FERTILIZATION IN CYPRIPEDIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 103

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(WITH PLATES XXIV–XXVII AND ONE FIGURE)

The orchids have attracted much attention on account of their striking habits and especially because of their remarkable adaptations to insect pollination, but the record of morphological investigation is a very scanty one. Morphologists have doubtless regarded them as highly specialized forms, whose interest probably lies within themselves rather than in any connection with the larger problems at present attracting attention. In the stages of development extending from the archesporium of the ovule to fertilization, STRASBURGER'S account (**43**) of *Orchis pallens*, in 1878, indicated that the familiar angiosperm stages were present in the family. Later, the same investigator (**44**) states that no parietal cell is cut off in *Gymnadenia conopsea;* and GOEBEL (**22**) makes a similar statement for *Orchis pallens*, probably based upon STRASBURGER'S original account of this species; but DUMÉE and MALINVAUD (**19**) report several genera with parietal cells. Recently NAWASCHIN (**35**) and STRASBURGER (**45**) have discussed triple fusion in the orchids and its relation to endosperm development, the former claiming that in tropical forms the nuclei remain in contact but do not fuse, the latter stating that fusion occurs in the forms he had investigated. In connection with the development of the embryo, especially in reference to certain remarkable suspensors of the group, there is a considerable body of literature, but it lies beyond the scope of this paper.

The greater part of the material for the present investigation was collected in the vicinity of Chicago in the summer of 1906, small collections having been made also in 1896 and 1898. The collections of 1906 were made June 17, July 4, 11, 17, and 27. *Cypripedium spectabile* and *C. parviflorum* furnished the greater part of the material, but *C. pubescens* and *C. candidum* were examined also in a few stages. The youngest material collected was from flowers just

opening, and later stages were collected from time to time until the seeds were almost mature.

MEGASPORE

As is well known, the ovary of orchids contains a great number of small ovules arranged in three double rows. The ovules are very slow in developing, but the archesporial cell is differentiated in the bud. No parietal cell is cut off, the archesporium functioning as the spore mother cell. The young ovule lengthens rapidly and a row of several cells is formed below the archesporium; these cells were often seen in mitosis, but the archesporium was never found dividing until the mother-cell stage. In flowers just opening the spirem of the mother cell is formed, and the synapsis stage is usually reached by the time the flower is in full bloom (*fig. 1*). While the division of the mother cell appears to be of rather long duration in most plants, it seems to be unusually prolonged in orchids as compared with other herbaceous plants. Certain cultivated orchids examined suggest the probability of no further development of the ovule unless pollination takes place. The cells of the ovule are all relatively large, with large nuclei, but the mother cell is quite striking because of its size. At this stage the whole ovule, which is always very small, is growing rapidly, the region of the integuments being especially active. The division of the mother cell takes place in the usual way (*figs. 1, 15, 16*).

Certain details of this division are particularly well shown in my material. After the integuments are started, the spirem begins to thicken, the chromomeres are very distinct, and the thread is quite long, so that it is hardly possible to do more than suggest its appearance in a drawing (*fig. 2*). A nucleus like that in *fig. 3*, in which only a few threads are drawn, shows them beginning to be paired; a little later stage is shown in *fig. 4*, where the threads lie closer together and pairing is more evident, the whole spirem being in this condition. In this stage the chromomeres were not so distinct, but it was evident that the threads were not entirely homogeneous. When the threads have approached each other until the chromomeres almost touch, or even seem to be in contact, the synaptic knot begins to form (*fig. 5*), usually around or close to the nucleolus, which often shows vacuoles at this time. As the threads are crowded more and more into the

knot, often several of them seem to be attached to the nuclear membrane (*fig. 6*); this was seen several times, so that it is probably the usual condition. The synaptic knot is at one side of the nucleus and gradually becomes a compact mass (*fig. 7*). Even when it is most compact, the constituent threads are apparently distinct, as is suggested in the figure by the rough edges of the knot and by the very evident lines across it. Hundreds of nuclei in this stage were examined and this seems to be the usual condition.

Upon coming out of synapsis, the threads are no longer in distinct pairs, but in many instances the double nature could be detected for short distances, and occasionally throughout much of the spirem. The threads gradually resume their position near the nuclear membrane (*figs. 8, 9*), and about this time the cytoplasm becomes arranged in lines which radiate from several centers (*figs. 8, 11*), which are evidently the first indications of the multipolar spindle. The chromomeres become less and less distinct, so that with the thick spirem an almost perfect homogeneity seems to exist (*fig. 9*); but even in this stage the double nature of the spirem is often evident, especially when cut ends are examined. There are eleven chromosomes at this reduction division (*figs. 12, 13*), but it was not possible to determine just how the splitting takes place—whether transversely or longitudinally. *Fig. 10* is at the right stage, but whether these pieces are to be regarded as having about completed the longitudinal splitting, or as the one long curved and twisted chromosome which will be cut transversely, could not be determined from my material. *Fig. 15* shows a typical spindle for the division of the mother cell. A cell in the wall of the ovule gave the expected count of twenty-two chromosomes for the sporophyte; these were scattered somewhat in the drawing (*fig. 14*).

When first formed, the daughter cells are alike in form and size (*fig. 16*); but very soon the inner cell shows that it is developing faster than the outer (*fig. 17*), which also begins to show signs of disintegration. This difference becomes more and more pronounced in the metaphase (*fig. 19*) and telophase (*fig. 20*) of the favored cell. Early indications of the spindle suggest the usual bipolar form (*fig. 18*), and longitudinal splitting of the chromosomes is shown in *fig. 19*, so that there is nothing unusual in this division of the daughter

cell (or cells) up to the telophase stage. There is no evidence of a wall forming between the two nuclei either in the telophase stage or later; and the two nuclei are thus left free in the cytoplasm. This stage was seen repeatedly, and never was there a wall separating the megaspores formed by the second division. *Figs. 20, 21, 23, 24* represent conditions common in the material studied, which was collected July 11, when the flowers had withered. This is the condition in most flowers when one looks for fertilization stages; but here the megaspores are just forming in many of the ovules, although in an occasional one there was some evidence of fertilization. The figures give a rather complete series of the stages that would be expected to show indications of wall formation; but there is no suggestion of an ephemeral wall even, as might be expected in case of its relatively recent elimination. *Fig. 22* shows two daughter cells in which the nucleus of the micropylar one is developing, but this was the only case of the kind seen.

EMBRYO SAC

Immediately after the division of the inner daughter nucleus, forming two megaspore nuclei with no wall separating them, these two nuclei organize the embryo sac (*fig. 23*), which increases rapidly in size and becomes very vacuolate. The two megaspore nuclei form spirems (*fig. 24*) which thicken, and the usual mitotic division follows (*fig. 25*). The spindles for this division, resulting in a four-nucleate sac, are small and apparently at right angles to one another. No evidence of another division was found, although at least three hundred slides with hundreds of ovules upon each were examined for this particular stage. When the sac is ready for fertilization, four nuclei are present, so that if other nuclei are formed they are very ephemeral. The micropylar daughter cell resulting from the division of the megaspore mother cell may still be distinguished, though it is evidently fast disintegrating (*figs. 23, 24, 25*). The four nuclei of the sac are usually of the same size (*fig. 26*), but in one instance the two micropylar ones were larger than the others (*fig. 27*). Probably *fig. 28* gives the explanation; in this sac the two megaspore nuclei are not dividing simultaneously, the antipodal one being in late prophase, while the micropylar one has reached the late telophase

and the two new nuclei are being organized. Not only has the embryo sac increased enormously in size, but the whole ovule is very active. The integuments have grown very rapidly, having lengthened until they reach far beyond the nucellus.

The usual egg apparatus, with its two synergids and egg, each with very distinct cytoplasm and *Hautschicht*, is organized in the micropylar end of the sac, the other nucleus being left in the center or toward the antipodal end (*figs. 29, 30*).

A few peculiar or "abnormal" forms were found. *Fig. 31* shows what appears to be two mother cells, which is suggested by the unusual development of that region of the ovule compared with that of the integuments and the remainder of the ovule; but the best evidence is in the chromosomes, which in both cells appear to be of the heterotypic form. In another orchid, in several cases, two mother cells (in synapsis) were seen in a single ovule. Usually the spindle in the division of the mother cell is parallel with the axis of the ovule; but *fig. 32* shows one at right angles to the usual position; only two of these spindles were seen, and they were near each other in the same ovary. No arrangement of megaspores seen could be related to this position of the spindle. Several examples of the division of both daughter cells were found (*fig. 33*). It is probable that in this case only one embryo sac is formed, for even in the very early spirem stage the two chalazal megaspores are developing at the expense of the others, or at least more rapidly than the two micropylar ones (*fig. 34*). The width of the ovule in *fig. 35* suggests the possibility of a transverse spindle, but it was evidently transverse in the second division, as suggested by the lines of protoplasm and by the position of the nuclei. These "abnormalities" are all rare in my material, not more than a dozen such cases appearing among thousands of the usual forms.

FERTILIZATION

The immense number of ovules suggested the possibility of parthenogenesis, or of failure to develop an embryo at all. The latter view is favored by the well-known difficulty in germinating orchid seeds, but the material studied gave no indication of either condition. All ovules of the right age had embryos, and in almost all of them evidences of the pollen tube were more or less distinct, traces of it

often being detected in the space between the wall of the ovary and the micropyle; and in most cases traces could be seen in the micropyle and continuing into the embryo sac (*figs. 43, 49*).

In the pollen grain the division into tube end generative cells takes place before shedding. In this division a wall forms in all cases examined, but it soon disappears. In one anther over one hundred late anaphases and early telophases were counted in which the wall was formed, and only about a dozen late telophases were without a wall. As these showed no traces of the spindle, the probability is that the wall was formed here as in the others, but had disappeared. The generative nucleus has a mass of cytoplasm about it that differs markedly in its staining reaction from that of the tube nucleus. The gametophyte number of chromosomes (eleven) was easily counted here. *Figs. 36–41* represent a series in this division. *Fig. 39* shows the wall forming in the telophase, while *fig. 40* shows it completed. The latter figure is also interesting in showing a chromosome left out of one of the nuclei at its organization.

The division of the generative nucleus was not seen; but as pollen from the bud to the time of shedding was studied, it must take place after pollination. When the pollen tube is passing from the ovary wall to the micropyle, the division has already taken place (*fig. 45*).

Only one attempt at pollination was made. Two flowers with stems about 25^{cm} long were brought into the laboratory, placed in water, and the whole put under a bell jar with some ventilation to keep them in as nearly normal condition as possible. Each was pollinated from the other. Four days later one of them was fixed, being apparently in good condition; but the other was dead six days after pollination. When the first was sectioned and stained, the ovules were found to have gone to pieces. The pollination was made in the hope of getting some idea of the development of the ovule in relation to the time of pollination. The stigma was sectioned and showed that the pollen grains had practically all developed tubes, many of them being traced into the conductive tissue, in some cases for some distance, and the grains were almost all empty. If the development of the tube was delayed, a condition like that in *fig. 41* was found, in which the tube is just forming and the generative nucleus is beginning to show signs of activity. Soon a complete

spirem is formed, and tubes less than the length of the pollen grain have the three nuclei in them; so that the generative nucleus probably divides into the male nuclei as the tube develops.

It will be remembered that the embryo sac ready for fertilization contains only four nuclei—the egg apparatus in the usual position, and the remaining nucleus near the center of the sac or toward the antipodal end (*figs. 29, 30*). As the pollen tube penetrates the sac, one of the synergids may be in front of it and a little lower than the other, as if it were being pushed from its usual position by the inrush of the contents of the tube, or moving for some other reason toward the antipodal end of the sac (*fig. 44*). The egg nucleus may be already in the spirem stage. The pollen tube here shows the tube nucleus in advance, with the two male nuclei elongated and near it. A stage just later than this shows two nuclei below the egg, and from the lines of cytoplasm one seems to be the synergid which has moved to that position (*fig. 42*). The two male nuclei are still somewhat elongated and are in advance of the tube nucleus. *Fig. 43* shows the polar nucleus and the synergid near the antipodal end of the sac, and the two male nuclei still elongated. The primary endosperm nucleus is formed in this case by the fusion of a male cell, the solitary polar nucleus, and the migrating synergid. In this fusion, as well as in the fertilization of the egg, the fusing nuclei often come into contact in the resting condition (*fig. 45*), the figure showing the male nucleus in contact with the egg still curved and elongated. Many views of this stage might be given with slight variation in the position of the nuclei; and while fusion in the resting condition was apparently the more common in my material, it was not at all uncommon to find spirems already well formed and in different stages of development.

The pollen tube was often traced from the ovary wall through the micropyle into the embryo sac. The male nuclei are usually elongated and sometimes in advance of the tube nucleus, but the tube nucleus usually precedes them (*fig. 46*). In no case does the pollen tube pass directly to the micropyle, but bends in various directions. This may be due to the attraction of other ovules, several of which will be almost as near as the one entered. It may be that after leaving the ovary wall the tube simply wanders in this space until it comes within the influence of the ovule it enters.

One sac indicated the possibility that the synergid may fail to unite in the triple fusion. *Fig. 47* is unusually interesting. Some of the nuclei show the tendency to become amoeboid, but the interest is due especially to the condition of the nuclei, all of those that take part in double fertilization having reached the spirem stage not only before fusion, but even before contact. The spirem is well formed in every nucleus, and shortened almost enough to segment into chromosomes. It would seem in this case that if fusion does take place, there could be no possibility of a fusion of the chromatin, which would certainly divide into chromosomes from each spirem as it is now formed. Well-formed spirems in different stages of development are common; in *fig. 48* the male and female nuclei have formed thick spirems which are evidently distinct, and while those of the triple nucleus are just as distinct, they are much younger. In the same figure an unusual development of the synergid, or possibly of the tube nucleus, is shown; but as no further indication of division was ever found, there is probably no significance to be attached to it. *Fig. 49* shows a pollen tube from its entrance into the micropyle to its ending in the sac, the male nuclei being not only elongated but curved. In a few instances there is some distance between the two male nuclei (*fig. 50*), but this seems to be rather uncommon. The very sharp bend in the tube shown in the last figure suggests some special attraction felt just at this point, directing the tube into the micropyle.

ENDOSPERM

The fertilized egg may divide before the primary endosperm nucleus (*figs. 51, 52*) and during this division the latter may be in the spirem stage (*fig. 51*), or resting, or with the spirem just forming; and in one instance the division of the egg was taking place before the second male nucleus had left the pollen tube. The endosperm nucleus, however, may divide first, *fig. 53* showing it in late prophase while the egg and male nucleus are barely in contact and the spirem is just beginning to be formed in each.

With the nuclei of both fusions in the spirem stage the endosperm nucleus may be farther advanced than the egg, one preparation showing its threads very distinct and much thicker than those of the

egg. *Fig. 54* shows a two-celled embryo and the primary endosperm nucleus in late prophase. At the three- or four-celled stage of the embryo, the endosperm nucleus shows a spindle forming (*fig. 55*), which is almost complete and is very broad; the chromosomes (approximately thirty-three) are probably in metaphase. On the other hand, the primary endosperm nucleus may reach the anaphase before the fusion of the male and female nuclei, as one of my sections shows, the male nucleus not having left the pollen tube, and both it and the egg nucleus with spirems forming.

The telophase of the endosperm spindle shows a wall (*fig. 56*), but it is probably ephemeral, as none was seen either in two- or four-nucleate endosperm. The endosperm nuclei are relatively large, as would be expected from the large amount of chromatin used in their formation (*figs. 57, 58*), one in *fig. 57* measuring 17×23 μ. For this reason they are not likely to be confused with other nuclei of the sac. The two-nucleate stage of the endosperm may be reached at any stage of the embryo between two and twenty or more cells. The figures for the second division of the endosperm were not seen, but the four-nucleate stage was found in a few sacs (*fig. 58*). From the appearance of these nuclei it is altogether probable that another division may take place, for they are apparently beginning the formation of the spirem for the next division, and are large, vigorous-looking nuclei.

While the presence of endosperm nuclei is quite common, it is not difficult to select a series that would indicate the possibility of their failure. An embryo of several cells with the primary endosperm nucleus still in the resting condition is rare (*fig. 59*); but we need not conclude that division may not take place later than this, for with a still older embryo (*fig. 60*) the endosperm nuclei have begun to form spirems, although there is no evidence of fusion further than the contact. In *fig. 61* one of the nuclei, probably the male nucleus, has been delayed in reaching the other two; yet some suggestion of the spirem is evident in all three. *Fig. 62* shows a sac which is almost filled by the embryo, and at the base are three nuclei which are probably the constituents of the triple fusion which has not taken place; it is barely possible that they are endosperm nuclei, but there is no evidence of a fourth nucleus. If these are not endosperm nuclei,

it is evident that in this case none will be formed, for all of them show signs of disintegration.

EMBRYO

The embryo is very simple. As stated above, the fertilized egg may divide before the primary endosperm nucleus, which seems to be the more common behavior in my material; or the primary endosperm nucleus may divide first (*figs. 51–55*). An inconspicuous suspensor of one or two cells is formed, but no other differentiation of regions could be made out in the younger stages (*figs. 55, 62*). The number of chromosomes in the segmentation of the egg seemed to be twenty-two (*figs. 51, 52*), and this number was counted several times in various parts of the ovule (*fig. 14*).

DISCUSSION

It is unfortunate that there is no definite name for the two cells resulting from the division of the megaspore mother cell. "Daughter cells" has been used in this paper to designate them. It has been customary to call the cell that develops the embryo sac a megaspore, and this is correct in all cases when the sac is organized from the chalazal cell in the "row of three" or from one of the cells in the "row of four." But if the name is applied to one of the "daughter cells" there is confusion, for in this case the reduction process is not complete.

MEGASPORE.—In angiosperms two types of embryo sac have been recognized: (1) the more common type, in which the mother cell gives rise to four megaspores, one of which develops the sac; (2) the Lilium type, in which the mother cell, "functioning as a megaspore," forms the sac directly. In the former case, the occasional "row of three" does not change the situation, for the functioning megaspore is derived from the mother cell by two successive divisions (COULTER and CHAMBERLAIN **16**).

The production of only two "megaspores" ("daughter cells") by the mother cell has been reported for a few plants that usually form megaspores in the normal way. SCHNIEWIND-THIES (**40**) reports two or four in Galtonia; GUIGNARD (**25**) two in Agraphis, Ornithogalum, and Commelina; MOTTIER (**31**) two in Arisaema (in one instance with no wall between them, which would present the

Lilium condition); CAMPBELL (**7**) two or three in Dieffenbachia; and JUEL (**29**) two in Taraxacum. The very few cases reported show the rarity of this condition among the angiosperms investigated. Four of the five families represented in this enumeration, to which the Orchidaceae are now added, are all monocotyledons; and this fact, taken together with the still greater reduction in the Lilium type, is in harmony with the theory that monocotyledons are a specialized offshoot from dicotyledons, as held by JEFFREY (**28**), QUÉVA (**37**), Miss SARGANT (**38**), and others.

However, SCHNIEWIND-THIES (**40**) has shown that in Lilium the heterotypic and homotypic divisions take place within the embryo sac; that is, there is no permanent wall formed in either of the divisions from mother cell to the four megaspore nuclei. It follows, therefore, that all four megaspore nuclei take part in forming such an embryo sac. In Cypripedium, on the other hand, a wall accompanies the first division of the mother cell, but no wall is formed in connection with the second division. In this case, therefore, two megaspore nuclei enter into the formation of the embryo sac, instead of four as in Lilium, or one as in most angiosperms. It is interesting to note here that if the usual megaspore walls were formed in Lilium and in Cypripedium, each sac would contain two nuclei—the egg and its sister, which is the polar that usually unites with the antipodal polar to form the primary endosperm nucleus.

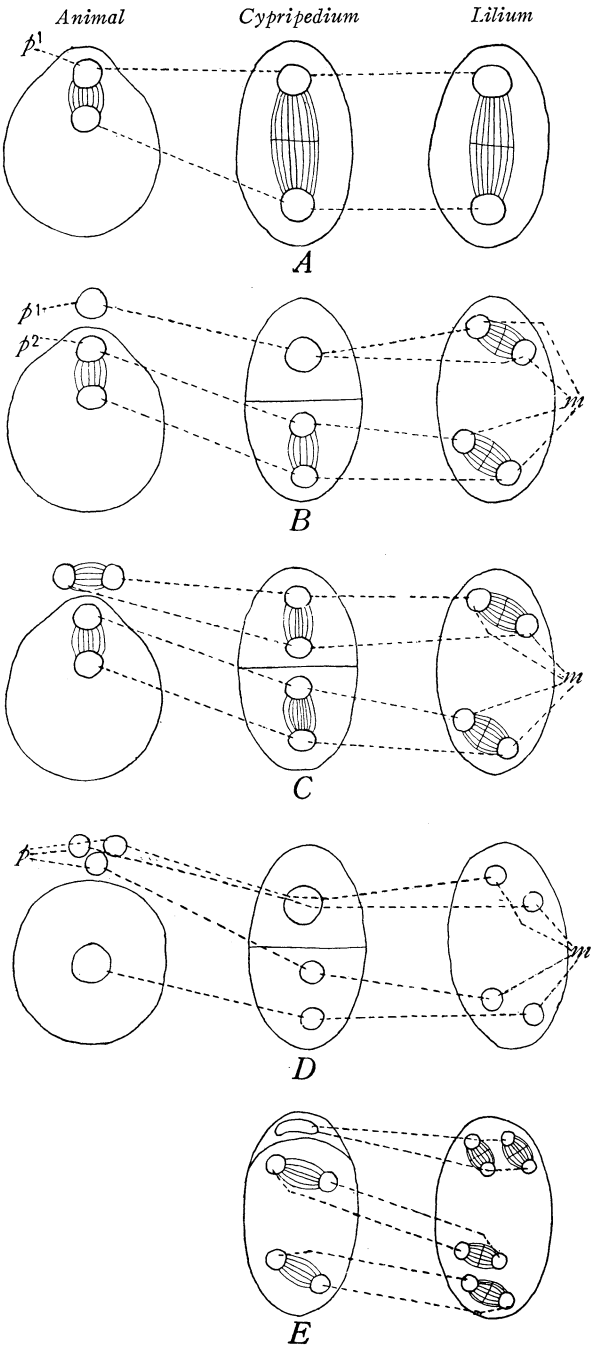
EMBRYO SAC.—An embryo sac with only four nuclei has not been reported before, but there is just the same number of divisions between the mother cell and the egg in this case as in Lilium. After all, the essential feature in the history of the egg is the distribution of the chromosomes in the reduction divisions. It is possible that the use of more than one megaspore in organizing the sac holds some necessary relation to "double fertilization," for in both Lilium and Cypripedium, if walls appeared in the usual way so that only one megaspore was used in forming the sac, and there were no additional divisions between mother cell and egg, there would be only two nuclei in the sac, and "double fertilization," at least "triple fusion," would be impossible.

It is interesting in this connection to compare this series with that in the animal egg. There the primary oocyte (mother cell)

divides and throws off a polar body, and forms the secondary oocyte. The polar body may divide; the secondary oocyte divides, forming another polar body and the mature egg (megaspore) (see BOVERI **6** and WILSON **47**). The two divisions are of the heterotypic and homotypic types found in plants in the formation of megaspores. In Cypripedium, if the last division in the embryo sac were omitted, there would be the same number of divisions in the formation of the egg as in the animal. If the wall were omitted between the daughter nuclei at the division of the mother cell, as in Lilium, and the last division were omitted, the sac would have four nuclei as now; and this would be true of Lilium if the last division did not take place. This situation was discussed by CHAMBERLAIN (**11, 12**) in connection with the usual eight-nucleate sac, and also in relation to the probable four-nucleate sac of Cypripedium.

The situation may be made more clear by means of diagrams. *Fig. A* represents the primary oocyte dividing in the usual way and the mother-cell division in Cypripedium and in Lilium; in all cases this is the heterotypic division. *Fig. B* represents the next division if the first polar body fails to divide, and if the micropylar nucleus fails as in Cypripedium. *Fig. C* represents the same stage as *fig. B*, but the first polar body and the micropylar cell in Cypripedium divide; this is the homotypic division. *Fig. D* represents the division indicated in *fig. C* completed, with the typical arrangement of nuclei in all three forms. This completes the animal series; but in Cypripedium and in Lilium there is another division, which is represented in *fig. E*. It is evident that in these forms there is even greater reduction in the number of divisions than in the male gametophyte if the mother cell be taken as the beginning of the gametophyte generation, as is done by COULTER and CHAMBERLAIN (**17**); for there the male nucleus represents two divisions beyond the microspore, while in Cypripedium and Lilium the egg is removed by only one division from the megaspore nucleus.

WALL IN POLLEN GRAIN.—The ephemeral wall in the pollen grain between the tube and generative nuclei is not difficult to explain according to present homologies. It has been reported in a few plants, as Neottia (GUIGNARD **26**), Sarcodes (OLIVER **36**), Naias and Sparganium (CAMPBELL **8, 9**), Populus and Lilium (CHAMBERLAIN



Successive stages in maturation of animal egg, and in development of megaspores in Cypripedium and in Lilium. *A*, the division of the primary oocyte to form the first polar body (p^1) and the secondary oocyte; and the division of the mother cell in Cypripedium and in Lilium—all heterotypic divisions. *B*, the division of the secondary oocyte to form the second polar body (p^2) and the egg; and the second division in Cypripedium and in Lilium, giving the megaspores (m)—the homotypic division in all. *C*, the same stage as the preceding, but the first polar body of the animal and the micropylar nucleus of Cypripedium dividing. *D*, the egg and the three polar bodies (p) of the animal, and the megaspores (m) of Cypripedium and of Lilium. *E*, not in the animal series; the division that gives the egg in Cypripedium and in Lilium.

13, 14), Sagittaria (SCHAFFNER 39), Convallaria (WIEGAND 46), Asclepias (FRYE 21), and Hamamelis (SHOEMAKER 41). If the tube cell represents the wall cells of the antheridium, this wall is a relict of the time when there was a complete separation between spermatogenous and wall tissue (COULTER and CHAMBERLAIN 18). This difference was shown not only by separating walls but by reaction to stains; and the latter difference still remains in the pollen grain after the wall disappears.

DOUBLE FERTILIZATION.—In looking over my material it seems strange that double fertilization was first reported less than a decade ago, when NAWASCHIN (33, 34) made his announcement. In my material double fertilization was observed in hundreds of instances; in fact there would be some difficulty in finding fertilization without some suggestion of triple fusion; it was as evident in every respect as the fertilization of the egg. However, it was stated above that in some cases it may fail to take place; and it would not seem unlikely that in some orchids triple fusion always occurs and in others it may be rare or omitted altogether, as indicated by STRASBURGER and NAWASCHIN. NAWASCHIN thought that in the failure of triple fusion he had found the explanation of the lack of endosperm in orchids, but it is evident that the little or no endosperm of this family must be referred to other causes. It may be accurate enough for taxonomic purposes to say "no endosperm" in the orchids, as in a recent manual (42), but it is not an exact statement.

DISTINCT PATERNAL AND MATERNAL CHROMOSOMES.—Some important evidence on this much-discussed question was obtained. This condition was first reported in plants by KRUCH (30), who worked with the liverwort, *Riella Clausonis;* but at that time (1891) it attracted little attention and seems to have been entirely overlooked by later workers. KRUCH showed the sperm entering the egg and segmenting in the upper part of the cytoplasm, while the chromatin of the egg-nucleus segments near the center, each showing eight chromosomes, and the nuclei were not even in contact. BLACKMAN (2), working with *Pinus sylvestris*, figured two groups of chromosomes in the first division of the fertilized egg and says: "there can be little doubt that these bodies are really the chromosomes of the two (male and female) nuclei." In his summary he makes the positive statement that they

are the chromosomes of the male and female nuclei. CHAMBERLAIN (**15**) in *Pinus Laricio* finds that "the chromatin appears as two distinct masses in the spirem stage. Perhaps segmentation of the two spirems occurs while they are still separate." MURRILL (**32**), working with *Tsuga canadensis*, says: "The chromatin of each (sperm and egg) nucleus collects in the form of a thick knotted thread near the center of the separating partition, and the two masses remain distinct until the spirem band begins to segment." Miss FERGUSON (**20**), using Pinus, finds "two groups distinct at time of segmentation of spirem and can still be made out during early development of chromosomes; but cannot be when they are being oriented on the nuclear plate." Recently HARPER and BLACKMAN have shown interesting features in some of the fungi that seem to be related to this independence of chromosomes. HARPER (**27**) has shown in Phyllactinia that "the material of each chromosome is in permanent connection with the central body throughout the stages of nuclear fusion and the resting condition, as well as in mitosis." BLACKMAN (**1**) says that rusts which have the aecidial stage have two nuclei in all cells from the aecidiospore to the teleutospore. The two nuclei fuse in the teleutospore and from there to the formation of the aecidiospores the cells are uninucleate. More recently GRÉGOIRE (**23**), working with roots of Allium, claims that the chromosomes do not unite to form a continuous spirem in the telophase, but always remain distinct bodies.

In my material, as shown above, it is quite common to find both the fertilized egg nucleus and the triple-fusion nucleus with distinct spirems in all the nuclei entering into the fusion (*figs. 42, 43, 44, 47, 48, 51, 53*). In one instance the spirems were all well advanced, so that the threads were quite broad, before contact of the nuclei (*fig. 47*). Spirems as far along as these will evidently be cut into chromosomes without fusing at all. If spirems are often formed separately, it would seem probable that when the nuclei fuse in the resting stage two separate spirems may still be present. Anyone who has tried to trace a spirem will at once recognize how difficult it would be to trace two of these long threads in the same nucleus; and as no one expected to find two, only one was seen until two were found distinctly separated. If there may be two spirems in the first

division of the sporophyte, why might it not be true of all the mitoses in the sporophyte generation? This would make half of the chromosomes paternal and half maternal in every sporophyte cell. Thus in the mother cell two spirems would be formed as usual, but here they would pair and fuse more or less completely in synapsis. If there are two phenomena in fertilization—stimulus to growth and the mingling of ancestral qualities—only the first would be felt in the sporophyte. The mother cell would give the opportunity for the second during the fusing of the pairs of threads.

The chromosomes of the fusing nuclei are often arranged in groups, and while these may not be definite enough to be positive evidence for distinct chromatin if taken by themselves, they are not only very suggestive but appear as distinct as the position of the nuclei in the spirem stage would lead one to expect (*figs. 51, 54*). In a form in which the paternal and maternal chromosomes differ, proof could be obtained; but even in Cypripedium, in such groups as those shown in *figs. 51–54*, the chromosomes are evidently of separate spirems. This seems very clear when these figures are studied in connection with those showing the spirems (*figs. 42, 43, 44, 47, 48, 51, 53*). The groups are just about as distinct as would be expected, for the nuclei usually overlap slightly in the spirem stage.

SYNAPSIS.—BERGHS (**3, 4, 5**), GREGORY (**24**), CARDIFF (**10**), and others have recently discussed synapsis and the stages leading up to and following this condition. It seems to be clearly established that in the forms studied by these authors there is a gradual pairing of the threads, beginning with the first suggestion of synapsis. The two threads are apparently united into a single thread by the time the nucleus comes out of synapsis. During synapsis itself and in early stages of the recovery from this condition some traces of the double nature of the thread are often seen, and the heterotypic chromosome is formed from this doubled thread. These stages were well shown in my material (*figs. 2–11*). BERGHS sees in this a device for reducing the number of chromosomes, but it may also furnish the opportunity for mixing of parts, or transfer of substance, or influence from the paternal and maternal chromosomes that have remained distinct throughout the sporophyte stage. CARDIFF (**10**) thinks the position

of the synaptic knot may be due to gravity. This does not seem to be true in Cypripedium, for the knot was found at all possible positions next the nuclear membrane in serial sections of the same ovary, and even in the same section. This was not only true for cross-sections, but also for longitudinal sections in which, since the ovary stands erect, gravity effects ought to be apparent. BURLINGAME (6a) has reached this same conclusion in his study of Ophioglossum.

SUMMARY

1. In Cypripedium two cells are formed by the mother cell, a wall not appearing in the second division, even in the rare instances in which the nuclei of both daughter cells divide. Two megaspore nuclei are used in forming the embryo sac, the Lilium type using four, and most angiosperms only one. The two megaspore nuclei in the sac of Cypripedium and the four in that of Lilium may be related to double fertilization.

2. Cypripedium has only four nuclei in the completed embryo sac. The Lilium type and Cypripedium have the fewest divisions from mother cell to egg reported among angiosperms. A comparison with the animal egg shows only one more division from the mother cell to the egg in Lilium and in Cypripedium than in the maturation of the animal egg.

3. So-called double fertilization is probably constant in Cypripedium. The primary endosperm nucleus (triple-fusion nucleus) is formed by the fusion of the polar, one synergid, and one male nucleus. Endosperm of four nuclei was found and the probability of other divisions taking place was noted.

4. In the presynaptic nucleus there is an evident pairing of the threads, probably of paternal and maternal origin. The gametophyte number of chromosomes is eleven, the sporophyte number is twenty-two, and the endosperm number is probably thirty-three. Distinct spirems were often found in all fusing nuclei, forming in one instance before contact. From these examples, the possibility of two spirems always forming in the sporophyte nucleus is suggested; this would secure the independence of the chromatin, and the fusing in synapsis would permit whatever exchange and mingling of parts there may be.

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EXPLANATION OF PLATES XXIV–XXVII

All figures were made with Bausch and Lomb camera lucida; *figs. 31* and *46* with Leitz no. 2 ocular and no. 7 objective; *figs. 61* and *62* with no. 4 ocular and no. 7 objective; all others with Leitz no. 4 ocular and B. and L. $\frac{1}{12}$ objective; details were filled in with a Zeiss apochromatic ocular and 2^{mm} objective.

The abbreviations used are as follows: *c*, chalazal daughter cell and nucleus; *e*, egg; *em*, embryo; *end*, endosperm; *g*, generative nucleus; *gc*, generative cell; *m*, micropylar daughter cell and nucleus; *meg*, megaspore nucleus; *p*, polar nucleus; *pe*, primary endosperm nucleus; *s*, synergid; *t*, tube nucleus; *♂*, male nucleus.

PLATE XXIV

FIG. 1. *C. spectabile*. Ovule with mother cell in synapsis.

FIG. 2. *C. parviflorum*. Mother cell with well-organized spirem showing distinct chromomeres.

FIG. 3. *C. spectabile*. Nucleus of mother cell, with a few threads showing beginning of pairing.

FIG. 4. *C. candidum*. Pairing of threads very evident throughout the nucleus, but only a few threads shown.

FIG. 5. *C. spectabile*. Synaptic knot forming; pairing of threads evident.

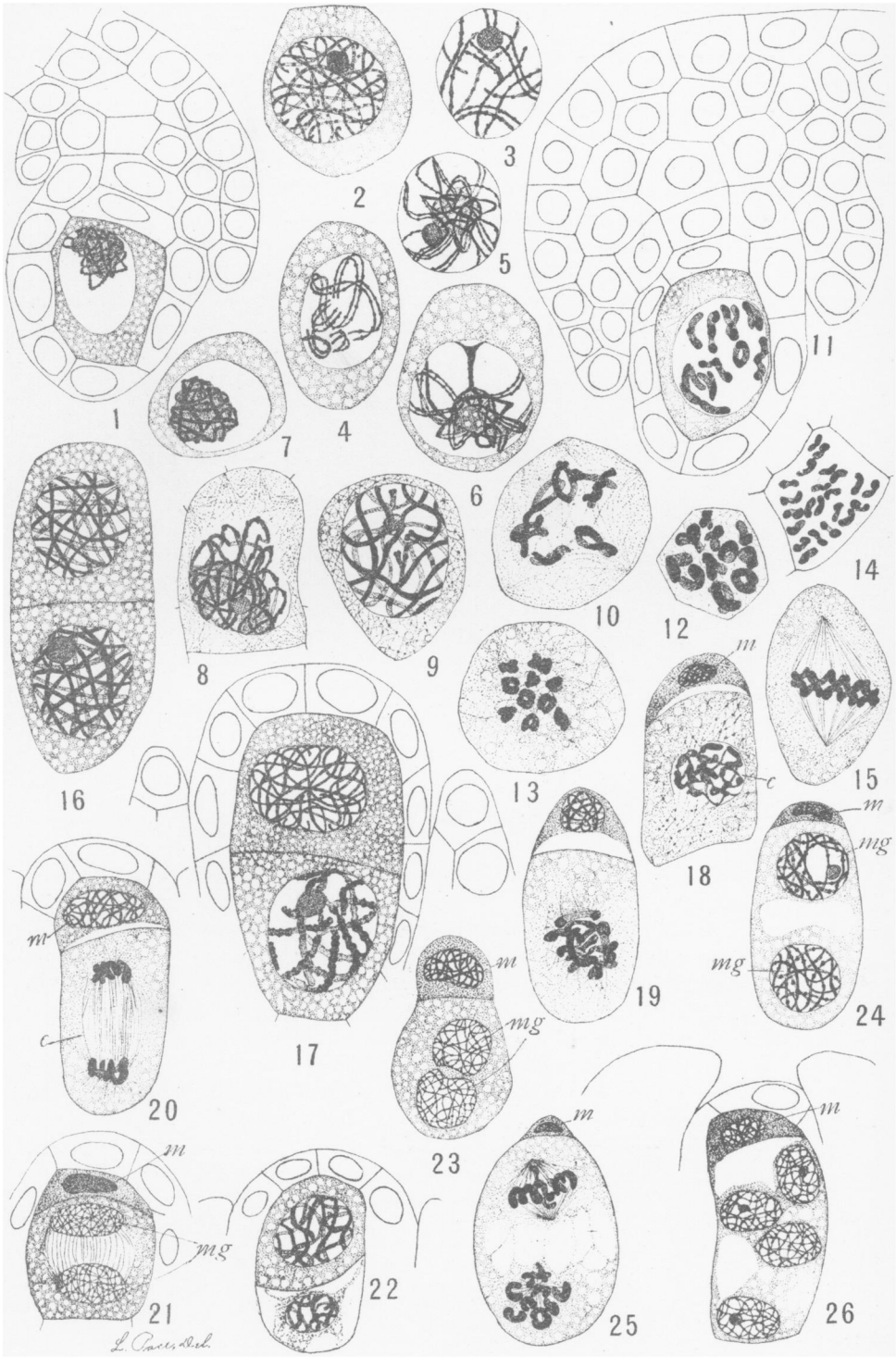
FIG. 6. *C. parviflorum*. Synaptic knot further advanced; threads in pairs and seemingly attached in places to nuclear membrane.

FIG. 7. *C. pubescens*. Synaptic knot complete.

FIG. 8. *C. spectabile*. Recovery from synapsis; lines of cytoplasm suggest multipolar spindle.

FIG. 9. Same. Shortening of thread; double nature evident at cut ends; lines of cytoplasm probably related to spindle formation.

FIG. 10. *C. pubescens*. Chromosomes formed.



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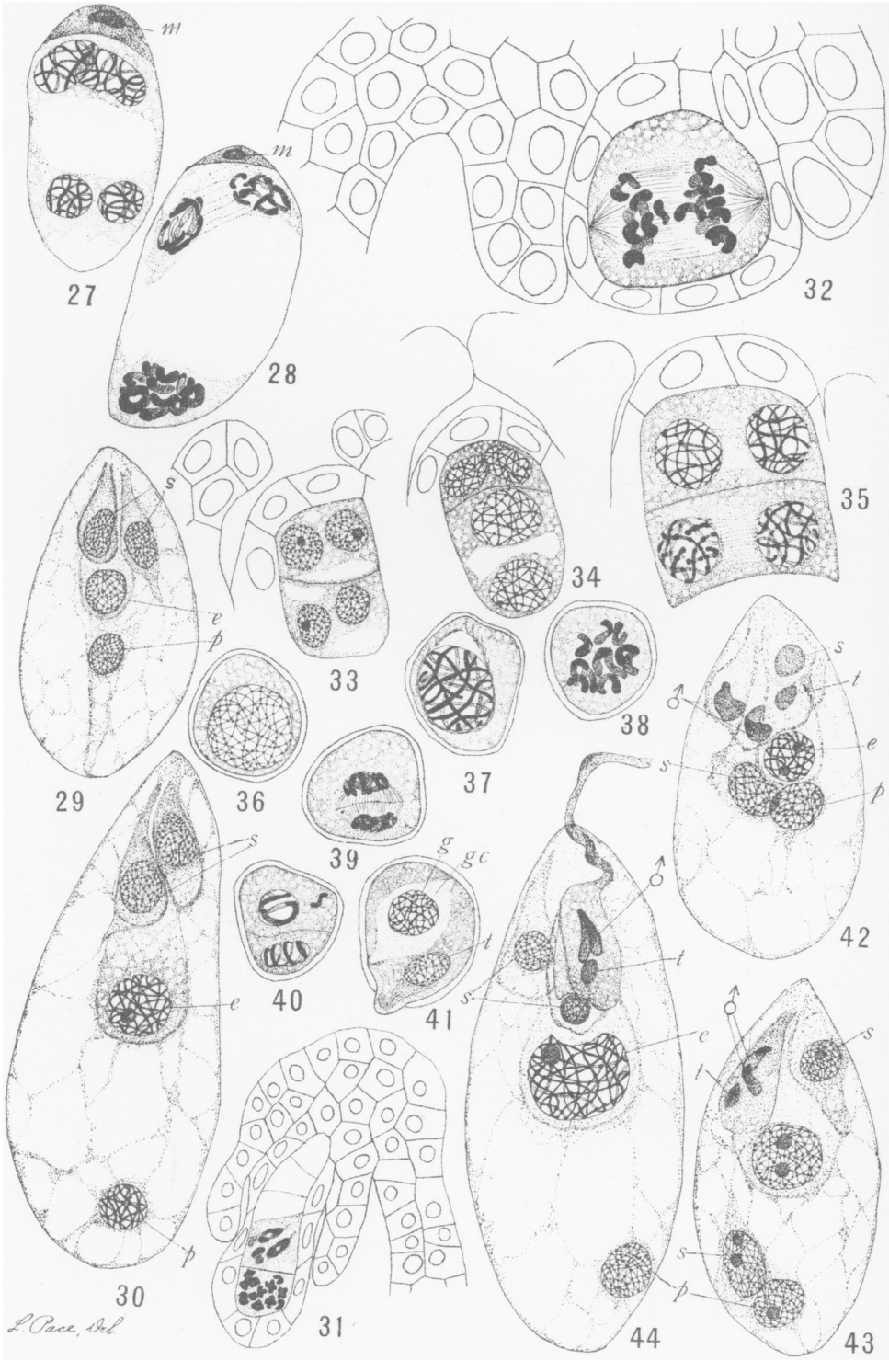

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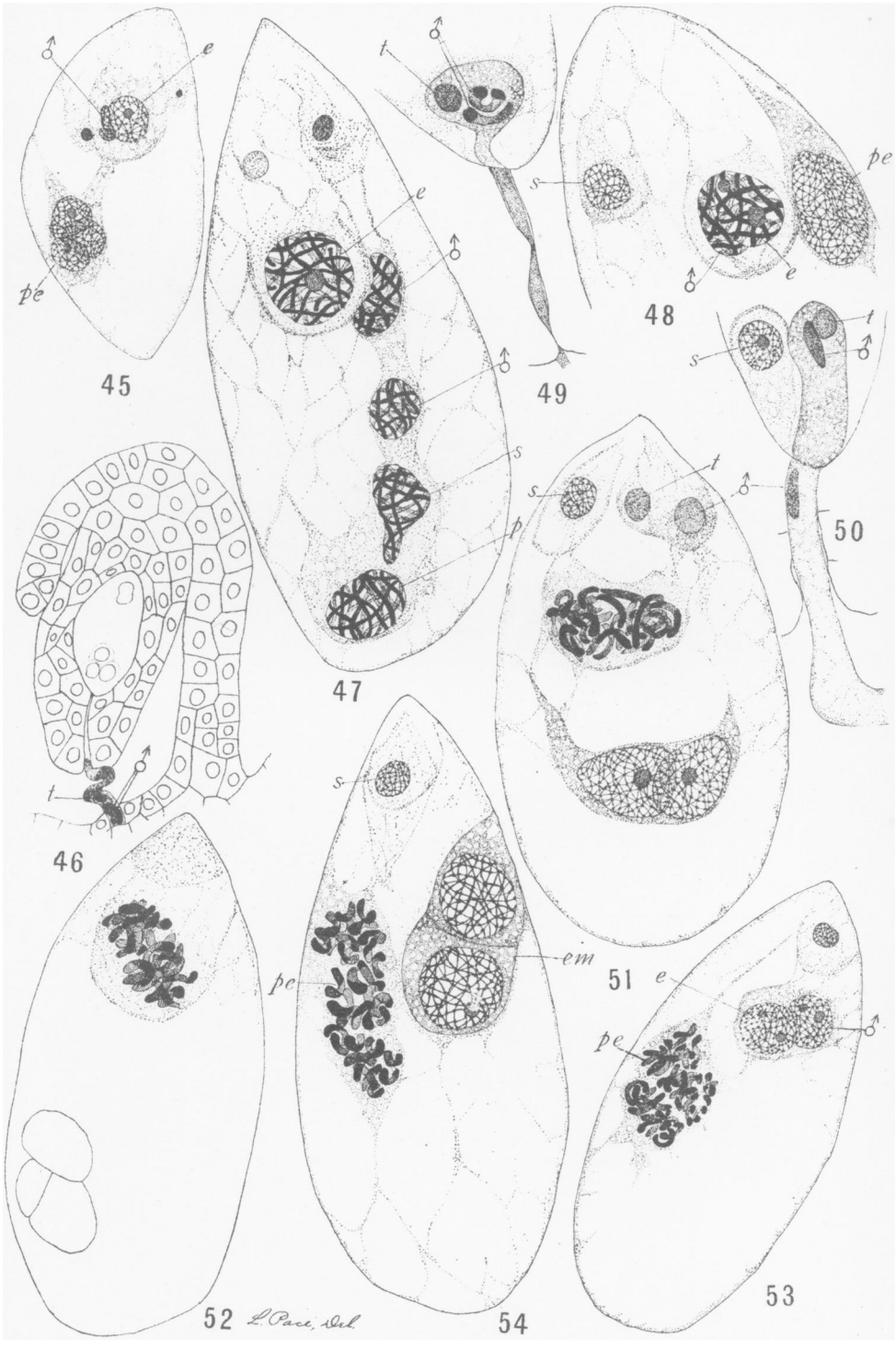

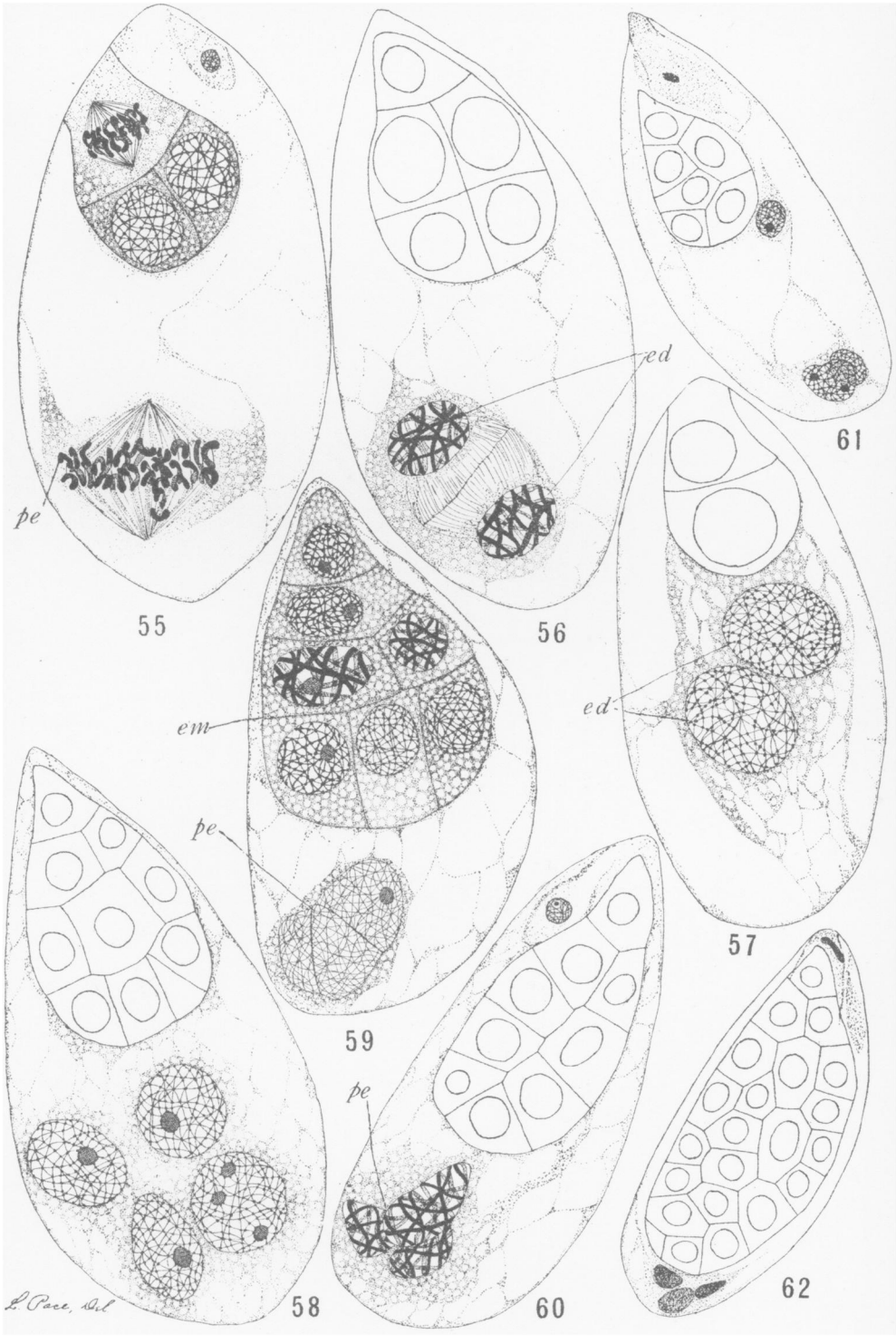

FIG. 11. Same. Ovule with mother cell in late prophase or in metaphase; origin of spindle indicated by lines of cytoplasm.

FIG. 12. Same. Eleven chromosomes.

FIG. 13. *C. spectabilis.* Eleven chromosomes.

FIG. 14. Same. Wall cell of ovule showing twenty-two chromosomes (sporophyte number).

FIG. 15. *C. pubescens.* Typical spindle of mother cell.

FIG. 16. *C. spectabile.* Daughter cells formed by division of mother cell.

FIG. 17. *C. pubescens.* Daughter cells, with chalazal nucleus farther advanced than the micropylar.

FIG. 18. *C. spectabile.* Suggestion of bipolar spindle.

FIG. 19. Same. Metaphase of second division, longitudinal splitting of chromosomes.

FIG. 20. Same. Telophase of chalazal daughter cell; no indication of wall.

FIG. 21. *C. parviflorum.* Division of chalazal daughter cell complete; remains of spindle, but no evidence of wall.

FIG. 22. *C. spectabile.* Micropylar daughter nucleus developing and the chalazal one disintegrating.

FIG. 23. *C. parviflorum.* Division shown in *fig. 21* completed; the sac slightly enlarged and containing two megaspore nuclei.

FIG. 24. *C. spectabile.* Early spirem for the division of the megaspore nuclei; micropylar daughter cell still recognizable.

FIG. 25. Same. Spindles for division of megaspore nuclei; micropylar daughter cell still persists.

FIG. 26. Same. Four-nucleate sac; micropylar cell still undivided.

PLATE XXV

FIG. 27. Same. Four-nucleate sac, the nuclei differing in size.

FIG. 28. Same. Nucleus at antipodal end of sac in late prophase; the other in late telophase.

FIG. 29. *C. parviflorum.* Embryo sac ready for fertilization, showing egg apparatus and "polar" nucleus (near center).

FIG. 30. Same. Another view of sac, showing polar nucleus near antipodal end.

FIG. 31. Same. Two mother cells.

FIG. 32. *C. spectabile.* Spindle of mother cell at right angles to its usual position.

FIG. 33. Same. Four megaspore nuclei of equal size; no walls at second division.

FIG. 34. *C. parviflorum.* Four megaspore nuclei, as shown in *fig. 33*, but the inner pair the larger.

FIG. 35. C. *spectabile.* Four megaspore nuclei; traces of spindle showing, but no trace of wall.

FIG. 36. Same. Pollen grain; early spirem for division to tube and generative nuclei.

FIG. 37. Same. Later stage; "cap" showing orientation of spindle.

FIG. 38. Same. Pollen grain showing eleven chromosomes.

FIG. 39. Same. Telophase; wall forming.

FIG. 40. Same. Wall completed; a chromosome omitted in the organization of one of the nuclei.

FIG. 41. Same. Pollen grain developing tube.

FIG. 42. *C. parviflorum.* Pollen tube within the embryo sac.

FIG. 43. *C. spectabile.* About same stage as *fig. 42.*

FIG. 44. Same. Earlier stage; tube within sac and one synergid below it.

PLATE XXVI

FIG. 45. *C. parviflorum.* Double fertilization.

FIG. 46. Same. Ovule with tube just entering, having passed from ovary wall to micropyle.

FIG. 47. Same. Spirems formed in all the fusing nuclei before contact.

FIG. 48. Same. Spirems of male and female nuclei farther advanced than of the nuclei to engage in the triple fusion.

FIG. 49. Same. Pollen tube entering sac; male nuclei elongated and curved.

FIG. 50. *C. spectabile.* Pollen tube unusually broad; one male nucleus some distance behind the other.

FIG. 51. *C. parviflorum.* Segmentation of egg; two groups of chromosomes.

FIG. 52. *C. spectabile.* Segmentation of egg; two groups of chromosomes.

FIG. 53. *C. parviflorum.* Male nucleus and egg just in contact; late prophase of primary endosperm nucleus, which shows thirty-three (approximately) chromosomes in three groups.

FIG. 54. Same. Two-celled embryo; primary endosperm nucleus in late prophase and showing three groups of chromosomes.

PLATE XXVII

FIG. 55. Same. Spindle forming for division of primary endosperm nucleus; three- or four-celled embryo.

FIG. 56. Same. Telophase of division of primary endosperm nucleus; wall forming.

FIG. 57. Same. Endosperm of two nuclei and a two-celled embryo.

FIG. 58. Same. Endosperm of four nuclei.

FIG. 59. Same. Embryo well advanced; triple-fusion nucleus still distinct but in the resting stage.

FIG. 60. Same. Older embryo; triple-fusion nucleus in spirem stage.

FIG. 61. Same. Embryo of several cells; nuclei of the triple fusion not yet in contact.

FIG. 62. Same. Much older embryo; probably a triple-fusion nucleus, but no evidence of division.