

GENERIC CROSSES.

Minor Thesis in Cytology.

submitted to

Dr. E. A. Smyth

for the degree of

MASTER OF SCIENCE IN HORTICULTURE

by

G. F. Gravatt

May 31, 1912.

Approved

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GENERIC CROSSES.

It is a general rule with organic forms that whenever their systematic differences are very great, they will not hybridize with each other. It is a hard matter to determine how great the systematic differences should be in order to prohibit hybridization. A large number of species will hybridize with other species of the same genera tho the product obtained is nearly always sterile. The great majority of species will not hybridize with other species of the same genera. In regard to crosses between different genera, a good many have been reported. Each of these successful crosses represents hundreds of unreported failures, owing to the great difficulty in effecting such a cross. These generic crosses have not been confined to any certain family or families, but have been effected in various different families, both in the Dicotyledons and in the Monocotyledons.

Burbank made several generic crosses one of which I will mention. The parents were tobacco and petunia which are so distant that as a rule, the pollination never results in good seeds. Among hundreds of seedless capsules, he found one in better condition and from its contents raised one single plant. This, as was expected, was absolutely sterile.

In the Cruciferae, I have found mention of only one generic cross, that between *Raphanus* and *Brassica*. Between the two species of *Raphanus*, *R. raphanistrum* and *R. sativus*, crosses have been made. Between three of the species of *Brassica*, (*B. napus*, *B. rapa*, and *B. campestris*) crosses have also been made.

Sageret, a German, reported a cross between *Raphanus sativus* and *Brassica oleracea* many years ago. However he tells very little about it. There seems to be some doubt about this cross in the mind of Fockle, who reports Sageret's work.

The Botanical Location of the Two Parents.

The family Cruciferae belongs in the dicotyledons. It is a fairly large family containing 127 genera and a large number of species. It is made up of herbs with a pungent watery juice and tetradynamous, regular flowers. One noticeable characteristic of the flowers is that the limbs of the four hypogynous petals form a cross. The fruit is a silique.

The two genera concerned in this thesis fall under the tribe Brassiceae. The chief distinctions between the genera Raphanus and Brassica are that the pod of the former is one celled or with spongy transverse partitions and indehiscent while that of Brassica is longitudinally two celled and dehiscent. Between the two species, Brassica campestris and Raphanus sativus, there are a good many differences such as color of petals etc. but these will be noted later on.

The Origin and History of the Parents.

The cultivated cabbage was developed from a small plant which grows wild on the sea cliffs of western and southern Europe, especially along the chalk cliffs of the English Channel. This wild plant is a perennial with a tough root, a diffuse habit of growth and thick, deep lobed leaves. The diffuse habit and deep lobed leaves are two characteristics very similar to the radish, which have been almost entirely bred out of the cultivated cabbage. This wild plant was in cultivation at the time of the earliest records and has been bred up since that time until today we have the finished produce in the form of the cultivated cabbage.

The origin of the common garden radish, Raphanus sativus, is not entirely clear. It is supposed by some that it was developed from some wild plant, native to the temperate part of Asia, which wild plant is now extinct. Other people think that the common garden

radish is just an improved form of the wild charlock, *Raphanus raphanistrum*, which grows wild in southern Europe. M. Carriere carried on experiments with this wild plant for five years and in that time he produced all the important radish shapes and colors. These experiments prove that it is possible for the radish to have developed from the wild charlock. The history of the radish dates back to 450 B. C. when Herodotus, in describing the building of the great pyramid of Egypt, mentioned an inscription which stated the amount expended in radishes for the workmen.

Thus we see that these two parents, both developed from native wild plants of Europe, have been in cultivation since the beginning of history.

Early History of the Cross.

In March 1910 a large number of flowers of three different varieties of radish were emasculated and bagged. Two days later at which most of the stigmas seemed to be in the proper condition, they were pollinated with pollen from a hybrid cabbage plant. This cabbage was a first generation hybrid between the varieties "Volga Russian" and "Curled Savoy". Perhaps the fact that the cabbage plant was a hybrid had something to do with the fact that this generic cross was effected. The bags were left over the pollinated flowers for two weeks. At the end of that time, all had dropped off except two small pods of the variety "Long Scarlet Short Top". A large number of cabbage flowers were emasculated and pollinated but none set.

In September 1910 the contents of these two pods consisting of several small shriveled seeds, were planted. Only one came up. From the first it could be seen that it was a cross. It was very vigorous and grew rapidly, being transferred from larger to larger pots until in the later part of January 1911, it had to be transferred from its

twenty inch pot to a central bed.

Growth Habit.

When still young the general growth habit of the radish and of the cabbage is quite similar. It is just a simple, spreading, diffuse growth. It is not long however, before the cabbage begins to head up and packs most of its leaves into a solid bud-like formation. Some time after, this head reaches maturity, a shoot begins growing in the center, by its development breaks the head open, and then grows upward rapidly, being nourished on the food stored in the thick leaves.

The radish differs from the cabbage in that it retains its open diffuse habit of growth. Instead of forming a solid head of leaves, it develops a tuber^{ous root} and in this tuber stores its surplus food. Then when the radish reaches maturity, a bloom stalk is sent up, being nourished mostly on food from the tuberous root.

This radish cabbage cross is very much of a mean between the two parents. Like both parents, at first it has an open, diffuse growth, as shown by Fig. 1. Later on while the cabbage is forming head and the



Fig 1.

radish its tuber, the cross forms a thick bushy growth. At first there is only a single elongated axis but sometime before blooming numerous side

branches start out. Some of these branches have just started in the plant shown in Fig. _____ The plant now becomes still thicker and many of the leaves die on account of lack of light. There is very little tuber development.

A bloom stalk grows up from the main growing point of the stem

just as in the case of the parents. This one is shortly followed by others from the side shoots. These bloom shoots attain a much greater length than those of either of the parents as shown by Fig. 2.

The plant

produced an enormous number of flowers throughout six months and seemed like it would have kept on producing them if it had not been killed by a bacterial stinking rot of the root.

Leaves.

This cross produced a great abundance of large leaves. In regard to the size and shape of leaves, they are much more similar to the cabbage than to the radish. The shape and general character of the leaves of the three plants are shown in Fig. 3. The largest leaf of the



Fig. 2 Mature Hybrid Plant.

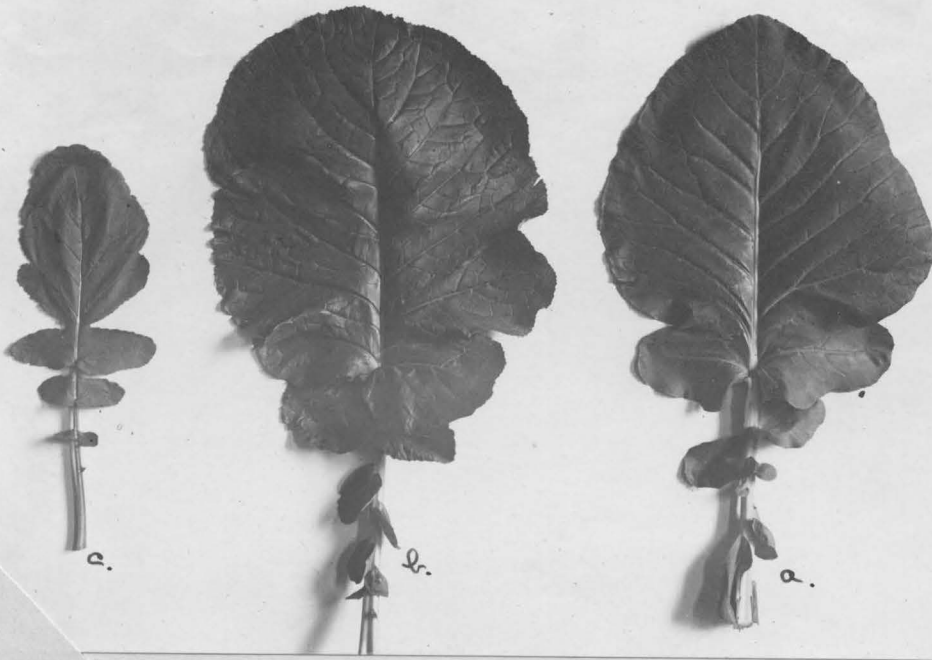


Fig. 3. a. Cabbage Leaf, b. Hybrid, c. Radish.
er than those of either of the parents.

In regard to color the cross is a mean between the two parents, the radish being a light green while the cabbage was a dark or blueish green. The taste of the cross is somewhat more like that of the ^{cabbage} radish and yet it retains some of the radish pungency.

The radish leaf is pubescent with stiff hairs while the cabbage leaf is glabrous. The leaves of the cross are just about glabrous.

In regard to thickness, the cabbage leaf is much thickened than that of the radish. The leaves of the cross are a mean between the two parents. However, the individual leaves vary somewhat in this respect.

The cabbage parent had a puckered leaf, the radish a smooth leaf and the cross has a leaf free from puckering.

The general microscopic features of the radish and cabbage leaves are not very different and the cross differs in no way from these.

cross was five feet nine inches long by one foot seven inches broad. This leaf was measured at about the time the plant started blooming. The leaves on the bloom stalk of the cross are somewhat broad-

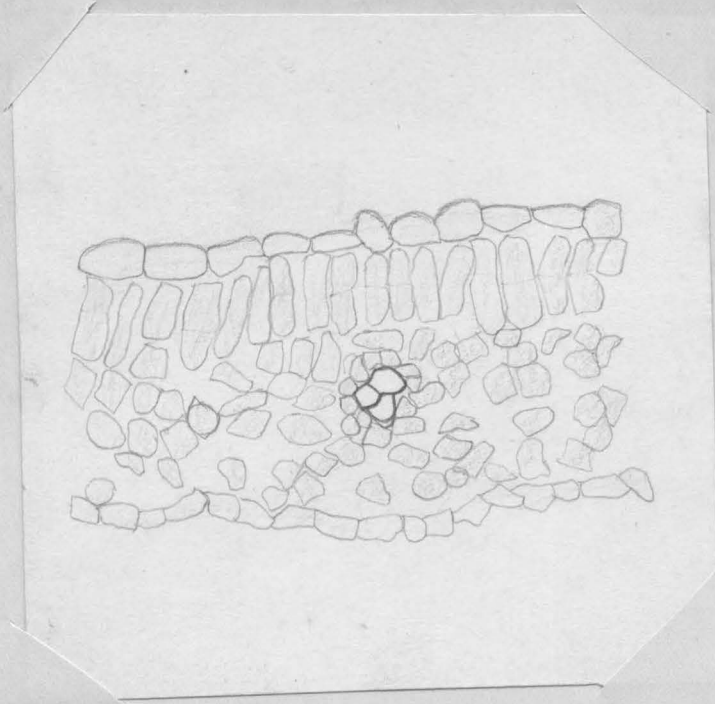


Fig. 4 Cross Section of Leaf.

the veins passed. The last layer is the lower epidermis.

Sections of the leaf of this cross were made at 6 and 8 microns. These were stained in haematoxylin and eosin. Fig. 4 shows the general features. The upper layer of cells is the epidermis. Below this is the palisade layer of elongated cells. In some cases, there seemed to be two layers of palisade cells. Next comes the region of the spongy cells of the mesophyll through which

Stem and Root.

It would seem likely that the hybrid would inherit some tendency toward tuber formation from its radish parent. However this does not seem to be the case since the thickness of the root where the tuber should be/ was no greater than the thickness of the stem. The hybrid does not even show a well developed main tap root as the radish does but divides into numerous branches at a short distance below the surface of the ground.

The stem of this hybrid is much larger than the combined stems of the two parents. When the hybrid reached the size shown in Fig. _____ the circumference of the stem was 13 1/2 inches, measured at a distance of one foot from the ground. The stem holds its size for some distance from the root. The lower part or about the first two feet of the stem is very thickly beset with leaves, being very similar to the cabbage in this respect.

These leaves drop off on account of shade from above leaving large and prominent scars. Starting from the root, the major portion of the stem is a purplish red color just the same as that of the radish. However, this purplish red color covers the stem of the hybrid much more thoroughly and extends further up than it does on the radish parent.

Sections of the smaller roots and of stems showed nothing peculiar about the hybrid in regard to the microscopic structure of these parts.

Flowers.

The pediceled flowers of this cross are borne on a greatly elongated axis. In some cases this axis attained a length of three feet. The flowers of the parents are borne in the same way though the flowering axis or raceme does not grow to such a length as with the cross. Perhaps this greater length of the raceme of the cross is due to the fact that no pods set and therefore all the food goes to the development of flowers.

The buds of the radish are obovate in shape while those of the cabbage are oblong elliptical and a good ^{much} bit larger and longer. The buds of the cross are a good mean between the two being somewhat elliptical in shape and about intermediate in size. Near the top of the bud of the radish, there are several prominent hairs growing out from the sepals. The cabbage and the radish cabbage cross do not have these hairs.

The cross has four sepals, two being a little smaller than the other and slightly overlapping as in both parents. The cross has four petals, about intermediate in size between those of the two parents. In regard to color they are much more similar to the radish than to the cabbage. The background color is white with a purplish tinge and veins,

giving the flower a purplish appearance. This is very similar to the radish while the cabbage flower has rich yellow petals.

Externally the pistil seemed to be normal and showed no characteristics differing from the parents. It always turned yellow and dropped off soon after the ^{petals} ~~petal~~ did.

The radish and cabbage flowers each have six stamens. Most of the flowers of the cross had six stamens but a good many have two extra stamens, making eight. Last year on the old parent plant, about 15% of the flowers had eight stamens. The two plants grown this year have not been near as vigorous as the parent due to attack of insects and of rot. As the consequence of this lack of vigor, these two extra stamens have not developed to full size as they did on the parent but are small and aborted. Most of them are about three fourths the height of the others and about one fifth the size or thickness. On a good many of the flowers the only indication of the extra stamens are two little filaments, about one third the height of the normal stamen.

These two extra stamens bring up some interesting questions. The normal number of stamens of the Cruciferae is six, two of these being inserted lower down and shorter than the others. However, in some genera there are only two or four stamens while in at least one genus there are ten stamens. Judging from these numbers, it would seem that at some time in the past, the present genera of this family were derived from plants that had many stamens and that these stamens have been gradually reduced in number.

The four sepals alternating with the four petals just about place this flower in the quaternary type but there are many contradictory theories as to how to explain the six normal stamens of this family if it is to be placed under this type. De Candolle and Saint-Hilaire admit the quaternary type for the sepals and petals and extend this

type to the stamens, claiming that each pair of long stamens represents a double stamen.

Some of the later botanists, among them Krause and Duchartre, say that there is no doubling of the long stamens. They claim that there are two quaternary whorls of stamens. The first of these contains only the two short stamens and there are supposed to be two others which are undeveloped. The second whorl is made up of the four long stamens. This view seems reasonable since the four long stamens are situated on a higher level or on a different plane from the two short stamens. As to the matter of two of the stamens being undeveloped, this is a very common state of affairs. Often stamens are changed into nectaries, petals or other parts. On the receptacle of the radish and of the cabbage, there are located two prominent nectaries just where the missing stamens should be. The following quotation from "Outlines of Classification" by Goebel is of interest at this point.

"Instances of this kind completely justify us in assuming abortion in cases also in which the absent organ does not disappear during development but is wanting from the beginning provided that a comparison of the number and position of the parts in nearly allied plants gives ground for supposing that there is something missing; but it is the modern theory of descent which supplied certain ground for the assumption of abortion of this kind."

However, these two leading theories do not explain very well the radish cabbage cross with its eight stamens. The two extra stamens seem to be located in the wrong place. Their position is indicated by the two small circles, the six normal stamens by dots, and the two large circles represent differences in elevation of the receptacle.

If these two extra stamens brought out by the disturbance of the equilibrium of the plant due to the gen-

eric cross, indicate the position of the undeveloped stamens, then the theory of De Candolle as to the four long stamens being just two double stamens, must be wrong.

The simplest way to explain these two extra stamens is to assume that in the past there were two whorls of stamens of indefinite number and that ^{these} ~~tests~~ stamens have been reduced to the present number of six. This guess may be strengthened by noting the fact that even now one genus has only two stamens. It would be hard to explain the arrangement of the eight ^{stamens} of the radish cabbage cross if we were working on the assumption that the original number of stamens were eight.

It was noticed that the pollen from this cross was not powdery and light as that of the radish and cabbage, but stuck together and had a tendency to ball up. There was often difficulty ^{in this} to get the pollen spread out on the stigma; it would stick together in clumps. On examination it was found that the pollen from the radish and that from the cabbage was very regular in size and shape while that of the hybrid was very irregular in both shape and size as shown in Fig. 8. Attempts to germinate this pollen from the hybrid in various solutions were a failure.



Fig. 6. Cabbage
Pollen.

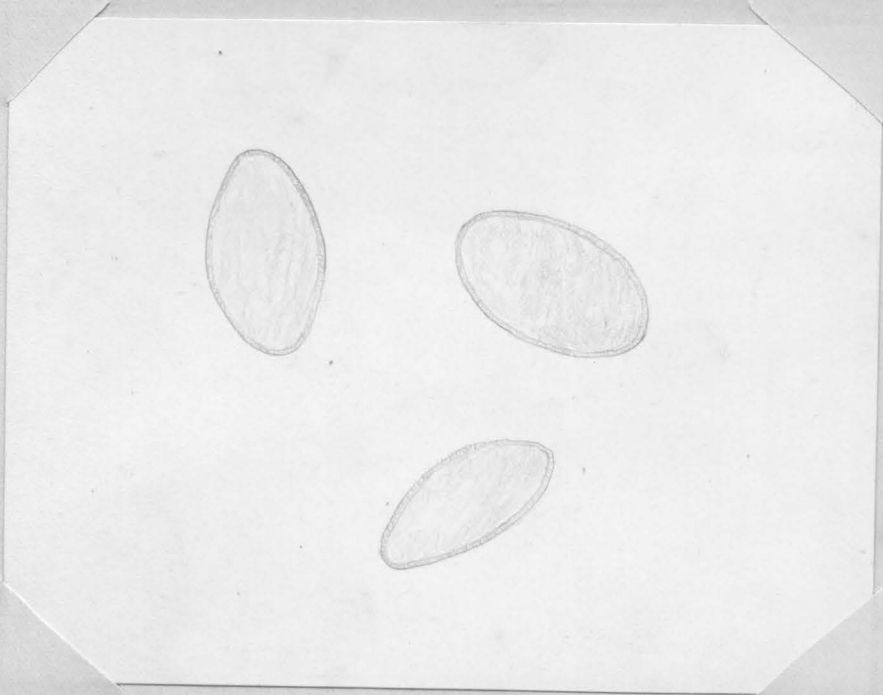


Fig. 7

Radish Pollen

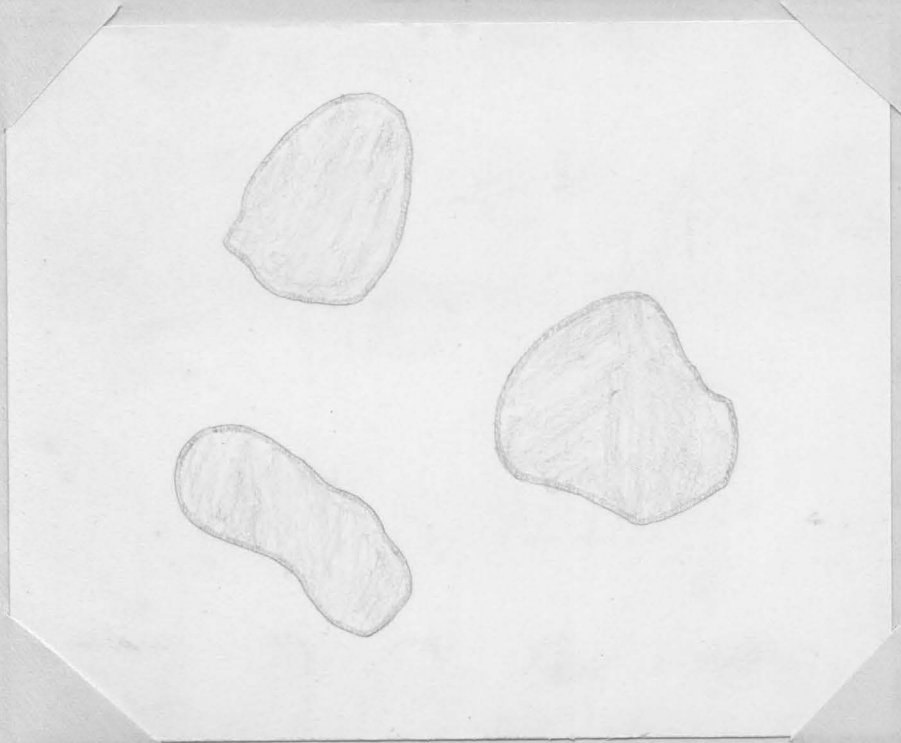


Fig. 8.

Hybrid Pollen.

Methods.

In making the drawings, a camera lucida was used to secure the general outlines and the proportional size of the cells.

Sections were made of the root, stem, leaf, bud and ovary, a good many being made of the latter two. The material was killed and fixed in chromo-acetic solution (25 cc. 1% chromic, 10 cc. 1% acetic, and 55 cc. water), washed in water, hardened and dehydrated in the grades of alcohol, cleared in xylol and imbedded in paraffin. Sections of the root and stem were made at 6 and 8 microns and stained in haematoxylin and eosin. Sections of the buds for pollen development were made at 4 and 6 microns. Sections of the ovary were cut at 10, 12 and 14 microns. These sections of the bud and ovary were stained in Flemming's safranin, gentian-violet and orange.

Formation of the Microspores.

The first indication of an anther is a very small outgrowth from the receptacle. This outgrowth is made up altogether of meristematic tissue or cells. Soon the outer layer becomes differentiated into the epidermis and then the outgrowth begins to take on the four lobed shape of the anther. A cross section of an anther at this stage shows in the center a single hypodermal cell or group of cells, of larger size, richer protoplasmic content, and with larger nuclei than the others. A longitudinal section shows this group is really a line or sometimes a plate of cells, extending nearly the length of the anther. This line of cells is called the archesporium.

This archesporium or hypodermal band of cells divides throughout its length by periclinal walls, producing an outer primary wall layer and an inner primary sporogenous layer.

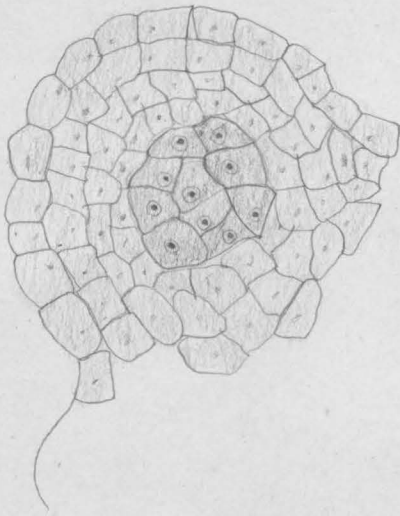


Fig. 9. Cross Section of Young Anther

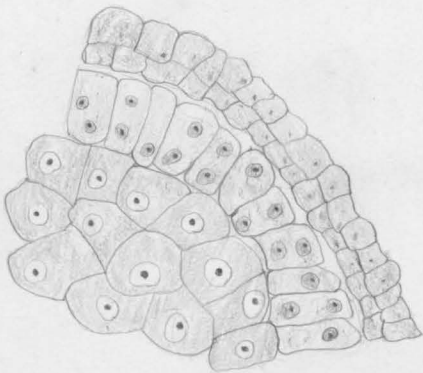


Fig. 10. Later Stage of Anther.

Fig. 9 shows this inner sporogenous layer in the center of the lobe of the anther. The outer primary wall layer divides rapidly and extends itself about $3/4$ of the way around the sporogenous layer. This outer layer now forms several wall layers. The innermost layer nourishes the sporogenous tissue and is known as tapetum. This layer is shown in Fig. 10. The cells of the tapetum are elongated in shape and very rich in protoplasm. They are often binucleate. Usually by the time the pollen tetrads have formed, the tapetal layer has given up all its food and just shrivels up. This shriveled layer is shown in Fig. 11. This appeared as a solid orange brown layer of matter, the cells or nuclei of which could not be made out.

On the outer side of the tapetal layer there are several layers of wall cells. These are known as the middle layers and usually

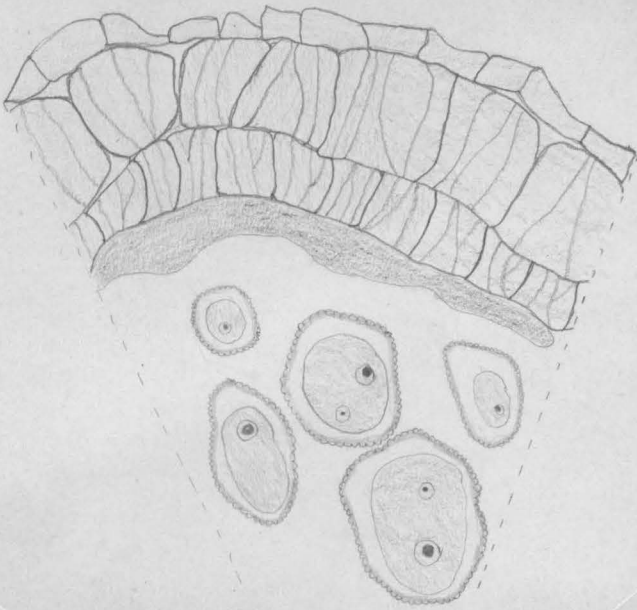


Fig. 11 Cross section of Anther Showing Pollen Grains, Tapetum & Wall.

become flattened and disorganized on account of the activity of the tapetum.

Surrounding the middle layers is the endothecium the cells of which are large and conspicuously banded as shows by Fig. 11. These bands take the gentian violet stain. These bands or fibrils seem to start from a line right next to the tapetal layer. In many

cases they divide and branch. These bands are not confined to the endothecium and on the back side of the anther, they are present on the cells for four or five layers inward. These thickened bands on the walls are hydroscopic and by their contraction effect the dehiscence of the pollen sacs.

On the outside is the epidermis, composed of very irregular and small cells. In some places it seems to be lacking.

While these surrounding walls are forming, the cells of the primary sporogenous layer divide several times and then stop for a while, forming the spore mother cells. These mother cells now go thru the various stages of division. At first the contents of the nucleus changes into threads, forming the spireme stage as shown in Fig. 12.

Then the nuclear threads become segmented into chromozomes which assemble at the equator. While the chromozomes are forming, the two poles with their spindle formation have made their appearance. Next comes the metaphase where the chromozomes separate just before journey-

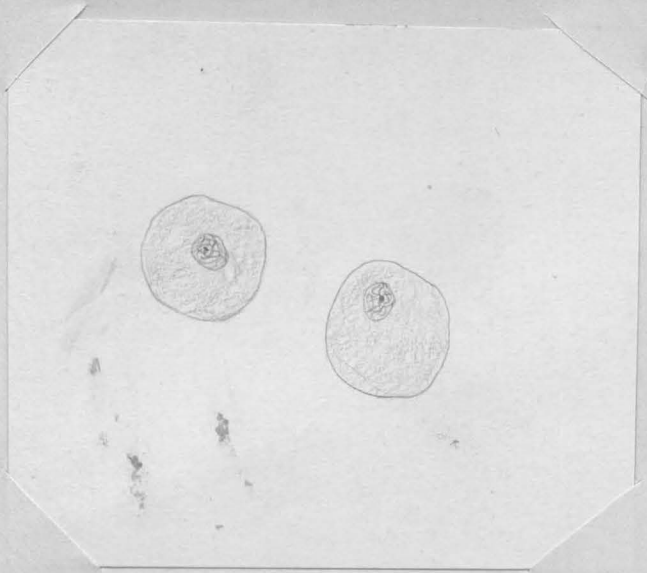


Fig. 12 Spireme Stage

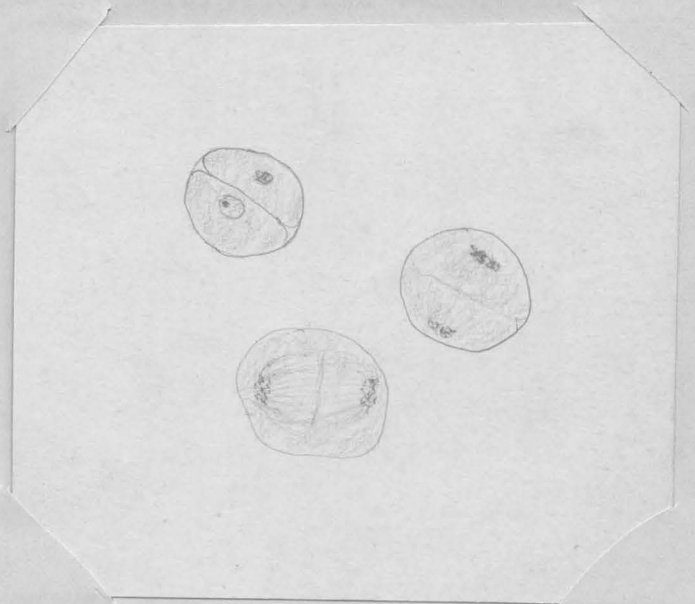


Fig. 13 Early Stage in Formation of the tetrads.

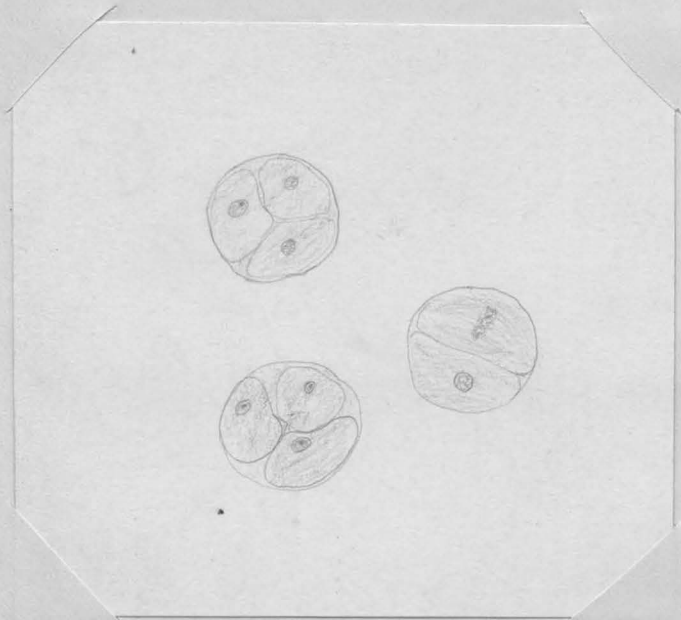


Fig. 14. Later Stage in Formation of Tetrads.

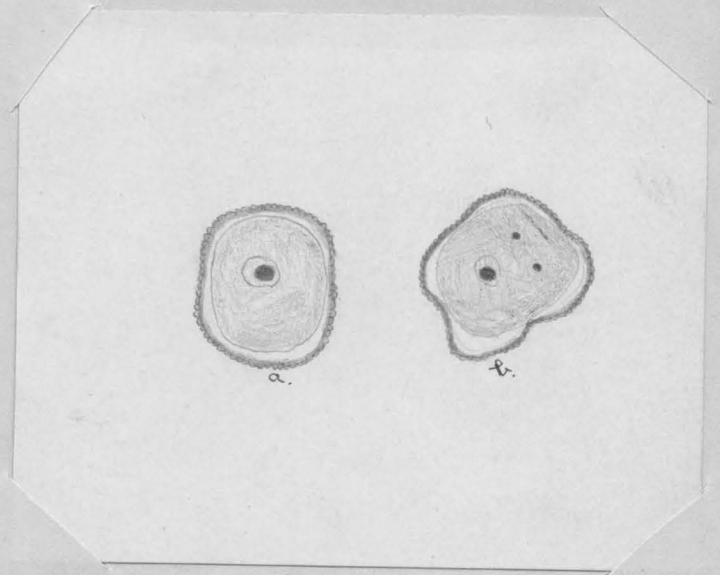


Fig. 15. Pollen Grains.

ing to the opposite poles. The chromosomes go to the opposite poles and a wall begins to form between the two as shown by Fig. 13. The chromosomes form a reticulum but this is hardly formed before it breaks up again into chromosomes preparatory for the second division. This second division takes place and we have the mother cell containing the four tetrads. Only three of these tetrads can usually be seen in each cell. These mother cells now break, releasing the tetrads which round off and become masses of pollen. The condition of the pollen grain at this time is shown in Fig. 15 a. It is just

simply a microspore with a single nucleus. Now this nucleus divides forming the generative and tube nucleii. The generative cell now divides again which results in the formation of two equal male cells. When the anther sheds, most of the pollen is in the two nucleated condition but many of the pollen grains had only one nucleus and some few had the tube nucleus and

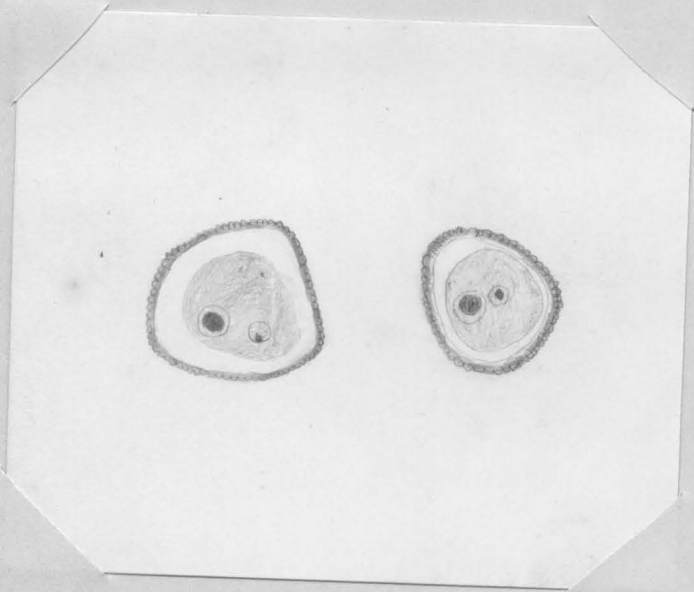


Fig. 16. Pollen Grains.
the two male cells.

Ovule Development.

The pistil of the radish cabbage hybrid seems to be perfectly normal, consisting of style, stigma and ovary. Sections of the stigma showed it to be similar to that of its parents. Fig. 17 is a portion of a cross section of a stigma showing the large outer cells which are characteristic of this region.

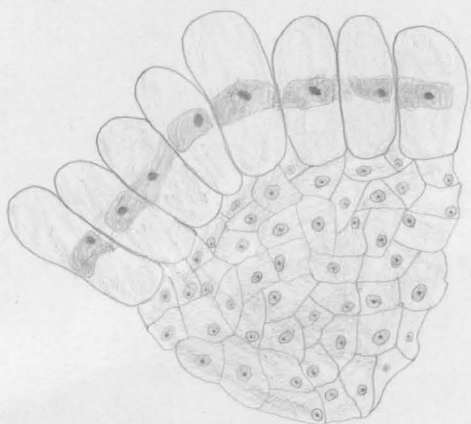


Fig. 17. Cross Section of Stigma.



Fig. 18. A Three Celled Ovary of
This Hybrid.

One of the chief generic distinctions between the two parents is that the ovary or pod of the radish is one celled or transversely several celled, the partitions being spongy or pithy which the ovary of the cabbage is longitudinally two celled as a rule and has two marginal placenta with the false partition running from one to the other.

Fig. 18 shows a three celled ovary of this hybrid. The slide showing this condition of the ovary had a good many sections on it, all of which showed the same three cells, and three placentae. I suppose that as in the case of the extra stamens, the hybrid has had its equilibrium somewhat upset on account of its distant parents, and consequently just produced an extra cell. It would be very interesting to know whether in the ripe pod, the three valves would separate from the placenta.

In regard to the development of the egg in the ovule, my slides were a failure, showing practically nothing. I am not sure where the failure lies, whether in the killing and staining or in the material itself.

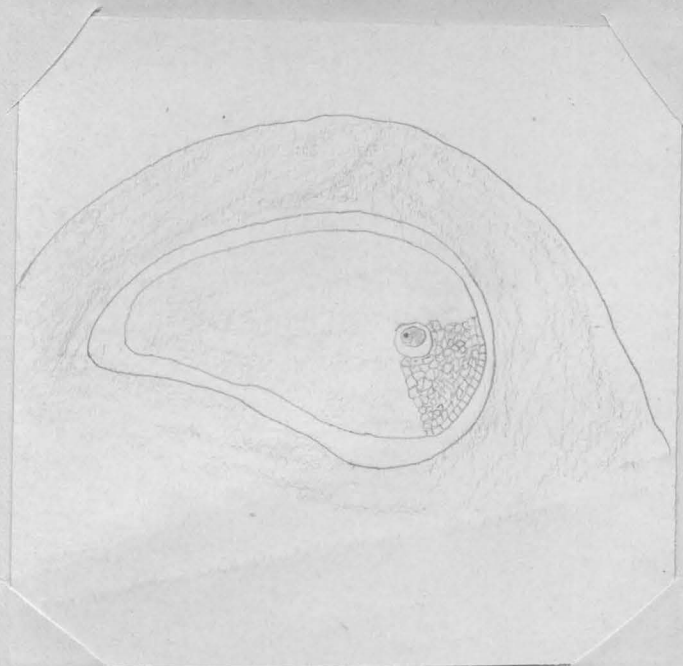


Fig. 19. An Ovule in one Cell
of the Ovary.

Fig. 19 shows an ovule in one of the cells of the hybrid ovary. The finer features were not at all clear. Out of a number of slides, this was the only ovule that showed signs of an egg. The ovules were plentiful enough and the surrounding walls seemed to be clear but that was all.

Perhaps it should be noted here that the cabbage ovary contains a good many more ovules than that of the radish. The hybrid seems to be more closely related to the cabbage

in this respect.

Sterility of this Hybrid.

In March 1911 this hybrid started blooming in great profusion. Nearly every day a large number of flowers were pollinated with its own pollen and pollen from the radish, cabbage, cauliflower, collard, Brussles sprouts, and kohl-rabi. Not a single flower set altho several thousand were pollinated during March, April and May. Flowers of the radish, cabbage and various brassica crosses were emasculated, bagged, and pollinated with radish-cabbage pollen. None of them set. Very much the same process has been gone through with a root cutting of the old parent plant during the past season tho not as large a number of flowers were worked with. This cutting was made in the summer of 1911 and started blooming sometime in January. Just the same as with the parent plant, no pods set. It was concluded that the Radish-Cabbage cross was sterile.

About distant crosses De Vries says, "Whenever their systematic differences are too great, the crosses will be infertile, or at least the produced hybrids will refuse fertilization, even with the pollen of their parents. Repeated crosses are impossible and no practical results can be obtained." De Vries was speaking of crosses between species when he said the above. Generic crosses are nearly always sterile. Most of them do not even produce flowers and if they do, the flowers are usually aborted in some way.

On account of its two celled ovary, this hybrid would probably fall in the genus *Brassica* tho the location of this plant would also depend somewhat on whether its pod would be dehiscent or indehiscent. Taken as a whole it is a blend of the two parents with the cabbage parent somewhat the stronger. As to the practical value of this cross, there seems to be none. If it were not sterile it would make a splendid hog feed on account of its great vigor and the production of a such a great quantity of leaves.