

## THE PHYSIOLOGY OF SELF-STERILITY IN PLANTS.

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Hermaphroditic animals and plants are known where fertilization of the eggs by the sperm or by the pollen produced by the same individual is very unusual. The extent of the phenomenon in the animal kingdom is unknown, *Ciona intestinalis*, an ascidian, having been the only species used in experimental work. In the angiosperms, the characteristic is widespread, and must be supposed to have originated many times. At least 50 families are represented, including both monocotyledons and dicotyledons.

The problem which this condition presents is interesting from several angles. The evolutionist wishes to know how and why self-sterility originated, since it often arose in groups where various provisions for cross-fertilization already existed. The geneticist wishes to know whether it is hereditary, and if so what method of inheritance is followed. The physiologist desires an interpretation in physiological terms. And finally, since many apples, pears, peaches, plums, cherries, and other useful fruits are self-sterile, the horticulturist hopes to find a practical method of dealing with the condition in orchard work.

Darwin (5) and his friends and correspondents, Müller (12) and Hildebrand (9), made a number of rather unsystematic cross-pollinations with the plants of several self-sterile species and obtained fruit in each instance. These results led Darwin to conclude that the sexual organs of every such individual have been specialized with respect to every other individual in the same sense in which the external characters of every individual are so specialized that no two are alike. He assumed that differentiation of some sort in the sexual elements is essential to fertilization. After making some 40 or 50 cross-pollinations, he even went so far as to say, "we may therefore confidently assert that a self-sterile plant can be fertilised by the pollen

of any one out of a thousand or ten thousand individuals of the same species, but not by its own." This is one of the few cases where Darwin allowed his enthusiasm to outrun his better judgment in issuing generalizations.

Jost (10), the first of twentieth century biologists to attack the problem, came to practically the same conclusion. He was able to show definitely that pollen-tubes from incompatible matings germinate and grow in the styles, but that they grow more slowly than do those from compatible matings. Darwin had suspected that this was the case from observations made on pollen-tubes dissected from living pistils. Jost proved the matter. Jost's hypothesis of individual specialization in the sex elements was the same as that of Darwin; but he recognized a little more clearly that the whole problem was one of differential pollen-tube growth, and he assumed that these differential rates are caused by specific substances which he termed *Individualstoffe*.

These early theories postulating such extreme sexual specialization were rather overdrawn, and were shown to be so by Correns' (4) experiments on the self-sterile crucifer, *Cardamine pratensis*. In this species cross-sterility of the same nature as self-sterility was found. Plants could be grouped into classes, each individual within a class giving the same mating reactions. Thus it was necessary to postulate only a limited number of inhibiting or accelerating agents. Correns endeavored to give a Mendelian interpretation to his results, but failed because of conflicting data. He crossed two plants B and G, and found that the resulting progeny fell into four groups, one group sterile with both parents, one group sterile with the B parent, one group sterile with the G parent, and one group fertile with both parents. His cross-pollinations within this population also show a certain degree of orderliness, but whatever regular order exists is probably masked by the classification as "fertile" of incompatible matings which produce some seed through what I have termed "pseudo-fertility."

In 1913 Compton (3) proved that the genetic difference between self-fertile and self-sterile races of *Reseda odorata* is monofactorial with self-fertility dominant. Self-fertile races are FF or Ff; self-sterile races are ff. That result was corroborated in this laboratory

in 1915 (6) by the results obtained from crossing the self-fertile species *Nicotiana Langsdorffii* L. with the self-sterile species *Nicotiana Sanderae* Hort. and *Nicotiana alata* Lk. and Otto var. *grandiflora* Comes, both of these crosses yielding fertile hybrids.

3 years later (8) a microscopical study showed that the pollen-tube growth-curves of compatible and of incompatible matings are different. As shown slightly diagrammatically in Fig. 1, compatible matings produce pollen-tubes whose curves, taken of course by averages, simulate the ordinary two constant autocatalytic curve.

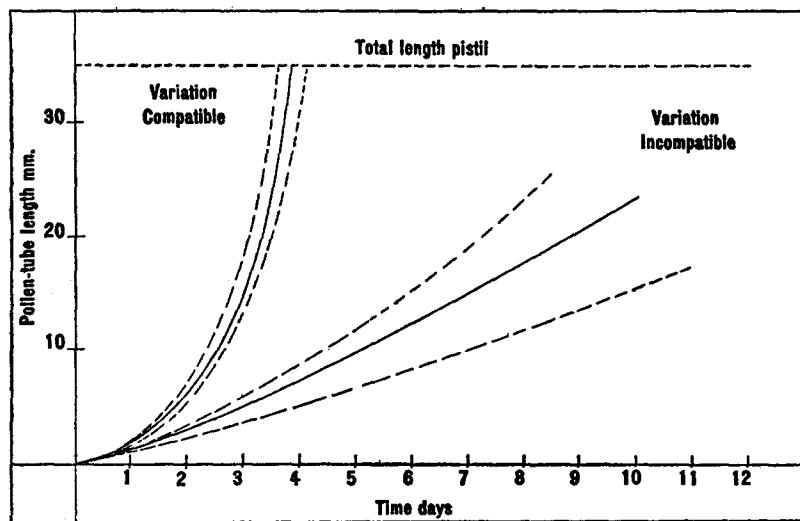


FIG. 1. Diagram illustrating pollen-tube growth-curves in compatible and in incompatible matings, *Nicotiana Sanderae* Hort.

They start slowly, but acceleration is rapid, and fertilization usually occurs between the 3rd and 4th day. Presumably if such curves could be followed further, as for example in a delayed pollination of the long styles characteristic of maize, it would be found that retardation would manifest itself later. Incompatible matings, on the other hand produce pollen-tube growth-curves with a very slight acceleration. They approximate a straight line.

In compatible matings, though there is considerable dispersion in the frequency distribution of pollen tubes within a single pistil at any

given time up to 30 hours after pollination, the pollen-tubes grow so rapidly when nearing the ovary that at any stated time later than 36 hours after pollination the dispersion is very low, probably not more than 2 hours elapsing between the fertilization of the first ovule and the last ovule. In contrast to the narrow variability shown by the growth-curves of compatible pollen-tubes, there is a very great dispersion among the pollen-tube growth-curves within a single pistil after an incompatible mating. At the time when the flower ordinarily withers and falls off, which averages about 8 days, the mean growth is about half the length of the style; but the major and minor extremes may be over a cm. apart. This is quite different from the frequency distribution of the compatible matings when the pollen-tubes are near the ovary. There they are bunched, with probably not over 2 or 3 mm. difference between the major and minor extremes. In passing it may be stated that there appears to be a compensatory growth in long styles. In the *Nicotiana* material the styles vary from 25 mm. to 70 mm., but the proportionate distance of the whole that is traversed in a given time seems to be about the same for styles of various lengths. This observation might perhaps be illusory when dealing with the rapidly growing tubes in late phases of compatible matings; but the same thing occurs in incompatible matings where more precise observations can be made.

Pollen-tube growth in compatible matings is influenced comparatively slightly by external variables such as moisture, fertility of the soil, and length of day, or by such matters as age of plant or time of flowering. Temperature changes have an effect, though they cannot be evaluated quantitatively as yet. One can say only that there appears to be an optimum at about 27°C. On the other hand variations in temperature and in the relative length of night and day have a marked effect on incompatible matings. Furthermore, toward the end of the flowering cycle the flowers often live from 10 to 16 days instead of the usual 7 to 9 days. At this time, with optimum temperature and light conditions, seed may be produced from incompatible matings. This is the occurrence termed *pseudo-fertility*. It is made possible by a correlation between flower persistence and flowering period rather than age of plant, for a second or even a third flowering cycle reenacts the first. And it is wholly a matter controlled

by the flowering cycle, for crosses between two incompatible plants one of which is at the height of the flowering period and the other at the end of the flowering period show pseudo-fertility only when the latter is used as the female parent.

It is clear, then, that self-sterility and self-fertility are two decidedly different conditions, and that these different conditions are hereditary. Nevertheless, since fertilization can occur after an incompatible mating if there is opportunity for gametic union, this difference is quantitative. Furthermore, there are numerous hereditary modifying factors within the species with which we are dealing. The diagram shown in Fig. 2 illustrates the complications that can ensue

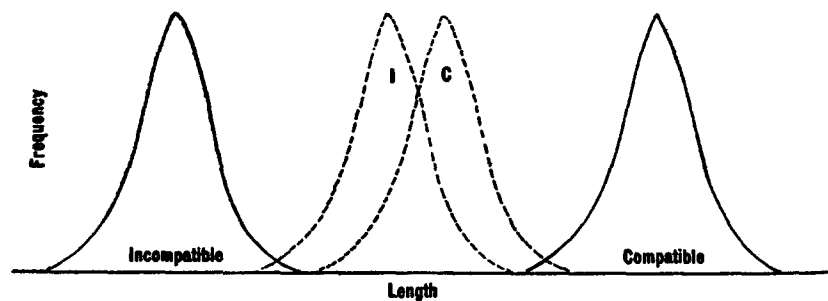


FIG. 2. Diagram illustrating frequency distributions of pollen-tubes after compatible and after incompatible matings at the period when the average of the compatible pollen-tubes is two-thirds the distance to the ovary. Single strains of *Nicotiana Sanderæ* have been found exhibiting curves like the unbroken lines under comparable conditions. Other individual strains under the same conditions exhibit overlapping curves like those shown by the broken lines.

because of this fact. Strains exist which under ordinary greenhouse conditions show pollen-tube frequency distributions in which the compatible and the incompatible matings yield curves as distinct as those which appear at the extreme right and the extreme left of the diagram. Other strains exist, however, where the frequency distributions from the two types of mating overlap. Fertility becomes confused with pseudo-fertility. In addition confusion can come about from any condition (*a*) which increases the life of the flower and therefore adds to the length of time during which a given pollen-tube has the opportunity to grow, or (*b*) any factor which

increases the rate of growth of the incompatible pollen-tubes more than it does that of the compatible pollen-tubes.

The proper set-up of the self-sterility problem, therefore, is to select material in which the growth-curves of compatible pollen-tubes are as different as possible from those of the incompatible pollen-tubes, and to keep external conditions constant except when it is desired to obtain seed from incompatible matings.

It is quite evident from an examination of Correns' results on *Cardamine pratensis* that compatible and incompatible matings in this species overlap much as they do in the two broken line curves of Fig. 2. The same statement can be made for the material used so extensively by Stout (13), *Cichorium intybus*. Stout has never appreciated the fact that he should eliminate as many variables as possible from his problems. He has been intrigued by the great complexity of his data. And for this reason he has been unable to show any orderliness in his results.

The first series of cross-pollination studies with the *Nicotiana* species as material was undertaken in 1911. A cross was made between strains of what was thought to be *N. Forgetiana* and *N. alata grandiflora* that had previously bred true to their respective characters. Twenty  $F_2$  plants were crossed in various ways until 154 matings had been made. Excluding selfings and pollinations with the pollen from a plant exhibiting true male sterility, there were 122 cross-matings, of which only three failed and failed repeatedly. While cross-sterility was found, therefore, having apparently the same nature as self-sterility, the frequency was so low that it seemed as if there was almost as much sexual specialization as was predicated by Darwin and Jost. This particular experiment does indeed throw some light on the amount of such specialization, but the conclusions to be drawn from it are more easily understood after a statement is made regarding the results obtained from later experiments carried on with the descendants of this or of similar crosses in which the data are less complex, presumably because of inbreeding.

Over 100 populations from one to fourteen generations removed from this original material have now been tested and retested as to their mating reactions. In every case cross-sterility comparable to self-sterility has been found, and the plants of any given family

have fallen into *not more than four* intrasterile, interfertile classes. These results, as shown later, can be interpreted by the hereditary behavior of four mutations of one chromosome locus called  $S_1$ ,  $S_2$ ,  $S_3$ , and  $S_4$ . If self-sterility is controlled by the distribution of genes in one locus only, it is a relatively simple matter genetically; if there are two or more loci involved, things become complicated, whether these loci lie in the same or in different chromosomes. The only evidence that two loci exist comes from the data on the  $F_2$  generation of the original cross, though material from other sources is now being tested, which it is hoped will disentangle the matter.

From a given cross, if but one locus is involved, no more than four intrasterile, interfertile classes can issue. And the size of these classes should be approximately equal. In the original cross described above three sterile matings were found out of 122 cross-matings. If there had been four classes of equal size, and the matings had been at random, 21 per cent of cross-sterility was to have been expected. Disturbance of the equality grouping should result in a rise in the percentage of cross-sterility. In other words, the lowest percentage of cross-sterility to be expected on the assumption that there could not have been more than four intrasterile, interfertile groups is twenty-one except for such slight disturbance as might come about from the fact that certain particular plants (four in number) were mated more often than were the others. The result obtained was approximately 2.5 per cent of cross-sterility. The most probable number of classes to be expected from such a result under the simplest postulates is eight. In addition, it can be shown in two different ways where all the possible matings were made involving certain plants, that six intrasterile groups *must have existed*, unless several errors were made in manipulation or record. This is indirect proof that there are at least two loci controlling the behavior of these self-sterile plants.

Early in the work it was decided to try to simplify genetic analysis by continued inbreeding. This can be done in any manner desired by taking advantage of the phenomenon of pseudo-fertility. Even selfings are usually fruitful if made in the young bud. Should self-sterility (and cross-sterility) be controlled by hereditary factors that segregate and recombine in normal Mendelian fashion, close inbreeding ought sooner or later to result in families where the factorial differences at the basis of self-sterility are reduced to a small number.

Following this method families were soon obtained which were composed of but one or of two classes, each plant within a class being sterile with all other members of that class, and fertile with all members of every other class. A number of two class families characterized by various combinations of the three classes X, Y, and Z have been studied intensively. A class X female mated with a class Y male always gave an approximately equal number of plants belonging to classes Z and Y. A class Y female mated with a class X male, the reciprocal of the above, always gave an approximately equal number of plants belonging to classes X and Z. Other matings gave similar results. Each type of cross produced two classes in nearly equal numbers, and the class of the female parent was never represented.

These odd results are easily interpreted if it is assumed that the active hereditary factors in these matings are  $S_1$ ,  $S_2$ , and  $S_3$ , that class X is  $S_1S_3$ , class Y is  $S_1S_2$ , and class Z is  $S_2S_3$ , and that a plant affords a stimulus to pollen-tube growth only when the pollen grain bears a sterility factor other than its own (7).

When a Z female ( $S_2S_3$ ) is crossed with an X male ( $S_1S_3$ ) only the pollen bearing the factor  $S_1$  is stimulated and functions. The resulting progeny consist therefore of the two classes  $S_1S_2$  (class Y) and  $S_1S_3$  (class X) in equal numbers. In the reciprocal cross, X female ( $S_1S_3$ ) by Z male ( $S_2S_3$ ), the mother plant ( $S_1S_3$ ) again affords stimulus only to gametes bearing factors other than its own,—this time the  $S_2$  pollen. The result is the production of the two classes Y ( $S_1S_2$ ) and Z ( $S_2S_3$ ) in equal numbers.

Practically all possible tests of this hypothesis have now been made and it has been substantiated in every case. By selfing young buds of the heterozygous classes, X, Y, and Z, it has been shown that ordinary Mendelian segregation occurs, and that the anomalous results usually obtained come about only because ordinarily the opportunity for union of like gametes is lacking. When a heterozygous class such as class Y ( $S_1S_2$ ) is selfed in the very young bud, however, equal opportunity is furnished for all possible combinations of gametes. The resulting progeny consist of plants having approximately the ratio 1  $S_1S_1$  plus 2  $S_1S_2$  plus 1  $S_2S_2$ , and these classes show the sterility reactions expected of them. For example,  $S_1S_1$  plants



crossed with  $S_2S_2$  plants give only plants of the one class  $S_1S_2$  no matter which way the cross is made. These  $F_1$  plants are sterile with both the parental types,  $S_1S_1$  and  $S_2S_2$ , when those types are used as males, because neither  $S_1$  pollen nor  $S_2$  pollen functions normally on  $S_1S_2$  plants.  $S_1S_2$  plants will cross with either parent when used as males, on the other hand, because  $S_2$  pollen functions on the  $S_1S_1$  parent and  $S_1$  pollen functions on the  $S_2S_2$  parent. The result in each case is the production of  $S_1S_2$  plants only.

Thus far four members of this allelomorphous series,  $S_1$ ,  $S_2$ ,  $S_3$ , and  $S_4$  have been proved to exist. Further, Brieger and Mangelsdorf (2) have shown that each is linked in inheritance with a certain flower color factor and gives a crossover percentage of about 18. This is the first time that hereditary factors having a typical physiological function have been shown to be linked with factors that are primarily morphological in their effect, and shows how arbitrary and illogical such a classification is. It should also be mentioned that  $S_3$ , when in the homozygous condition, changes the appearance of the whole plant.

Now what physiological conclusions can be drawn from these results? To discuss this question in its simplest form, it is more convenient to eliminate the effect of variable external conditions on pollen-tube growth and to consider only the effect of heredity in a constant environment. One must admit that such variables as light, temperature, and age of plant are effective, and it is to be hoped that later they can be measured precisely; but this is impossible today.

When thus simplified it is clear that self-sterility, and its corollary cross-sterility, is controlled by a limited number of hereditary factors which function in producing differential pollen-tube growth. Self-sterile plants are not specialized individually in this regard, as Darwin and Jost assumed; but they are specialized as groups. The number of groups is unknown. One allelomorphous series containing four members, has been proved definitely. Three other possibly different members of this same allelomorphous series are now being investigated in order to discover whether they are really distinct from the four already isolated. We will not be certain, however, until many other matings have been completed. In addition, our own early experiments and the experience of Darwin and others

wherein an extraordinary amount of fertility was found in crosses made at random, makes it highly probable from a mathematical standpoint that at least one other locus with several allelomorphs exists. Presumably, then, at least two allelomorphic series exist, and the minimum number of different substances affecting pollen-tube growth is six.

The next point to be considered is whether the difference between compatible and incompatible growth-curves is to be interpreted by assuming that *like* factors produce substances that inhibit growth, or by assuming that *unlike* factors produce substances which accelerate growth. The second assumption is more probable. If only one allelomorphic series affecting pollen-tube growth were necessary to account for all data, it would make no difference which interpretation was adopted. With only one active locus, a single mating can give only four intrasterile, interfertile groups. Such a result would be obtained when  $S_1S_2$  plants are mated with  $S_3S_4$  plants. Now it may be that no further complexity need be assumed, since thus far no single mating has given direct proof of more than four of these fertility groups. Yet the earlier experience with material now dead and unusable gives circumstantial evidence of a larger number of groups. Let us postulate a second locus effective in self-sterility and cross-sterility, therefore, which may be called X. If this locus also has four allelomorphs, then a cross between  $S_1X_1S_2X_2$  and  $S_3X_3S_4X_4$  would give sixteen intrasterile, interfertile classes provided a single factorial *difference* gives the compatible type of growth-curve. If a single factorial *similarity* gives the incompatible type of growth-curve, results would be expected which differ from those thus far obtained. In fact unless a genetic difference promotes pollen-tube growth, no greater complexity is possible than that which yields four intrasterile groups. This conclusion is easily seen to be valid when one examines the results with such crosses as class  $S_1S_2$  by class  $S_2S_6$ . If another locus exists which affects pollen-tube growth, both of these groups must be homozygous for it. The complete genetic composition of these classes as regards self-sterility and cross-sterility must be  $X_1X_1S_1S_2$  and  $X_1X_1S_2S_3$ . The weight of the evidence, therefore, is in favor of postulating two series of multiple allelomorphs, though only one has been identified, and of assuming that *any genetic*

*difference* in these factors promotes pollen-tube growth of the compatible type.

A second question of some interest is: How do these pollen-tube growth factors act? There is critical evidence on this point. The presence of compatible pollen-tubes in the same style with incompatible pollen-tubes does not change the latter growth-curve materially. Pollen-tube growth being intercellular, it must be assumed, therefore, that the reaction between the stylar tissue and the compatible pollen-tubes is mutual. There is no accelerating agent as such secreted by the cells of the style, called forth by the presence of the compatible pollen-tubes. If this were the case, the incompatible pollen-tubes might be expected to use it and thus grow more rapidly, for experiments on pollen-tubes grown on artificial media indicate that their actual nourishment is of a simple and rather general nature. The indications, therefore, are that easily diffusible substances pass out of the cells of the style and into the membrane of the pollen-tube where they can be manufactured into the necessary growth substances, if the two kinds essential for the final reaction are complementary.

The light shed by these experiments on the evolutionary side of the question is not very dazzling, yet perhaps it may prove to be a serviceable beacon. One has reasonable grounds for several more or less hypothetical conclusions. It is not known for certain whether the factor FF, present in such self-fertile plants as *Nicotiana Langsdorffii*, and in which a change to ff produces self-sterility, is a mutation at the same locus as the allelomorphous series S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, and S<sub>4</sub>. If this were the case, then the first mutation from FF to ff (equal to S<sub>1</sub>, let us say) would result in a factor that could only be carried along in the heterozygous condition, masked by the fertility factor, and usually under those extraordinary combinations of circumstances which lead to pseudo-fertility. If sterile individuals were formed in rare cases, they would all be cross-sterile with each other, S<sub>1</sub>S<sub>1</sub>, and would either be eliminated by natural selection or again absorbed into the fertile group by mating with gametes carrying the fertility gene. Perhaps this reasoning can be followed more easily if the factor F is called S<sub>f</sub> and its first mutation S<sub>1</sub>. The first combination would be the production of fertile heterozygotes, S<sub>f</sub>S<sub>1</sub>. By analogy with the matings that have been observed, crosses S<sub>f</sub>S<sub>f</sub> × S<sub>f</sub>S<sub>1</sub> and

$S_t S_1 \times S_t S_t$  ought to be possible. The factor  $S_1$  might thus be preserved. But matings  $S_t S_1$  by  $S_t S_1$  might be expected to produce the same results as matings  $S_t S_1$  by  $S_t S_t$ , because  $S_1$  pollen-tubes should grow too slowly to compete with  $S_t$  pollen-tubes. Actual establishment of a self-sterile strain under this postulate, therefore, would necessarily await a second mutation at this locus giving an additional sterility factor  $S_2$ .

What actually happens when the self-fertile species *N. Langsdorffii* is crossed with the self-sterile species *N. Forgetiana* is the production of self-fertile heterozygotes which yield one self-sterile plant to every three self-fertile plants after selfing. Since the factors exhibit no tendency to cause differential pollen-tube growth, one must suppose either that the dominant fertility factor does away with such a tendency, that *N. Langsdorffii* carries other sterility factors, or that the pair of allelomorphs  $F$  and  $f$  are not of the  $S$  series. The latter choice might obtain even if one of the other postulates were true. Experiments are now under way to test these points critically by crossing *N. Langsdorffii* with homozygous  $S_1$ ,  $S_2$ , and  $S_3$  plants. At present there is only one bit of evidence on the subject. Previous experiments have shown that self-steriles extracted from such a cross are of two types showing different reactions. Only one-fourth of the self-steriles, that is to say one-sixteenth of the  $F_2$  population, showed the same degree of self-sterility as was exhibited by the self-sterile grandparent. The remaining three-fourths of the self-steriles appeared to be modified into a decidedly lower grade of self-sterility; and this modifying factor must have been received from the self-fertile grandparent. True, this modifying factor may not be a true self-sterility factor, but it is a factor at a second locus influencing self-sterility. And if one type of self-sterility factor can be carried by a self-fertile plant, it is probable that others can also be carried. The presumption, therefore, is that self-sterility factors can be formed at one or more loci,  $S$  or  $X$ , in plants homozygous for a fertility factor  $F$ , which is wholly independent. If at any time  $F$  mutates to  $f$ , self-sterile strains can become established immediately, for any tendency of the constantly crossed and recrossed self-sterile plants to show heterosis would influence their survival in competition with the self-fertile plants.

It may be objected that I have here drawn rather far-reaching conclusions from experiments covering only three species, and these from a single genus. There is a certain validity to this criticism, but the situation is not quite so hard as it seems. The experiments of Stout (13) on *Cichorium intybus*, of Correns (4) on *Cardamine pratensis*, of Lehmann (11) on *Veronica syriaca* and of Baur (1) on various Spanish *Antirrhinums* show a regularity of the *type* which this hypothesis demands. The species used by Stout and by Correns are so pseudo-fertile that a critical reanalysis of their results is impossible, but no data were obtained that are directly opposed to the demands. Lehmann, who had better material, did not make the matings needed to determine the points at issue. Baur, on the other hand, made the crosses essential for an evaluation according to the scheme described here, using a species he called *Antirrhinum hispanicum*. (In a recent letter, he calls this material *A. Segovii* N.S.) He obtained two intra-sterile, interfertile groups as the result of crossing two plants E II and E V. Yet as he did not give the exact parentage of the members of the  $F_1$  generation in his published paper, his work could not be reanalyzed. E II was fertile with both classes of the progeny. E V was sterile with all of the plants of one of the classes. If then E II were the female parent belonging to class  $S_1S_2$  and E V were the male parent belonging to class  $S_1S_3$  (his class "a") then the cross should have produced equal numbers of  $S_2S_3$  (a new class fertile with both parents) and of  $S_1S_3$  (his class "a," the assumed class of the male parent and sterile with it). What he actually obtained was 13 plants of class "a" ( $S_1S_3$ ), the same as the assumed class of the male parent; and 13 plants of class "b", a class different from either parent. In other words, if his cross were E II female by E V male, the results corroborate the hypothesis described in this paper; if E V were the female and E II the male in the cross, the results are antagonistic to the hypothesis. In his letter of February 19, 1926, Professor Baur states that the cross was E II female by E V male. Thus there is complete corroboration of the hypothesis on plants belonging to the family *Scrophulariaceae*, a family which, while closely related to the family *Solanaceae*, is sufficiently removed from it to make it improbable that the same mechanism would control self-sterility in both cases if it were not a rather general type.

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