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POLLEN DEVELOPMENT IN THE GRAPE WITH SPECIAL REFERENCE TO STERILITY

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POLLEN DEVELOPMENT IN VITIS WITH SPECIAL REFERENCE TO STERILITY*

By M. J. Dorsey

INTRODUCTION

Sterility in plants has been the subject of much investigation, both from the standpoint of its bearing upon the evolution of plants and from that of its relation to the setting of fruit. These investigations have established the fact that many complex and closely related factors have a bearing upon it. In general these factors may be grouped under two heads: (a) those which are "inherent" in the plant and (b) those which result from an unfavorable environment. Sterility of the first type is deep-seated and cannot be controlled while that of the second can often be avoided by methods which counteract the effect of the unfavorable factors.

STERILITY IN PLANTS

Knowledge of sterility has been developed with that of functional differences in the floral structures. Swingle ('04) states that "artificial pollination of the date palm was doubtless discovered by the ancient Assyrians, and has been practiced, probably, for at least three or four thousand years." Interest in the general question of fertilization in plants received its greatest impetus in 1862 when Darwin published his work on The Various Contrivances by Which Orchids Are Fertilized by Insects. In this investigation he showed that there are various contrivances by which orchids are fertilized which are primarily designed to bring about cross-pollination. Since the appearance of this work it is probable that too much emphasis has been placed upon the multitude of special floral structures that are supposedly designed to bring about cross-pollination. Darwin ('76) emphasized the importance of cross-pollination in plants and showed that some forms are much reduced in vigor by continual self-pollination.

While there seems to be an "inherent tendency" in some genera toward such a differentiation and specialization of the floral parts as tend to favor cross-pollination, in others this differentiation is not

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found and close-pollination habitually occurs. This specialization, or lack of specialization, as the case may be, results in a complex series of conditions with respect to a differentiation of floral structures when both flower and plant are taken into consideration. If the stamens and pistils are borne in the same flower, it is perfect, bisexual, hermaphroditic, or monoclinous; if in separate flowers, the flower is imperfect, unisexual, or diclinous. Declinous species may be monœcious, i.e., the two kinds of flowers are borne on the same plant, or dicœcious, i.e., the pistillate and staminate flowers are borne on separate plants.

With respect to the source of the pollen, cross-pollination occurs when a stigma is pollinated with pollen from an anther borne on a different plant; close-pollination, when pollen borne by a perfect flower is transferred to its own stigma. Between these two extremes the intermediate type occurs when pollination takes place by the transference of pollen from the anther of one flower to the stigma of another on the same plant. Cross-pollination, or at least pollination of the intermediate type above mentioned, must occur (a) when the species is dicœcious, (b) when special morphological structures prevent close-pollination, (c) when the pollen is matured and shed either before or after the stigma reaches the receptive stage, and (d) when the pollen borne by the stamens of a flower is either sterile or for various reasons does not germinate when placed upon the stigma.

Sterility, it should be remembered, can result from defects leading up to the formation of pollen or lack of functional power in it or in some of the structures within the ovary. It may be associated with various types of morphological abnormalities in the stamen or pistil. A distinction should be made, however, between sterility resulting from morphological causes, such as aborted structures, and that resulting from physiological causes, such as differences in the time of maturity of pollen and stigma or "lack of affinity," whatever this may mean, between pollen and stigma.

The relation of parthenocarpy and parthenogenesis to seed- and fruit-production should not be overlooked. Parthenogenesis is the development of an egg into a plant without fertilization. In parthenocarpy fruit is developed without pollination. The stimulus resulting from pollination seems necessary in some cases in which fertilization, however, does not occur. These are cases, therefore, of parthenogenesis but not of parthenocarpy. Common instances of parthenocarpy are found in certain varieties of the cultivated grape, orange, apple, and banana. It is clear that parthenocarpic development, especially in some varieties of grapes, may invalidate bagging tests of sterility unless carefully checked.

STERILITY IN CERTAIN ECONOMIC FRUIT-BEARING PLANTS

A discussion of the general question of differentiation and sterility in the floral parts of the higher plants has been introduced in order to show that sterility in cultivated fruits is not unlike that in other plants. In common with other plants, cultivated fruits are brought into contact with a variable environment at critical periods in their development, especially at the time of blooming and at the close of the growing season. Many varieties, however, especially those which have been introduced from abroad, are not so well adapted to encounter severe extremes as the native plants.

Sterility in fruits has been investigated rather extensively. The most important fact brought out by this work is that certain varieties are self-sterile to a greater or less extent when self-pollination takes place. A corollary to this is the further fact, which is now followed in common practice, that varieties require mixing in planting in order to facilitate the setting of fruit. The work in most cases has not been of such a nature as to determine the cause of sterility, but cytological investigations of sterility by some investigators have resulted in bringing out many interesting facts regarding structural and functional defects in essential organs.

The bagging method has been used extensively in testing the potency of pollen in fruits when self-pollinated. The unopened flower is enclosed in a bag made of paper, or other material. This method gives contradictory results in some cases and is undoubtedly more reliable with some fruits, as the grape, than with others.

The idea of enclosing blossoms in paper bags for crossing is by no means new. N. B. White, of Norwood, Massachusetts, states in a personal letter that he first used it "some time in the sixties," and that he got the idea from France. A number of the early grape-breeders adopted this method. Goff ('85) enclosed clusters of Concord in paper bags to determine whether they would produce fruit as well when self-pollinated as when crossed. He states that with this variety those "enclosed were quite as well formed as those not enclosed."

The first important work in this country, however, in testing self-sterility in fruits was begun independently by Beach ('92 b, '94, '95, '98, and '99) working with the grape, and Waite ('94), with the pear and apple. The classical work of Waite was begun in 1890. He found that many varieties of the cultivated pear are self-sterile but cross-fertile. The cause of self-sterility was attributed to the "lack of affinity" between the pollen and the ovules of the same variety rather than to any deficiency in the pollen. The work of Beach with the grape showed that many varieties of this fruit are also self-sterile. The results of

Green ('93) and Whitten ('99) with the grape agreed with those of Beach.

Bailey ('92) states that "the chief difficulty in the growing of the native plums is the fact that some varieties do not fertilize themselves." He adds that this peculiarity seems to be due to "impotent" pollen rather than to any "imperfection in the flowers." Waugh ('96) carried the work with plums further and showed that many varieties are self-sterile as in the grape, apple, and pear. Reimer and Detjen ('10), using the bagging method, found that some of the varieties of *Vitis rotundifolia,* such as the Scuppernong, were also self-sterile in varying degrees.

Hedrick ('08) among others, has emphasized the relation of weather to setting of fruit, and he points out that weather conditions in which the temperature falls low enough to be harmful to blossoms are usually associated with frost or rain. It is well known that the pistils of most fruits are less hardy than the stamens. Unfavorable weather, besides being accompanied by low temperatures which often may result in injury, lessens the activity of insects in pollination and often results in direct harm from heavy rains and winds. Fairchild and Beach ('92) subjected a Mount Vernon pear tree and a vine of Dutchess grape to a constant spray for nine and twelve days respectively. Although the conditions of rainy weather were only partially imitated, since the light, temperature, and wind relations would be different in this experiment, nevertheless unfruitfulness resulted. The pollen, however, seemed to be but little affected. On the other hand, Fletcher ('00) found that apricot pollen was injured by a prolonged rain in 1898. Lewis and Vincent ('09), working with the apple, and Fletcher ('09-'10), with the pear, found that climatic conditions influence the self-fertility of a variety. These investigators also note that a variety may vary in sterility in different localities. Other investigators in Europe, as well as in this country, have extended the list of self-sterile fruits. The value of investigations of the general subject of sterility in plants warrants the recent interest taken in this problem and should insure a still more thorough investigation into the causes which produce it, and, if possible, the discovery of methods of overcoming it.

STERILITY IN THE GRAPE

We have seen that a number of varieties of American grapes are self-sterile when pollinated with their own pollen, but that they set fruit when pollinated with potent pollen from another vine. It would seem, then, that the cause of sterility is to be found in the pollen rather

than in the pistil. The relation of hybridity, flower type, and dioeciousness to impotency in the grape pollen is important, and will be treated more at length later in this discussion.

The problem before us in the grape, then, is the determination of the cause of sterility and whether it is of such a nature that it can be overcome. The solution of this problem involves a cytological study of pollen development in both the sterile and fertile forms.

This work was suggested as a result of the author's experience with the grape while assistant horticulturist at the New York State Experiment Station and was submitted to the Graduate Faculty of Cornell University in June, 1913,* as major thesis for the degree of Doctor of Philosophy. This work has been completed at the University of Minnesota Agricultural Experiment Station as an Adams Fund project.

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MATERIAL AND METHODS

Brighton, which was made the basis of this investigation, is typical of a number of sterile or nearly sterile cultivated varieties which have attained considerable commercial importance. It is a hybrid between Concord and Diana Hamburg. This variety was originated by Jacob Moore, of Brighton, Monroe County, New York, and fruited for the first time in 1870.

Brighton is a vigorous grower, is hardy in the milder climates, and has proved a good producer when properly pollinated. The berry is dark red at maturity, round, and of very good quality. The stamens are recurved, and extensive bagging tests, especially those of Beach

* Since the work was completed, Gard ('13) has published the results of some cytological investigations of sterility in the grape, which confirm the results reported in this paper. Gard worked with other varieties than those made the basis of this work. In the varieties with reflexed stamens he found the pollen nearly or quite empty and lacking a generative cell, or the nucleus of the latter, when present, was found deformed or degenerated.

('98 and '99), as well as numerous observations where vines have stood alone, have shown it to be self-sterile or nearly so.

Concord, the pollen parent of Brighton, is a variety of *V. labrusca.* It has been carefully compared with Brighton with reference to the development of the microspore, the degeneration of the tapetum, and the chromosome number. Diana Hamburg, the other parent of Brighton, is a hybrid between *V. labrusca* and *V. vinifera.* It could not be obtained and this hybrid is probably no longer in existence.

Barry is also a hybrid between *V. labrusca* and *V. vinifera,* and it was chosen for comparison with Brighton on this account. Mature pollen has been sectioned and examined from the following varieties also: Adirondac, Amber Queen, America, Beta, Black Eagle, Black Hamburg, Canada, Catawba, Clinton, Croton, Dracut Amber, Dutchess, Eumelan, Gaertner, Herbert, Hubbard Seedless, Lindley, Marion, Massasoit, Mills, Northern Muscadine, Rogers No. 5, and Vergennes. In this list a number of species are represented in many different combinations.

Material has also been studied from 2 wild species—*V. bicolor* and *V. vulpina.* Of the former, 4 staminate and 3 pistillate vines were selected, and of the latter, 24 staminate and 28 pistillate vines.

Material of the varieties and species mentioned has been fixed from vines growing under a variety of conditions. That of Brighton and Concord has been taken from vines growing in the experimental vineyards of the Stations at Cornell University, Ithaca, New York, Geneva, New York, and the University of Minnesota, at Minneapolis. The material of *V. bicolor* was obtained only from the region about Ithaca, New York. That of *V. vulpina* was obtained extensively from wild vines growing near both Ithaca, New York, and Minneapolis, Minnesota. Mature pollen of most of the varieties was sent to the writer from the Geneva Experiment Station by Richard Wellington.

In order to compare pollen-formation in Brighton with that of other self-sterile forms, as well as with the self-fertile, 788 vials of material have been fixed and embedded, and over 1,800 slides of various stages have been made.

Flemming's killing fluids have been used in three strengths, strong, medium, and weak. The first two and chrom-acetic, in which equal parts of a one-per-cent solution of each acid was used, gave good results. Flemming's triple stain was used most extensively, although Heidenhain's iron-alum-haematoxylin also gave good results. The paraffin method of embedding was followed throughout. The sections were usually cut five microns thick, although a number were cut which varied from this thickness.

POLLEN DEVELOPMENT IN VITIS

MORPHOLOGY OF THE FLOWER PARTS

The early stages of the flower were investigated with the object of determining whether there are any morphological abnormalities which might influence later development. These stages were compared in Concord, Brighton, and *V. vulpina* ♀, and from all appearances growth differentiation occurs normally.

The cluster.—The embryonic grape cluster, in the bud, is enclosed by scales. When growth starts in the spring it breaks through some of these and leaves them behind. The young growths at this time vary in color and are covered with masses of pubescence. Longitudinal sections show that each secondary division of the cluster occupies a position axillary to smaller scales on the central axis.

The flower.—The grape flower is pentamerous, although there is considerable variation (Dorsey, '12) from the usual number of floral parts. The petals, which are united throughout their entire length, usually break first at the bottom when the flower opens, being united to the receptacle at this point by a constricted area composed of from six to eight layers of small, thin-walled cells. The petals also are united at the suture by two rows of small, thin cells, where the separation takes place at blooming-time. The calyx, in the early stages, extends well up over the young bud. *Vitis* presents three types of flowers which are borne on separate vines: (1) stamens upright with abortive pistil, (2) stamens upright with functional pistil (perfect flowers), and (3) stamens reflexed, with more or less abortive pollen, and a fully developed functional pistil.

The anther.—The anther in the early, bud stages has a very short filament, and in cross-section shows four areas of rather homogeneous tissue, with numerous cells undergoing division. The archesporial cells in these areas, which mark the position of the anther sacs, are easily distinguished by their larger size. Each anther has four pollen sacs or loculi, varying from 40 to 50 μ in diameter, at the stage of the young pollen mother-cells. Only rarely do variations from this number of sacs occur. The anther sac enlarges rapidly as pollen development takes place, so that at maturity it has enlarged to about 120 μ in diameter.

When dehiscence takes place, the walls of each lateral pair of loculi break away by their inner, narrow, thin-walled margins, throwing the pollen of both together. The pollen has much the same appearance in all the loculi, and when dehisence takes place in the bud it is liberated toward the stigma. Beach ('92 b) observed that in a num-

ber of varieties the anthers dehisce before the flowers open. Anthers occur rarely in the cutivated varieties in which there is no pollen.

The filament.—The filament in those flowers with upright stamens presents a distinct morphological difference from those with reflexed stamens. Cross-sections of both are elliptical or circular. The epidermal cells in filaments of the upright stamens are of equal or nearly equal size on the outer and inner surfaces (Figure 4), while the outer epidermal cells of the recurved type are much smaller than the inner (Figure 3). The result of this difference in the strength of opposite sides of the stamens of the reflexed type is the curving backward which occurs in many wild and cultivated pistillate forms. The fibrovascular bundles in both the upright and reflexed filaments seem normal and present no constant differences. Figures 3 and 4 represent cross-sections of Brighton and Concord respectively; sections representing the reflexed and upright filament in a number of varieties and species show this same difference. This difference in the filament in this connection is interesting in that the reflexed stamen is associated in varying degrees with pollen sterility.

TISSUES AND CONTENTS OF THE YOUNG ANTHER

In the stage of the young pollen mother-cells the tissues of the anther are well defined and distinct. The mother-cells are surrounded by a single layer of tapetum, three layers of parietal cells, and a rather thick-walled epidermis (Figure 1).

The epidermis.—The epidermal cells of the anther, up to the time of the young mother-cells, are thick-walled, longer than wide, and have rather large nuclei and scant cytoplasm (Figure 1). At this time their radial diameter is about 20 μ. When the second division of the mother-cells is completed, they have become narrower and more elongated (Figure 2) and have much thicker outer walls. The nucleus in many cells is now more or less flattened, but in others it appears normal. At the time of the division of the microspore nucleus the epidermal cells of many loculi are present only as thick irregular borders to the outer layers of parietal cells or the endothecium (Figure 5). At this time the epidermal cells are about 7 μ thick. Very few epidermal cells at this stage contain cytoplasm. The lumen of the cell is usually completely closed. At all stages the epidermal cell walls stain deeply with orange G.

The parietal cells.—In the typical young anther there are three layers of parietal cells (Figure 1). The cells composing the two inner layers are slightly smaller than those in the outer layer and at the early mother-cell stages have the appearance of being slightly

compressed. They are readily distinguished from the cells of the tapetum at this time by their smaller size. The cells of the outer parietal layer and the epidermal cells are now about equal in size.

Beginning at the time of the young mother-cell, important changes take place in the parietal cells. The two inner layers gradually become compressed (Figure 2) so that in the late mother-cell and tetrad stages they appear as narrow bands of irregular cell walls between the endothecium and the tapetum (Figure 5). Later, when the tetrad nucleus is dividing, and still later, when the pollen is mature, they form a ragged border on the inner surface of the endothecium, disappearing completely in many loculi by the time dehiscence takes place.

The endothecium.—The outer layer, however, undergoes quite a different development (Figures 1, 2, and 5). Its cells enlarge, increasing in radial diameter from about 7μ in the early mother-cell stage, when they are about equal in size to the epidermal cells, to 13μ at the time of mitosis in the microspore nucleus and to 30μ at the time of dehiscence. They now function as the principal tissue in the walls of the loculi, the epidermal cells forming an irregular strengthening border on their outer surfaces. These are measurements of the cells in question in Brighton, but the other forms resemble it closely in general proportions except *V. bicolor,* in which the endothecium is nearly twice as thick.

The tapetum.—The function of the tapetum as a nourishing layer immediately surrounding the pollen mother-cells, its morphological origin and subsequent growth and degeneration, are well known, and any departure from its normal behavior is of interest when associated with sterility.

The importance of studying the tapetal cells in connection with sterility and degeneration in pollen has been emphasized by Gates ('07 a) and others. Bonnet ('11), who has made extensive investigations of the evolution of the tapetum, states that the phenomenon of amitosis does not appear to exist in the nourishing cells. All of the appearances which have been attributed to them are believed by Bonnet to be explained by mitotic irregularities and nuclear fusions. Gates ('11) found the tapetal cells in *Oenothera gigas* uninucleate up to about the time of synapsis. Duggar ('00), working with *Symplocarpus,* found that the tapetal cells wandered in among the mother-cells.

In the stages of the young pollen mother-cell the tapetal cells are not as large as the pollen mother-cells; they have at this stage one large nucleus, in which there is usually one nucleolus (Figure 1). The chromatin is granular in structure and stains deeply. The cytoplasm

is very irregularly granular, and uneven in its distribution in the cell, and resembles the cytoplasm of the parietal layers in its staining reaction. At these early stages there is no evidence of degeneration or division of the nuclei, or of conspicuous vacuoles in the cytoplasm.

From the time of synapsis to that of diakinesis in the pollen mother-cells the tapetal cells have grown to about the size of the mother-cells or even larger, and now have from two to three nuclei, which usually vary much in size and shape; or, in some cells, there is only one large nucleus with an irregular or lobed outline. The nuclei of the tapetal cells at this time (Figure 6) have usually two or more nucleoli, which generally vary in size. In most cells vacuoles occur, which vary much in size and shape.

Degeneration may be much further advanced in some tapetal cells in a loculus than in others; or the different loculi in an anther or bud may show considerable variation in this respect. In some loculi there is a tendency for the tapetum on one side of the loculus to break down much faster than on the other—a condition similar to that observed in *Oenothera* (Gates, '07 a).

During the division stages in the pollen mother-cells, degeneration becomes very evident in the tapetum. Further divisions take place, resulting in from two to four or more smaller nuclei, which in cross-section may be circular, elongated, elliptical, or irregularly lobed. The nuclei are either irregularly scattered in the cell or are aggregated near the center. The nucleoli have increased in number, so that from two to four or more may be seen in each nucleus. Large vacuoles appear in the cytoplasm, which has lost much of its granular structure and appears to be of a more homogeneous nature.

By the time the divisions are completed, the degree of disintegration in the tapetum varies much in different buds. While in some cells the nuclei appear to be well organized and the cytoplasm is more or less vacuolated, and less granular in appearance than in the earlier stages, in others degeneration has advanced so far that no trace of nuclei is evident and the cell contents are quite disorganized. The nucleus may be completely broken down or very much compressed and irregular in outline with the contents staining deeply (Figure 5). Large vacuoles occur in the cytoplasm, which now has the appearance of being drawn out in irregular, ragged strands. The tapetal cell walls have become more irregular by this time and are often broken apart. In many sections the tapetum is broken away from the parietal layers, the two inner rows of which have become much compressed and enclose a circular clear area between them and the tapetum. The fixation in anthers showing this is good, which would indicate that this condition is due to the breaking down of the two inner parietal

layers and the enlargement of the loculus rather than to shrinkage during fixation.

After the liberation of the microspores by the dissolution of the thick wall of the mother-cell, the tapetal cells rapidly lose their contents, so that by the time of the division of the microspore nucleus their contents have almost completely disappeared in most cells, while in others only ragged strands of protoplasm partly border the cell walls and show more affinity for the orange stain than for the violet. In some of the protoplasmic strands still remaining in the tapetum, traces of the nuclear membrane and contents appear as more deeply staining, elongated areas.

While a few cases of tapetal cells of unusual size, which seem not to be undergoing the usual degeneration, have been observed in Brighton, in general, in the forms of *Vitis* investigated, the tapetum undergoes the course of development and disintegration usual in the dicotyledons.

Other changes now occur in the loculus. Upon the division of the microspore nucleus, rapid enlargement of the pollen grain takes place, and during further growth and adjustment, the tapetal cells are gradually compressed (Figure 5) against the endothecium. When the pollen is mature, and before dehiscence takes place, the tapetal cells form a narrow, ragged, and irregular border to the loculus and, together with what remains of the two inner rows of parietal cells, lie close to the inner walls of the endothecium. In some anthers, at the time of dehiscence, very little remains of the tapetal cell walls.

The anther sap.—The term anther sap will be used to designate the liquid medium surrounding the microspores after their liberation from the common mother-cell wall. In discussing the degeneration of the tapetum and its bearing upon sterility the relation between it and the anther sap should not be overlooked. The degenerating tapetum and the thick mother-cell walls no doubt contribute largely to its formation.

The anther sap first becomes evident after the mother-cells have rounded up. At this time, as we have seen, disintegration has begun in the tapetum. While in the earlier stages the anther sap stains only lightly, upon the breaking down of the thick, orange-staining, mother-cell walls it stains more easily and readily takes the orange and violet. At about the time of the division of the microspore nucleus or later, it stains more deeply and is of a very homogeneous nature (Figure 37). It fills the intervening spaces between the microspores and the tapetum and where the tapetal cells are broken apart, it enters between them and occupies the whole space within the loculus. During the time of the later pollen development it seems to become vacuolated, or

drawn out into irregular strands, and by the time dehiscence occurs it has been absorbed in some anthers, while ragged strands of it still remain in others.

The foregoing account of the degeneration of the tapetum in *Vitis* is based upon the study of the variety Brighton. It should be remembered that the pollen of this variety is impotent. Careful comparisons have been made of the degeneration of the tapetum in Brighton with that of some other sterile or partially sterile varieties, such as Barry, Northern Muscadine, Massasoit, and *V. vulpina* ♀, with some of the self-fertile ones, as Concord, Black Hamburg, and *V. vulpina* ♂. Here we have an opportunity to compare the history of the tapetum in sterile and fertile varieties, among the latter being Concord, the pollen parent of Brighton. The normal appearance of the tapetum up to the late pollen-mother-cell stage, its subsequent disintegration, and consequent nourishment of the microspores, indicate that in both the sterile and the fertile forms of *Vitis* studied it undergoes the normal course of formation and disintegration common to the higher plants. This being the case, sterility in *Vitis* does not seem to be due to abnormalities in the functioning of the tapetum.

THE HETEROTYPIC AND HOMŒOTYPIC DIVISIONS

A thorough investigation of the development of the microspores up to and including the homœotypic division was made in order to determine whether there was any cytological evidence of degeneration leading to sterility during these stages. These stages were carefully compared in the sterile and fertile forms. The following account is based upon Concord and Brighton.

The pollen mother-cells.—In *Vitis* the anther tissue, during the stages of the young pollen mother-cells, does not differ in any important feature from that already recorded in other higher plants. The epidermis, parietal layers, and tapetum are well differentiated, and the mother-cells have the characteristic polyhedral shape (Figure 1), with rather thick walls. During this period, the different loculi in the anthers of a bud show great uniformity of development, although the later stages, as the division stages, which take place much more quickly, do show a slightly progressive development. These variations, however, are not great.

The mother-cell nucleus is large, quite uniform in size, and roundish or slightly oval in cross-section. The chromatin in the earlier stages is noticeably scant (Figure 7) and the delicate reticulum is spread out about the periphery of the nucleus, leaving large clear areas of nuclear sap near the center, unoccupied by either chromatin or

nucleolus. Irregular areas of chromatin occur, which are rather uniform in size and stain a little more deeply than the fine, loose network connecting them. These are not as distinct and definite in outline as the prochromosomes of Overton ('05), Stout ('12), and others.

The mother-cell nuclei in Brighton have from one to four or more nucleoli, the usual number being two or three. There is one large nucleolus, quite uniform in size in different nuclei, usually lying near one side of the nuclear cavity, and one or more smaller ones which in some sections stain much more lightly (Figure 7). The number of nucleoli varies in different species. The mother-cells of Concord, representing *V. labrusca,* usually have only one nucleolus in each nucleus; *V. vulpina* has one large one and frequently one or more smaller; *V. bicolor,* one large and one smaller, although in some sections there are more than one of the smaller nucleoli; *V. vinifera* has as many as from four to six. Brighton resembles its *V. vinifera* parent in this respect.

The chromatin increases considerably in amount during the presynaptic stages and the network becomes more drawn out into irregular granular fibers, which are not confined to the periphery of the nuclear cavity so much as is the reticulum in the earlier periods of development. These fibers in many places approach each other and run more or less parallel for varying distances, much resembling the condition in *Lilium* (Allen, '05) in this particular. The larger, deeper-staining chromatin granules are still evident, and the fibers radiating from these still give the whole structure the appearance of a distinct reticulum. The slender fibers gradually change to larger strands, so that previous to synapsis a very irregular, loose spireme is formed, which is granular in nature and still contains many somewhat larger chromatin masses.

Synapsis.—The spireme in *Vitis* at the time of synapsis is slender, dense, and quite even in thickness. The nucleoli are closely associated with the synaptic mass, sometimes lying outside of it, but more frequently more or less embedded in it. In synapsis the spireme is very much bent and folded upon itself, forming a dense, compact mass which, with the nucleoli, occupies a position at one side of the nuclear cavity close to the membrane. The synaptic mass is very irregularly roundish or oval in general outline, with many irregularities upon its surface in the form of folds or projecting loops. The threads forming the projecting folds are so dense and compact that their exact nature cannot be determined.

In *Vitis* the spireme undergoes a distinct contraction period during synapsis, differing in this respect from the condition reported in a few other forms among which is *Smilacina* (Lawson, '11 a). Just previous to synapsis the more or less distinct spireme, as has been mentioned, is well distributed through the entire nuclear space. Fifty measure-

ments of the nuclear diameter at this stage gave an average of 7.6 μ. During synapsis 50 nuclei had an average diameter of 8.2 μ, while the average diameter of the synaptic aggregation of these same nuclei was 4.1 μ. Median sections, including most or all of the synaptic mass, were selected for these last measurements. Three hundred nuclei, at the late open-spireme stage, had an average diameter of 8.4 μ, while the same number, at diakinesis, averaged 8.3 μ. These measurements are all based upon Brighton, and only median sections were used.

The open spireme.—When loosened from the synaptic coil, the spireme is quite uniform in thickness, and granular in appearance, but is still compact. It becomes irregularly distributed throughout the nucleus, but occurs mainly near the periphery and is coiled and folded upon itself (Figure 8), resulting in many irregularly twisted and tangled loops. No free ends can be seen. It is circular in cross-section and only occasionally are stages found which show its double nature. This period lasts for some time and the spireme becomes noticeably thicker in the later stages. The nucleoli are found to lie either near the center of the nucleus or at the side. The open spireme has much the same appearance in all of the species examined. It is during this period that the mother-cells become rounded-up and more or less separated.

Diakinesis.—The spireme thread can be seen in some sections more or less completely segmented into chromosomes, which are at first very ragged and irregular but soon become uniformly scattered about the nucleus, lying close to the nuclear membrane. The nucleolus still persists and the mother-cells have become much more rounded. The chromosomes are distinctly two-parted at this stage, agreeing in this respect with the condition recorded in many other forms, and are quite uniform in size. There are apparently no unpaired chromosomes, as in *Drosera* (Rosenberg, '03, '06 b, '09). Later in diakinesis the double chromosomes become more compact and lose the ragged appearance of earlier stages.

Decided changes in the mother-cells take place simultaneously during the later stages of diakinesis. The mother-cells, which now have thicker walls, become more spherical in form. They first break away at the angles and as the rounding-up proceeds become free and independent of each other. At this stage delicate granular strands which radiate outward from the nucleus, appear in the cytoplasm. The nucleolus becomes flattened or elongated against the nuclear membrane and in some sections stains more lightly. In the region of the nuclear membrane fibers appear (Figure 10) which are more or less. tangential to it. The nuclear plasmic membrane becomes more ir-

regular in outline and gradually disappears. At the same time the fibers elongate. At first the fibers lie close together in the region of the nuclear membrane, then gradually separate and extend in all directions in the cytoplasm, in pointed bunches, giving the appearance of a distinct multipolar spindle, characteristic of this stage.

The nucleoli disappear at this time, assuming various shapes as they do so, some being much flattened and elongated, or drawn out to a long point in one direction; or in the later stages they sometimes break up into a flattened mass with parts extending out in finger-like projections (Figure 10). The spindle fibers change from the irregular multipolar condition to that of the distinct bipolar spindle by extension in two directions. During this change the two-parted chromosomes come to lie more nearly in one plane. These changes take place quickly, some anthers showing a progressive development from diakinesis to the late prophase of the heterotypic division, while in other anthers in the same bud, stages from diakinesis to the completion of the homœotypic division may be represented.

The heterotypic division.—The chromosomes in the prophase of the heterotypic division, in Brighton, are so compact that their double nature can be detected only in a very few cases. They are quite uniform in size, regular in outline, and slightly oval in shape. Toward the poles the spindle is either conical or slightly rounded; but it is always distinctly bipolar and extends nearly across the cell, either pole terminating in a rather sharp point which lies in the cytoplasm free from the plasma membrane. The average length of 10 spindles of this division was 12.3 μ. The fibers extending from pole to pole are very distinct and thicker than those attached to the chromosomes (Figure 11).

In the equatorial-plate stage the chromosomes assume a position nearly in one plane. In well-fixed preparations they are separate and distinct and can be easily counted. They are compact and regular and stain deeply. No trace of nucleolus is to be seen. In polar views, in which the chromosomes can be seen, they are found to be of nearly equal size and none lie apart from the others or show any evidence of abnormality. The shape and size of the chromosomes at this stage are quite similar in the different varieties and species examined.

There is in most divisions some irregularity in the separation of the chromosomes at the equatorial plate and their passage to the poles (Figure 11). This irregularity is not marked and occurs to about the same extent in both the fertile and the sterile forms. No chromosomes have been observed to have been left behind in the cytoplasm.

At the conclusion of the anaphase, the chromosomes lie in a close,

compact group at the poles, well toward the sides of the mother-cell. The changes which take place during the telophase are rapid. The intrapolar spindle fibers become less and less distinct, but are still visible in the cytoplasm even after the daughter-nuclei are well organized. After the formation of nuclear membranes about the chromosomes, the daughter-nuclei increase in size rapidly, but the chromosomes still exist in masses quite uniform in size and are distributed mostly about the periphery of the nucleus. One or more nucleoli appear in the daughter-nuclei. The chromosomes become more irregular in outline and less dense, but they do not anastomose, or lose their identity.

The daughter-nuclei undergo a period of growth and reorganization after the heterotypic division, but a cell plate is not laid down between them. The chromosomes are distributed evenly to the daughter-nuclei, and the subsequent development, leading up to the homœotypic division, is much the same in the fertile and the sterile forms.

The homœotypic division.—The period between the two divisions of the mother-cell is short, and even though there is considerable growth and reorganization of the daughter-nuclei following the heterotypic division, sections are common in which both divisions are found in the same bud or even in the same anther. The cytoplasm of the spore mother-cell during the early prophase of the homœotypic division has the same general appearance as in the earlier stages. The daughter-nuclei of the first division are usually slightly oval or compressed, the long diameters being perpendicular to the axis of the heterotypic spindle.

At the time of the appearance of fibers in the region of the nuclear membrane, the chromosomes are distinct and distributed quite uniformly about the periphery of the nucleus. This division does not have the distinct multipolar-spindle stage characteristic of the previous one, but as the preparation for division proceeds, the fibers become massed into irregular regions at opposite sides of the cell which determine the axis of the spindle. As the nuclear membranes disappear, the nuclei become compressed and irregular. The spindles are formed at each side of the mother-cell perpendicular to the axis of the heterotypic spindle, but are narrower, more pointed, and often curved or bent to one side. The chromosomes at the equatorial-plate stage of the homœotypic division, like those in the previous division, are separate, very compact, and quite uniform in size. The two nuclei divide simultaneously, the axes of the two spindles lying in different planes in most cells. To all appearances the chromosomes are distributed equally to the daughter-nuclei in this division also, and come to lie in a close compact mass at either pole. The four nuclei in each mother-cell are

reorganized with marked regularity, and none have been observed to degenerate at this stage or earlier (Figure 12).

Four microspores are formed in each mother-cell in *Vitis,* as is usual in most genera. Exceptions, however, to the usual number occur in some genera, which may or may not be associated with sterility in the pollen. Tischler ('08) found from none to four developed in *Potentilla,* while Coulter and Chamberlain ('09), setting forth the results of other workers, show that in some species from three to six or more microspores, equal or unequal in size, may be formed. While particular reference has been made to Brighton, material representing *V. labrusca, V. vinifera, V. vulpina, V. bicolor,* and a number of hybrid varieties has been carefully compared with this variety. The development up to this point in each form studied proceeds normally. It is clear, then, that with respect to sterility in *Vitis* the chief interest centers around the development of the microspore into the mature pollen.

GROWTH AND DEVELOPMENT OF THE MICROSPORE

Organization of the microspore nucleus.—A nuclear membrane soon forms about the close, compact mass of chromosomes after the homœotypic division. The chromosomes still persist as definite, regular masses, but distribute themselves about the nuclear cavity close to the membrane when growth begins (Figure 12). The nuclear sap increases in amount rapidly, and the enlarging nuclei remain regular and turgid during the early-growth period.

The nuclei become large and nearly spherical, but the chromatin has undergone some changes, becoming less dense and more drawn out into an irregular, granular network rather than being in masses more or less uniform in size. There are large clear areas in the nuclear sap. The nucleoli appear soon after the reorganization of the nucleus and increase in size, so that before the tetrads are separated they are quite large, and spherical in shape. The nuclei are centrally located in the microspores while united in the mother-cell as well as for some time after being set free.

The nucleus enlarges rapidly during the period of rapid growth of the microspore wall. In the microspores of some anther sacs the nucleus remains spherical, but in other cases it becomes much elongated or oval.

The chromatin is scant and is distributed about the periphery of the nucleus in irregular granular strands. The nucleolus is nearly always located at one side of the nucleus. Previous to the division of the microspore nucleus considerable growth takes place in both the

stainable cytoplasm and chromatin and although the vacuoles become much smaller they do not disappear.

The liberation of the microspore.—While the mother-cells have become much thickened by the time the division stages have been reached, their thickness becomes much greater after the homœotypic division. During the later stages of the tetrad period after the microspores become rounded but before the mother-cell wall breaks away and sets them free, they become separated by a partition of the mother-cell wall varying in thickness and outline, which seems to flow in between them (Figure 9). Transitional stages of this change can be seen when the walls are being formed about the microspores and they begin to round up, where the apparently more or less viscous material entering into the composition of the common mother-cell wall begins to enter between them.

The rapid increase in the thickness of the material in the mother-cell walls occurs previous to its dissolution, or breaking down. It should be noted that the microspores are liberated by a gradual dissolution of the thick mother-cell wall rather than by a breaking process due to their growth. Transitional stages of this process can be seen in some sections where the mother-cell is only partly dissolved. The material entering into the mother-cell wall, from the earliest stages up to its disappearance upon the liberation of the microspores, is of a very homogeneous structure and stains deeply. During the process of dissolution the staining reaction changes, so that it stains much more faintly, and finally there are left only irregular patches in the anther sap which react to the stains. The outer margin of the mother-cell wall usually persists after the inner part is more or less broken down or dissolved. During the process of dissolution the mother-cell walls in some cases seem to be vacuolized, in others drawn out into shreds or irregular strands, and in some others all that remains is a very irregular network about the microspores. The material making up the mother-cell walls is added after its dissolution to the anther sap in which the liberated microspores are embedded and nourished.

The liberated microspore.—The internuclear fibers of the homœotypic division which radiate outward in the cytoplasm from each nucleus are quite distinct (Figure 12) previous to the appearance of the delicate granules upon them which mark the position of the membrane laid down simultaneously between the microspores. Each microspore soon becomes surrounded by a thin membrane, which is independent of that of the common mother-cell. As long as the microspores are held together in the tetrad, their individual cell walls remain thin and undifferentiated.

The arrangement of the four nuclei in the tetrad is that charac-

teristic of the simultaneous divisions in the dicotyledons, in which three nuclei occupy a triangular position in one plane, with the other above or below, as the case may be.

The microspores continue to grow while enclosed within the mother-cell wall, becoming more rounded in the later stages (Figures 9 and 13). The cytoplasm becomes more dense and has a granular structure similar to that of the early stages of the mother-cells.

One of the most evident changes occurring in the microspore after being set free by the dissolution of the mother-cell wall is the more rounded shape assumed. The nucleus is still centrally located. Following the stage in which the rounding occurs, there is a period in which the microspore enlarges rapidly, and the stainable cytoplasm appears much more scant when spread out in the increased area. The walls continue to enlarge, increasing the area of the cell so rapidly that the stainable cytoplasm becomes distributed around the periphery, the greater portion, with the nucleus, occupying a position at one side of the microspore (Figure 14), a position which is retained until after the division of the microspore. During this stage there are invariably one or more large, irregular vacuoles into which the stainable cytoplasm projects in ragged uneven strands. Owing to the rounding of the microspore immediately after separation from the tetrad and the central location of the nucleus at this time, the relative position of the nuclei to those walls which were adjacent in the mother-cell cannot be determined, as Duggar ('00) found to be possible in *Symplocarpus*.

The growth of the microspores continues, although not so rapidly, after their nuclei divide. Their increase in size occurs at the same time that the tapetum and the two inner rows of parietal cells undergo further compression or degeneration, and the growth of the endothecium enlarges the anther sac. These changes occur simultaneously and increase the space within the loculus to accommodate the increased volume of the microspores.

Division of the microspore nucleus.—During the period of growth of the microspore, after being set free from the common mother-cell wall, the nucleus enlarges rapidly and at the same time changes take place in the chromatin, which, as we have seen, remains in rather definite uniform masses for some time during the early organization of the microspore nucleus.

As the microspore grows, the chromatin gradually spreads out into a delicate granular network (Figure 14). The amount of the chromatin increases greatly and the network becomes denser and more uniform in thickness. The chromatin exists in this form for some time, but before division a continuous spireme is formed which at first, in

some sections, contains large granules and is very ragged along the margins, but later becomes thicker and more regular in outline.

The spireme is long and well distributed throughout the nuclear area, coming to lie, in the later stages, near the nuclear membrane (Figure 15). Before segmenting into chromosomes, the spireme is much bent into segments (Figure 16), as in *Symplocarpus* (Duggar, '00), which correspond to the chromosomes into which it is to segment. The chromosomes of this division when just formed from the spireme are very large compared with those of either the heterotypic or the homœotypic division. They are uniformly distributed about the periphery of the nucleus (Figure 20) and lie close to the nuclear membrane. The nucleolus, which is usually situated at one side of the nucleus, is large and at this stage becomes slightly irregular in outline. Before disappearing as the time of division approaches, the nucleoli become flattened or elongated and stain much more lightly. The chromosomes become much shorter and thicker (Figures 17 and 20) as they pass to the equatorial plate.

The spindle fibers appearing in the division of the microspore nucleus are much less conspicuous than those of the heterotypic division. In preparations showing stages immediately preceding the arrangement of the chromosomes on the equatorial plate, there are delicate strands radiating outward into the cytoplasm from the nuclear membrane on the side opposite that nearest to the microspore wall, similar to those in the heterotypic division, except that owing to the scantiness of the stainable cytoplasm they are fewer in number and less conspicuous. The fibers are very inconspicuous in the region of the nuclear membrane previous to its breaking down and the disappearance of the nucleolus. This division differs from the heterotypic in this respect as well as in the greater size of the chromosomes.

Before the nuclear membrane disappears, the chromosomes, which are usually curved and bent, lie close to it. The nucleolus becomes flattened or drawn out in many cases before the nuclear membrane shows any irregularities or evidences of breaking down. Sections showing the nuclear membrane irregular and much compressed in the plane parallel to the microspore wall, and the chromosomes thus brought into a close group, still show no distinct spindle. It is not until the chromosomes are grouped very nearly in one plane at the equatorial plate that the developing spindle becomes plainly visible.

In the division of the microspore nucleus, the spindle is short and broad (Figure 22). The division figure in *Vitis* is also located well toward one side of the microspore, as in *Symplocarpus* (Duggar, '00) and *Nymphaea* (Lubimenko and Maige, '07), with the axis of the spindle perpendicular to the microspore wall to which it is adjacent

(Figure 18). The fibers of the spindle on the side nearest the microspore wall originate very near to it, are short, and show little or no convergence into a single sharp pole. The fibers on the other side have a "blind ending in the cytoplasm," and in some division figures, while they converge somewhat toward the pole, they are never brought to a point (Figure 22). The chromosomes in the equatorial plate lie separate nearly in one plane, and in well-fixed preparations are easily counted. They are uniform in size and have become shorter, thicker, more nearly round, and more compact (Figures 17 and 22).

Just previous to their separation in the metaphase the chromosomes seem still more nearly round than a little earlier and they are so compact at this stage that no longitudinal splitting can be seen. Irregularities in their separation in the metaphase are not so pronounced as in other divisions and they approach the poles quite uniformly in two close groups (Figure 18). In some divisions the chromosomes which go to form the generative nucleus lie farther apart than those at the opposite pole. No cases have been observed, however, of chromosomes being left behind in the cytoplasm.

The chromosomes which form the generative nucleus are brought very near to the microspore wall at the completion of mitosis. In the early telophase the daughter chromosomes lie close together at their respective poles.

Organization of the generative and vegetative nuclei.—Following the division of the microspore nucleus, the stainable cytoplasm increases in amount until in most cases the larger vacuoles disappear and the cytoplasm becomes quite evenly distributed. A nuclear membrane is formed simultaneously about each group of daughter chromosomes (Figure 24). While the nuclear sap increases in amount, both nuclei at first are flattened or elongated. The chromosomes become distributed about the nuclear cavity mostly near the periphery of the nucleus. Early in its organization the vegetative nucleus becomes larger and more rounded in outline, and at the same time moves inward toward the center of the microspore. The difference in size between the vegetative and generative nuclei becomes greater as growth proceeds (Figures 24, 26, and 33).

The cell plate laid down between the nuclei first becomes evident soon after the nuclear membranes are formed, and is quite distinct before the nucleoli appear. The fact that the mitotic figure in the division just described is located well toward one side of the microspore makes it possible for a small generative cell to be formed, because the cell plate laid down after division is much shorter than the diameter (Figure 24). A wall is soon formed about the generative cell, which assumes a position against the microspore wall, becoming flattened,

oval, or sometimes nearly spherical in shape (Figures 21, 26, 30, 33, and 61). The chromatin of the generative nucleus remains in rather uniform masses for some time during the early periods of its organization, but in the fertile forms, as Concord, and in *V. vulpina* ♂ and *V. bicolor* ♂, it spreads out into a reticulum or into irregular strands before the dehiscence of the anther.

Usually each generative nucleus contains one nucleolus, but frequently two occur. The cytoplasm of the generative cell appears less dense in most cases than that about the vegetative nucleus. The generative cell varies in size as well as in shape. In the fertile forms the generative nucleus is only about half the diameter of the vegetative nucleus. The generative nucleus has not been observed to divide in the pollen grain.

As has been stated, the vegetative nucleus enlarges much more rapidly than the generative nucleus. As in the generative nucleus, however, the chromosomes remain in quite uniform masses for some time, but as growth proceeds they stain less densely and gradually spread out into irregular granular strands. This condition prevails in the vegetative nucleus at the time of dehiscence. It is at this time quite large, spherical in shape, and with large clear areas in the nuclear sap. While the vegetative nucleus usually has one large nucleolus, it sometimes has two or even more. The vegetative nucleus so far as observed in *Vitis* does not fragment, as has been recorded in a few genera, although, as will be shown later, it becomes irregular and sometimes lobed in some of the sterile forms.

Before the pollen is shed the vegetative and generative nuclei in the fertile forms assume the typical resting condition (Figures 21 and 33). They have a normal reaction with the stains, differing in this respect from the deeply staining nuclei in the sterile pollen.

The growth and organization of the vegetative and generative nuclei have been described as they take place in the fertile forms, where both are developed and function normally. It now remains to describe their history in the sterile and aborted pollen.

STERILITY IN VITIS

With respect to the period at which degeneration resulting in sterility takes place in *Vitis,* we have two general types: (1) aborted pollen grains, in which sterility results from degeneration previous to the division of the microspore nucleus, and (2) pollen in which degeneration first appears in the two-celled pollen grain.

ABORTED POLLEN

The regularity with which four microspores are formed in each

mother-cell has been pointed out. No morphological evidence of degeneration has been observed in these before being liberated from the common mother-cell wall, although there may be some varieties of the grape in which degeneration processes take place during these early stages. The subsequent development in many microspores after being liberated from the mother-cell wall is arrested to such an extent that the nucleus does not undergo mitosis. In some cases the microspore wall only partially undergoes the usual thickening and growth and only slight traces of stainable cytoplasm remain. In the varieties studied a few completely empty microspore walls were found which had generally undergone the usual thickening. This condition has been observed also in *Mirabilis jalapa* (Tischler '08). In other instances, while the wall is cutinized and the germ pore developed or not, as the case may be, the nucleus, which seems normal except for size, does not divide. This is the usual condition in the aborted pollen and still exists as late as dehiscence, so that further development is improbable.

The empty walls found in some instances are interesting and suggest the possibility that the contents of some microspores may be absorbed and contribute to the general nourishment of the others. If such a process does take place the anther sap could act as a medium of exchange.

The stainable cytoplasm in the normal microspores during the period of rapid growth and enlargement subsequent to being liberated from the mother-cell wall, is scant. Some grains during this period, however, are much smaller than others, more irregular in outline, and more or less collapsed. The nuclei in some of these smaller grains appear normal, but in others they are irregular and the staining is often diffused. These conditions are undoubtedly the earliest stages of arrested development which result in the aborted pollen. In many instances the stainable cytoplasm in the late stages of the aborted grains is more coarsely granular than the normal pollen; in others the stainable contents are disorganized and diffused.

Aborted microspores occur in various percentages in the native forms, as well as in the cultivated varieties. While in the end the result is the same, a distinction should be made between aborted and sterile pollen. The former occurs in both sterile and fertile forms and seems to be due to arrested development soon after being liberated from the tetrad, while the latter results from disintegration processes subsequent to mitosis in the microspore nucleus, and occurs associated with the reflexed type of stamen and the absence of the germ pore. The aborted pollen is easily distinguished from the normal when placed in liquids. The typical appearance, when mounted in lactic acid, is shown in Figures 64 and 65. The Clinton pollen (Figure 64) is nearly

all perfect, while in the Niagara (Figure 65) about 50 per cent is aborted.

Booth ('02 a) found that the aborted pollen in a number of cultivated varieties varied in amount and failed to develop pollen tubes when placed in a 20-per-cent sugar solution, while the normal pollen borne in the same anthers germinated. Jeffrey ('14) emphasizes the relation of aborted pollen to hybridity. In an extensive survey of the pollen of many genera, he found that "in good species the spores or pollen is invariably perfect morphologically," while "known hybrids on the contrary are characterized by a greater or smaller number of abortive spores, which have little or no protoplasmic contents." So it would seem that the condition which prevails in the grape is not exceptional.

In Tables I and II data are presented to show the percentage of aborted pollen in 121 staminate and 50 pistillate vines of *V. vulpina.* In Table III data are presented to show the same in 52 cultivated varieties.

TABLE I.—PER CENT OF DEFECTIVE POLLEN IN 121 WILD VINES OF *V. vulpina* ♂

Per Cent Defective	0	1	2	3	4	5	6	7	8	9	10	11	12–20–23–41–45	Total	Average Per Cent Defective
Number of Vines	22	26	18	15	9	6	2	4	3	6	2	2	1 2 1 1 1	121	4.08

TABLE II.—PER CENT OF DEFECTIVE POLLEN IN 50 WILD VINES OF *V. vulpina* ♀

Per Cent Defective	0	1	2	3	4	5	6	7	8	9	10	11	12	13–16–18	Total	Average Per Cent Defective
Number of Vines	7	13	8	8	4	0	1	1	2	0	1	0	1	1 1 2	50	3.70

TABLE III.—PER CENT OF DEFECTIVE POLLEN IN 52 CULTIVATED VARIETIES

Variety	Species	Per Cent Defective	Variety	Species	Per Cent Defective
Adirondac ☿	*Lab. Vin.*	37	Black Eagle ♀	*Lab. Vin.*	1
Agawam ☿	*Lab. Vin.*	12	*Brighton ♀	*Lab. Vin.*	18
Amber Queen ♀	*Vin. Vulp. Lab.*	8	Brighton ♀	*Lab. Vin.*	3
America ♀	*Lin. Rup.*	9	Brilliant ☿	*Lab. Vin. Bourq.*	5
*Barry ♀	*Lab. Vin.*	20	Campbell		
Barry ♀	*Lab. Vin.*	11	Early ☿	*Lab. Vin.*	38
*Berckmans ☿	*Vulp. Lab. Bourq.*	60	Canada ☿	*Vulp. Lab. Vin.*	43
*Beta ☿	*Vulp.*	1	Catawba ☿	*Lab. Vin.*	12

TABLE III.—*Continued*

Variety	Species	Per Cent Defective	Variety	Species	Per-Cent Defective
Clinton ♀	*Vulp. Lab.*	2	*Janesville ♀	*Lab. Vulp.*	3
Concord ♀	*Lab.*	12	Jefferson ♀	*Lab. Vulp.*	16
*Concord ♀	*Lab.*	4	Lindley ♀	*Lab. Vin.*	4
Croton ♀	*Vin. Lab. Bourq.*	69	Manitou ♀	*Lab. Vin. Bourq. Lin. Rup.*	31
Delaware ♀	*Lab. Bourq. Vin.*	37			
Diamond ♀	*Lab. Vin.*	19	Marion ♀	*Vulp. Lab.*	17
*Diamond ♀	*Lab. Vin.*	20	Massasoit ♀	*Lab. Vin.*	35
Dracut Amber ♀	*Lab.*	51	Mills ♀	*Lab. Vin.*	36
			Moore Early ♀	*Lab.*	16
Dutchess ♀	*Vin. Lab. Bourq. Aest.*	57	*Moore Early ♀	*Lab.*	19
			Niagara ♀	*Lab. Vin.*	50
*Early Ohio ♀	*Lab.*	7	Northern Muscadine ♀	*Lab.*	32
Empire State ♀	*Vulp. Lab. Vin.*	9	Rogers No. 5 ♀	*Lab. Vin.*	8
Eumelan ♀	*Lab. Vin. Aest.*	42	*Vergennes ♀	*Lab.*	49
Gaertner ♀	*Vin. Lab.*	26	Vergennes ♀	*Lab.*	39
Gold Coin ♀	*Aest. Lab.*	47	V. bicolor ♂	*Bic.*	4
Hartford ♀	*Lab. Vin.*	28	V. bicolor ♂	*Bic.*	8
Herbert ♀	*Lab. Vin.*	10	Wilt ♀	*Vulp.*	8
Hubbard Seedless ♀	*Lab.*	2	*Winchell ♀	*Lab. Vin. Aest.*	48
Janesville ♀	*Lab. Vulp.*	3	Winchell ♀	*Lab. Vin. Aest.*	41
Average per cent defective.....		22.83			

* Pollen collected from the Station vineyard, St. Paul, Minnesota.

The pollen upon which Tables I and II are based was collected in 1914 from wild vines of *V. vulpina* growing in the region around St. Paul, Minnesota. Table III is based upon pollen obtained from the Experiment Station vineyard at St. Paul, and from J. W. Wellington, of the New York State Experiment Station, Geneva, New York.

In making the counts, pollen was mounted on a slide in a one-per-cent solution of Methyl green. The per cent of aborted pollen recorded for each vine or variety is in all cases based upon counts of 200 grains. Pollen was taken from only one cluster on each vine and while such a limited amount, even though taken at random, cannot be regarded as typical for the vine, it does serve as an index to the condition of the pollen produced.

The amount of aborted pollen which occurs in the grape varies much in different vines. In the 52 cultivated varieties the average per cent of aborted pollen is 22.83, compared with 4.08 in 121 wild staminate vines of *V. vulpina* and 3.70 in 50 wild pistillate. There is no significant difference between the amount of aborted pollen in the pistillate and staminate vines of *V. vulpina*. Of the 52 cultivated varieties only 10 have less than 5 per cent of aborted pollen, while of the

171 wild vines of *V. vulpina,* there are 130. There was perfect pollen in the mounts from 29 wild vines of *V. vulpina.*

The difference between the per cent of aborted pollen in known hybrids and the pure forms, among the cultivated varieties, is only slight. The average per cent of aborted pollen from 10 vines, of varieties generally regarded to be pure *V. labrusca,* is 23.10, while that for 38 of the hybrid varieties is 24.60. There are some instances, however, among the hybrids, as in Black Eagle, where the amount of aborted pollen is small.

In the data set forth in Tables I, II, and III there are many exceptions among both wild and cultivated forms to the results of Jeffrey ('14). There was no evidence of hybridity in any of the wild vines which produced the larger quantities of aborted pollen. In fact hybridity probably occurs only rarely in this region since *V. vulpina* is the only species found in the wild.

Since aborted pollen occurs in much the same relative amounts in the self-fertile and self-sterile varieties, from the standpoint of fertilization and the setting of fruit it would seem that the aborted pollen is unimportant in the grape, because in the fertile forms there is still an abundance of potent pollen.

DEGENERATION OF THE GENERATIVE AND VEGETATIVE NUCLEI

Sterility resulting from nuclear degeneration in the two-celled pollen grain of *Vitis* assumes two general extreme forms: (1) sterility in which degeneration occurs only in the generative nucleus (Figures 32, 40, 42, and 57), and (2) sterility resulting from degeneration, to a greater or less extent, in both generative and vegetative nuclei (Figures 31, 36, 38, and 52). Forms intermediate between these two extremes present a variety of relations between degeneration in the generative and vegetative nuclei, as well as the relation of the generative cell wall to the generative cell. Both general types of sterility occur in the material fixed from each vine of Brighton and Barry.

Degeneration of the generative nucleus.—While degeneration in the generative nucleus has been studied particularly in Brighton, the conditions in the mature pollen of other forms, such as Barry, a hybrid between *V. labrusca* and *V. vinifera,* and also *V. vulpina* ♀ and *V. bicolor* ♀, as well as several varieties bearing sterile pollen in varying degrees, have been carefully compared with it. Degeneration leading up to sterility has much the same general character in all the forms studied.

The first evidences of degeneration in the generative nucleus vary somewhat in different microspores. In some, in which the nuclear

membrane becomes irregular in outline or compressed, the chromatin is often massed in various shapes (Figures 29, 35, and 38). In others the nuclear membrane is quite regular in outline but the whole nucleus is small and shows evidences of arrested growth (Figure 58). There is frequently an appearance of diffuse staining in the whole area of the generative cell.

Some preparations of both Brighton and Barry show the generative cell to be nearly devoid of cytoplasm and the nucleus to contain little chromatin (Figure 37). The nuclear membrane and the nucleolus, however, usually remain, but frequently both stain much more lightly than in normal grains.

The condition of the chromatin in the generative nucleus varies much in different instances during the process of degeneration. In cases of degeneration occurring late after the organization of the generative nucleus, the chromatin becomes spread out in strands, or in some forms in a granular, irregular network, resembling the appearance of the chromatin in resting nuclei (Figures 25 and 58). In other nuclei, the chromosomes retain their dense, uniform structure and the nuclear membrane seems to break down, resulting in a dense, irregular area which stains very deeply, showing little differentiation except for darker-staining masses scattered throughout (Figures 32, 40, 49, and 56). The general breaking down resulting in this condition occurs either before or after the appearance of the nucleolus.

The stainable cytoplasm of the generative cell in most cases is drawn toward the region of the generative nucleus (Figures 32, 34, 40, and 54), leaving clear areas in many places near the generative cell wall, adjacent to the tube nucleus. The deeply staining area gradually becomes denser toward the region of the broken down nucleus. The appearance of the degenerating mass and the staining reactions suggests that the nuclear material is being dissolved and diffused outward from the dense, deeply staining area (Figure 41). When the generative nucleus breaks down in this way, the disintegrated mass of nuclear material usually lies very near or against the microspore wall.

The tube nucleus, during this type of degeneration in the generative cell, in many cases appears normal (Figures 32 and 42). In others, there are some evidences of degeneration, such as smaller size, dense chromatin masses which do not become spread out, and a very small nucleolus (Figures 35 and 56).

There are a very few microspores in both Brighton and Concord in which the nucleus divides and although both generative and vegetative nuclei are organized, they remain similar in size, and approach the center of the pollen grain, but there is no evidence of a membrane separating the generative and vegetative cells. The chromatin in both

nuclei under these conditions, as well as the stainable cytoplasm, is usually very scant. These cases, however, are very rare.

It will be remembered that the cytoplasm at the time of division of the microspore nucleus contains large vacuoles, and that division takes place, typically, at one side of the microspore. After division the stainable cytoplasm in some pollen grains evidently does not increase in amount, and both nuclei break down and become flattened and compressed against the microspore wall. Degeneration of this type suggests an intimate relation between the cytoplasm and the development of the nuclei.

Degeneration of both vegetative and generative nuclei.—When both nuclei degenerate, the tube nucleus occupies a position near the center of the microspore, as usual, while the generative cell is found either very near to it (Figures 31, 32, 36, and 49), or at one side against the microspore wall.

This variation in the position of the generative cell has been observed in many other forms not associated with degeneration. Chamberlain ('97), for instance, shows that the generative cell in *Lilium auratum* may be in the body of the microspore instead of being pressed against the side. The tube nucleus of degenerating pollen grains is in some cases quite regular in outline but more frequently irregular, flattened, elongated, and lobed. In many pollen grains in which the two nuclei are very close together, the generative nucleus is more or less embedded in the vegetative nucleus (Figures 36 and 48); the tube nucleus in such instances becoming flattened and bent upon the generative nucleus in the form of a crescent.

There is often a clear area about the vegetative nucleus (Figures 34, 35, 51, and 56), or an area of less densely staining cytoplasm. This does not have the appearance of a vacuole, and the strands of stainable cytoplasm extending across this zone suggest a contraction which does not seem to be due to fixation.

The chromatin in the degenerating vegetative nucleus sometimes becomes finely divided and granular, with little evidence of a reticulum in some cases; in other cases showing less disorganization it still exists in long, irregular granular strands. In some cases the chromosomes do not anastomose but remain in rather uniform masses, distributed throughout the small, irregular nucleus (Figures 23, 28, 29, and 58). If degeneration has advanced still further, the nuclear contents in many cases stain deeply and show little or no differentiation (Figures 29, 56, and 57). When the nucleolus is present in either the vegetative or generative nucleus, there is often a clear area about it in the nuclear sap in which there is no chromatin.

The absence of the nucleolus (Figure 38) in many nuclei indi-

cates that degeneration may begin before it appears after division in either the tube or the generative nucleus. When present in the degenerating nuclei, the nucleolus is frequently small and irregular and stains densely, although in some cases it is normal in size and appearance.

In some genera the tube nucleus fragments or divides, giving rise to two or more nuclei which may vary in size. In *Vitis,* while the tube nucleus undergoes the usual enlargement, more than one has not been observed in the microspores of any of the forms examined. It is, however, often lobed and irregular in both Brighton and Barry.

Degeneration in the generative nucleus generally assumes a somewhat different form when associated with the same condition in the tube nucleus. In this case, as has been mentioned, the generative cell usually moves more or less away from the microspore wall to a position nearer the vegetative nucleus, or against it, at the central part of the microspore (Figures 31, 38, and 52). Its size varies and is often much reduced. The outline may be spherical, elongated, flattened, or lens- or crescent-shaped (Figures 19, 25, 35, 47, and 49). There is frequently no nucleolus, but when present it has much the same appearance as in the degenerated vegetative nucleus. The whole nuclear area in many cases, as well as the strongly contracted generative cell, stains deeply.

The generative cell.—After the division of the microspore nucleus in both sterile and fertile forms, a generative cell membrane is laid down between the daughter-nuclei in all cases in which this stage has been observed. There are instances, however, in the sterile pollen, in which the two nuclei occupy a position near the center of the microspore, when the membrane becomes so contracted about the generative nucleus (Figures 38 and 52) that it cannot be distinguished with certainty. In these cases the nucleoli generally fail to appear after mitosis. This fact, together with the contracted condition of the generative cell wall and the position of the two nuclei near the center of the microspore, suggests that degeneration began very early in the organization of the nuclei.

In the mature pollen of the sterile forms, the generative cell membrane varies much in position and outline. In some it is irregularly contracted close about the generative nucleus, bringing the cytoplasm into a compact dense area against the microspore wall (Figures 34, 35, 36, 49, and 55), and the generative nucleus, although compressed or irregular, is still evident. In other cases the generative cell is small, stains densely, and moves toward the center of the microspore near the vegetative nucleus. Some generative nuclei, however, instead of becoming smaller, enlarge abnormally (Figure 37), the chromatin con-

tent becoming very diffuse and the nuclear membrane irregular. This condition, accompanied by an unusual enlargement of the generative cell, advances so far in some microspores that the chromatin and nucleolus lie scattered throughout the generative cell. This condition probably explains the appearance in the cytoplasm of masses staining deeply similar to chromatin. The generative cell becomes so deranged in some microspores that its wall, as well as the nuclear membrane, breaks down. Chromatin masses under these conditions are found free in the cytoplasm. These are not nuclear extrusions of the same nature that Digby ('12) found in *Primula,* in that they are associated with degenerative processes.

The generative cell wall seems to have much to do with the position of the generative nucleus after degeneration. When it is well developed, as in the fertile forms (Figures 33, 61, 62, and 63), the generative cell is situated at one side of the microspore, and degeneration occurs frequently only in the generative nucleus. In other cases, instead of remaining in a position at one side of the microspore, the generative cell becomes spherical, flattened, or lens-shaped, and moves toward the center, near the vegetative nucleus. The position assumed, as well as the size of the generative cell, suggests that the formation and development of its wall is sometimes influenced by the forces resulting in degeneration and consequent sterility.

The cytoplasm of the vegetative cell.—After mitosis the stainable cytoplasm of the vegetative cell increases rapidly in amount, usually becoming evenly distributed, but sometimes vacuoles varying in size and number appear in the vegetative cells in the mature pollen grains of both the sterile and the fertile forms. The cytoplasm of the mature pollen grain very closely resembles that of the pollen mother-cell in structure and staining reaction in all the forms in which this stage has been examined. There seems to be no constant difference between the cytoplasm in the sterile and that in the fertile forms, and it is probable that in general, except in the aborted microspores, degeneration occurs too late to have much influence upon the growth of the cytoplasm.

The germ pore.—A characteristic difference between fertile and sterile pollen, besides the shape, is found in the germ pore. For some time after the young microspores are liberated from the tetrad, they undergo a period of growth in which the wall becomes thicker but remains undifferentiated. The germ pores become evident in the fertile forms, such as Concord and *V. vulpina* ♂, before much thickening takes place in the exine and some time previous to the division of the microspore nucleus. In the sterile forms, such as Brighton, Barry, or *V. vulpina* ♀, no germ pores are evident during the same period. In

none of the forms studied has "mixed pollen" been observed, that is, some pollen with and some without germ pores. While this difference is quite constant the exine in both becomes thickened and cutinized. The position of the generative nucleus has no constant relation to the germ pore.

In Table IV, the relation of the type of stamen and fertility of the pollen to the germ pore is set forth in a number of varieties and species.

TABLE IV.—RELATION OF THE TYPE OF STAMEN AND FERTILITY OF THE POLLEN TO THE GERM PORE

Variety	Species	Stamens	Germ Pore	Pollen*
Adirondac	*Lab. Vin.*	Upright	Present	Nearly sterile
Black Hamburg	*Vin.*	Upright	Present	Fertile
Canada	*Vulp. Lab. Vin.*	Upright	Present	Nearly sterile
Concord	*Lab.*	Upright	Present	Fertile
Croton	*Vin. Lab. Bourq.*	Upright	Present	Fertile
Dracut Amber	*Lab.*	Upright	Present	Fertile
Dutchess	*Vin. Lab. Bourq. Aest.*	Upright	Present	Fertile
Mills	*Lab. Vin.*	Upright	Present	Partly fertile
Northern Muscadine	*Lab.*	Upright	Present	Partly fertile
Vergennes	*Lab.*	Upright	Present	Partly fertile
V. bicolor ♂	*V. bicolor*	Upright	Present	Fertile
V. vulpina ♂	*V. vulpina*	Upright	Present	Fertile
Amber Queen	*V. vulp. Lab.*	Reflexed	Absent	Self-sterile
America	*Lin. Rup.*	Reflexed	Absent	Self-sterile
Barry	*Lab. Vin.*	Reflexed	Absent	Self-sterile
Black Eagle	*Lab. Vin.*	Reflexed	Absent	Self-sterile
Brighton	*Lab. Vin.*	Reflexed	Absent	Self-sterile
Eumelan	*Lab. Vin. Aest.*	Reflexed	Absent	Self-sterile
Gaertner	*Vin. Lab.*	Reflexed	Absent	Self-sterile
Herbert	*Lab. Vin.*	Reflexed	Absent	Self-sterile
Lindley	*Lab. Vin.*	Reflexed	Absent	Self-sterile
Marion	*Vulp. Lab.*	Reflexed	Absent	Self-sterile
Massasoit	*Lab. Vin.*	Reflexed	Absent	Self-sterile
V. bicolor ♀	*V. bicolor*	Reflexed	Absent	Self-sterile
V. vulpina ♀	*V. vulpina*	Reflexed	Absent	Self-sterile

* Based upon the work of Beach ('98 and '99).

The relation of the germ pore to sterility and type of stamen is interesting. In sequence of development it is formed, or not, as the case may be, previous to the occurrence of degeneration in the generative nucleus, which results in sterility. Just what influence, if any, the absence of the germ pore has upon subsequent pollen development from the standpoint of nutrition or stimulation would be difficult to determine. As has been shown, the tapetum, to all appearances, functions normally in both sterile and fertile forms, and the pollen in each is surrounded by the anther sap from which it is undoubtedly nourished. It would seem, since the development of the microspore in *Vitis* is much the same in the sterile and fertile forms as to rate or

time of development, size attained, up to and including nuclear divisions, and to a certain extent in the organization of the generative and vegetative nuclei, that the failure to develop a germ pore in the sterile pollen is not of immediate consequence. However, it may be that pollen possessing a germ pore may be more readily nourished in some ways from the anther sap. The reflexed type of stamen, the absence of the germ pore, and degeneration in the generative nucleus present a series of closely related phenomena characteristic of those forms bearing sterile pollen. So it would seem that the failure to develop a germ pore in the sterile pollen is closely associated with the factors, whatever they may be, which result in the reflexed stamen and degeneration in the generative nucleus.

The mature pollen of Vitis.—When dry, the pollen grains of the fertile forms of *Vitis* are oblong with flattened ends, and those of the sterile forms are more oval, irregular, or misshapen. The exine is thick in both the sterile (Figure 40) and the fertile forms (Figures 59 and 63) and except for very slight irregular ridges bears none of the peculiar markings found in many genera. There are three deep sutures in the exine (Figures 59 and 60) which extend the entire length of the fertile pollen grain, midway in each of which a single germ pore appears as a small oval or nearly round pit, which projects slightly into the cytoplasm. No variations in the number of sutures and germ pores have been observed. The sutures do not occur in the sterile forms such as Brighton or Barry. Booth ('02 a) and Reimer and Detjen ('10) point out the differences in shape between sterile and fertile pollen and the relation of each shape to sterility. It seems probable that the sutures extending lengthwise in the fertile pollen render the exine of this type more rigid, so that it does not assume the various irregular shapes of the pollen lacking these. If this is true, then the relation of shape of pollen to sterility is largely mechanical.

When placed in liquids, such as killing fluids, water, or sugar solutions, both types of pollen quickly become spherical or oval. In fixed preparations, the long diameter of one hundred mature pollen grains of Brighton averaged 18.95 μ and the short diameter of the same number, 16.89; in another bud the long and short diameter of the same number was 20.25 and 17.26 μ respectively. This change of shape is noted by Booth ('02 a) in the grape, and Halsted ('90 a) and Salaman ('10) record the same phenomenon in *Solanaceae*. Bateson and Saunders ('05) observe that sweet pea pollen, when dry, "is irregularly kidney-shaped or *round*, while normal pollen is long with straight sides. Treated with reagents, normal pollen swells to an *elliptical* shape with a distinct long axis, and shows *three* pores set evenly round the short circumference. Round pollen thus treated be-

comes nearly *spherical* and usually has only *two* pores." White ('86) shows that pollen grains obtained from a wreath in an Egyptian tomb, probably 3,000 years old, became rounded when placed in water. The writer has also tested this change in outline in other forms besides the grape and the same rounding occurred as noted above.

It is difficult to determine exactly what influence this change in shape has upon the relative arrangement of the cells within the pollen. Well-fixed sections show a uniformity in the distribution of the cytoplasm, and a normal appearance and position of other structures, which would point to no serious disturbance. This condition indicates that pollen is naturally adapted to the absorption of liquids—a function necessary to further development.

A number of workers, as Engelmann ('94), Munson ('99), Beach ('98, '99, and '02), and others, have shown that the pollen produced by the reflexed type of stamen is more or less impotent. A classification based upon stamen type, according to the work of Beach ('98, '99, and '02), of 132 of the most important commercial varieties described by Hedrick *et al.* ('08) and Dorsey ('09), shows that there are 95 varieties with upright and 37 with reflexed stamens. Of the 95 only 11 are classed as self-sterile or partly so, while only 2 of the 37 having reflexed stamens were partly fertile, the remainder being sterile. Eight species are represented in 22 different combinations. In the cultivated varieties with upright stamens, the flower is perfect rather than staminate, as in the wild vines, which have upright stamens. The cultivated varieties with reflexed stamens are essentially the same as the wild vines, in that the pollen is generally sterile in both.

Interesting variations in the nuclei of mature pollen are recorded. According to Chamberlain ('97), "Hartig was the first to describe two nuclei in the mature pollen grain," although in 1884 Strasburger greatly extended the knowledge of pollen by describing it in a number of species. Chamberlain ('97) found that the male gametophyte in *Lilium tigrinum* at the time of shedding may contain as many as six tube nuclei, and *L. auratum* has in some cases three nuclei in the generative cell. The position of the nuclei in the pollen also varies. Lubimenko and Maige ('07) illustrate a mature pollen grain of *Nuphar luteum,* in which there is a tube and a generative nucleus, and also two small heavily staining bodies. The cytoplasm in the pollen grain illustrated contains several large peripheral vacuoles. Kirkwood ('07) shows that the generative nucleus in *Micrampelis* is much elongated and located with the vegetative nucleus near the center of the cell.

The cause of sterility in Vitis.—Sterile pollen in the grape undoubtedly results from disintegration processes, which occur either before or after the division of the microspore nucleus. When degener-

ation occurs subsequent to this division the structures within the microspore are affected differently. In some cases the degeneration seems to be more general and deep-seated, and both the generative and the tube nuclei are affected, as well as the generative cell membrane and cytoplasm. In other cases all of the structures appear normal except the generative cell, the nucleus of which undergoes degeneration. In all cases, however, the result is the same, in that degeneration occurs in the generative nucleus, which precludes the possibility of normal functioning of the microspore. Degeneration in the generative and tube nuclei is associated with the reflexed form of stamen and the absence of germ pores.

In Brighton, Barry, *V. vulpina* ♀, *V. bicolor* ♀, and a number of varieties reported as sterile, or nearly so, there are a few pollen grains in which the generative nucleus does not appear to undergo degeneration. The appearance of the generative nucleus in the forms mentioned shows a series, with respect to the degree of degeneration, from pollen grains in which the generative nucleus is apparently normal to those in which both the tube and the generative nuclei are disorganized and degenerated. Typical cases are shown in Figures 19 and 26, where the generative nuclei in Brighton appear normal. Later stages of such instances, however, generally show evidences of degeneration as a diffused or deeper-staining reaction or contracted and irregular nuclei. Whether any degeneration can take place in the generative nucleus without impairing its functional activity is problematical. Bagging tests conducted by Beach ('98 and '99) show that a few berries set when Brighton and others usually classed as self-sterile are self-pollinated. Such results, in which only a few berries set, can easily be confused by accidental cross-pollinations. The few microspores, nevertheless, in which the generative nuclei appear normal, may still be capable of germination and so may explain the results of these bagging tests. The absence of germ pores, however, would seem to preclude this, leaving accidental crossing as the most probable cause.

TYPES OF STERILITY

Cytological studies have disclosed a variety of defects in essential organs which have gone far toward presenting a more accurate understanding of the nature and causes of sterility.

Gates ('07 a) shows that in *Oenothera lata* the pollen may break down in different ways. Degeneration may occur as early as the pollen-mother-cell stage, and to such an extent that the tapetal cells, as well as the pollen mother-cells, may more or less completely disappear, in which case the "middle layers" then grow inward and fill the cavity

of the loculus, or the tetrads may be formed only to break down later, owing to a lack of nourishment by the tapetum.

An interesting type of sterility in the pollen of *Oenothera gigas* has been described by Gates ('11). In this form, owing primarily to "a failure of the surrounding tissues of the anther to grow and form a cavity which allows sufficient space for the mother-cells to round off during synapsis and float freely in the cavity of the anther," they retain their archesporial nature, and remain a compact tissue with the characteristic polyhedral cells of this stage.

Juel ('00), working with a hybrid of *Syringa vulgaris* x *S. persica* known as *S. rothomagensis,* shows that degeneration begins in some pollen mother-cells as early as synapsis, but that usually the heterotypic and homœotypic divisions are completed. In this form extra nuclei occurred in the tetrads.

Irregular pollen grains and "frequently twin cells" were found in *Solanum* by Salaman ('10). He observed that the irregular grains are either aborted or immature.

In hybrid pigeons, Guyer ('00), finds that degeneration occurs in the testes of all sterile forms, but that it is much more pronounced in those hybrids between the most widely divergent individuals. He describes irregular spindles, unequal pairing of chromosomes, and degenerating primary spermatocytes. He also finds ('12) in guinea-chicken hybrids that the testes are normal in size, but that in the case of the pigeon hybrids the "critical point seems to be the synaptic phase" when the chromosomes of different parentage appear unable in many instances to "unite normally."

Sterility in hybrids between different species of *Mirabilis, Potentilla,* and in three species of *Syringa,* were studied by Tischler ('08). In these he found different types of pollen degeneration. In *Mirabilis jalapa,* anthers are found in which the pollen grains are empty; in other cases in the same species some pollen is not broken down to such an extent and the cytoplasm is much vacuolated, especially about the periphery. In *Potentilla* hybrids he found irregularities in the division of the pollen mother-cells, resulting in some cases in the suppression of one of the daughter-nuclei and the unusual enlargement of the other. In other cases, the two divisions occur normally and one or more or none of the tetrad nuclei break down. This irregularity results in the formation of some pollen grains with little or no stainable contents, which consequently vary much in size in the mature anther.

Gregory ('05) found that in the sweet pea degeneration occurred in the pollen mother-cells before the formation of the chromosomes. He found irregular spindles or incomplete and abnormal division resulting from a constriction of the pollen mother-cell.

Tischler ('09), working with some tropical plants, describes a very interesting type of sterility, which differs from those cases already mentioned in that there is a lack of functional activity instead of a degeneration or breaking down of tissues. In some of these there is a starch period, occurring late in pollen development. The time of digestion of the starch varies greatly in different species. In *Cassia fistula,* for instance, the pollen is sterile, owing to the fact that the starch is not digested. Tischler believes that sterility in this case is due to the absence of diastase. He found that when diastase was added to the media in which the pollen was being germinated, a prompt and beautiful development of the tubes resulted.

Carnation hybrid No. 72, produced by Webber at the Cornell Experiment Station, is self-sterile, yet its pollen is fertile with other varieties. When pollinated with pollen from other varieties, No. 72 produces seeds in abundance. Both pollen and pistil appear normal functionally. The causes of "incompatibility" of this nature between pollen and pistil probably vary and may be due to either pollen or pistil, or both. Such cases need further investigation.

Osawa ('12) found much irregularity in the development of the gametophyte in Unshu, a variety of *Citrus nobilis.* In some anthers no sporogenous tissue formed, the anther being filled with parenchyma, while in others tapetal cells completely filled the loculus. Some mother-cells degenerated before division, others divided normally and while many microspores degenerated at various stages subsequent to their liberation from the common mother-cell wall, occasionally normal pollen was produced. In the Washington Navel Orange, which is a variety of *C. aurantium,* greater abnormalities take place. The sporogenous tissue is formed in young anthers, but in some loculi "the sporogenous cells have large vacuoles and relatively small nuclei"; these cells may soon lose their sporogenous appearance and disintegrate. Generally, however, the sporogenous cells develop into normal mother-cells, the resting nucleus being large, and remaining in this condition "a relatively long time." Further growth of the mother-cells is not accompanied by a corresponding increase in cytoplasm, a condition followed by vacuolization. The nucleus becomes less chromatic and the mother-cells show evidences of degeneration. The nucleus becomes irregular; the cytoplasm becomes more granular and more deeply staining; and the chromatin "never passes into the synapsis stage." The mother-cells lose their turgidity and become shrunken, deeply staining masses, and gradually disappear.

It will be seen from the few types mentioned that sterility occurs as a result of various causes. The physiological and ecological factors leading up to it are complex and closely related and consequently

are difficult to isolate and study separately. Besides sterility resulting from defects in the essential organs and unfavorable ecological factors, self-sterile strains occur within species and some forms are sterile in one region and not in another.

The terms sterility and fertility have been used loosely in botanical and horticultural literature. In horticulture the usage has generally been in the sense of a plant being self-sterile or self-fertile. Pollination and fertilization have also been confused. Sterility and fertility have a general application and the usage of these terms includes self-sterility and self-fertility. Self-sterility is generally used in the sense of a plant being unable to produce fruit when pollinated with its own pollen, and, so used, has no reference to whether it is sterile with pollen borne on other individuals of the same variety or species, or whether the fault lies with the pistil or pollen. Sterility is often applied to unfruitfulness due to unfavorable ecological factors—a usage which has no reference to defects in either pollen or pistil. A self-sterile plant may be sterile with the pollen from other individuals of its own species or two self-sterile plants may be mutually fertile. In the grape, as in other plants, where the fault lies with the pollen, self-sterility or sterility occurs whenever this pollen is used. Sterility in this sense has no reference to the fact that the pistil may be normal functionally. Where sterility results from defects in the pistil, unfruitfulness occurs when pollinated with any pollen. It will be seen, then, that a plant may be self-sterile as a result of defects, occurring at various stages of development, in either pollen or pistil.

There is a series of growth processes between pollination and fecundation. Considerable growth may occur in the pollen tube and for various reasons fertilization may fail to occur. Observations of sterility should determine whether pollen has been applied to the stigma and whether any growth of the pollen tube takes place. Self-fertility may be possible when only a small percentage of the pollen is potent or functional.

In speaking of sterility with reference to a lack of functioning in the pollen or failure to produce it, the term so used may include a failure to produce sporogenous tissue, degeneration at various stages in pollen formation, or failure to form pollen tube and gametes.

The loose usage of the terms sterility and fertility has, no doubt, largely resulted from their inadequacy to express all the conditions and cases which necessarily exist. Further introduction of terms at the present state of our knowledge would probably lead to confusion later, even if studies from the viewpoint of cytology, physiology, and heredity have shown much concerning the causes and nature of sterility.

In this connection it is interesting to note those cases where there seems to be a stimulus resulting from pollination, or partial growth and functioning of the pollen, without fecundation being accomplished. Instances of sterility, parthenogenesis, and parthenocarpy show that the relation of pollen to pistil is varied and complex. This interaction and stimulus seems to be intimate and necessary in some cases and not in others.

Studies of flower types have shown a remarkable complexity of structure and differentiation which result in a variety of relations with respect to the source of pollen which reaches a stigma. These variations are significant from the evolutionary standpoint. Sterility has its setting in these complex structures and relations.

THE RELATION OF THE CHROMOSOMES IN HYBRIDS AND PARENT FORMS

The relation between the chromosome number in parents and hybrid is important. Digby ('12) points out, however, that "the fact that a hybrid may be either sterile or fertile does not therefore appear to depend on the pairing of an equal number of parental homologous chromosomes, nor can the irregularity of the spindle figures be called a determinant of hybridity." An instance of a fertile hybrid pea has been reported by Cannon ('03 b) when the two varieties Fillbasket and Debarbieux were crossed, each having the same number of chromosomes, namely, 14 (2x). The hybrid was fertile, had the same number of chromosomes as each parent, and matured its pollen as did the parent varieties. Rosenberg ('03, '06, and '09), on the other hand, shows an interesting relation between the chromosomes of a *Drosera* hybrid, the parents of which have unequal numbers of chromosomes. The parents, *D. rotundifolia* and *D. longifolia,* have 10 and 20 chromosomes, respectively, in their germ cells. Upon the equatorial plate of the heterotypic division, there appear 10 double and 10 single or unpaired chromosomes. Rosenberg regards the 10 unpaired chromosomes as derived from *D. longifolia,* and he found that they were often taken into one or the other or both daughter-nuclei instead of being left free in the cytoplasm. This latter process, however, was irregular.

Gates ('07 a) records an interesting case of the behavior of a portion of the chromatin in *Oenothera lata.* One or two "heterochromosomes" are formed after synapsis "by the cutting off of a portion of the spireme thread before the remainder breaks up into chromosomes," although they are not found in some mother-cells. These bodies do not divide and by the end of the heterotypic division they

disappear. Gates suggests that "they probably represent discarded chromosomes."

Digby ('12) found that the hybrid *Primula kewensis* has 18 chromosomes (the 2x number). Both parents, *P. floribunda* and *P. verticillata,* have the same number. *P. kewensis* occurs in both a fertile and a sterile form, the latter by some means having doubled its chromosomes and having 36 as the diploid number. An improved strain of *P. kewensis* (fertile) also has 36 (2x) chromosomes. An irregularity in the usual chromosome number of the hybrid occurs, however, when *P. floribunda isabellina,* having 18 (2x) chromosomes, is crossed with the fertile hybrid *P. kewensis,* having 36 (2x) chromosomes. In this cross the hybrid has 18 chromosomes as its diploid number.

Chromosomes in hybrids and species in Vitis.—The reduced number of chromosomes in Brighton is 20 (Figure 17). This variety, it will be remembered, is a hybrid between Concord and Diana Hamburg. Concord, which is generally considered a variety of *V. labrusca,* also has 20 chromosomes as the reduced number. Diana Hamburg is a cross between *V. labrusca* and *V. vinifera.* It is probable, then, since *V. labrusca* is represented in both parents of Brighton, that each parent had 20 chromosomes. Since material could not be obtained from Diana Hamburg, an attempt was made to determine the chromosome number of Black Hamburg representing *V. vinifera,* but the chromosomes could not be counted in any of the sections showing the division stages of this variety. It is possible, however, for the chromosome number to vary in different varieties of a species so that if the chromosomes could have been counted in Black Hamburg, this would not have given the chromosome number of the other parent of Brighton with certainty, although it would seem that since Barry, another hybrid between *V. labrusca* and *V. vinifera,* has 20 (x) chromosomes, *V. vinifera* probably has no more than 20 (x).

The haploid chromosome number of *V. vulpina* is 20, the same as that of *V. labrusca.* The chromosomes in *V. vulpina* were counted in a pistillate vine which bore impotent pollen. While in *V. bicolor* the chromosomes could not be counted with assurance of accuracy, in the preparations showing the division stages of this species there were a few sections in which the number was not far from 20.

The chromosomes were counted in Concord in the heterotypic division, and in Brighton in both the heterotypic division and the division of the microspore nucleus. The counts in *V. vulpina* ♀ and Barry were both made during mitosis in the microspore nucleus.

The chromosomes in Brighton and Barry, two hybrids between *V. labrusca* and *V. vinifera,* are the same in number and present no

abnormalities during division. The same can be said of Concord, representing *V. labrusca* and *V. vulpina* ♀.

In the forms of *Vitis* in which the division stages have been examined there seemed to be no unpaired chromosomes, and the divisions to all appearances were, as we have seen, normal in both the parent and the hybrid forms. Inequality in the chromosome number, resulting in an unequal pairing in the reduction division, therefore, does not necessarily appear to be a factor in sterility in the grape.

THEORIES AS TO THE CAUSE OF STERILITY

The causes of sterility are undoubtedly variable and complex. There is an extensive literature bearing upon the subject and many theories have been advanced attributing the causes to various factors.

Darwin ('75) recognized the fact that exotic plants may become sterile when grown under the unnatural conditions of regions other than the native habitat. Ludwig ('00), however, points out that the reverse may be true. Darwin shows also that sterility in plants may result from an aborted condition of either the male or female organs, or from modifications in structure ("monstrosities"), which affect the organs in various ways.

Gaertner (Darwin, '75, Vol. II, p. 149) has applied the term contabescence to the shriveling of anthers when they become brown or tough and bear no good pollen. This condition resembles that found in most sterile anthers, and Gaertner regards it as an "inherent tendency of the species to become dicœcious."

Bailey ('96) holds that "impotency among cultivated plants is the beginning of a potential tendency toward unisexuality."

Ludwig ('00) says: "Self-sterility I have found to occur with special regularity in the case of such plants as propagate themselves vigorously by means of rhizomes, offsets, or bulbils." He also points out that within the same species self-sterile individuals may be found in one place and self-fertile ones in another.

Guyer ('00), speaking of hybrid pigeons, says that "it would seem that the conflicting tendencies of the two parental plasmas frequently render the union of the single chromosomes to form the double (bivalent) types impossible or abnormal."

Tischler ('02) believes that sterility in hybrids does not depend upon chromatin repulsion. Neither does he believe that irregularities in the tetrad division can be considered a characteristic of hybrids. Where they occur they do contribute to sterility, but an abnormal number of chromosomes does not necessarily preclude the possibility of further development. Sterility is dependent, according to Tischler, upon the coming together of two sexual cells which do not possess

an identical developmental direction or tendency. Upon the coming together of the sex cells, he holds that the attraction is sometimes too strong and sometimes too weak.

Allen ('05) believes that when two species are crossed, "an exact correspondence between the two idioplasms is no longer to be expected." Their number and arrangement may vary, so that "the applications of the idioplasms to each other and the consequent interchange of pangens is made difficult." When the parents are closely related, the difficulty is less serious, but as the "relationship becomes more distant, the difficulty increases until the processes leading to the formation of the germ cells become impossible, and the hybrid is necessarily sterile."

Gates ('07 a) says that "it is conceivable that the maternal and paternal chromatin after entering the same nucleus in fertilization could continue in this condition throughout the sporophytic history without interfering with a development, but that the intimate union which occurs in synapsis, where an interchange of material between the parental idioplasms probably takes place, might lead to the development of 'incompatibilities' between the plasms, such as are exhibited in irregularities and disturbances in the reduction of divisions, and finally more or less complete failure of later development."

With respect to inheritance, Salaman ('10) finds male sterility in the potato a dominant Mendelian character, while Bateson and Punnett ('08) found the character of sterile anthers in the sweet pea recessive to the fertile character.

Correns ('12) made detailed inheritance studies of sterility in *Cardamine pratensis* and ascribed sterility in this form between individuals of distinct origin to be due to the presence of inhibitory substances.

The writer has shown ('12) that sterility in *Vitis* does not occur as a result of doubling where there is a multiplication of floral envelopes or a more or less complete transformation of some parts into others.

DISCUSSION

Sterility resulting from degeneration in the generative nucleus, as in *Vitis,* or at earlier stages, as in *Oenothera* (Gates, '07 a) and *Potentilla* (Tischler, '08), should be distinguished from that resulting from unfavorable weather conditions which occur at flowering-time during many seasons.

In *Vitis,* sterility is associated with both hybridity and dicœciousness. In the wild the grape is dicœcious, and fertile pollen borne by the pistillate flower with reflexed stamens is rare, if it occurs at all.

Sterility cannot necessarily be attributed to hybridity, however, since we have both fertile and sterile hybrids. The perfect flower, with upright stamens bearing fertile pollen, may or may not be a hybrid. The relation of the reflexed type of stamen, dicœciousness, and sterile pollen, suggests that degeneration in the generative nucleus resulting in pollen sterility is only a step toward functional dicliny. Taking into consideration the fact that sterile or fertile pollen is borne by either hybrids or pure forms, and the fact of the relationship between sterility and dicœciousness, it seems that the factors causing sterility in *Vitis,* whatever these may be, are independent of hybridity and are closely related to the fundamental influences operating to produce diclinism and dicœciousness.

Considering only the wild types of *Vitis,* which are dicœcious, we have on the one hand a suppression of the pistil and on the other, a suppression of the stamen. Forms intermediate between these occur (Dorsey, '12) which might be interpreted as in accord with Booth's contention (Booth, '02) that the grape is "in a state of evolution from an older hermaphrodite form to forms that are essentially staminate and pistillate." The cytological evidence of this paper, however, does not obviously affect the question of evolutionary development.

The fact that there is a close relation between such phenomena as dicœciousness, the reflexed type of stamen, the absence of the germ pore, and degeneration of the generative nucleus, togther with the fact that degeneration takes place so regularly in the generative nucleus in the sterile pollen, suggests that the idioplasm is fundamentally affected. The two chromatin elements must have a very intimate relation in the cells of the sporophytic, as well as during the gametophytic generation, but any "incompatibilities" between them do not gain expression in *Vitis* until late in the development of the microspore. It would be difficult to determine whether or not the fault lies with the chromatin element derived from the male gamete or from the female, or both. If synapsis be the "critical point" in the series of developmental changes leading up to the formation of the gametes, since it is clear that sterility is known to result in various genera of plants from degeneration both before and after this stage, it would seem evident that there are deep-seated influences at work which finally express themselves at different stages.

SUMMARY

Sterility in economic fruit-bearing plants occurs in many forms and is an important consideration from the commercial standpoint. It may be a result of defects or degeneration in essential organs or of unfavorable ecologic factors. Its occurrence has been known for a

long time and it has been investigated extensively, especially in plants bearing edible fruits.

Investigation of sterility in the economic fruit-bearing plants has shown that many varieties are unfruitful when self-pollination takes place and that cross-pollination must be resorted to. These investigations have also shown that some varieties are more effective as pollinizers than others and that "mixing" in planting, if properly done, will result in fruitfulness by bringing about the necessary cross-pollination.

Many varieties of American grapes have been shown by a number of investigators to be unfruitful when self-pollinated. Cross-pollination brought about by "mixing" at planting time has been successful in overcoming this. In the grape, sterility has been found to be due to the pollen rather than to the pistil. In this investigation a cytological study of pollen development has been made with the object of determining why the pollen borne by some of our American varieties is not functional.

Brighton, a hybrid between *V. labrusca* and *V. vinifera,* was made the basis of this investigation. Concord, its pollen parent, which is a variety of *V. labrusca,* and Barry, another hybrid between *V. labrusca* and *V. vinifera,* were carefully compared with it. Pollen was also studied from pistillate and staminate vines of *V. bicolor, V. vulpina,* and a number of cultivated varieties, which represent various different combinations between species.

Early bud stages in the varieties examined showed no morphological abnormalities which might influence later development. The embryonic cluster is enclosed in scales in the bud through which it breaks as growth proceeds. The calyx in the young stages extends well up over the young bud. The tissues in the young anther are distinct. The mother-cells are surrounded by a single layer of tapetum, three layers of parietal cells, and an epidermis which has a thick outer wall. The outer layer of the parietal cells functions as the principal tissue of the endothecium.

In cross-section, the filament of a reflexed stamen has small epidermal cells on its outer surface, differing in this respect from the upright in which the epidermal cells of the two surfaces are practically equal. This difference in strength between the two surfaces in the reflexed stamen results in the recurving of the filament.

The tapetum in the grape undergoes the usual course of formation and disintegration, found in other higher plants. It functions normally, to all appearances, in both sterile and fertile forms. The term anther sap has been applied to the liquid medium which first makes its appearance after the mother-cells begin to break apart and which later

completely fills the space in the anther sac between the pollen grains. The tapetum and anther sap function alike in the sterile and fertile forms.

The series of stages occurring in the pollen mother-cells previous to the heterotypic division take place normally. Before synapsis a loose, irregular spireme is formed, which is well distributed throughout the nuclear area. As synapsis approaches, the spireme becomes more even in thickness. The synaptic mass is close and compact. After this stage the spireme becomes well distributed throughout the nuclear area. Its double nature is now apparent. At diakinesis the chromosomes are distinctly two-parted and usually lie near the nuclear membrane. The mother-cells at this time become rounded up and separated.

The heterotypic and homœotypic divisions take place normally and, as usual, in rapid succession. The spindles of these two divisions are distinctly bipolar and are formed from the multipolar spindle fibers which arise in the region of the nuclear membrane. The chromosomes are compact and relatively short and their distribution to the daughter-nuclei appears to be equal in each division. No nuclear membrane is laid down after the heterotypic division.

Four microspores are regularly formed in each mother-cell and while still embedded in the common mother-cell wall they become rounded in outline and their nuclei become well organized. Their liberation from the mother-cell wall appears to be due to a dissolution process. After being liberated, the microspore wall undergoes a period of rapid growth and enlargement before much thickening takes place. It now becomes spherical in shape and the larger portion of the stainable cytoplasm assumes a position toward one side of the microspore. One large vacuole, or sometimes two or more, are found in the cytoplasm. Germ pores and sutures appear in the wall as the thickening takes place. These are not formed in the sterile pollen.

The nucleus becomes larger as growth proceeds and is nearly always located toward one side of the microspore in the larger mass of stainable cytoplasm, a position which is retained until division. The chromatin increases in amount and a spireme is formed, which, before segmenting into chromosomes, becomes bent into segments which correspond to the chromosomes into which it is to segment.

As the division approaches, the chromosomes become shorter and thicker. The spindle is short, broad, and relatively inconspicuous, and has a blind ending in the cytoplasm. Mitosis is completed quickly and the chromosomes reach the poles in two close groups. The daughter-nuclei are much flattened when first organized, but soon become spherical in shape. As they enlarge, the vegetative nucleus moves

toward the center and occupies a central position. The stainable cytoplasm increases in amount rapidly at this stage and soon fills the whole area of the microspore. The vegetative cell is cut off at one side, assuming various shapes in the mature pollen.

In the formation of the sterile and fertile pollen of the grape the heterotypic and homœotypic divisions and the division of the microspore nucleus take place normally. Sterile pollen in the grape results from degeneration processes in the generative nucleus or arrested development previous to mitosis in the microspore nucleus. Where degeneration begins early after the division of the microspore nucleus, both the generative and vegetative nucleus may be affected. If the generative cell is well organized before disintegration begins the vegetative nucleus may remain normal.

Aborted pollen is found in varying quantities with both sterile and fertile pollen. It occurs in pure forms as well as in hybrids but is more abundant in the latter. Since pollen is produced in abundance by the grape, aborted pollen is relatively unimportant from the standpoint of fertilization or the setting of fruit.

The reduced number of chromosomes in Barry, Brighton, Concord, and *V. vulpina* ♀ is 20. The number in *V. bicolor* could not be definitely determined, but is near 20. Since *V. vinifera* is one of the parents of both Barry and Brighton, the reduced number of chromosomes in this species probably does not exceed 20. *V. labrusca* is represented in both parents of Brighton, so that each parent probably had 20 chromosomes, which would result in an equal pairing in this variety at the reduction division. In fact, an unequal pairing of chromosomes at this time has not been observed in any of the forms in which this stage has been studied; therefore this condition is not necessarily a factor in sterility in the grape.

Since both fertile and sterile hybrids occur among the cultivated varieties of American grapes, hybridity is not necessarily a cause of sterility. The relation of the sterile pollen to the absence of the germ pore, the reflexed type of stamen, and the tendency toward dicœciousness, suggest that pollen sterility in the grape is only a step toward functional dicliny.

It is evident, then, that the cause of sterility in the grape is deep-seated and intimately concerned with the functional activity of pollen and the oft-recurring question from grape-growers as to whether sterility can be overcome by cultural conditions will have to be answered in the negative, leaving another method of avoiding it at their disposal, namely, "mixing" varieties in the vineyard at planting-time.

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DESCRIPTION OF PLATES

Figures 1-33 were outlined with the aid of an Abbe camera lucida. For the higher magnifications the following lenses were used: Leitz 2 mm. Apochromatic objective N. A. 1.32 and compensating ocular 8; Bausch and Lomb 1.5 mm. objective N. A. 1.30 and Hughenian oculars 10 and 12.5. The micro-photographs were taken with a large Bausch and Lomb apparatus and Zeiss compensating ocular and objective. All are magnified 916 times.

PLATE I

Fig. 1. Brighton. A section through an anther, showing pollen mother-cells, tapetum, middle layers, and epidermis. x 400.

Fig. 2. Brighton. A later stage than Figure 1 showing changes in epidermis, parietal layers, and tapetum. x 550.

Fig. 3. Brighton. A cross-section of filament, showing smaller epidermal cells of outer surface; a, outer surface. x 120.

Fig. 4. Concord. A cross-section of filament. Epidermal cells of practically equal size on both surfaces; a, outer surface. x 120.

Fig. 5. Brighton. A section of an anther sac previous to division of the microspore nucleus, showing epidermis, endothecium, the remnants of the two inner rows of the middle layers, and the tapetum. x 550.

Fig. 6. Brighton. Tapetal cells just previous to the heterotypic division, showing the binucleated stage. x 550.

Fig. 7. Brighton. A pollen mother-cell previous to the spireme stage. x 2000.

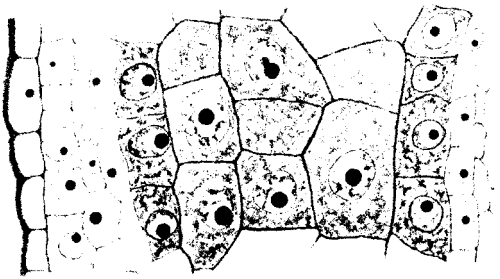
Fig. 8. Brighton. The open spireme stage in a pollen mother-cell. x 2340.

Fig. 9. Concord. A section through a tetrad, showing the thick mother-cell wall. x 1560.

Fig. 10. Brighton. A transitional stage during late diakinesis, showing the breaking down of the nuclear membrane and nucleolus and the arrangement of the spindle fibers into a bipolar spindle. x 2000.

Fig. 11. Brighton. A spindle figure during the heterotypic division. x 2340.

Fig. 12. Brighton. The organization of the microspore nuclei in a mother-cell, subsequent to the homoeotypic division. x 2340.



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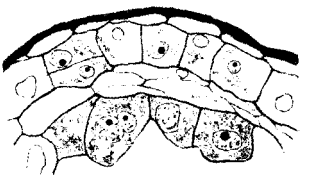

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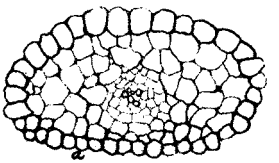

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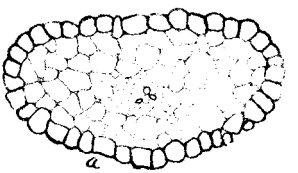

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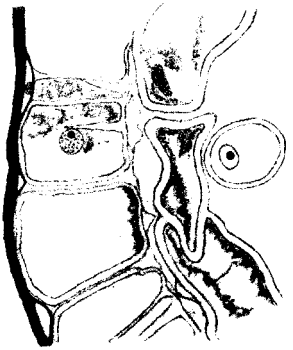

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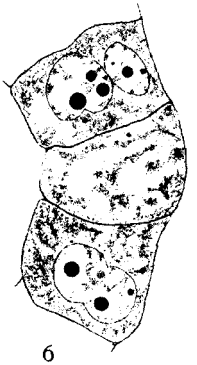

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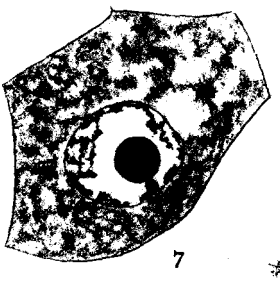

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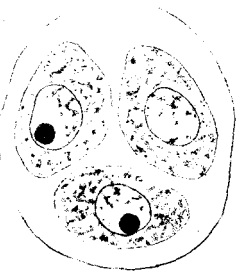

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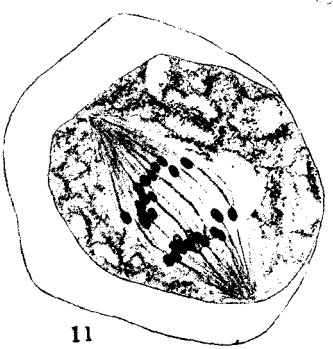

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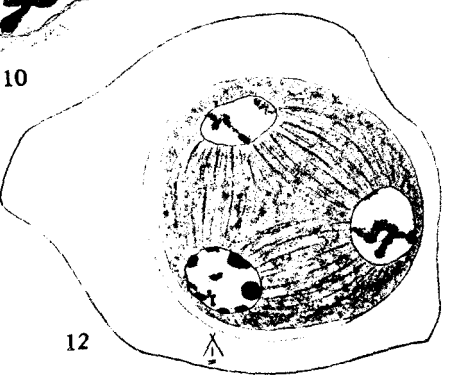

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PLATE II

Fig. 13. Brighton. One of the young microspores of a tetrad previous to being liberated from the mother-cell wall. Note thin wall and spreading out of the chromatin. x 2340.

Fig. 14. Concord. A young microspore, showing the large nucleus, vacuole, and thickened wall. x 2340.

Fig. 15. Brighton. The spireme stage in the microspore nucleus. x 2340.

Fig. 16. Brighton. Late spireme stage in microspore nucleus, showing spireme segmenting into chromosomes. x 2340.

Fig. 17. Brighton. Equatorial plate stage in the division of the microspore nucleus, showing haploid number of chromosomes. x 2340.

Fig. 18. Brighton. A late division stage in the microspore nucleus, showing the spindle figure located well toward the cell wall. x 2340.

Fig. 19. Concord. The generative nucleus in a fertile form soon after division. x 2340.

Fig. 20. Brighton. Diakinesis in a microspore nucleus. x 2340.

Fig. 21. *V. vulpina* ♂. A generative cell in a normal mature pollen grain. x 2000.

Fig. 22. Brighton. Metaphase in the division of the microspore nucleus. x 2340.

Fig. 23. Brighton. An early stage of degeneration in the generative nucleus. x 2000.

Fig. 24. Brighton. The cell-plate stage after division of the microspore nucleus. x 2340.

Fig. 25. *V. bicolor* ♀. An early stage of degeneration in the vegetative nucleus and generative cell. x 2000.

Fig. 26. Brighton. An instance of extreme organization in the generative cell. x 2340.

Fig. 27. *V. bicolor* ♀. An advanced stage of degeneration in the vegetative and generative nuclei. x 2000.

Fig. 28. *V. vulpina* ♀. An early stage of degeneration in the generative cell. x 2340.

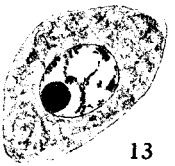
Fig. 29. *V. vulpina* ♀. An instance where degeneration is taking place only in the generative cell. x 2340.

Fig. 30. Concord. A normal generative cell. x 2000.

Fig. 31. Brighton. Degeneration in both vegetative and generative nuclei. x 2340.

Fig. 32. Brighton. Degeneration in the generative cell only. x 2340.

Fig. 33. Concord. The normal generative cell and vegetative nucleus. x 2000.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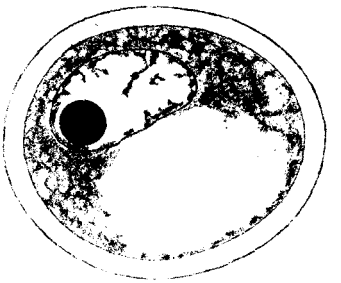
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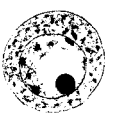
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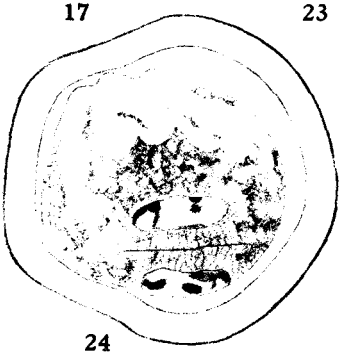
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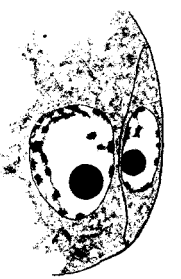
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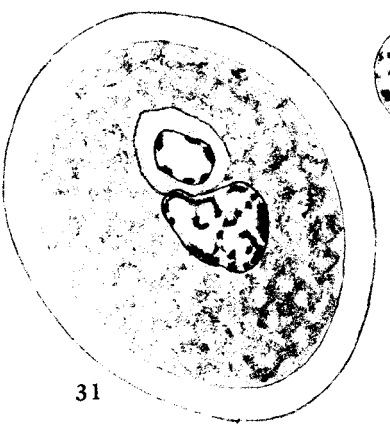
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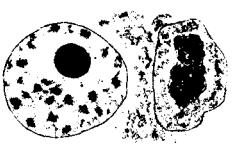
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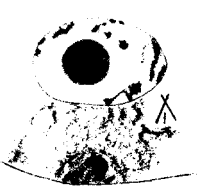
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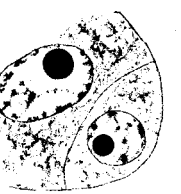
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PLATE III

Fig. 34. America. An advanced stage of degeneration in the generative cell.

Fig. 35. Barry. Degeneration in both nuclei in the pollen grain.

Fig. 36. Brighton. Both nuclei are degenerated and located near the center of the pollen grain. No nucleoli have been formed in this instance.

Fig. 37. Barry. A section through the anther showing the anther sap, and an unusually large generative cell with scant cytoplasm and nuclear contents.

Fig. 38. Brighton. A condition similar to Figure 36, in which the generative cell is even more densely stained.

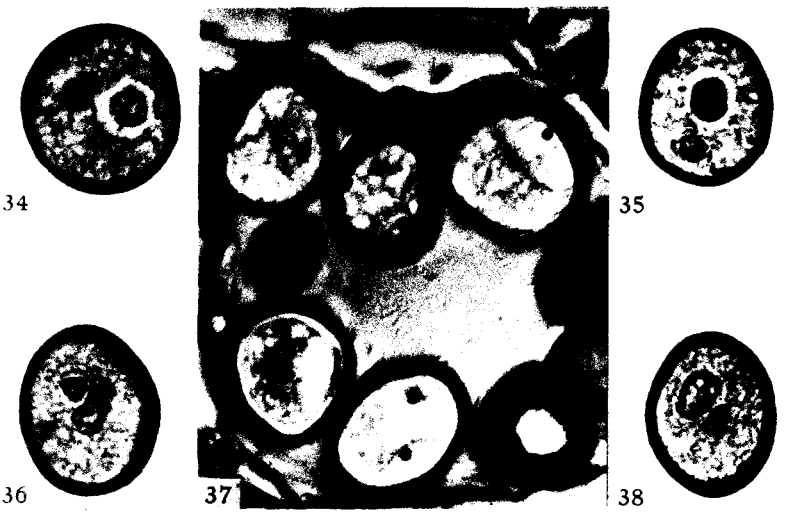
Fig. 39. Brighton. A pollen grain in which the generative nucleus has a diffused staining reaction.

Figs. 40, 41, 42, and 43. Brighton. Pollen in which degeneration is taking place in the generative nucleus only. Contrast with Figures 36, 38, and 39.

Figs. 44 and 45. Croton. Lens-shaped generative cells.

Fig. 46. Gaertner. Generative cell and vegetative nucleus degenerated and surrounded by a clear area in the cytoplasm.

Figs. 47 and 48. Lindley. Generative cells degenerating. The vegetative nuclei located near the center and surrounded by a narrow clear area in the cytoplasm.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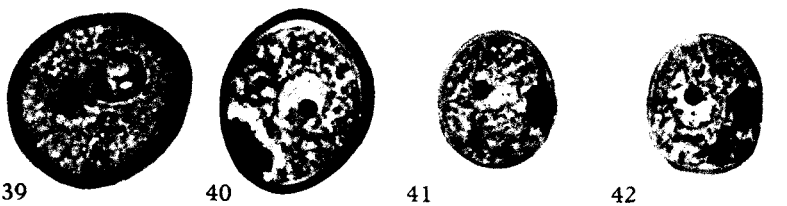
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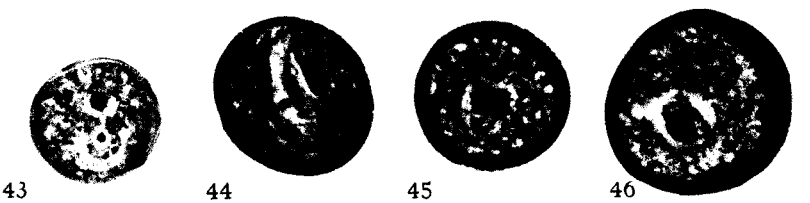
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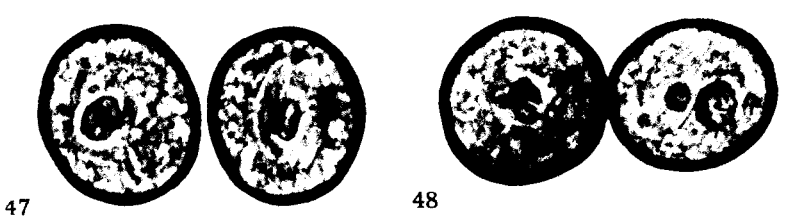
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PLATE IV

Figs. 49, 50, and 51. Marion. Advanced degeneration in both nuclei. No nucleolus present.

Figs. 52 and 53. Northern Muscadine. This variety has upright stamens and germ pores and is partly self-fertile. Degeneration is found in some nuclei, as shown.

Fig. 54. Massasoit. Generative cell small and degenerated and the vegetative nucleus irregular.

Fig. 55. *V. bicolor* ♀. Degeneration in the generative cell and vegetative nucleus in a sterile pistillate vine.

Figs. 56 and 57. *V. vulpina* ♀. An advanced stage of degeneration in the generative cell characteristic of pollen borne by the wild pistillate vines.

Fig. 58. *V. vulpina* ♀. An earlier stage of degeneration in the nuclei than shown in Figs. 56 and 57.

Fig. 59. *V. vulpina* ♂. A section, showing the germ pores on fertile pollen borne by a wild staminate vine.

Fig. 60. *V. vulpina* ♂. An external view of the suture and germ pore in fertile pollen.

Figs. 61, 62, and 63. *V. vulpina* ♂. Sections, showing the normal generative cells in fertile pollen.

Fig. 64. Clinton. Photomicrograph of pollen mounted in lactic acid, showing the normal potent pollen. x 280.

Fig. 65. Niagara. A photomicrograph of a mount in lactic acid, showing about one-half of the pollen aborted. x 280.

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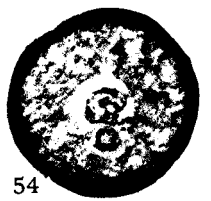

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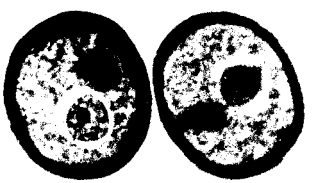

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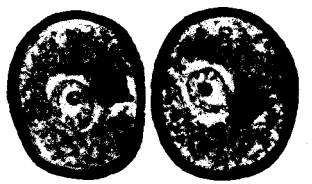

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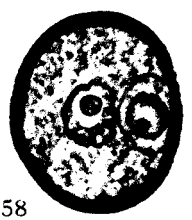

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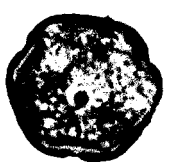

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