Oenothera rubrinervis* states that the spirem constricts into fourteen chromosomes which break apart in pairs and then form the bivalent chromosomes by a folding together and fusion of the parts of each pair. Davis says there are ring-shaped chromosomes, some of which are linked together in *O. grandiflora* (1). He says these are present as soon as the sporocyte passes out of the synizetic stage. In *O. biennis* (2) he finds a chain of fourteen chromosomes breaking into seven pairs from which seven chromosomes are formed by fusion. This method of chromosome formation of course is essentially the same as that of loop formation, but I have found the loops definitely formed and just as definitely contracting until there are seven chromosomes formed from the seven loops. These results are the same as were found in *Fuchsia* (8). The loops frequently form quite definite rings as is seen in figure 16.

In figure 19, the chromosomes still show something of their ring and loop character and there are two nucleoli shown. The next figure (Fig. 20) shows a certain amount of loose material in the nucleus which may be derived from the nucleolus although there is no direct evidence for this conclusion. The next two figures (Figs. 21 and 22) show the chromosomes broken apart and the cytoplasm flowing into the nuclear space. The nuclear wall has entirely disappeared. In the cytoplasm are seen great numbers of prominent granules. These remain in the cytoplasm throughout the reduction process. Whether these are starch or not was not definitely determined. Figure 23 shows the beginning of the formation of the spindle with the chromosomes being drawn into the equatorial plane. Figure 24 is the mother star stage at the time when the chromosomes begin to be segregated into the univalents. The next two figures (Figs. 25 and 26) do not show the full quota of chromosomes but show the beginning of the true reduction in those that can be seen. The next two drawings (Figs. 27 and 28) represent metaphase stages with the chromosomes half way to the poles. Figures 29 and 30 are daughter star stages. The lower pole of figure 30 shows a slight beginning of the nuclear wall. The seven univalent chromosomes are about half the size of those appearing on the mother star. The number can be easily counted at this stage.

Following this stage the nuclear membrane develops rapidly and the daughter nuclei swell to a much larger size. The chromosomes remain as distinct bodies although there is some distribution of the chromatin material (Fig. 31). Even in the resting condition the chromosomes in the two daughter nuclei remain as seven distinct bodies and there is no real reticulum developed (Fig. 32). At this stage all traces of the spindle have disappeared.

Soon after, the second division begins (Fig. 33) and the chromosomes in the mother star are again distinctly visible as small

bodies of the same general shapes as appear in the first division but much smaller. The tetrad (Fig. 34) appears normal, irregularities not being so abundant as in *Fuchsia*.

SUMMARY.

1. In very early stages of the microsporocytes the chromatin material is scattered throughout the nucleus on a loose reticulum.

2. There are seven protochromosomes formed, some of which show a double nature.

3. These protochromosomes are transformed into a spirem.

4. There is a period of contraction or synizesis during which loops of the spirem project out from the contracted mass. The spirem shows a granular nature.

5. The spirem is continuous and becomes thrown into loops seven of which are shown in many preparations.

6. These seven loops contract until seven separate bivalent chromosomes are formed. About this time the nuclear membrane disappears.

7. The univalent chromosomes remain as seven distinct bodies in the daughter nucleus and are easily distinguishable until the beginning of the second division.

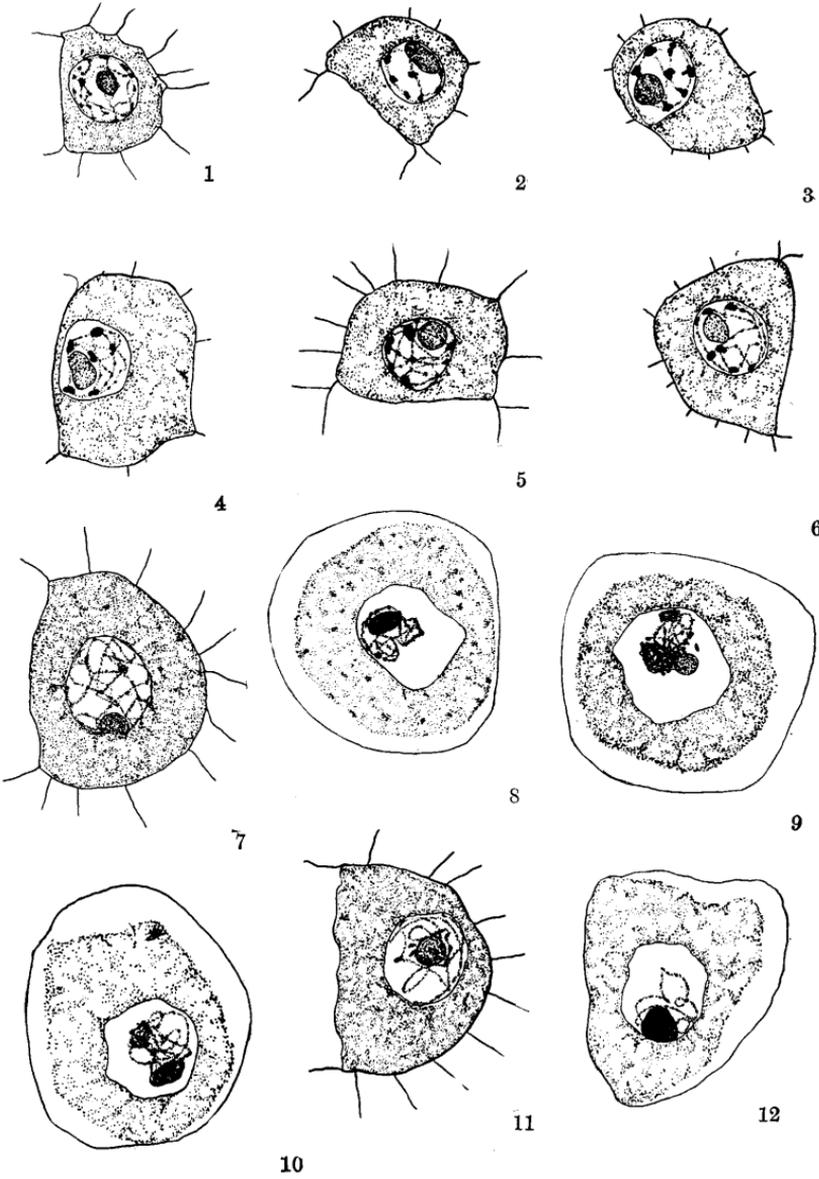
8. The second division follows and results in the formation of normal tetrads. The seven chromosomes are again easily counted in this division although they are much smaller.

LITERATURE CITED.

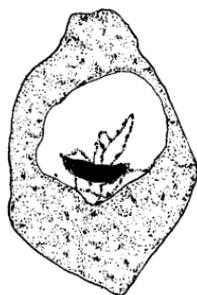
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DESCRIPTION OF PLATES IX, X, XI.

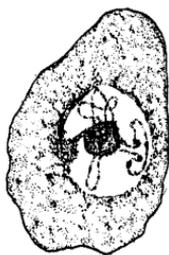
- Fig. 1. Microsporocyte in early stage showing the chromatin material.
Figs. 2, 3. Microsporocytes showing 7 protochromosomes.
Fig. 4. Microsporocyte showing 6 protochromosomes and some reticulum.
Figs. 5, 6. Microsporocytes in which some of the protochromosomes have been used up in the formation of the spirem.
Fig. 7. Fully formed spirem before synizesis.
Figs. 8, 9, 10. Different stages of synizesis.
Fig. 11. Spirem beginning to show a disposition to loop.
Fig. 12. Microsporocyte which shows a continuous spirem that is thrown into loops, three of which are plainly visible.
Fig. 13. Spirem showing loops.
Figs. 14, 15, 16, 17, 18. Microsporocytes showing the spirem thrown into loops.
Fig. 16. Spirem thrown into seven loops, two of which are double.
Fig. 19. Microsporocyte showing the contracted loops which are forming the bivalent chromosomes.
Fig. 20. Bivalent chromosomes still fastened together.
Figs. 21, 22. Microsporocytes showing the seven bivalent chromosomes completely formed.
Fig. 23. Chromosomes being drawn into the equatorial plane.
Fig. 24. Mother star stage.
Figs. 25, 26. Microsporocytes in which the chromosomes are separating.
Figs. 27, 28. Metakinesis stages.
Fig. 29. Daughter star stages.
Fig. 30. Beginning of the formation of the nuclear membrane around the lower daughter nucleus.
Fig. 31. Daughter skein stage in which the spindle has not disappeared, showing the seven daughter chromosomes in each nucleus.
Fig. 32. Daughter nuclei before the second division showing the chromosomes as seven distinct bodies.
Fig. 33. Mother star of the second division.
Fig. 34. Microspore tetrad.



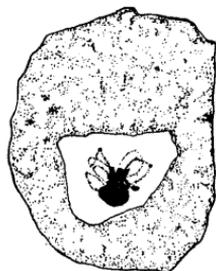
McAvoy on "*Oenothera biennis*."



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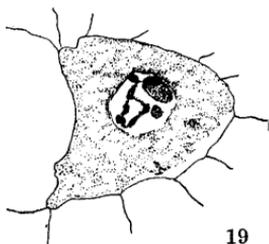
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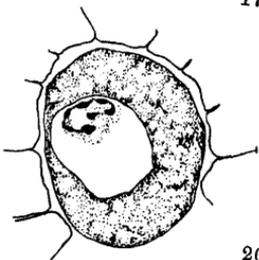
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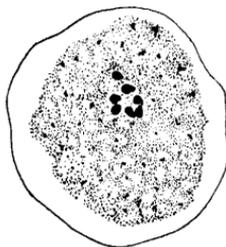
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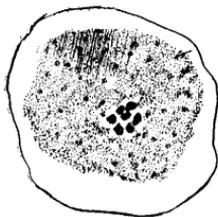
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