# STUDIES IN THE DEVELOPMENT OF THE PIPERACEAE[1]

## I. THE SUPPRESSION AND EXTENSION OF SPOROGENOUS TISSUE IN THE FLOWER OF PIPER BETEL L. VAR. MONOICUM C. DC.

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SEVENTY-ONE FIGURES[2]

In an account of the seed development of Heckeria umbellaia (L.) Kunth (Piper umbellata C. DC) and of Piper medium Jacq., published eight years ago (Johnson, '02), these forms were compared with other angiosperms, and especially with Peperomia pellucida. A study of the plant here described was begun at the same time. This species of Piper was chosen because, from all species available at my collecting ground, the east end of Jamaica, this one was markedly distinguished by its climbing habit, its unisexual flowers and its immersed ovaries and seeds.

The detailed investigation of the present species confirms certain conclusions reached in the study of the genus Piper, but also shows several important peculiarities, especially in the development of stamens and ovaries.

The material used was collected in Jamaica by W. C. Coker in 1900, by the author in 1903 and 1906, by R. K. Miller in 1905 and by I. F. Lewis in 1906. A fifth lot of material was collected in the East Indies by D. H. Campbell in 1907. Obligation is here acknowledged to the above mentioned gentlemen, and also to M. Cassimir de Candolle for identifying the plants from Jamaica,

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on which the results here given are primarily based. The material was fixed in chrom-acetic or in chrom-osmo-acetic.

The flowers of Piper betel are formed in long terminal spikes. The individual spike may be made up partly or wholly of pistillate flowers with occasional staminodia, wholly of staminate flowers, or, more rarely, almost wholly of hermaphrodite flowers (fig. 13).

The mature staminate flowers are about two millimeters in diameter and five centimeters or more in length. The female and hermaphrodite spikes are often twelve millimeters thick when mature, and as long as the males. The number of flowers on a spike may be as great as 500 or 600. On either a male, female or hermaphrodite spike there may be from four to ten sterile bracts at the base. Above these, the lowermost fertile bracts, on the hermaphrodite spike, may bear hermaphrodite flowers just as often as male or female ones. The conditions at the tip of the spike are apparently similar, but the number of sterile bracts is more variable. Sometimes all but two or three of the terminal bracts have flowers in their axils, while, in other cases, twenty or more of the terminal bracts may be sterile.

Each hermaphrodite flower consists of two stamens, which stand side by side, at the same level on the spike. The carpels, with the three or four stigmatic lobes, stand between them (figs. 4, 5, 13, 44). Each flower is subtended by a bract, which is at first rather bracket-like with a short thin stalk (fig. 1). Later it becomes somewhat mushroom-shaped, with a thick stalk and a nearly circular terminal scale (figs. 34, 50).

The development of the flower is initiated in the usual way, by the bulging out of the periblem to form the stamen. Each stamen soon shows a swollen sporogenous tip, flattened on the side toward its mate, and a short but distinct stalk below (figs. 3, 4). The archesporium of the stamen arises as usual from groups of periblem cells, and is already well differentiated when the carpellary ring is closing above the ovarian cavity (figs. 2, 4).

When first clearly distinguishable, the primary archesporium of each microsporangium consists of a rounded group of 50 or more cells (figs. 8, 9). These divide later to form 1000 or more

spore-mother-cells in the larger microsporangia (fig. 11). The development of the tapetum and tetrads of microspores shows no unusual features. The ripe microspores are spherical, 7–9$\mu$ in diameter, with a smooth outer wall. They are at this time unicellular, but have two nuclei. When the nuclei are first formed, a delicate evanescent wall is visible between them. One of the cells thus formed is about twice as large as the other (figs. 18, 19).

The wall of the anther is three cells in thickness, except at the top, where it may be but two, outside the tapetum. The opening of the anther for the escape of the pollen is a slit or gap, which is terminal to the stamen and longitudinal to the spike (fig. 45). With the ripening of the microspores, the stalk of the stamen elongates considerably and pushes the opening anthers out beyond the bracts. The bracts, meantime, have been separated markedly by the increase in thickness of the axis (figs. 33, 45).

The most interesting peculiarity of the stamens of Piper betel is the variability in the degree of development attained by the stamen as a whole, and by its sporangia. This phenomenon occurs in Satureia, as is briefly noted by Correns ('08, p. 667). Often one of the two stamens of a male or hermaphrodite flower in P. betel, is much smaller than the other, as may be seen from a surface view, or section of the spike (fig. 13 at x). Not infrequently one of the stamens of an hermaphrodite or male flower is reduced to a sterile stalked knob, or mere peg, without a trace of a microsporangium in it (figs. 32, 34). In all female flowers, of both female and hermaphrodite spikes, both stamens are reduced to this latter condition (fig. 32).

In the hermaphrodite flowers of Piper betel monoicum, the appearance of the stamens and ovules in section indicates that both are functional in the same flower. If this be true, the plants should be spoken of as polygamous, rather than monoecious.

If we now consider the stamens which do bear microsporangia, we find that the number and extent of these sporangia differ greatly in different stamens, or even in the same stamen. A count of the sporangia in 225 stamens, from five spikes with hermaphrodite flowers and five with male flowers, showed that only 13½

per cent of these stamens had the usual complement of four sporangia (figs. 10, 16, 20). About 22 per cent of them had three sporogenous masses each (figs. 21, 22, 31). The larger proportion, 37½ per cent of all the stamens, had only two groups of sporogenous cells, which may both be in the same theca (figs. 26, 27, 28), or on opposite sides of the connective (figs. 23, 24, 30). In a very considerable proportion of the stamens but a single pollen mass is formed. This was found in 17½ per cent of those counted. The sole remaining spore mass may be on either the acroscopic or the basiscopic side of the connective (figs. 27, 28), or it may extend past the connective from one theca to the other (figs. 25, 29, 31). In all cases of this sort seen, the mass was continuous across the ventral side of the stamen (figs. 29, 31). Finally, 10 per cent of the stamens counted were devoid of any sporogenous tissue whatever (figs. 32, 51).

Not only does the arrangement of the sporogenous tissue differ in different stamens, but serial sections show that it differs at different levels in the same anther (figs. 22, 23—fig. 23 being nearer the top of the anther).

Two types of reduction in the number of spore masses can be distinguished among the stamens illustrated in figs. 20 to 32. There is one series of cases in which each of the masses present is in the position of one of the four sporangia of the normal stamen, while one or more of the regions usually occupied by a sporangium remains entirely sterile (figs. 9, 26, 28). In a second series of cases the regions usually occupied by two or three distinct sporangia, together with the larger or smaller sterile region between these, is occupied by a continuous sporogenous mass (figs. 21, 23–25, 27–31). No stamens were seen in which a continuous sporogenous mass seemed to fill the regions occupied by all four of the sporangia of the normal stamen.

The first type of reduction mentioned is evidently an example of the phenomenon referred to by Goebel ('05, p. 554) as the "arrest or suppression of pollen sacs," and spoken of by Bower ('08, p. 127), as the "abortion of the sporangia." Cases of this

sort were shown by Engler ('76, p. 297), to occur in the Asclepiadaceae, and, in Asclepias syriaca, where the two posterior sporangia are wanting, this absence is due to the complete failure of the archesporium to appear even at the earliest stages of development.

The second sort of reduction mentioned is clearly the kind referred to by Goebel as a "confluence of pollen sacs," which process, he says (p. 554) "may take place by the subsequent breaking down of sterile tissue, or by the development of fertile tissue in places where otherwise sterile tissue should be." Bower refers to this method of decrease in the number of sporangia as the "fusion of sporangia."

It is to be noted that this decrease in the number of sporogesous masses may not mean a decrease in the number of spores formed, if the size of the persisting spore masses is sufficiently increased. This is evident from a comparison of figs. 12 and 15, or 20, 21, 24 and 26, all of which are from stamens of about the same age and are magnified to the same degree.

The important questions to be answered concerning these peculiar stamens of Piper betel are: 1. At what stage of development is the suppression of one, two, three or all four of the microsporangia normally present, first discoverable? 2. At what stage is the body of sporogenous cells, that in certain stamens seems the topographic equivalent of two or three normal sporangia, first recognizable as a continuous mass? 3. In what manner are these peculiar modifications of structure brought about? In other words, to put all three questions in one, at what time, in what manner, and from what cause, does the trend of development in the various tissues of the anther depart from the usual development of these in the normal stamen?

The shape of the upper pollen sacs in the stamens shown in figs. 23, 24, 27, suggests that the continuous sporogenous mass there shown is a result of the partial disappearance of a septum, which at an earlier stage separated two distinct archesporial groups in the upper theca. The same conclusion might sometimes seem warranted by the appearance of sections of the same stamen taken at different levels (figs. 22, 23). The former of these figures

is from nearer the base of the anther, the latter from nearer the tip. That is, at the base of the theca in question, there seem to be two distinct sporangia, while a little higher up there is a continuous sporogenous mass across the whole width of the theca. Each of the two sporogenous masses shown in figs. 24 also seems the equivalent of the two usually present in each theca. So also the sections from different stamens shown in figs. 23, 27, 28, appear to indicate different stages of a progressive fusion of two sporogenous masses like those which have remained distinct in the stamen shown in fig. 26.

That no actual fusion of primarily distinct sporogenous masses really occurs during development is clearly seen from a study of sections of younger stamens. For example, I see no reason for doubting that the stamen shown in fig. 9 would have matured into one of the sort shown in fig. 26, or that the one shown in fig. 10 would have matured into one such as is shown in figs. 16 and 20. There is no evidence, from the development of Piper betel, that originally distinct sporogenous masses may become continuous, either by the degeneration of the sterile partition separating them, or by the cells of the partition becoming sporogenous.

A third possible explanation of the origin of this variability in the number of pollen masses present in the mature stamen of Piper betel is that discovered for Lemna, in which Caldwell ('99), has shown that certain portions of the primarily sporogenous tissue fail to form spores, and, instead, give rise to three sterile septa, dividing the sporogenous mass into four. In a stamen like that shown in fig. 8, for example, the belated appearance of a septum of sterilized archesporial cells in the upper theca would give rise to a stamen like that in figs. 9 or 26. But here also the study of development shows that nothing of the sort occurs.

In brief, all available evidence goes to show that the number of pollen masses present in the mature stamen is the same as the number of archesporial groups first laid down in the young anther. There is no fusion of fertile tissues, nor any secondary sterilization, to form septa, of sporogenous tissues once delimited. Hence it is evident that, whatever the terms "confluence of pollen sacs" and "fusion of sporangia" may be intended to mean, they cannot

be used, in the case of Piper betel, at least, to indicate any ontogenetic process of union occurring after the sporogenous cells are once distinguishable.

If the normal stamen of angiosperms is to be regarded as possessing four microsporangia—a view generally accepted—then it is evident that in the case of certain of these abnormal stamens we are warranted in saying that the development of one or more of the microsporangia is completely suppressed. The tissue, which in the normal stamen organizes the sporogenous mass, may in another fail to show any such specialization of its cells. On the other hand, we have seen that the tissue of the region which usually forms the connective may occasionally give rise to sporogenous cells, and thus form a continuous pollen mass across from theca to theca (figs. 25, 29, 31).

In the above description I have spoken generally of sporogenous masses, instead of sporangia. The question naturally arises here whether each distinct sporogenous group, with its continuous tapetum and wall, is to be regarded as an individual sporangium. In stamens, such as are illustrated in figs. 9 and 26, one must admit it would seem, that two microsporangia are present, and that each corresponds exactly, in location and development, to one of the four sporangia of the ordinary stamen. When however, we attempt to determine the number of sporangia in stamens such as are shown in figs. 21, 24, 25, 27, 28 and 29, the solution of the problem is not so evident. If we accept the definition of sporangium given by Bower ('08, p. 112), that an individual sporangium, in vascular plants, consists essentially of "an isolated spore-mother-cell, or a connected group of them, or their products—together with its protective tissues," then in the stamen shown in fig. 27, for example, there is but a single microsporangium present. For there is a single continuous mass of sporogenous cells completely surrounded by a tapetum. If, on the other hand, we note the position and extent of the sporogenous mass, and its partial division by a septum, it then seems evident that it is in position and extent the equivalent of two of the sporangia of an ordinary stamen. For the same reasons, the sporogenous mass of the stamen shown in fig. 29 may be considered the equivalent,

topographically, of three microsporangia, though according to Bower's definition it would be a single sporangium.

The outcome of this consideration of the facts stated must be to emphasize the lack of any definiteness in our conception of the sporangium as an individual organ, a lack which has been most clearly indicated, perhaps, in recent literature, by Bower's definition, above quoted.

The suppression or reduction in development of microsporangia may, as we have seen, be carried so far that the stamen is left quite sterile (figs. 32, 34). In still other cases, as in some female flowers, the stamen is apparently not represented by any structure, not even a staminodium. Even where pollen is formed it appears sometimes not to be functional, if one may judge from the withered microspores seen in flowers where other tissues seem well fixed.

The question of the greatest importance in connection with the present study is that concerning the factors which change the course of development of certain stamens in such a way that the normally fertile portions remain sterile, or, in other cases, the cells of normally sterile regions become fertile. This question will be considered after we have noted the development of carpels and ovules.

The wall of the ovary in Piper betel arises as a ring-like but lobed outgrowth, at a point just above the subtending bract of the flower. It is therefore between the two stamens, in the case of the hermaphrodite flower (figs. 2, 4, 13, 33, 44). Soon after this circular wall has closed in above the ovarian cavity, the ovule appears as a slight mound at the bottom of this cavity (fig. 2). The lobes of the ovarian wall elongate considerably as growth proceeds, and give rise in the mature flower to the three- or four-lobed stigma (figs. 13, 25, 35, 36, 37). The lobes of the ripe stigma are somewhat pointed and papillate (fig. 44), but after pollination, they thicken at the base and shrivel at the point, till in the mature fruit nothing but three or four blunt warty tubercles remain (figs. 65, 66). The number of these lobes usually present at the tip of the carpellary wall, and the presence of three partial divisions in this wall, some distance below the tip, indicates

that this wall is composed of three carpels (figs. 36). This view is strengthened by the fact, that even when a larger number of stigmatic lobes is present, the structure of the wall lower down is like that of ovaries with three stigmas. For example, a transverse section nearer the base of the ovary from which the four stigmatic lobes in fig. 37 are taken, shows a structure exactly like that illustrated in figs. 35 and 36, which latter figures are taken from an ovary with a distinctly three-lobed stigma. The number of vascular bundles in the wall of the ovary also indicates that it is made up of three carpels. All of this evidence supports the interpretation of the structure of the ovary given for the close relatives of Piper betel by Eichler ('78, 2, fig. 4).

From a very young stage of development of the flower, the tissues of the axis continue to expand radially on all sides, keeping pace with the elongation of the ovary. The result of this is that the cavity of the ovary becomes completely buried in the fleshy axis (figs. 4, 33, 44, 63, 68). Another result of this growth is that the stamens are carried outward, and often in the mature flower, seem to stand upon a mound of carpellary tissue (figs. 44, 45, 65).

The structure of the ripe fruit of Piper betel differs markedly from that of Piper medium (Johnson, '02, p. 322, figs. 14, 15). In the first place, the immersion of the seed of P. betel in the axis leaves little of the tissue about it to be derived from the carpels proper. The latter apparently form only the roof of the ovarian cavity (figs. 65, 66). The three layers of the carpellary tissue of the fruit of P. medium are distinguishable in P. betel only at the upper end of the fruit. At the sides, and near the base, only the two inner layers are present. There are four vascular bundles running longitudinally in the outer of these two layers (fig. 67), instead of six as in P. medium. The oil-secreting cells are three or four layers thick in the upper third of the fruit (fig. 66), they form a single broken layer at the sides of the fruit, and become more numerous again at the base. The whole of the tissue surrounding the seed is relatively soft, and the seed apparently must be set free by the decay of these.

While the normal course of development of the fruit in a female or hermaphrodite flower, is that just described, there are scattered

over the male, female and hermaphrodite spikes, female flowers, which show a wide range of stages of degeneration (figs. 46-51). Moreover, in any flowers with normal ovaries, the ovules seem to degenerate after reaching various stages of development. This often begins before the time of pollination and hence cannot.in these cases, at least, be attributed to the lack of a stimulus due to this process. The first stage noted in the series showing progressive degeneration of the ovary itself is one in which, though the carpels have closed together above the ovarian cavity, and the stigmas seem normally developed, the ovary is without a trace of an ovule (fig. 46). In other cases the ovarian cavity may be as reduced as in those just mentioned, and in addition, the stigmatic lobes markedly retarded in growth (fig. 47). In the flower shown in figs. 48 and 51 the ovarian cavity has entirely disappeared, while the stigmas are represented by a small spine, with no trace of three distinct constituent lobes. The stigmas of the flower shown in fig. 49 are represented merely by a slight mound. Finally, in the flower shown in fig. 50, there is no trace whatever of carpellary tissues.

The above mentioned cases were taken from spikes in which most of the flowers seemed functionally hermaphrodite, and in all the flowers figured the pollen seemed to be perfectly normal.

Of the functionally male spikes studied, only two or three spikes were found in which the ovary was not represented by a slightly or considerably developed rudiment, in from 5 per cent to 30 per cent of the flowers. Often an ovarian cavity is present, and rarely a well-organized ovule. No case, was seen, however, where fertilization seemed to have occurred in an ovule on a male spike.

The ovule of P. betel arises as a mound on the floor of the ovarian cavity. It is first distinguishable just after the carpels have closed in to surround the cavity (figs. 2, 4).

The inner integument is initiated at about the time the primary sporogenous cell is undergoing its first division (figs. 33, 52). The tips on the carpels have at this time closed tightly above the ovule to form a rather long stylar canal (figs. 45, 52). The outer integument starts soon after the inner (fig. 53), but never grows above the latter to take part in the formation of the micropyle,

as it does in P. medium (Johnson '02, figs. 5, 11, 14). A striking feature in the rate of growth of the integuments in P. betel is the inequality in rate of growth on the different sides of the ovule. That this difference is very considerable is evident from many longitudinal sections of the ovule (figs. 44, 54). The minor irregularities are still more clearly shown by the study of a series of successive transverse sections, such as are shown in figs. 38 to 43. This series shows that the inner integument has two distinct lobes, while the outer one has at least four. The longest lobe of the outer integument is pushed up tightly against the lower end of the stylar canal (figs. 44, 54).

In the ripe seed, the outer integument is made up of four or five layers of thin-walled cells (fig. 61). The inner integument forms the chief seed coat. It consists of three or four layers of cells throughout most of its extent. The innermost of these is slightly thickened and brownish in color, while the outermost layer has its outer walls greatly thickened by a granular deposit against their inner surface (fig. 68).

The escape of the seed from the fleshy spike, and the germination have not been seen. It is hoped that these will be found in material to be collected this spring.

The embryo-sac arises from the single, hypodermal, sporogenous cell in each ovule. This cell cuts off a thin parietal cell at the micropylar end, which may form six or seven layers of tapetal cells at the tip of the mature nucellus (figs. 52, 54, 57). The lower of the two descendants of the primary sporogenous cell enlarges, and finally develops directly into the embryo-sac (fig. 54). The first mitosis in the sac has not been seen, but when the two daughter nuclei prepare for division (fig. 55), it is evident that only sixteen, the reduced number of chromosomes, is present.

The four nuclei resulting from this second division of the embryo-sac may sometimes remain near the ends of the sac, where they are formed, or there may be a single nucleus at one end and three at the other (figs. 56, 58). Often, however, perhaps in half the cases seen, these nuclei may be closely grouped near the middle of the embryo-sac (fig. 57). In these cases the nuclei are as closely grouped as the nuclei of a microspore tetrad. It is of

course possible that some of these variations from the usual arrangement of nuclei in the four-nucleate stage, are connected with the phenomenon of degeneration of the sac which is of not infrequent occurrence, to judge from shrunken sacs found in the older spikes.

In an early eight-nucleate stage the nuclei are found gathered in two groups of four each, one at the upper, one at the lower end of the sac (fig. 59). Somewhat later than this an egg apparatus, of the usual three-celled type, is found at the upper end of the sac. Three antipodals are grouped at the base, and two polar nuclei meet at the middle of the sac (fig. 60).

Pollen tubes, and the details of fertilization, have not been seen, but, from the number of cases where two nuclei were seen in the egg, (fig. 54), there seems no reason to doubt that fertilization is accomplished in the normal manner. No evidence of a triple fusion has been noticed. The early stages of development of the embryo and endosperm have not been found, but in seeds that have grown to twelve times the diameter of one containing a just-ripe sac, the fertilized egg is still undivided, though the endosperm nucleus has given rise to scores of free nuclei in the peripheral cytoplasmic layer of the sac (fig. 63). The antipodals at this time have already multiplied to a number which may be as great as 35 in a single section of the sac. They occupy a large space at the base of the sac (figs. 61, 62). In the ripe seed the antipodals, somewhat crushed, can still be seen in a depression below the endosperm (figs. 65, 70).

The endosperm develops cell-walls after about 100 or more free nuclei have been formed, the walls apparently arising in the ordinary way. In the mature seed the endosperm forms an irregularly globular mass about 700$\mu$ in diameter, and showing about 150 cells in a median longitudinal section of the seed and embryo sac. The cells of the ripe endosperm contain little, if any, starch but have rather dense protoplasmic contents, which may serve as a store of nitrogenous material. The chief carbohydrate supply of the seed is stored in the starchy perisperm, which makes up 99½% of the bulk of the seed (fig. 65).

## DISCUSSION

The most interesting questions raised by the foregoing work on Piper betel are those concerning the different degrees of suppression of sporangia and sporophylls.

From the progressive series of reductions above noted, it seems fair to assume that whatever influence is at work to suppress sporangial development, is also concerned with the complete suppression of the stamens or carpels, and thus determines the formation of male or female flowers, which in many cases, make up the whole spike, to the exclusion of hermaphrodite ones. In other words, it is this influence that determines whether gametophytes of one or both sexes shall be formed at the next step in the life cycle of this plant, and of which sex they shall be.

We can take up these questions in regard to Piper betel more profitably after reviewing what is known of other plants, first concerning the time, and second, concerning the immediate cause of the differentiation of sexes in gametophyte or sporophyte.

Of well-known forms among the algae it is clear that in Vaucheria, Nemalion and some species of Spirogyra, Œdogonium, Coleochaete and Fucus, the individual plants are distinctly hermaphrodite, since the two kinds of sexual cells may arise close together on the same plant. In other species of Spirogyra, Œdogonium, Coleochaete and Fucus and in many Chlorophyceæ, Phaeophyceæ and Florideæ, the sexual plants bear one kind of sexual organs only.

In such a form as a dioecious species of Œdogonium, *e.g.*, the hermaphrodite condition can be supposed to exist, if at all, only up to the formation of the zoöspores from the zygote. It is not known whether all zoöspores from one zygote give rise to plants of the same sex or not. In dioecious species of Fucus there is no evidence to show that the thallus is not unisexual from the time of its origin from the oöspore. Hence the hermaphrodite condition must be supposed to exist in the oöspore only, and for a very short time. In hermaphrodite species of Fucus we must conceive of the thallus as being hermaphrodite up to the time of differentiation of antheridia and oögonia. If Yamanouchi's ('09)

view is correct, that the so-called antheridia and oögonia of Fucus are really sporangia, and that the male and female cells are really the daughter cells of spores that germinate directly in the sporangium, then the segregation of the sexes here, at the initiation of the microsporangia and megasporangia, is very like that to be noted later in the heterosporous ferns.

The case of Nemalion differs from that of the monoecious species of Fucus in the intercalation of a process of fragmentation of the oöspore (carpospore-formation), during which process, however, no segregation of the sexes occurs. This is evident from the fact that each carpospore gives a plant bearing antheridia and carpogonia on neighboring branches. This fact is particularly interesting in view of the statement of Wolfe ('04), that meiosis does occur during carpospore-formation.

In the brown alga Dictyota dichotoma the oöspore gives rise to a hermaphrodite plant which bears tetraspores (Williams, '03 and Hoyt, '10.) The latter germinate to male and female plants, perhaps two of each from each tetrad, according to Hoyt. The segregation of sexes apparently occurs in this species at meiosis.

In most of the Florideæ the conditions existing are probably the same as in Dictyota, as is indicated by the work of Yamanouchi ('06) and Lewis ('09). That is, the gametophytes are dioecious, the sporophyte is hermaphrodite, and segregation occurs along with meiosis at tetra-spore-formation.

In the fungi, little work has been done on this problem, except the very interesting work of Blakeslee on the moulds. In the genus Sporodinia, Blakeslee ('06) has discovered that the mycelium from both sporangiospores and zygospores is hermaphrodite, and thus that the sexual substances fusing in the zygote must become distinct, if at all, at about the time of the formation of the gametes. In Phycomyces nitens, he found that the mycelium from the zygote is hermaphrodite, while that from the sporangiospore is generally unisexual, and the segregation of sexes occurs during the development of the spores. Finally, the same worker found that in Mucor mucedo the mycelium from each zygote, like that from the sporangiospores, is preponderatingly male or female,

and the segregation of sexes, or suppression of one sex must therefore occur between the fusion of gametes and the germination of the zygospore, and must result in the suppression now of the male, now of the female tendency, in the mycelium coming from the zygospore.

Among the Bryophytes, it seems clear from the work of Blakeslee on Marchantia ('06), the Marchals on mosses ('06, '07), and of Strasburger ('09) on Sphaerocarpus, that the segregation of sexes takes place at spore-formation, probably during meiosis.

In homosporous ferns each sporophyte is usually clearly hermaphrodite, each spore also, and the prothallus coming from it, is usually bisexual, for though the male organs only may be developed at first, the female organs are usually formed later, on all well-nourished prothalli. Segregation in this case, if it can be called such, evidently takes place during the later development of the gametophyte.

The heterosporous ferns and Selaginella give the first clear indication, after that noted in Mucor mucedo and possibly Fucus, of the segregation of the sexes at a point in the development of the sporophyte other than sporogenesis. In Marsilia, *e.g.*, the gametophyte is never hermaphrodite, but distinctly male or female. The sporophyte, on the contrary, is hermaphrodite and retains this condition, so far as can be seen, up to the time of the separation of the three marginal cells of the seventh grade, in the sporocarp, which give rise to all the thirty or forty microsporangia and megasporangia of each sporus (Johnson, '98, figs. 34, 38). Shattuck ('10, p. 23) states that all the sporangia of Marsilia have the same development up to the time that spore-mother-cells are formed, after which the microspores and megaspores are markedly different in character. He states further that changing the conditions surrounding the plant, at the proper time of development, may cause the young spores in certain of the microsporangia to become enlarged, and to assume somewhat the type of spore wall of the normal megaspore. He has not, however, yet been able to germinate these enlarged spores, and has therefore failed to demonstrate conclusively that these have

become really female in character. It seems to the writer, especially in view of the early distinction of the micro- and megasporangium initials in Marsilia (Johnson '98), that the view of Strasburger ('09, p. 12), is still tenable—that all the spore-mother-cells formed in the microsporangium are essentially male and those in the megasporangium are essentially female.

Seliganella resembles Marsilia in that the gametophytes are unisexual and the sporophyte hermaphrodite, but it differs in that the sexes become distinct considerably earlier in the development of the sporophyte than in Marsilia. In fact, the sporophylls bear one kind of sporangium each. In erect spikes, those sporophylls at the base of the spike bear megasporangia only, those at the tip, microsporangia only. In the prostrate spikes of other species, megasporangia occur on the sporophylls turned toward the earth, while in species with drooping spikes they are found at the tip only (Hieronymus ('00), p. 659).

In such conifers as Pinus and Larix, and in monoecious angiosperms, the gametophytes are male and female, and the sporophyte is hermaphrodite, but the separation of the sexes is evident at a still earlier stage than in Selaginella. Microsporangia and megasporangia are borne not merely on different sporophylls but even on different branches, that is, in male and female cones or flowers.

In Cycas and Ginkgo and in dioecious angiosperms, the segregation of the sexes occurs at a still earlier stage, and male and female flowers are developed on entirely separated sporophytes, that is, the sporophyte, as well as the gametophyte, has become apparently unisexual, like the zygosporic mycelium of Mucor mucedo. In these angiosperms the hermaphrodite condition exists, if at all, only in the fertilized egg, or, possibly, on into the early stages of the sporophyte. It is certainly evident that the sex of the sporophyte is fixed, once for all, at some stage of the sporophyte before that at which the first crop of flowers is borne. For, so far as the writer has been able to learn, perennial plants of this type bear flowers of the same sex year after year.

In most angiosperms, while the gametophytes are unisexual, the sporophyte is hermaphrodite, so far as can be seen, up to the

time of formation of the sporophylls, when a separation of the sexes is evident. Here, as in the case of the gametophyte of the fern, the male influence or substance, seems dominant at first, as is evidenced by the earlier origin of the microsporophylls in most instances. These angiosperms differ in this respect from those Selaginellas with basal, (i.e. older), megasporophylls and terminal microsporophylls, though they resemble these selaginellas in having both megasporangia and microsporangia on the same axis. The monoecious Araceae, among angiosperms, also offer an example, or, at least, evidence, of the earlier dominance, of femaleness, in the usually basal position of the female flowers, on the spadix. Cases have also been noticed by Correns ('08), in Satureia, and by Strasburger ('09[2]) in Mercurialis, where purely female flowers are the first to develop in the inflorescences of these plants, the stamens not developing until much later.

We now come to the second part of our question, namely, the cause of the segregation of the sexes at the particular point where it does occur.

Experimental work on a number of plants has shown pretty clearly that the distribution of nutrient substances in the plant, together with the external conditions affecting the nutrition of the plant as a whole, are among the important factors concerned in the expression or suppression of certain organ-building tendencies in plants. We may recall in this connection only such of this work as bears on the causes of development of the reproductive organs.

Klebs has shown that the development or non-development of the sexual reproductive organs of Vaucheria, some other algae, and certain seed plants can be determined by changing such external factors as the osmotic or chemical character of the nutrient solution, or of the light or temperature affecting the plant. These facts strongly suggest that such factors may also determine which sex may be assumed by any individual in dioecious plants.

Among higher plants, such as ferns and Equisetum, many observers have asserted that the smaller, poorly nourished gametophytes are always male. In view of the fact that the antheridia appear on normal prothallia before the archegonia, the persistent

absence of the latter organs in certain cases may simply mean that these starved prothallia fail to attain that degree of maturity (whatever that may mean) at which archegonia are normally produced. This view seems distinctly confirmed by the experimental work of Miss Wuist ('10), on the prothallia of Onoclea struthiopteris. Campbell ('05, p. 314) has stated that these prothallia are constantly dioecious. Miss Wuist finds that ordinary soil cultures show about 1 per cent of protogynously monoecious prothallia. When prothallia, from a soil culture, which have borne only archegonia, are grown in Beyerinck's fluid for five to seven days they begin to bear antheridia. Similar results were obtained when prothallia bearing archegonia only were transferred from distilled water to Knop's solution. Miss Twiss ('10, p. 168) has shown that the prothallia of Aneimia and Lygodium are not really dioecious but merely protandrously monoecious. Goebel ('05, p. 220) suggests that the evidence for persistent dioeciousness in the ferns is everywhere inadequate.

It seems possible, from what has been said, that all the cases of dioeciousness described among homosporous pteridophyta are attributable to external factors. This belief is strengthened by the work of Correns ('08, p. 661) on Satureia hortensis. He has shown that, in this gynomonoecious species, individuals that under normal conditions produce 15 per cent of normal females, 7 per cent of imperfectly hermaphrodite and 78 per cent of functionally hermaphrodite individuals can, by cultivation on poor soil, or with insufficient illumination, be induced to form a larger percentage of pure females. By the combined action of these two agencies the male tendency may be so greatly inhibited that but 17 per cent of hermaphrodite flowers are formed while the proportion of pure females rises to 79 per cent. Correns also notes that the percentage of hermaphrodite flowers is greatest at the height of the blooming season, while, at the beginning and end of the flowering period, female flowers preponderate.

Another interesting case with a bearing on this question, and one that perhaps brings us a step nearer the immediate causal factor, is that of Mercurialis annua, studied by Strasburger ('09[1], p. 507). He found that certain isolated plants of this species

persisted for months in bearing female flowers only. Finally a few reduced stamens appeared, and then, after the process was once initiated, the same plants continued to bear considerable numbers of short-lived, but functional, stamens along with the female flowers. Certain of the plants, bearing female flowers only, had these accidentally pollinated from a male plant in another greenhouse. In consequence, they not only formed seeds, but, shortly after, began to develop male flowers, and did this much earlier than did the unpollinated female plants mentioned above. These facts led Strasburger to the conclusion that the lack of pollination, and the consequent lack of seed-development in this species, leads to a gradual increase of a tendency to initiate stamen rudiments, probably by the accumulation of some material substance. The same process may be induced even more quickly by the influence on the female plant of pollination and seed-production.

The works of Williams ('03) and Hoyt ('07) on Dictyota suggest a similar accumulation of some activating substance as the cause of the very regular periodic initiation and discharge of the gametangia of this alga.

In Melandryum rubrum, Strasburger ('00) found that the presence of the parasite, Ustilago violaceae, may cause plants that have hitherto borne only female flowers to develop staminate ones. Goebel ('07), suggests that, in such cases as this of Melandryum, the course of nutritive substances in the infloresence is changed, in consequence of some stimulus produced by the Ustilago. Strasburger ('09[1], p. 19) suggests that a substance activating male development, and one activating the development of female organs, are always present in the infloresence, and that in hermaphrodite flowers the two substances come into play separately, while in male or female flowers the male substance alone, or the female substance alone, completely preponderates.

Whether the explanations suggested by these unusual types can be assumed to indicate the relation of the sex-determinig substances in the case of permanently unisexual plants is, however, less certain. This is shown, *e.g.*, by the work of Noll on Marchantia, of which he propagated the unisexual plants by the gemma

for over thirty generations without any indication of change of the strictly unisexual condition. It must also be remembered that such perennial dioecious plants as Cycas, Ginkgo, Populus, etc., have been under continuous observation for years, without any completely authentic case of change of sex being recorded, so far as the writer has been able to learn. It may be considered as doubtful whether the apparent microspores found by Chamberlain ('97) on the carpel of Salix petiolaris, the apparent spermatogenous cells found in the archegonia of Mnium by Holferty ('04), or the apparent megaspores found in the microsporangium of Marsilia by Shattuck ('10) are really capable of functioning as reproductive cells.

We may now consider the case of Piper betel in the light of the observations just reviewed, to see whether these help in interpreting the facts recorded concerning this species, and also whether these facts support or controvert in any degree the views reached from the study of other forms.

It is clear, from what has been said of Piper betel, that the sporophyte of this species is distinctly hermaphrodite, and also that the sexual character of each constituent of the hermaphrodite flower is already determined at the time of initiation of the stamens and carpels. It is likewise evident that the tissue of the young spike that is to bear perfect flowers must be potentially hermaphrodite in character. If this latter be true, then it seems probable that the tissue of those spikes, all of whose flowers are functionally unisexual, is likewise hermaphrodite at first. We must either admit this as proven by the fact that staminate flowers often bear some rudiments of megasporophylls, and the carpellate flowers nearly always bear rudimentary stamens, or else we must assume that, in the latter case, *e.g.*, the male-determining substance is absent but the female-determining substance is capable of causing, or at least allowing, the development of the abortive stamen-like structures. Of these two possibilities, the evidence available seems to make the former view far more probable. In other words, it seems clear from the different degrees of suppression of microsporogenous tissue described in P. betel, that we must assume the male-determining substance to be present in all flowers, but more

or less inhibited in certain flowers from expressing itself as a controlling factor in the development of tissues in the flower rudiment. That is femaleness is unusually more or less dominant over the male tendency. (Shull, '10, p. 119.) Or, we must assume that an absolute segregation of the sex-determining substances may occur at very different times in different spikes, in different flowers of the same spike, or even in different stamens of the same flower. Of these two views the former seems much more probable from the facts above given. It also agrees with the results, previously referred to, of the work of Correns ('08) and Strasburger ('09) on gynomonoecious angiosperms. The same condition is clearly indicated also by the work of the Marchals ('07) on those diploid gametphores of the mosses, in which, while some individuals bore both antheridia and archegonia, *i.e.*, the flowers were hermaphrodite, other individual gametophores, as long as kept, bore only one kind of sexual organ each.

From all the evidence now available we are warranted in assuming the possibility of the presence of the second sex in many of those angiospermous sporophytes where only one sex has thus far been detected. The best evidence for this assumption being found in the fact that in certain known cases the application of the proper stimulus may cause this second sex to become evident, by the development of its proper reproductive organs. (Morgan, '09, p. 337, 346.)

If then the flower of Piper betel is potentially hermaphrodite, what is the cause of the suppression, partially or wholly, of the sporogenous tissue or even of the sporophyll itself, now of the stamens, now of the carpels or now of both? What, in certain stamens, is the cause of the extension of the sporogenous tissue across the whole width of the anther? That space relations, or the crowding of the parts of the flower in the bud, do not constitute the determining factor seems evident from the fact that the sporogenous tissue of the microsporangium, *e.g.*, may be suppressed or extended now in the upper, now in the lower theca of the stamen, while, less frequently, it may occur in both, or extend across from one theca into the other (figs. 9, 24-31). There is likewise no indication of the localization of these abnormal

flowers along the axis of the spike. They occur with equal frequency at the base, middle or tip of the spike. Finally, it is possible that the immediate cause of the failure of a microsporangium, *e.g.*, to develop in any quarter of the anther is the absence of the necessary nutritive or spore-determining material from this region. How such an unusual distribution of this substance may be brought about it is not easy to see, though it is perhaps no less understandable than the cause of the usual distribution which results in the formation of the four microsporangia in the normal stamen.

Probably any factor that disturbs the mechanism for the normal process sufficiently may bring about the suppression or extension of sporogenous cells. Such a disturbance of the normal movement of either nutritive, stimulative, or possibly of inheritance-bearing substances in the plant seems the most probable immediate cause of the phenomena here recorded for Piper betel. Moreover it seems evident that the change which occurs in this sex-determining or stamen-determining substance is progressive and quantitative. (See Morgan, '09, p. 336.) Whether this distribution of material is ultimately conditioned by external factors acting on the plant, must here, as elsewhere, be determined by experiment. For an investigator working in a region where this plant can readily be brought to flower and fruit, it may be expected to yield results of great interest concerning the segregation of the sexes in this species, with a bearing on the problem of the distribution of sexes in angiosperms generally.

## SUMMARY AND CONCLUSIONS

The distribution of flowers of Piper betel may be either dioecious, monoecious, or monoeciously polygamous. The degree of development of the stamens and pistils often differs markedly in different flowers of the same spike, or even in different stamens of the same flower.

The details of the development of the stamens and pistils of the perfect flowers are those usually found in angiosperms. An evanescent wall separates the two nuclei formed by the first

division of the microspore nucleus. The ripe microspore is unicellular and binucleate. The primary archesporial cell of the ovule cuts off a single parietal or tapetal cell above. Then the lower half, which is perhaps to be considered a megaspore mother-cell, gives rise immediately to an eight-nucleate embryo-sac of the usual angiospermous type.

Fertilization and endosperm-formation take place in the normal way. About 100 free, peripheral nuclei are formed before cell walls appear in the endosperm. The antipodals multiply to 100 or more. In the ripe seed the embryo consists of a globular undifferentiated mass of about 500 cells. The endosperm is of 150 cells in median longitudinal section. Its cells contain little stored carbohydrate. The numerous antipodals persist in the seed, but seem to have little stored food-material in them. The abundant and starchy perisperm is the chief storage tissue. The fruit is immersed in the axis, but aside from the differences in structure connected with this fact, it resembles the fruit of Piper medium.

The most striking peculiarity of this species is the extreme variability in the development of the microsporangia and megasporangia on different spikes or in different flowers of the same spike. The number of microsporangia in a stamen *e.g.*, may vary from none to four, and the extent of a single sporangium may be such as to fill one-quarter of the anther, or, in others, as much as three-quarters. The number and relative extent of the sporogenous masses in the stamen is constant from the time of their initiation. There is no breaking down later of sterile septa to throw two sporogenous masses into one. There is no secondary fusion or confluence of sporangia, nor is there any evidence of the abortion or suppression of sporogenous tissues once initiated, unless it be found in the possible infertility of the well-formed pollen of some of the ripe anthers.

The cause of the differences in development of the sporogenous tissue seems not to be connected in any way with the space relations of the flowers on the spike, since any type may occur at any point on the spike, *i.e.*, at base, middle or tip. The real cause is probably to be sought in those factors, internal or external,

that disturb the normal production or course of movement of material in the plant.

The evidence from this study of Piper betel concerning the distinction of sexes in the sporophyte, seems to show that the tissue of the young spike, and often of the individual flower, must be hermaphrodite in character. The differentiation of the sexes, by separation or by the suppression of one of them, must take place at or after the initiation of the rudiments of the parts of the flower. This view is in accord with the conclusion gained from experimental work on a number of species of angiosperms, as well as on certain lower plants.

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## EXPLANATION OF FIGURES

All figures are camera drawings and all are from microtome sections except figs. 13, 25, 65, 68.

The magnification given in the description of each figure is that actually shown by the figure as printed on the page.

Abbreviations used: *Ant*, antipodal; *Asp*, archesporial cell or cells; *Br*, subtending bract; *Cp*, carpel or carpellary tissue; *E*, egg; *Em*, embryo; *Esp*, endosperm; *Iin*, inner integument; *Oc*, oil-containing cell; *Oin*, outer integument; *Pa*, parietal cells; *Ps*, pollen-sac; *Psp*, perisperm; *St*, stamen; *Vb*, vascular bundle.

1 Part of longitudinal section of young spike, showing bracts and rudiments of stamens. × 75.

2 Part of similar section of slightly older spike, showing rudiment of carpels and ovule. × 75.

3 Part of section like last, through a stamen showing beginning of differentiation of the archesporium. × 350.

4 Longitudinal section of flower through stamens and carpels. × 110.

5 Transverse section of a bract and the two stamens of its flower. × 160.

6 Transverse section of very young stamen. × 600.

7 Transverse section of slightly older stamen, showing beginning of differentiation of archesporial and vascular tissue. × 350.

8 Similar section of slightly older stamen, showing one archesporial group of cells at each end of the section. × 350.

9 Similar section of still older stamen, showing two archesporial masses in one loculus and none in the other. × 350.

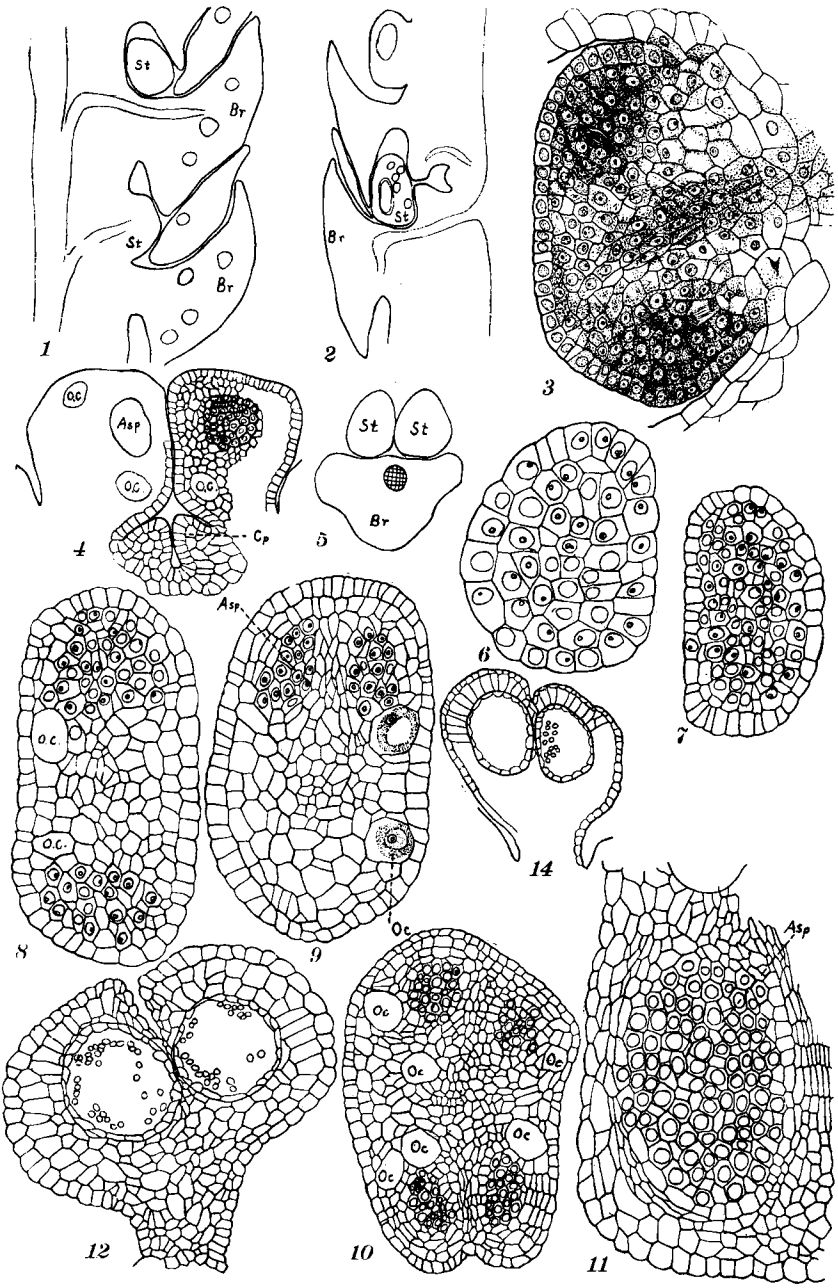
10 Similar section of slightly older stamen, showing two archesporial masses (microsporangia) in each loculus. × 210.

11 A single microsporangium from the stamen shown in fig. 26. × 350.

12 Longitudinal section of a stamen, from a transverse section of a spike, showing microspores, tapetam, wall and line of dehisence. × 150.

13 Surface view of part of spike, showing overlapping of bracts and the variable number of stamens and stigmas. × 10.

14 Longitudinal section of stamen, from transverse section of spike. × 75.

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EXPLANATION OF FIGURES

15 Section of the stamen shown in the last figure, several sections removed from the one there shown. × 160.

16 Transverse section of a nearly mature stamen with four microsporangia. × 75.

17 Section of nearly ripe pollen-grain, showing mitosis of first division. × 1000.

18 Section of older, two-celled pollen grain. × 1000.

19 Ripe pollen-grain, in which the wall has disappeared. × 1000.

20 Transverse section of half-mature stamen with four microsporangia. × 100.

21 Transverse section of stamen with three pollen sacs, of a staminodium and of a bract. × 100.

22 Transverse section of stamen, showing two sporogenous areas in the upper loculus and one in the lower. × 100.

23 Another section of the same stamen, showing the continuity of the sporogenous mass in the upper loculus. × 100.

24 Transverse section of stamen showing one sporogenous mass in each loculus. × 100.

25 Surface view of spike, showing bracts, stigmas and variety in distribution of the sporogenous masses. × 38.

26 Transverse section of stamen with two sporangia in the lower loculus and none in the upper. × 100.

27 Transverse section of a stamen with one sporogenous mass in the upper loculus and none in the lower. × 100.

28 Transverse section of stamen with one sporogenous mass in the lower loculus and none in the upper. × 100.

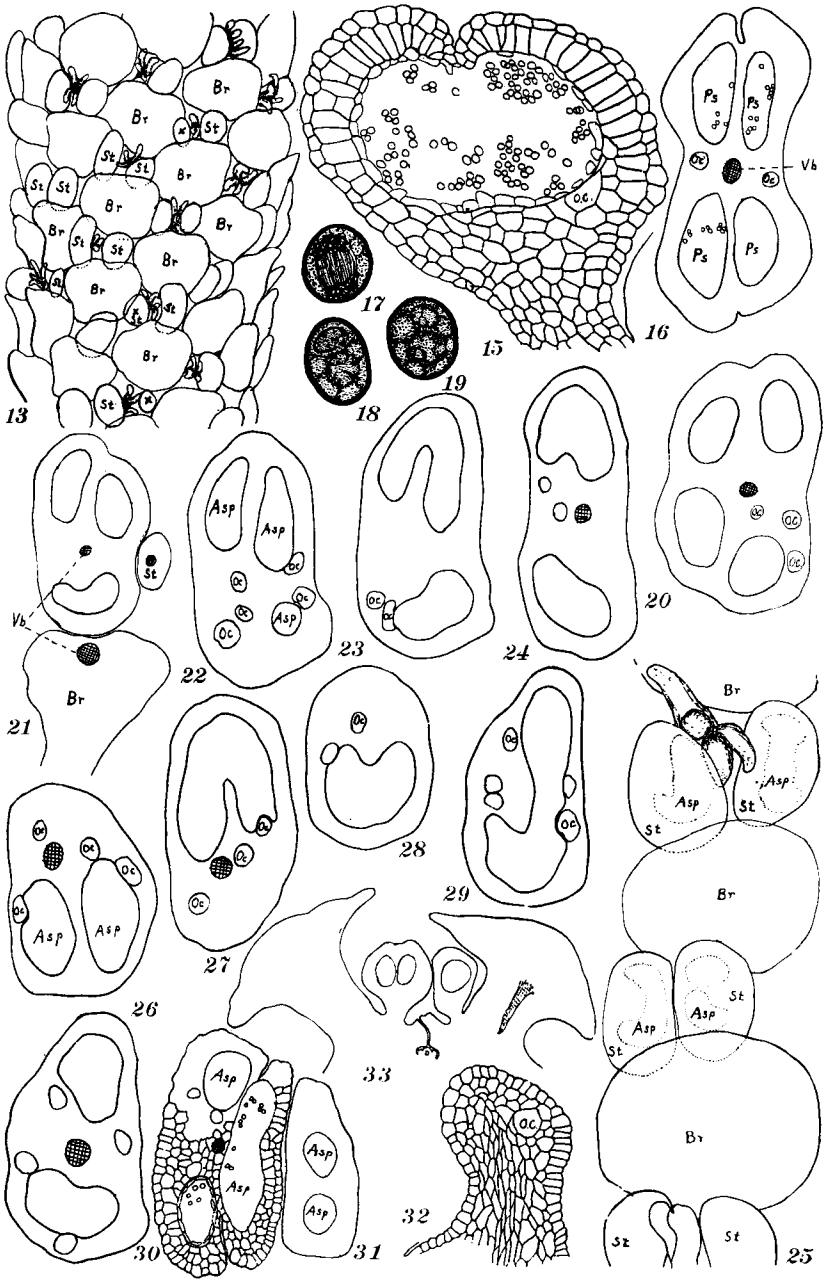
29 Transverse section of stamen with one continuous sporogenous area. × 100.

30 Transverse section of the same anther at a level nearer the base, apparently showing two sporogenous masses. × 100.

31 Transverse section of anther showing continuous sporogenous mass across the anterior face of anther, while one sporangium in each loculus remains distinct. × 75.

32 Longitudinal section of staminodium, of about same age as the stamens shown in figs. 20–30. × 160.

33 Longitudinal section of flower and two bracts, showing relation of these structures and the complete submergence of the ovary in the axis. × 40.

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EXPLANATION OF FIGURES

34 Longitudinal section of flower with one staminodium. ×40.

35 Transverse section of tip of carpels showing three styles. × 75.

36 A lower section of the same ovary. × 75.

37 Transverse section of another ovary with four styles. × 75.

38–43 Series of successively lower transverse sections of an ovule and its integuments, showing the different height of the integument on different sides of the ovule. × 160.

44 Longitudinal sections of flower with mature stamens and stigmas and with four-nucleate embryo-sac. × 35.

45 Longitudinal section of flower, and two bracts, in which the pollen has been discharged from the single stamen. × 35.

46 Longitudinal section of flower with two stamens, well-developed stigmas, but no ovules in the cavity of ovary. × 40.

47 Section of another flower like last but with partially aborted styles and stigmas. × 35.

48 Section of similar flower but with no ovarian cavity and a mere spine in place of styles. × 40.

49 Section of similar (but younger) flower with still more reduced styles. × 75.

50 Longitudinal section of flower from male spike, with no trace of carpellary tissues evident. × 40.

51 Longitudinal section of flower, from middle of male spike, in which both tamens and carpels are rudimentary. × 75.

52 Longitudinal section of young ovary, with ovules showing inner integument. × 350.


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53 Longitudinal section of young ovule with two integuments, parietal cell already formed. × 350.

54 Longitudinal section of older ovule, with integuments completed and the megaspore mother-cell preparing for its first division. × 350.

55 Embryo-sac preparing for the second division, each spindle shows about eighteen chromosomes. × 600.

56 Section of a four-nucleate sac, showing a pair of nuclei at each end. × 750.

57 Section of a four-nucleate sac, showing the nuclei grouped near the middle. × 600.

58 Section of four-nucleate sac with one nucleus at the micropylar end and three at the antipodal end. × 600.

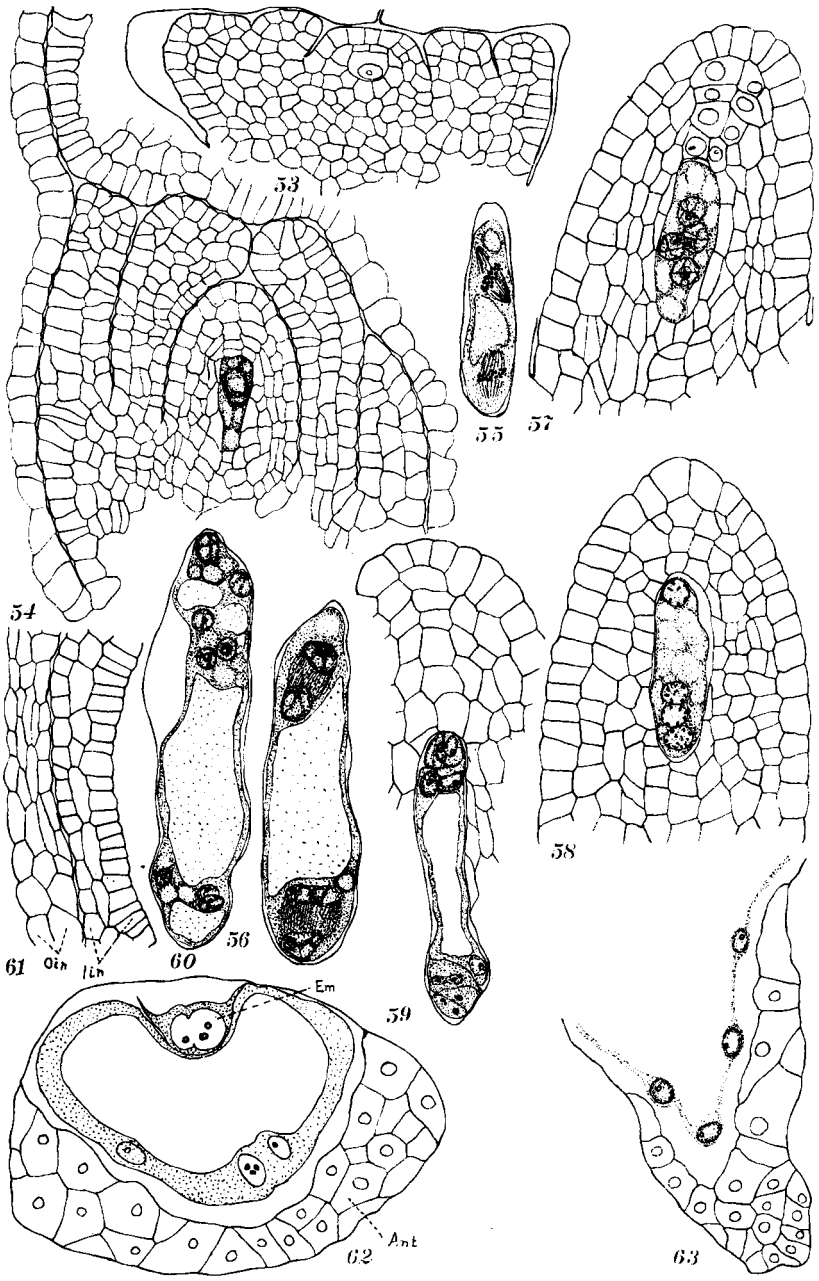
59 Longitudinal section of nearly mature sac, in which the polars have not yet moved to the middle. × 600.

60 Longitudinal section of nearly mature, somewhat abnormal embryo-sac. × 600.

61 Part of longitudinal section of integuments of an ovule with a nearly mature embryo-sac. × 350.

62 Longitudinal section of sac, with fertilized egg, free endosperm nuclei, and 25 antipodals in the single section. × 170.

63 Part of a similar section, showing endosperm nuclei, and numerous antipodals in a deep pocket at the base of the sac. × 180.




D. S. J., del.

EXPLANATION OE FIIURES

64 Part of longitudinal section of seed, showing one-celled embryo, free endosperm nuclei, antipodals, tapetum, inner integument and part of the perisperm. × 75.

65 Transverse section of spike, showing number and arrangement of ripe fruits. × 10.

66 Longitudinal section of a mature fruit, showing structure of fruit and seed. × 40.

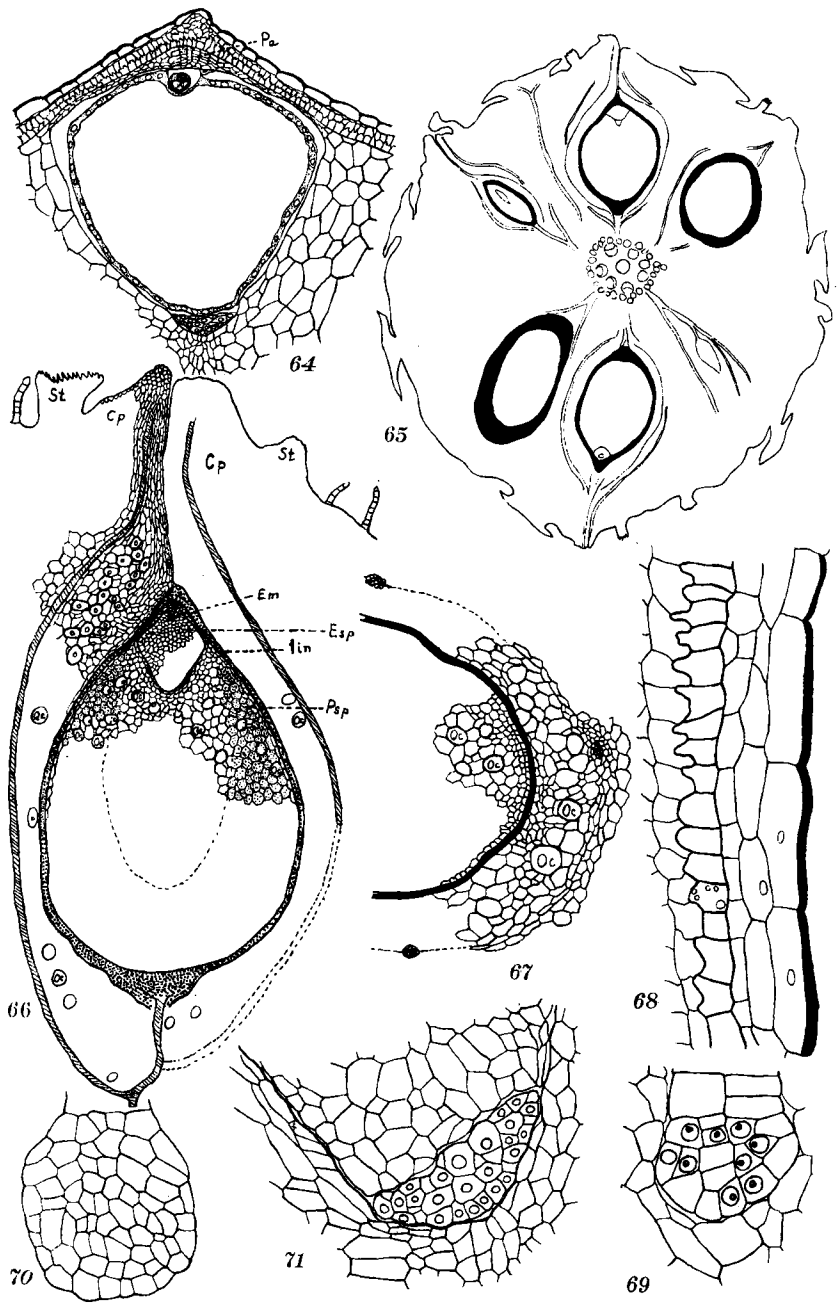
67 Part of transverse section of mature fruit. × 40.

68 Part of longitudinal section of inner integument, showing structure. × 350.

69 Longitudinal section of a half-grown embryo. × 350.

70 Similar section of mature embryo. × 350.

71 Part of longitudinal section of ripe seed, showing perisperm and persistent antipodal mass. × 170.




D. S. J., del.