A CONTRIBUTION TO THE LIFE HISTORY AND CYTOLOGY OF ERYTHRIONIUM.

CONTRIBUTIONS FROM THE BOTANICAL LABORATORY, OHIO STATE UNIVERSITY. VIII.

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(WITH PLATES IV–IX)

Several years ago, while studying at the University of Chicago, the writer made a special investigation of the reduction nucleus in the ovule of Lilium Philadelphicum, maintaining that a true reducing division occurs in this plant (29). Immediately after the completion of the investigation, search was made for another plant in which to continue the study of reduction, together with other points of interest in the life history. The type finally selected was Erythronium, and both E. albidum and E. Americanum have been studied. The work was carried on for some time at the University of Chicago, and has been continued for the past three years at the Ohio State University. The material was collected mainly near Chicago and in the vicinity of Columbus, but some was also obtained from Kansas. It is exceedingly difficult to procure the earlier stages of the flower, since bulbs with flower buds are very rare when compared with the numerous younger sterile ones.

The usual methods of killing and staining were used; the killing fluids being chrom-acetic acid and Flemming's weaker fluid; and the stains anilin-safranin and gentian-violet, Heidenhain's iron-alum-haematoxylin, Delafield's haematoxylin, and
anilin-safranin and picro-nigrosin. The sections were mostly cut 10, 12, and 18 μ, and stained on the slide.

GENERAL CHARACTERS.

The deeply buried bulbs begin to develop the incept of the flower early in the summer. By the first of September the incipient flower bud is considerably advanced, and the carpels are developing the ovules. Usually before the first of October the single hypodermal archesporial cell can be distinguished, and the integuments are just beginning to make their appearance. The anther wall shows five layers and the pollen mother cells are enlarging. By December first the tetrads are formed, but separation of the four microspores may be delayed for some time later in certain flowers. The cells of the anther are filled with starch grains and the tapetum is still active, some of its nuclei being in stages of direct division. At this time there is no sign of the division of the nucleus of the microspore, but the exine of the wall is developing. The nucleus divides some time between December 1 and April 1, but the time was not ascertained. In the meantime, the archesporial cell in the ovule has been increasing in size and activity, and has formed the continuous spirem from the chromatin network. In this condition it passes the winter. The cell in which the reduction takes place, therefore, has a period of development extending over six months. In some years it cannot be much less than eight months. It will also be observed that while the reduction division in the anther takes place in the fall, in the ovule it is delayed until early spring.

The flowers are growing rapidly long before the frost is entirely out of the ground, and during this time the divisions in the embryo sac occur, so that when the flowers come out of the ground the divisions are usually completed.

Very few flowers appear to develop ovules of any size, and ripe seed is very scarce. In fact I have seen very little during the past three years. Propagation is effected largely by means of the multiplication of the bulbs. About the first of June, at
Columbus, most of the leaves have wilted away. The plant, therefore, is rarely much more than two months above ground.

Erythronium is an ideal example of the retreating bulb. The retreating stems are axillary buds which are carried downward by growth and division of the cells above and beside the apex of the bud. *Fig. 1* is a section of such a young bulb, and the dotted region shows where active cell division is going on. The development of these offsets has been described several times recently, so that it is unnecessary to refer to the subject further.

The deep burrowing is probably not only to place the plant in deep soil, but also to keep the flower protected in the warm earth during winter. The advantage of retreat for nine or ten months underground must be decided, and the causes for the habit complex. The leaves come out before there is any danger of shading from other plants, and before the leaves of the higher stratum of trees shut out the light. The plant is thus well adapted to forest conditions.

**Karyokinesis in the Bulb.**

The division stages in the bulb were studied in order to trace out the development of the spindle. The resting nuclei usually have a rather dense chromatin network with numerous nucleoli imbedded in cavities (*fig. 2*). Often the nucleoli take on fantastic shapes, probably due to budding and division. Some of these are shown in *figs. 3, a, b, c*, etc. Farther up, beyond the division region, the nuclei elongate in the cells of the developing vascular bundles. After the continuous spirem begins to form, two caps of fibers appear on opposite sides of the nucleus, which are the incepts of the future spindle. These are dome-shaped or cone-shaped in appearance, and often end in definite granules around which there is sometimes a system of radiations (*fig. 5*). Similar incipient spindles, but farther advanced, are shown in *figs. 6, 7, and 8*. In *fig. 7* the chromatin granules are plainly visible in the spirem, forming a single chain. *Fig. 9* represents a loose mother skein some time before the formation of the mother star. The spindle at this stage is
sharply pointed and ends in small centrosomes. After the daughter star stage there are sometimes two bodies at each pole, which may represent divided centrosomes (fig. 10). The cells of the bulb are often packed with starch. In some cases the starch is so abundant that the spindles are very much crowded by it (figs. 11, 12). It will be seen from an examination of the figures that the development of the spindle proceeds as in the roots of Allium (30) and Pinus (12). It is never multipolar. This seems to be the normal course of division in vegetative cells, and represents the way in which the spindle is developed during the normal quantitative karyokinesis in the higher plants.

Some time after the appearance of the Cytologische Studien, Němec, studying in the Bonn laboratory, announced that in vegetative cells the spindle is bipolar from the beginning. It is doubtful, however, whether the classification he made of bipolar and polycentric spindles will be found to hold good in general.

THE MALE GAMETOPHYTE.

The nucleus of the microspore probably divides early in the spring, for the pollen grain is well developed before the frost has left the ground. The tube nucleus takes a very light stain and is comparatively small, while the generative nucleus is large and is surrounded by dense-staining cytoplasm which is organized into a cell amoeboid in form (figs. 16-18). These generative cells are very striking in appearance. They can hardly have a cellulose wall because of the peculiar shapes they assume. The dense coat of cytoplasm is very different from that of Sagittaria and Alisma, where there is very little cytoplasm, and it is difficult to see anything but the nucleus.

Fig. 19 shows a germinated pollen grain on the stigma. The tube nucleus and generative cell are still side by side in the body of the grain, but are evidently preparing to pass into the tube. In the tip of the tube there is some dark-staining material which becomes very abundant as the tube elongates, forming dark masses or plugs (fig. 20). The tips of tubes in very favorable preparations showed no definite nuclei (fig. 21). They must be
farther up among the masses of dark-staining material and are thus difficult to identify.

In the liliaceous types the division of the generative nucleus takes place in the tube; in many Helobiae and some other monocotyledons, in the grain before it is shed. In Typha the generative nucleus does not divide in the grain, while in Silphium (22), one of the highest types, the division is in the grain and the sperm cells are elongated and even coiled like spermatozoids. Such elongated sperm cells are also common in Alisma. It appears, therefore, that progressive reduction of the male gametophyte has not been uniform in the various lines of angiosperms. Search was made for the division of the tube nucleus, as is common in certain species of Lilium (8) and Hemerocallis (13), but nothing was found. Such a division probably never occurs in Erythronium.

The style has a large continuous canal, from the stigma to the cavities of the ovulary, for the conduction of the pollen tube. This canal is lined by a layer of glandular cells for the nourishment of the tubes (figs. 13, 14). The pollen tube does not grow through any tissue until after it passes into the micropyle. It is not difficult to see how such an angiosperm could develop from a gymnospermous condition.

THE DEVELOPMENT OF THE MEGASPORANGIUM AND THE REDUCTION DIVISION.

As stated before, the archesporial cell begins to enlarge about the first of October, and by December first the chromatin network is very distinct and is being transformed into the continuous spirem (figs. 22-24). In the following stages the nucleus becomes very large, and the same is true of the chromosomes. This makes Erythronium a favorable subject for the study of these structures. After December the nucleus probably goes into a partial state of rest until early in the spring, at which time development and division continue. During this period it will be convenient to call the cell a megaspore. The spirem is at first very long and slender and the chromatin granules are
never so prominent as in *Lilium Philadelphicum* (figs. 25-25a). In *L. Philadelphicum* the chromatin granules divide and the chromatin band undergoes longitudinal splitting before much shorten-ing and looping take place, but in *Erythronium* the division of the granules seems to be somewhat later, and they do not appear double until the band has twisted into the twelve loops (figs. 26, 27, 28, 37). The granules are large and more or less irregular in shape. A little later the chromosomes appear homogeneous throughout. This would certainly give support to the belief that the division of the chromatin granules is a mechanical contrivance for bringing about the longitudinal division of the linin band, although this might not at all interfere with their supposed function as bearers of hereditary tendencies.

The breaking apart of the twelve loops to form the twelve individual chromosomes (fig. 29) appears to be accomplished by the twisting and contracting movements of the band. The chromosomes are usually of various sizes, some being much larger than others. They often appear as single coiled loops (figs. 37, 38), but usually their double nature can be readily observed. The chromatin loops are not so closely coiled as in *Lilium Philadelphicum*, and when they are arranged in the mother star the twisted condition can still be distinguished. The chromosomes appear to be attached to the spindle threads near the two free ends of the loop, the closed end extending outward (see figs. 39-48, chromosomes before division, and figs. 49-57, chromosomes after division). During metakinesis the loops are uncoiled, and the two free ends are gradually pulled apart until each chromosome breaks in the middle, thus accomplishing a transverse division, one end of the original chromatin loop going to the one pole, and the other to the opposite one. This would be a true reducing division. It is exceedingly difficult to follow out the course of events at this point, and there is always room for doubt as to correct interpretation. There is a possibility that the loops are attached at the closed ends. However, some of the examples are quite convincing, and another than a transverse division seems out of the question (see
especially figs. 46 and 47, also compare the undivided chromosome, fig. 44, with a chromosome in the daughter star, fig. 57). There is not a single example which will not agree with the supposition of a transverse division, while many of the figures could not be explained on the supposition of a longitudinal one. And while it may perhaps be granted that a transverse division has not been absolutely established, it may be said that there is much less evidence in favor of a longitudinal one. In the next division the chromosomes are V-shaped and the longitudinal splittings perfectly apparent (figs. 69, 70).

Although there is no way known to the writer of tracing the origin of the reduction chromosomes in this nucleus to two previous ones, theoretically one might consider it possible that the reduction chromosome represents two normal chromosomes, and the closed loop the point where the usual transverse break should have taken place. Were this the case, the points of attachment of the spindle fibers at or near the two free ends would represent the heads of the two simple chromosomes, and the break at the head of the loop during metakinesis simply the delayed division bringing about the usual number of pieces. But such a process would necessarily result in a qualitative division.

The process here described is essentially the same as that reported for Lilium Philadelphicum, and the interpretation is similar, since it appears to the writer, after a long and careful study of the objects, that no other interpretation seems possible.

On account of the contradictory character of the investigations so far published, it appears that one or the other set of observations has been wrongly interpreted, or else there is more variation in the phenomenon of chromatin reduction than is generally supposed. There may not be so much uniformity in the manner in which reduction is brought about as our present ideas in regard to the nature of chromatin seem to demand; and once the hypothesis is accepted that the chromatin organs are not the only bearers of heredity, there is no reason why a large amount of variation should not be present. There is
still room for entirely new hypotheses, and care should be taken lest newer and perhaps better suppositions be rejected by the too common appeal to authority.

Shortly before the publication of my paper on Lilium, but not until the investigation had been completed, articles on the subject of reduction were published by Calkins (6), Mottier (23), and Strasburger (34). Each of these authors presented evidence favorable to the hypothesis that a transverse splitting of the chromosomes occurs during the reducing divisions of the plants studied. Miss Sargant (27) had also published a paper somewhat earlier, in which some facts were presented favorable to the supposition of a transverse division. Calkins, however, seems to be the only one of these investigators who has not reversed his published opinion. More recently Belajeff (2) has asserted the transverse division, while Stevens (32) holds that in the ferns studied by him both divisions which go to form the spore tetrad are longitudinal. Guignard (14) has lately also published articles on the subject, maintaining that there is only a longitudinal division. Atkinson (1) has published the results of his investigation of sporogenesis in the anthers of Arisaema triphyllum and Trillium grandiflorum. In the case of Arisaema he states that a qualitative division takes place in the first division, while in Trillium it occurs in the second. Duggar (11) also believes that a transverse division occurs in the first division in Symlocarpus fœtidus. In studying the development of the microspores of Convallaria majalis and Potomogeton foliosus, Wiegand (35) was unable to determine whether the division was longitudinal or transverse, but he inclines to the belief that it is transverse in the second division. Thus it appears to be very doubtful in which division the reduction normally occurs. Here, as in many other problems of cytology, the personal element is still very large.

The zoologists also report these variations. Paulmier (25), in his study of the spermatogenesis of Anasa tristis, says that the chromosomes have a twisted appearance, and that the first division is transverse and a true reduction division, while the
second is an equation division. Some zoologists have found that in certain animals the second is the reduction division.

Some of the nuclei of Erythronium are of enormous size. Those in the walls of the ovule are usually from 15 to 20 μ in diameter, while the large reduction nucleus often measures from 40 to 50 μ. In many cases, where the sections were cut 18 μ thick, the spindle was distributed through three sections. In such cases the spindle threads not only have their terminals cut but they are often more or less distorted. The same is true of the nuclei before the spindle is formed. If such a large spindle were cut into sections 5 μ thick it would be distributed through nine or ten sections!

In the sliced spindles of Erythronium multipolar figures are very common. Examples are shown in figs. 31, 32a, 33, 34, and 35. In fig. 31 one pole is intact and ends in a dark body, while the other is cut off. Fig. 32a is a multipolar spindle representing a tangential section. The other part of the spindle is little injured and shows well-developed centrosomes at the poles (fig. 32). In fig. 34, a strand of spindle threads has been displaced, so that it projects beyond the limits of the cytoplasm. In this material no multipolar spindles were found which were not sectioned, and they are therefore not regarded as being the result either of normal or diseased conditions, but simply due to the method of preparation. Indeed, the nuclei and spindles were so large that it was difficult to obtain the chromosomes in their normal positions, as they were frequently displaced by the knife.

Unfortunately, the stages were not at hand for tracing out the origin of the spindle. Fig. 28 represents the general appearance of a section of the nucleus some time before the final looping takes place. The nucleus usually has an enlarged or expanded appearance, with the spirem lying free in the cavity. Fig. 27 shows a large number of false poles produced as the result of contraction. In fig. 26 the spirem has looped up into the twelve loops, but no sign of a spindle appears either on the inside or outside of the nuclear membrane. The loops have not
broken apart, but were cut by the knife. It is probable that the spindle begins to form rapidly at about this stage, although it might already have passed its incipient stage and not be detected, if it lies closely applied to the nuclear membrane.

In the study of *Lilium Philadelphicum* the writer was unable to discover the origin of the radiations which appear around the daughter nuclei, but subsequent study of Ranunculus demonstrated conclusively that they originate around the poles. *Fig. 56* in Dr. Coulter's article on *Ranunculus* (10) was furnished by the writer as a good example of this. It is from the endosperm of *R. multifidus*, which is a very favorable object for the study of such radiations. In the root tips of *Allium Cepa* the same origin was traced step by step. A comparison of *figs. 30, 32, 36, 58, 59*, and *60* will show conclusively the origin of the remarkable radiations to be seen in well-prepared material of *Erythronium*. The radiations have their origin from the poles, and only later do the daughter nuclei push outward and give to the radiations an apparent nuclear origin. The radiations at first appear to be very straight and regular (*fig. 36*), while later they become more or less distorted before they begin to disappear (*fig. 60*). In favorable sections centrosomes are visible, as appear in *figs. 31, 32, 36, 58*, and *59*. In the stage represented in *fig. 36*, the attraction sphere appears to form a rather indefinite area from which the radiations arise. As to whether these bodies are built up temporarily or are permanent, the present study gives no information. In either case it is proper to call them centrospheres. At least they are the centers for the spindle threads and polar radiations.

The fate of the nucleolus was not discovered. It is still present at the time of the looped mother skein (*fig. 26*). In later stages, at the beginning of the daughter skein, spherical bodies were seen in the cytoplasm which may be extruded nucleoli (*fig. 59*). No figures were seen in the entire study which could be interpreted as a synapsis stage. The writer has maintained that what is usually called synapsis is a mere artifact which can be produced at will by using proper reagents. At the beginning of the formation of the spirem, however, the chromatin thread
becomes free and continues to orient itself and contract until the looped mother skein is formed. There is a continuous shortening and thickening and often twisting up of the entire spirem, but the contraction is not one-sided, and it does not appear to have any special relation to the nucleolus.

THE SECOND AND SUBSEQUENT DIVISIONS.

The division of the reduction nucleus gives rise to the first two cells of the gametophyte. The daughter nuclei go into a resting stage and form a network from which a new spirem is developed (figs. 61–63). The network at first shows granules which are visible in a single chain in the spirem (fig. 66), but they are not visible after the mother star is formed (figs. 68, 69, 70). The chromosomes are distinctly V- and U-shaped, and the daughter chromosomes are formed in the ordinary way by longitudinal splitting (figs. 69, 70). This is a normal quantitative karyokinesis, therefore, which is quite similar to the sporophytic quantitative karyokinesis except that there are only half the number of chromosomes formed by the transverse breaking of the spirem. Several countings indicate about twelve chromosomes in the daughter star. In one case the chromosomes were all distinct and plainly twelve in number.

The spindle in fig. 68 has been sectioned, and this may account for the lack of poles. The relation of the large vacuoles to the position of the poles of the incipient spindle should also be noted (figs. 65–67). There are often remarkable radiations around the mother nucleus. These have nothing to do directly with the formation of the spindle, however, and are the radiations normally present at this stage in both plant and animal karyokinesis. In some cases it appears that they may have their origin at the dome-shaped caps of the spindles (figs. 63, 64). There are also numerous strands of the central spindle left between the daughter nuclei of the first division, and it is probable that some of the radiations around the daughter skeins may also be left and be preserved to the beginning of the following division. The third division which gives rise to the
eight-celled embryo sac appears to be of the same nature as the second. *Fig. 71* shows the position of these spindles. The uppermost nucleus gives rise to the two synergids, the one below this to the egg and upper polar nucleus. A typical arrangement of these divisions is shown in *fig. 72*. The old spindle has survived in this instance, and has separated into two limbs below. As is usual in many of the Liliaceae, the egg apparatus is not very definitely organized. A nearly mature sac is represented by *fig. 73*. In this case, however, the nuclei are larger than usual.

Some interesting features were observed in the second division. In some cases the cytoplasmic radiations around the nucleus at the beginning of the formation of the spirem did not extend to the incept of the spindle, but ended in a rather dense cytoplasmic zone surrounding this (*fig. 62*). This of course may not be of any special significance, but merely an individual peculiarity. The fate of the central spindle of the first division, however, deserves special mention. This, as stated, persists usually until the following division is well under way. Then it often appears to mass up into two very dense irregular bodies which stain very deeply (*figs. 65, 66, 68*). Whether these masses represent a special substance distinct from the general cytoplasm and that part of the cytoplasm which alone is used in the formation of spindle threads and radiations it would be difficult, of course, to tell at present. There is little question as to the origin of the masses, and if there is a special substance for the formation of spindle threads and radiations, distinct from the cytoplasm proper, these masses must represent such a substance.

**THE DEVELOPMENT OF THE EMBRYO.**

No stages of fertilization were discovered, nor any in which the polar nuclei were conjugating. When the pollen tube enters the micropyle it increases enormously in size and is exceedingly distinct. It is very different in appearance from the tubes in Alisma and Sagittaria. The definitive nucleus begins to divide about the time of the union of the male
and female gametes. Fig. 74 shows an embryo sac with the three antipodals, the dividing definitive nucleus, the oospore, two synergids, with an extra nucleus which may be a sperm cell, and a nucleus in the pollen tube, probably the tube nucleus. During the first few divisions of the embryo, the formation of the endosperm proceeds very rapidly (fig. 76). The oospore divides first by a transverse wall (figs. 75, 76), and then each resulting cell divides by a vertical wall, forming a four-celled embryo (figs. 77, 78). These divisions are almost simultaneous, although the upper one usually leads. Sometimes, however, the divisions are more irregular (fig. 81).

The young embryo lies free in the endosperm some distance from the upper end of the sac, and the synergids disappear very early. After the first few divisions of the embryo the sac enlarges greatly below, while the upper part remains narrow and may even contract (figs. 76, 77, 79). Fig. 79 represents a five-celled embryo, one cell being cut from the upper tier and one from the lower. Fig. 80 is a six-celled embryo in which the two lower cells have each divided by a transverse wall, while fig. 81 represents a six-celled embryo with two cells of the lowest tier cut away. Fig. 82 is a twelve- to fifteen-celled embryo, and fig. 83 about a twelve-celled one. These examples will show how very irregularly the development proceeds. Up to this time and later there is usually a distinct difference between the cells which came from the upper and lower cells of the first division. This difference is shown not only by a difference in the contents of the cell, but especially by the staining reaction. Thus in fig. 83 the upper or suspensor cells have a bright yellowish cytoplasm, while the embryo cells are very granular and deep red. This is also present in Lilium Philadelphicum, sometimes being very prominent in the older embryos (9). Figs. 84 and 85 represent later stages of the embryo of E. albidum. The suspensor region is rather large and often very irregular in shape and much lobed, but the whole structure gives rise to only a single embryo, as was verified by numerous examples. The embryos were very badly shrunken, however,
because imbedded in the horny endosperm which is not easily penetrated by killing fluids. In _E. Americanum_, as shown by Jeffrey, the suspensor is much larger. _Figs. 87 and 88_ represent sections of two embryos from this plant. These show well the large umbrella-like suspensor. The lowermost lobe is the one which develops the embryo, but if any of the other lobes of the suspensor should become separated from the main mass it would probably develop an independent embryo. This would be only an accidental case, however, as may frequently happen in any embryonic tissue. This is probably not to be regarded, therefore, as an ordinary case of polyembryony, but the large suspensor is especially developed as an embryonic absorbent organ, as suggested by Coulter (9).

_Columbus, Ohio._

LIST OF PAPERS ESPECIALLY CONSIDERED.

The figures are reduced to three eighths of their original size. All the figures not especially indicated are from *Erythronium albidum*. The combination of objective and ocular is given for each case, the following being used: Zeiss compensating oculars, 4, 6, 12, 18; Zeiss 8.0 mm apochromatic objective; Leitz $\frac{1}{4}$ oil immersion and 1 objective; Leitz ocular 8; Bausch and Lomb $\frac{1}{4}$ oil immersion and $\frac{2}{3}$ objectives. The drawings were made with the aid of an Abbé camera lucida.

Fig. 1. Section of young bulb showing region of cell division dotted. B. & L. $\frac{1}{4}$ Z. 6.

Fig. 2. Normal resting nucleus in growing tip of bulb. B. & L. $\frac{1}{2}$ Z. 12.

Fig. 3. a, b, c, etc. Nucleoli of various shapes from nuclei in growing bulb. B. & L. $\frac{1}{2}$ Z. 12.

Fig. 4. Elongating nucleus in region of developing vascular bundle. B. & L. $\frac{1}{2}$ Z. 12.

Fig. 5. Close mother skein with incipient spindle, from young bulb; fuchsin iodin-green. B. & L. $\frac{1}{2}$ Z. 12.

Fig. 6. Dome-shaped spindle from bulb with granular areas at the tips of the domes; anilin-safranin gentian-violet. B. & L. $\frac{1}{2}$ Z. 4.

Fig. 7. Dome-shaped spindle with centrospheres, from bulb; chromatin granules distinct; early close mother skein; anilin-safranin gentian-violet. B. & L. $\frac{1}{2}$ Z. 12.

Fig. 8. Early close mother skein with dome-shaped spindle, from bulb; anilin-safranin gentian-violet. B. & L. $\frac{1}{2}$ Z. 12.

Fig. 9. Beginning of looped mother skein with bipolar spindle and centrosomes, from bulb; anilin-safranin gentian-violet. B. & L. $\frac{1}{2}$ Z. 4.

Fig. 10. Spindle with centrospheres from nucellus; anilin-safranin gentian-violet. L. $\frac{1}{2}$ Z. 12.

Fig. 11. Cell with starch, from bulb. B. & L. $\frac{1}{4}$ Z. 4.

Fig. 12. Dividing cell, packed with starch, from bulb. B. & L. $\frac{1}{4}$ Z. 12.

Fig. 13. Cross section of style showing glandular cells lining the style canal. B. & L. $\frac{3}{4}$ Z. 4.
SCHAFFNER on ERYTHRONIUM