STUDIES ON SELF-STERILITY I. THE BEHAVIOR OF SELF-STERILE PLANTS

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INTRODUCTION

The occurrence of self-sterility

Among both hermaphroditic animals and plants forms are known in which fertilization of the eggs by sperm or by pollen of the same individual is difficult or even impossible. This condition is known as self-sterility,[1] although the term is not a happy one, since both the male and the female gametes are morphologically perfect and are functional with the complemental gametes of other individuals.

Self-sterility is probably a widespread phenomenon though its presence has been proved experimentally in comparatively few plants and in only one animal. The result, one might even say the aim, of self-sterility, however, is cross-fertilization. Regarded from this standpoint, it is to be classed with the various other specializations of animals and plants, such as morphological differences in the accessory sexual organs, dichogamy, monœcism, diœcism, etc., which tend toward the same end; and since these obvious contrivances for cross-fertilization are so numerous and so dispread, it is difficult to believe that the less easily detected self-sterility is rare, particularly as it has arisen independently in widely separated groups.

The important rôle played by cross-fertilization in the evolution of animals and plants may be attributed in some degree, therefore, to the phenomenon of self-sterility; hence, any light thrown upon its meaning is a contribution toward an explanation of the significance of cross-fertilization in general.

Among animals only *Ciona intestinalis* has been proved to be self-sterile (CASTLE 1896), though the condition is suspected in several other forms.

Among Angiosperms self-sterility is rather generally distributed. KNUTH (1898, Vol. I, pp. 42-45) gives a list of 134 self-sterile species representing 46 families and including both monocotyledons and dicotyledons. This list is the best compilation of recorded cases and may be considered fairly complete to-day as very few additional records have

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[1] The words self-incompatibility and self-impotence have been substituted for self-sterility by various writers. These terms seem to us to be neither more nor less objectionable than self-sterility, since neither takes into consideration the fact that the same type of infertility may exist between different individuals. The important point in the matter is that one should not confuse the phenomenon with any of those types of true sterility where there is either complete or partial incapacity for the production of gametes functional *per se*. For a discussion of the differences between self-sterility and true sterility see KRAUS (1915) and STOUT (1916).

appeared in subsequent publications. It is naturally somewhat inaccurate, inasmuch as several cases are recorded in which cross-pollination was merely prevented by bagging the inflorescence or by isolating the plants and self-pollination not insured. Nevertheless, at least 70 percent of the records are properly proved cases of a self-sterility that is something more than an ephemeral condition due to environmental changes or to a fleeting period of reproductive inactivity that is normal in the life history of so many plants.

There remain, then, in the neighborhood of 100 well endorsed instances of self-sterility scattered over some 35 families. These families are so different in their modes of reproduction that no general conclusion can be drawn regarding the development of self-sterility. There are legumes which are usually self-fertilized, and orchids that have developed quite wonderful floral mechanisms favoring cross-fertilization; there are showy flowers, and flowers peculiarly inconspicuous; there are flowers with perfume, and flowers without it; there are anemophilous plants, and plants that would be classed as strictly entomophilous. In certain genera, such as Passiflora, there is a general tendency toward self-sterility; in other genera, for example Verbascum and Nicotiana, closely related species behave very differently.

In other words self-sterility has arisen many times, and often in groups where there was apparently no need for it if the necessity is assumed to be that of cross-fertilization. Not only is this an irresistible argument in favor of the idea already expressed that only a small fraction of the cases of self-sterility have been discovered and that self-sterility has been a much more important factor in plant evolution than has previously been suspected, but it also indicates that certain of the mechanical devices that have received great credit for promoting cross-fertilization were inadequate for the needs of many plants.

EARLY WORK ON SELF-STERILITY

The discovery of self-sterility in plants probably should be credited to KÖLREUTER, the first[2] real student of hybridization, although his case is somewhat doubtful. KÖLREUTER (1764) found that during two years three plants of *Verbascum phœniceum* set no seed with their own apparently good pollen, although they seeded readily with pollen of *V. Blat-*

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[2] THOS. FAIRCHILD crossed *Dianthus caryophyllus* with *D. barbatus* in 1719, and LINNEUS brought his hybrid between *Tragopogon pratensis* and *T. porrifolius* into flower in 1759, but neither of them contributed to the world any important facts regarding hybridization.

taria, V. nigrum, V. phlomoides and *V. Lychnitis*. Later these plants showed sporadic fertility alternating with sterility of pollen or of eggs or of both sex-cells, so that this instance may be only one of induced true sterility due to conditions. It seems to deserve priority as an instance of self-sterility, however, for DARWIN (1872, p. 341) found *V. phœniceum* and *V. nigrum* to be self-sterile, although the related species *V. Thapsus* and *V. Lychnitis* were self-fertile.

SPRENGEL (1793), the other important hybridist of the 18th century does not mention the subject.

Several true instances of self-sterility were discovered by HERBERT (1837) in his experiments with the Amaryllidaceæ. He says:

> "Nine very fine crosses of Hippeastrum were flowering [there] at the same time; one a natural seedling from *Johnsoni* or *Regio-vittatum*, two, *Johnsoni-pulverulentum*, one *Johnsoni-vittatum*, one *psittacino-Johnsoni* crossed again by *vittato-Johnsoni*, one from *Johnsoni* by *solandriflorum*, and two from *vittato-Johnsoni* by the same. Being desirous of blending again these plants which were all cross-bred, different flowers were touched with pollen from their several neighbors and ticketed, and other flowers were touched with their own pollen. Almost every flower that was touched with pollen from another cross produced seed abundantly, and those which were touched with their own either failed entirely or formed slowly a pod of inferior size with fewer seeds, the cross impregnation decidedly taking the lead."
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> "It is only from the superior efficacy of the pollen of another plant that we can account for the circumstances of some hybrid plants, which breed freely with plants of either parental stock and fecundate them, not producing seed readily when left to themselves; for if their pollen is able to fertilize and their ovary to be fertilized, there can be no positive sterility in the plant, though there may be a want of sufficient energy under certain, or perhaps under ordinary, circumstances."

These observations of HERBERT referred to hybrids, though he also found self-sterility in the species *Zephyranthes carinata*, and DARWIN in discussing them very properly sets them apart from the cases of self-sterility in pure species. We shall show later, however, that absolute self-sterility exists both in pure species and in hybrids, and is one and the same phenomenon. In fact HERBERT himself very nearly demonstrated this. In a letter to DARWIN (1875) written in 1839, he states that after a duplication of these experiments with like results, he was led to make similar trials on a pure species. He selected a plant of *Hippeastrum aulicum* which he had recently imported from Brazil. Three of its flowers he selfed without result; a fourth flower he crossed with pollen of a triple cross between *H. bulbulosum*,[3] *reginæ* and *vittatum* and obtained good seed.

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[3] Probably *H. rutilum* Herb.

Later work cited by DARWIN (1875) also supports this idea. BIDWELL in New South Wales found *Amaryllis belladonna* to be partially self-sterile, though fertile to the pollen of other species. E. BERNET, of Antibes, a man having a wide experience in crossing species of Cistus, found that their hybrids when fertile (he does not mention the pure species) were completely self-impotent. His statement is that, quoting Darwin, "the flowers are always sterile when the pistil is fertilised by pollen taken from the same flower or from flowers on the same plant." "But," he says—without the italics—"*they are often fertile if pollen be employed from a distinct individual of the same hybrid nature, or from a hybrid made by a reciprocal cross.*" A. RAWSON, a well known English horticulturist, found the same absolute self-sterility in various named varieties of Gladiolus that were said to have descended from Gandavensis, an old race produced by crossing *G. natalensis* by *G. oppositiflorus.* The interesting point in RAWSON's work was that none of the plants of the same variety would set seed when interpollinated. As each variety had been propagated asexually by bulbs, he was of course actually dealing with plants of the same germinal constitution, though under somewhat different environmental conditions. For this reason it is extremely improbable that these were cases of induced true sterility.

> "Altogether, Mr. RAWSON, in the year 1861 fertilised twenty-six flowers borne by four varieties with pollen taken from other varieties, and every single flower produced a fine seed capsule; whereas fifty-two flowers on the same plants, fertilised at the same time with their own pollen [which had been proved to be good by the crosses], did not yield a single seed capsule."

Returning to the phenomenon as exhibited in pure species, WM. MOWBRAY, gardener of the Earl of Mountnorris, in a letter to the Secretary of the HORTICULTURAL SOCIETY (England), dated October 29, 1830, states that he could get fruit only from *Passiflora alata* and *P. racemosa* by reciprocal fertilization.

Observations on self-sterility in this genus continued to be made later by a number of observers. The most important work was done by ROBERTSON MUNRO (1868). MUNRO found *P. alata, P. racemosa, P. cœrulea, P. Bellottii, P. kermesina, P. holosericea* and *P. fulgens* to be self-sterile, while DARWIN obtained evidence that *P. laurifolia* and *P. quadrangularis* were in the same condition. The evidence of perfect self-sterility in the first three species is incontrovertible, in the remaining species it is highly probable.

Some of the details from MUNRO's work are exceedingly interesting.

In the first place he found plants of *P. alata* to be highly fertile with their own progeny as the following quotation shows.

> "I impregnated a considerable number of these flowers with their own pollen, everyone of which proved abortive. But on impregnating eighteen flowers on the mother plant with pollen from her own self-impotent seedlings, I got eighteen fine plump ovaries full of seed."

Again, MUNRO found that self-sterile plants were sometimes cross-fertile and sometimes cross-sterile with plants of the same species and presumably of the same generation. For example, three self-sterile plants of *P. cœrulea* all produced seeds with pollen from one other plant. The same experiment on *P. alata* showed cross-sterility in two instances and cross-fertility in one instance.

A curious case of a return to self-fertility in *P. alata* through grafting was also reported by MUNRO. Mr. DONALDSON, gardener at Keith Hall, grafted a self-sterile plant upon stock of an unknown species. Though its pollen still refused to fertilize certain other plants of the same species, it was markedly self-fertile and fertile with at least one other plant. Seedlings from this plant were all self-sterile but were fertile with the mother plant.[4]

GÄRTNER (1849), who was among the most reliable of the early hybridizers, found a number of self-sterile species. *Dianthus japonicus* was sterile both with its own pollen and with the pollen of *D. barbatus*. Two plants of *Lobelia fulgens* likewise proved self-sterile. Their pollen was good on *L. cardinalis* and *L. syphilitica,* their ovules could be fertilized by the pollen of these species, but self-pollination yielded nothing. A plant of *Verbascum nigrum* was also completely self-impotent though fertile as a male with *V. Lychnitis* and *V. austriacum* and fertile as a female with *V. Thapsus.*

Similar conditions in certain exotic orchids were reported by SCOTT (abstract 1863, complete paper 1865). A duplicate of a table in his paper and a summary of his conclusions follow.

SCOTT and MUNRO (DARWIN 1875) each independently found *Oncidium sphacelatum* also to be wholly self-sterile after some three hundred attempts at self-pollination, though the species was fertile reciprocally with other Oncidiums. MUNRO in addition confirmed SCOTT's observations on *O. divaricatum* and added *O. flexuosum* to the list of self-sterile plants.

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[4] It is likely that this phenomenon is similar to the pseudo self-fertility due to conditions, which is discussed later in this paper.

Unions between *Oncidium microchilum, O. divaricatum* var. *cupreum* and *O. ornithorhynchum.*

	Number of flowers fertilized	Total number of capsules produced	Number of good capsules	Estimated number of seeds	Estimated number of good seeds	By calculation	
						Total seeds	Good seeds
1. *O. ornith.* × *O. micro.* (No. 2)	8	3	3	20200	4242	or as 1000 to 210	
2. *O. micro.* (No. 2) × *O. ornith.*	12	0					
3. *O. ornith.* × *O. micro.* (No. 1)	8	5	4	23360	3737	or as 1000 to 160	
4. *O. micro.* (No. 1) × *O. ornith.*	12	2	0				
5. *O. divar. cup.* × *O. micro.* (No. 2)	6	3	3	22050	7938	or as 1000 to 360	
6. *O. micro.* (No. 2) × *O. divar. cup.*	18	2	0				
7. *O. divar. cup.* × *O. micro.* (No. 1)	6	4	4	26240	8922	or as 1000 to 340	
8. *O. micro.* (No. 1) × *O. divar. cup.*	6	2	2	17700	1434	or as 1000 to 420	
9. *O. micro.* (No. 1) × *O. micro.* (No. 2)	6	5	4	45800	34350	or as 1000 to 750	
10. *O. micro.* (No. 2) × *O. micro.* (No. 1)	18	0					
11. *O. micro.* (No. 1) × own pollen	24	1					
12. *O. micro.* (No. 2) × own pollen	24	0					

"By a summary comparison of these results we have the following highly interesting facts disclosed. First, we see that the male element of *O. microchilum* (No. 1) will fertilise the female element of the two distinct species *O. ornithorhynchum* and *O. divaricatum* var. *cupreum* and yet be completely impotent upon its own female element; nevertheless the susceptibility of the latter (female element) to fertilisation is shown by its fertile unions with another individual of the same species, and likewise by a fertile union with an individual of a distinct species, namely *O. divaricatum* var. *cupreum.* Secondly, the male element of *O. microchilum* (No. 2) will fertilise the female element of *O. ornithorhynchum* and *O. divaricatum* var. *cupreum,* and likewise another individual of its own species, though on its own female element it is utterly ineffective."

These observations, together with similar ones on *O. Cavendishianum* recorded by LECOQ (1862) from the experiences of RIVIÈRE were made on hot-house plants and DARWIN originally attributed their self-sterility to the peculiar conditions under which they were grown. He was forced to modify his conclusions, however, through information received from FRITZ MÜLLER. The latter self-fertilized over one hundred flowers of *Oncidium flexuosum* at Desterro, Brazil, where it is native, without obtaining a single seed, but he did discover the important fact[5] that each plant *was fertile with the pollen from any other plant.*

SCOTT and MÜLLER each independently made the further discovery that the tissue of the style of the self-sterile plants was penetrated freely by the pollen tubes after selfing, though fertilization did not subsequently occur.

As DARWIN noted:

> "Another observation made by FRITZ MÜLLER is highly remarkable, namely that with various orchids the plant's own pollen not only fails to impregnate the flower, but acts on the stigma, and is acted on, in an injurious or poisonous manner."

We have not been able to find any confirmation of these results, and it seems entirely probable that the apparently poisonous action of the pollen after an "illegitimate" pollination, might have been due to the action of bacteria or fungi, since the work was done under tropical conditions. But the facts are so exceptional that we give DARWIN'S (1875, vol. 2, p. 112) account.

> "FRITZ MÜLLER observed the poisonous action of the plant's own pollen in the above mentioned *Oncidium flexuosum, O. unicorne, pubes* (?), and in two unnamed species. Also in two species of Rodriguezia, in two of Notylia, in one of Burlingtonia, and of a fourth genus in the same group. In all these cases, except the last, it was proved that the flowers were, as might have been expected, fertile with the pollen from a distinct plant of the same species. Numerous flowers of one species of Notylia were fertilised with pollen from the same raceme; in two day's time they all withered, the germens began to shrink, the pollen masses became dark brown, and not one pollen grain emitted a tube. So that in this orchid the injurious action of the plant's own pollen is more rapid than with *Oncidium flexuosum* Eight other flowers on the same raceme were fertilised with pollen from a distinct plant of the same species; two of these were dissected and their stigmas were found to be penetrated with numberless pollen tubes; and the germens of the other six flowers became well developed. On a subsequent occasion many other flowers were fertilised with their own pollen, and all fell off dead in a few days; whilst some flowers on the same raceme which had been left simply unfertilised adhered and long remained fresh. We

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[5] It is probable that cross-sterility existed, but was not discovered.

have seen that in cross unions between extremely distinct orchids the pollen long remains undecayed; but Notylia behaved in this respect differently; for when its pollen was placed on the stigma of *Oncidium flexuosum,* both the stigma and pollen quickly became dark brown, in the same manner as if the plant's own pollen had been applied."

MÜLLER suggests an explanation of this phenomenon which must be pleasing to the minds of strict Natural Selectionists. He believes it to be an advantage to the species to have its pollen positively deleterious rather than simply neutral, because the flowers would then soon drop off, and the energies of plants no longer be directed toward nourishing a part which would not finally function.

Another quotation from DARWIN (*ibid.*, p. 113) is interesting both for the facts contained and for the deductions of MÜLLER.

> "The same naturalist found in Brazil three plants of a Bignonia growing near together. He fertilised twenty-nine flowerets on one of them with their own pollen, and they did not set a single capsule. Thirty flowers were then fertilised with pollen from a distinct plant, one of the three, and they yielded only two capsules. Lastly, five flowers were fertilised with pollen from a fourth plant growing at a distance, and all five produced capsules. FRITZ MÜLLER thinks that the three plants which grew near one another were probably seedlings from the same parent and that from being so closely related, they acted very feebly on one another. This view is extremely probable for he has since shown in a remarkable paper (MÜLLER 1873) that in some Brazilian species of Abutilon, which are self-sterile, and between which he has raised some complex hybrids, that these, if near relatives were much less fertile *inter se,* than when not closely related."

This work of MÜLLER (1873) consisted in noting the fertility of various matings of 8 species of Abutilon that he denotes by the letters A, C, E, F, M, P, S and V, the individual plants being distinguished by subscripts. Thus the plants $EF.F_1$ and $EF.F_2$ are similar combinations formed by crossing species E with species F and crossing the first generation hybrids thus formed with F_1 and F_2. The principal results were as follows:

Number of flowers	Mother plant	Source of pollen	Number of fruits	Average No. of seeds
9	$F.EF_1$	Others of same stock		
20	$F.EF_1$	$F.EF_2$, $EF.F_1$ and $EF.F_2$	3	1.3
10	F.EF	FE and FE_2	10	4.5
11	F.EF	EF_2 and EF_3	10	4.6
10	F.EF	F.	9	4.6
6	F.EF	$F.CF_1$ and $F.CF_2$	6	4.5
1	F.EF	FS_1	1	4.7

He says that the results following the intercrossing of sister plants were not due to bad pollen, as on other plants it was completely potent; the pollen of F.EF$_2$ producing fruit full of seeds on FS$_1$, that of EF.F on FE$_2$, that of EF.F$_2$ on F, and that of F.EF$_1$ on F, F.CF$_2$, FS$_1$ and FS$_2$. In explaining the phenomenon he follows DARWIN in supposing inbreeding to be the cause.

Most of these observations and investigations were known to DARWIN who not only published historical accounts in the "Origin of species" and "Variation of plants and animals under domestication," but between 1860 and 1880 carried out numerous experiments on the subject which were reported in a series of papers in the JOURNAL OF THE LINNEAN SOCIETY and other places and were brought together in the three classics, "On the various contrivances by which British and foreign orchids are fertilised by insects" (1862), "The effects of cross- and self-fertilisation in the vegetable kingdom" (1876), and the "Different forms of flowers on plants of the same species" (1877).

DARWIN'S investigations on fertilization in the orchids are only remotely related to the subject in hand, but his experiments on heterostyled dimorphic forms are, we believe, concerned with an analogous phenomenon. The "illegitimate" unions according to DARWIN include certain matings other than self-pollination, but the greatly decreased fertility after self-pollination in practically all of these species as well as the absolute self-sterility of so many forms indicate that the condition is one like ordinary self-sterility though complicated by a linkage with style length and with pollen size. The work of BATESON and GREGORY (1905) on the inheritance of heterostylism in Primula has done something toward clearing up these relationships, but much remains for the future. As these investigations of DARWIN are readily available and cannot, at present, add materially to our discussion of self-sterility on account of moot points, they will not be described further; but we shall abstract from the experiments on those plants usually considered to be genuinely self-sterile.

DARWIN (1876) investigated rather thoroughly the conditions in five self-sterile species, *Eschscholtzia californica, Abutilon Darwinii, Senecio cruentus, Reseda odorata* and *R. lutea.*

A plant of *Eschscholtzia californica* had been accidentally found to be self-sterile by FRITZ MÜLLER (1868, 1869) while working in southern Brazil. This induced him to investigate its behavior through six generations, during which time he found all of the plants to be completely self-sterile though fertile between themselves. As DARWIN had found

English plants comparatively self-fertile and as HILDEBRAND had discovered no complete self-sterility in plants grown in Germany, he obtained from MÜLLER seed of the Brazilian plants known to be self-sterile and from them raised seedlings. These while not wholly self-fertile, tended toward fertility, which fact DARWIN attributed to the lower English temperature. A second generation of seedlings proved to be still more self-fertile. Conversely, seed of English stock sent to Brazil proved to be more self-fertile than the native race, though one plant thus exposed to the climate of Brazil for two seasons, was wholly self-sterile.

These results were paralleled by the behavior of *Abutilon Darwinii* which is self-sterile in its native Brazil, but became moderately self-fertile *late in the first flowering season* in DARWIN'S greenhouse.[6]

DARWIN made no extensive experiments on self-sterility with Brazilian plants in collaboration, so to speak, with FRITZ MÜLLER; but this was not for the lack of material, for in a letter to FOCKE (1893), MÜLLER says the number of self-sterile species of plants in Brazil is very large, and that different species of the same genus often behave differently in regard to self-pollination. He observes that self-sterility is often associated with unusual vegetative vigor and that species of Oxalis having trimorphic flowers which are all self-sterile make unusually vigorous growths. This condition observed by MÜLLER is doubtless merely another example of the hybrid vigor or heterosis so common among both plants and animals, and shows the reason, of course, why self-sterility has been maintained by natural selection.

DARWIN'S experiments on *Senecio cruentus* are noteworthy only because the varieties used were descendants of garden hybrids.

Two plants of a purple-flowered and one plant of a red-flowered variety were found to be self-sterile and cross-fertile.

The experiments with *Reseda odorata* were more detailed. Those of 1868 are shown in tabular form, the letters representing individuals and the subscripts pollinations. As may be seen, the seven plants used were absolutely self-sterile. The number of pollinations made allow us no doubts about the matter, F and G being selfed many times as well as the others, though in these two cases no figures were reported. Sixteen cross-matings, on the other hand, were all fertile.

In the spring of 1869, four other plants were raised from fresh seed and isolated under nets. Three of these proved to be wholly self-fertile, while the fourth was not completely self-sterile.

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[6] Cf. our results on flowers late in the season.

DARWIN'S experiments on *Reseda odorata* in 1868.

Male parents

Female parents	A	B	C	D	E	F	G
A	S_{16}	F	F				
B	F	S_{18}	F	F			
C	F	F	S_{19}	F	F		
D	F	F	F	S_{18}	F		
E	F		F	F	S_8		
F						S	
G							S

Much surprised at these divergent results DARWIN raised six more plants in 1870. Of these, two were almost self-sterile and four were completely self-fertile. The former produced altogether five seeds, which were grown the following year. These plants made a luxuriant growth, but were almost completely self-sterile like their parents [an indication of pseudo-fertility]. The progeny of the self-fertile plants was not followed.

These varying results were attributed by DARWIN to a difference in inherited sexual constitution. He says in his general conclusions (1876, p. 346):

> "Finally, the most interesting point in regard to self-sterile plants is the evidence which they afford of the advantage, or rather of the necessity, of some degree or kind of differentiation in the sexual elements, in order that they should unite and give birth to a new being. It was ascertained that the five plants of *Reseda odorata* which were selected by chance, could be perfectly fertilised by pollen taken from any one of them, but not by their own pollen; and a few additional trials were made with some other individuals, which I have not thought worth recording. So again, HILDEBRAND and FRITZ MÜLLER frequently speak of self-sterile plants being fertile with the pollen of any other individual; and if there had been any exceptions to the rule, these could hardly have escaped their observation and my own. 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. Now it is obviously impossible that the

sexual organs and elements of every individual can have been specialised with respect to every other individual. But there is no difficulty in believing that the sexual elements of each differ slightly in the same diversified manner as do their external characters; and it has often been remarked that no two individuals are absolutely alike. Therefore we can hardly avoid the conclusion, that differences of an analogous and indefinite nature in the reproductive system are sufficient to excite the mutual action of the sexual elements and that unless there be such differentiation fertility fails."

These inductions are cleverly drawn and clearly expressed, but they are not all justified by the data in DARWIN'S possession. The matings between self-sterile plants made by HILDEBRAND, MÜLLER and DARWIN were neither individually nor collectively sufficient to establish the point that "a self-sterile plant can be fertilized by the pollen of any one out of a thousand or ten thousand individuals of the same species," and it is upon this supposition that the generalization is based. Further, MUNRO, whose work was known to DARWIN, had found cross-sterility in Passiflora.

As it is not proposed to make this review a check list of species which are, as a whole or in part, self-sterile, but rather to set forth the known facts concerning the behavior of self-sterile plants and to outline the various theories that have been suggested to interpret the phenomenon, we shall pass DARWIN'S conclusions without further comment. His work properly stands as the outpost of advance in the subject until the rediscovery of Mendel's Law in 1900. The method of analysis of pedigree cultures foreshadowed by VILMORIN but really initiated by MENDEL has made a methodological revolution. It seems fitting, however, to close this part of our paper with the work of a botanist who, though making no outstanding contributions to the subject, was a contemporary of and an aid to DARWIN, and who from the chronological standpoint links the work of DARWIN to that of the present day.

HILDEBRAND worked and wrote indefatigably upon questions of fecundation in plants from 1863 until 1908. His first paper (1863), on dimorphism in *Primula sinensis* appeared almost simultaneously with that of DARWIN, and since that time in the neighborhood of seventy contributions on similar subjects have appeared under his name.

HILDEBRAND (1866) published some rather extensive experiments with *Corydalis cava* in which he showed that the plants were absolutely self-sterile although both pollen and ovules were functional. But his investigations were noteworthy with respect to the large number of species in which he established a high probability of self-sterility, rather than for any fundamental researches on the genetic problem concerned. We will mention only one other paper, therefore, merely to show the large numbers of self-sterile plants that are sometimes (possibly often) to be found in a single family when said family is even partially investigated.

In 1896 he published on the Cruciferæ and found *Hesperis tristis, Lobularia maritima* (=*Alyssum maritimum* Lam.), *Cardamine pratensis, Rapistrum rugosum, Iberis pinnata* and *Sobolewskia clavata* fully self-sterile, *Aethionema grandiflorum* and *Hugueninia tanacetifolia* (=*Nasturtium tanacetifolium* Hook.) nearly self-sterile, and only *Draba verna* and *Brassica rapa* fully self-fertile.

RECENT WORK ON SELF-STERILITY

The work of the last decade on self-sterility has been less concerned with the discovery of new cases than with an interpretation of the phenomenon in keeping with modern biological thought. Several noteworthy investigations on both plants and animals have appeared.

JOST (1907) repeated HILDEBRAND'S experiments on *Corydalis cava,* and unlike the latter, observed a small percentage of self-fertility. In his experiments 93 selfed plants yielded 6 capsules, whereas 42 crossed plants produced 30 capsules. Self-sterility was also noted in *Secale cereale* (a variety *montanum*) and *Lilium bulbiferum.* The immediate cause of the different behavior of these plants after self-pollination and after cross-pollination was found to be the difference in rate of pollen-tube growth. In Secale, pollen tubes were found to have penetrated the micropyle in about eight hours after cross-pollination, although after self-pollination the tubes had merely reached the base of the pistil after twenty-four hours. Pollen tubes also appeared to grow somewhat faster than after self-pollination when crosses (?) were made between flowers on the same plant, but in view of the fact that asexually propagated plants from a single seed appear to behave very similarly this observation may not be correct. In this connection it should be mentioned that FOCKE (1890 and 1893) found that *Lilium bulbiferum* plants of the same clonal variety were completely cross-sterile, although sister seedlings were cross-fertile. Similar observations on asexually propagated pome fruits have been made by WAITE (1895) and LEWIS and VINCENT (1909), but in these cases "fruitfulness" rather than "fertility" was noted.

To explain his results JOST had recourse to the old concept of "Individualstoffe." He believes that individuals not only of the same species but of the same family differ qualitatively in their chemical composition, that the gametes of any plant possess the "Individualstoff" of that plant, and that pollen tubes grow well only in tissues having a different "Individualstoff."

In 1912 a very important paper by CORRENS appeared in which a Mendelian interpretation of results was proposed. His experimental work began with a hybrid between *Petunia nyctaginiflora* and *Petunia violacea* that had been produced in 1901, and of which 11 individuals had passed through the winter. Six of these plants were found to be self-fertile, three completely self-sterile and two nearly self-sterile. Among the self-sterile plants certain combinations proved easy to make, while others were impossible. It was sometimes impossible even to cross the self-sterile with the self-fertile plants [probably pseudo-fertile]. For several reasons, however, CORRENS found Petunia unsatisfactory and the work was dropped until 1910; it was then recommenced with *Cardamine pratensis,* a Crucifer that had been shown to be wholly self-sterile by HILDEBRAND (1896).

Concerning the "cause" of self-sterility, borrowing the term from the author, he gives the following facts: The pollen grains germinated on the stigma of the self-pollinated flowers, but produced only short tubes that did not penetrate the tissues of the stigmas, while after cross-pollination the pollen tubes were found in the upper part of the ovaries after only 48 hours.

The pedigree culture investigations began with two plants, B having very light lilac flowers, and G having flowers of a more intense lilac. These plants were crossed reciprocally, the combination B♀ × G♂ being designated No. 1 and the other No. 2. From each of these matings, 30 plants were raised, and formed the basis of the remaining experiments. They were numbered 1a, 1b, 1c, - - - - 2a, 2b, 2c, etc.

These plants were first tested for their fertility when used as females by crossing each individual with the pollen of two unrelated plants from Lake Zürich and Schwabia respectively. These pollinations were successful without an exception, proving that pollen from a single plant could fertilize each of the 60 F_1 sibs.

From 3 to 15 pollinations were then made upon every F_1 plant with the pollen of each parent B and G. About half of these pollinations were uniformly fruitful or uniformly unfruitful as the case might be, but the other half showed variations in behavior that made classification of the results difficult. For example out of ten pollinations of plant 10 with the pollen of B, 6 were successful and 4 unsuccessful. This plant was classed as fertile with B. Again, plant 1k pollinated 7 times with the pollen of G yielded 3 good capsules, 2 poor capsules and 2 failures CORRENS classes this plant as sterile with G with a question mark. These results seem at first sight to indicate a definitely graduated fertility in Cardamine. This is not impossible; but, arguing from our own experience (*Nicotiana alata*), it appears to be more probable that the plant is in a rather unstable condition physiologically and can be influenced easily by external conditions.

CORRENS did endeavor to test the question of the influence of age of plant on fertility by (1) making 17 duplicate pollinations the next year with pollen from a plant raised from a cutting of B, and by (2) making 18 reciprocal pollinations from the F_1 plants upon B and G. The pollinations with pollen from the cutting of B made in 1912 checked with those made in 1911 with pollen from the original plant B in a remarkable manner. Of the reciprocals, 7 were successful both ways, 5 failed both ways, 4 were rather indefinite but similar, while only one showed a conflicting result (2 failures one way and 3 successes the other).

In spite of these facts, however, it is apparent from CORRENS'S account that the plants were at all times kept in as fine condition as possible so that the behavior under a poor environment or during different phases of the flowering period was really not determined. What these experiments did do was to prove beyond a reasonable doubt the physiological similarity of cuttings with respect to cross-fertility and cross-sterility, and to indicate that reciprocal crosses always behave in the same manner. Unfortunately for the latter thesis, however, there are a few conflicting results in his table 8, though this he does not mention. Of the 53 reciprocals recorded there, 31 give the same results, 17 give different results, while 5 are questionable.

CORRENS concluded that the behavior of the F_1 individuals with the pollen of the parents was such as to indicate equal-sized classes of definitely fertile or definitely infertile plants, the behavior of the reciprocals being the same. His classification gave the following groups:—fertile with B, 32; sterile with B, 28; fertile with G, 30; and sterile with G, 30.

He further concluded that the action of an F_1 individual toward one parent was wholly independent of its action toward the other, and that the population could be divided into 4 classes with reference to the behavior of the individuals toward both parents, as follows:

Fertile with both B and G, type bg,	16 plants
Fertile with B, sterile with G, type bG,	16 plants
Fertile with G, sterile with B, type Bg,	14 plants
Sterile with both B and G, type BG,	14 plants

An explanation of these facts was sought by assuming that each parent B and G carried at least one transmissible factor, *B* and *G* respectively, which actively inhibited pollen-tube growth, besides at least one inactive factor, *b* and *g* respectively. The formulae for these plants would then be *Bb* and *Gg,* and when they are crossed four equal-sized classes of zygotes will be formed *BG, Bg, bG* and *bg,* because *B* and *b,* and *G* and *g* segregate at reduction. These four F_1 classes should behave when backcrossed with each parent in the manner shown above.

There seems to be no reason in his hypothesis why plants of the type *bg* should not be self-fertile though this is not the case. In fact all of the 60 F_1 plants are assumed to be self-sterile although two cases showing some self-fertility (probably pseudo-fertility) are shown in table 8c. But this discrepancy is probably due to an imperfect description of the hypothesis by the author, as the relation between self-fertile and self-sterile plants is evidently meant to be left out of consideration.

The intra-class and inter-class pollinations between the F_1 plants of which he made about 700 (tables 8a-8d), hardly come up to expectations, but there is a regularity that cannot be overlooked.

COMPTON (1913 a) confirmed DARWIN's report that both self-fertile and self-sterile plants occur in the mignonette, *Reseda odorata.* From experiments on crossing these two races he obtained the following facts:

(1) Self-sterile plants when bred *inter se* threw self-sterile offspring only. This was thought to indicate that self-sterility is a Mendelian recessive. (2) Certain self-fertile plants, when self-fertilized gave self-fertile offspring only. When crossed with self-sterile plants the same result was obtained. These plants COMPTON regarded as homozygous dominants. (3) Other self-fertile plants, when self-fertilized, gave approximately 3 self-fertile to 1 self-sterile offspring. The same plants crossed with self-sterile individuals produced about one-half self-fertile and one-half self-sterile progeny. These he regarded as heterozygous. All of these facts are satisfactorily interpreted by the hypothesis that self-fertility is a simple dominant to self-sterility.

In a later paper COMPTON (1912) suggests, as JOST had previously done, the presence in the pistil of diffusible substances which stimulate or retard pollen-tube growth after cross- or self-pollination respectively.

The growth of pollen tubes in the style and the growth of fungus hyphae in a host appealed to Compton as analogous, and he suggests that self-sterility may be due to agents similar to those which govern immunity or susceptibility in animal or plant.

These results confirm a Mendelian hypothesis already suggested by Baur (1911) without reporting detailed results. He crossed the self-sterile *Antirrhinum molle* with the self-fertile *A. majus* and obtained only self-fertile offspring. The F_2 generation consisted of both self-fertile and self-sterile plants, the former being in the majority. Baur gave these hybrids to Lotsy (1913) who raised a large F_2 generation with similar results although he was inclined to believe that the plants showed variable degrees of self-fertility and self-sterility. Neither Compton, Baur nor Lotsy touched the question of the behavior of self-sterile plants among themselves.

Since self-sterility was discovered in the Ascidian *Ciona intestinalis* by Castle (1896), its reproductive behavior has been studied by Morgan (1905, 1910), Morgan and Adkins (Morgan 1913), and Fuchs (1914a). Morgan and Adkins showed that these animals vary in degree of self-sterility. Perfectly self-sterile individuals were the exception, but self-fertility never equaled cross-fertility. Individuals also varied in the ease with which their eggs might be fertilized by the sperm of other individuals. The following matings were made with the results noted in percentage of eggs fertilized:

♂ ♀	A	B	C	D	E
A	0	87	92	84	96
B	38	0	35	98	97
C	93	96	0	97	96
D	91	98	77	0	89
E	96	92	60	74	0

FUCHS (1914 a), however, has criticized MORGAN'S work, maintaining that 100 percent of segmenting eggs can be obtained in every cross with normal ova if sufficiently concentrated sperm suspension be used. He showed, among other things; that (1) an increased concentration of sperm suspension caused an increase in the number of eggs self-fertilized, (2) a greater concentration of sperm was usually necessary to bring about any self-fertilization than would cross-fertilize 100 percent of foreign eggs, and (3) contact with suspension of own sperm decreased the ease of later cross-fertilization.

The work of FUCHS suggests a physico-chemical basis for self-sterility, since contact of eggs with their own sperm appears to cause changes in the egg membranes which inhibit entrance of own sperm and to some extent of foreign sperm, yet his criticism of MORGAN'S statements is not to the point for by the submission of the eggs to different sperm concentrations he has increased the number of variants under investigation.

MORGAN (1913, p. 217) explained his facts by means of this hypothesis:

> "This failure to self-fertilize, which is the main problem, would seem to be due to the similarity in the hereditary factors carried by the eggs and sperm; but in the sperm, at least, reduction division has taken place prior to fertilization, and therefore unless each animal was homozygous (which from the nature of the case cannot be assumed possible) the failure to fertilize cannot be due to homozygosity. But both sperm and eggs have developed under the influence of the total or duplex number of hereditary factors; hence they are alike, i.e., their protoplasmic substance has been under the same influences. In this sense, the case is like that of stock that has long been inbred, and has come to have nearly the same hereditary complex. If this similarity decreases the chances of combination between sperm and eggs, we can interpret the results."

This interpretation of self-sterility endeavors to give a modern rendering of DARWIN'S idea that the condition is analogous to the decreased fertility often resulting from other modes of inbreeding. From his other numerous observations on cross- and self-fertilization, DARWIN felt instinctively that such an analogy should exist, even though self-sterile plants were continually cross-pollinated and must of necessity have a mixed ancestry. MORGAN'S contribution was to show in a general way how such a similarity might come about. His suggestion is unquestionably stimulating and we have been glad to acknowledge our indebtedness to it (EAST 1915).

One should not ascribe more breadth to the hypothesis than the author really intended, however; for certain coördinate problems that may or may not have the same underlying cause, were not included in its scope. For example, it assumes nothing regarding the origin of self-sterility or the difference between self-sterility and self-fertility. At first sight one feels that there is a great weakness in its failure to account for self-fertility, since the eggs and sperms of self-fertile races also develop under the influence of the total or duplex number of hereditary factors, and it is difficult to see why this should decrease the attraction between eggs and sperm in some cases and not in others. But the *difference* between self-fertile and self-sterile organisms is not of necessity the same problem as the *behavior* of self-sterile organisms.[7] This distinction is manifest if one refers to COMPTON's work. In his material the difference between self-fertility and self-sterility is that of a single Mendelian factor,— self-sterility being recessive. But COMPTON does not attempt to account for the behavior of his self-sterile plants.

DARWIN, on the other hand, made no serious attempt to interpret the behavior of self-sterile plants, or to describe the fundamental difference between self-fertile and self-sterile races. He was concerned chiefly with the origin of self-sterility. The basic reason for the evolution of self-sterility, he thought, lay in a *necessity* for cross-fertilization. In this we believe he was unwise. The benefits of cross-fertilization, no one doubts. With the vigor of heterozygosis as the immediate advantage for natural selection to grasp, with the immense ultimate advantage of multiplicity of forms brought about by Mendelian recombination, one can see reason in all the host of devices for producing cross-fertilization in animals and plants,—including even bisexuality itself. But this does not mean that cross-fertilization is an inevitable need, as DARWIN believed was so clearly demonstrated by his observations on the deleterious effects of inbreeding. It is rather merely an asset in the struggle for existence, as recent experiments have shown.[8] Consequently emphasis should be placed on the assured benefits of cross-breeding and not on the doubtful evils of inbreeding. One can understand therefore why self-sterility might be desirable, and why it should be retained by natural selection after coming into existence, but the cause of its origin must still be denoted by that useful word *chance,* the veil of ignorance.

In view of these facts—and all of the important facts regarding self-sterility have been cited—the fundamental questions involved are almost as obscure now as they were when DARWIN left them. But the work of

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[7] STOUT (1916) continually confuses these two problems.

[8] See EAST and HAYES (1912) and the papers there cited.

MORGAN, CORRENS and COMPTON encourages the hope that their solution, if one may use that term for scientific description, will be accomplished. An interpretation in harmony with modern biological conceptions which will in its turn be helpful, ought at least to be possible when all of the facts are at hand.

Since the historical part of this paper was written, STOUT (1916) has published a bulky memoir on self-sterility in *Cichorium intybus*. A large portion of this paper is devoted to destructive criticsm. DARWIN and his contemporaries, BAUR, COMPTON, CORRENS, EAST, JOST, LOTSY, MORGAN and SHULL are "placed upon the carpet" and dealt with severely. One wonders whether all of these writers can be wholly wrong in the views that have been assailed, and if not, just wherein the differences of opinion lie. We cannot help but feel that they are due largely to his misconceptions of the views of the various writers concerned.

As examples of what is meant by this statement, let us mention two of the points on which STOUT lays great stress. He feels strongly that self-sterility is a markedly variable character, and that this has not been recognized by previous writers. But since the existence of variability in the somatic expression of self-sterility has been admitted unanimously by the writers with whom we are acquainted, the true point at issue is not this, but rather the question whether any considerable part of the variation in this character is the result of genetic differences. This question has been investigated in Nicotiana, and there the variation seems to be almost wholly due to environmental changes, as is shown later in this paper. Considered with this point in mind, a reasonable and constructive interpretation of our own and many other self-sterility data can be given. Where before there was chaos a certain order appears. STOUT'S failure to recognize these truths is probably the reason why he has been unable to make any constructive analysis of his own numerous data for the fact that some of his families arising from selfed seed behaved exactly as the families arising from crossed seed shows that he is often (at least) dealing with a pseudo self-fertility (see p. 531).

Now this argument of STOUT'S, we gather, is meant to be only a particular instance advanced in favor of his general view that characters are (always?) too variable genetically to be represented properly by fixed Mendelian factors. The justice or injustice of such a contention cannot be discussed here, but we should like to point out that in assuming—as is so often done—that geneticists commonly believe in an elemental stability of characters, the attitude of the great majority of such workers is misconstrued. If we have interpreted Mendelian investigators' views correctly, they believe that characters are variable, but in different degrees in different species; and that there is adequate evidence to show that most characters in most species are so constant throughout the number of successive generations ordinarily available for experimental purposes when viewed under the conditions most likely to eliminate variables other than heredity, that the *abstract* idea of fixed germinal factors can be used properly and helpfully in genetic analysis.

As a second case where we believe STOUT has not represented fairly the views of the writers criticized, the section of his paper entitled "Relation of vegetative vigor and fertility to inbreeding and cross-breeding" may be cited. STOUT criticizes in particular the views of DARWIN, SHULL, and EAST and HAYES on this subject. He rests his case on a paper by BURCK (1908) in which the writer holds, that (quoting STOUT):

> "(1) plants that are regularly self-fertilized show no benefits from crossing, (2) that nowhere in wild species is there evidence of an injurious effect from self-fertilization, and that there is abundant evidence of continued vigor and high fertility resulting from long-continued self-fertilization, and (3) that the advantage derived from crossing within or between garden varieties appears when there is doubtful purity; and is due to the fact that both vigor and fertility have already been decreased by hybridization, and that when crosses do give increased vigor and fertility the cross has restored in increased measure the original nuclear organization of the parent species."

The logic of the third statement is too delightful for comment, being worthy indeed of Mother Eddy. Vigor is decreased by hybridization. Vigor is increased by hybridization. It is increased by restoring "nuclear organization." Not only is nuclear organization restored, but it is restored in *"increased measure."*

The second statement has never been denied by modern writers, to our knowledge. It was emphasized by EAST and HAYES (1912), who pointed out why the advantage of cross-fertilization in plants should be stressed rather than the disadvantage of self-fertilization. This advantage, if one may recall it, lies in the fact that n inherited variations can produce but n forms under self-fertilization, and may produce 2^n forms under cross-fertilization by Mendelian recombination.

The first statement is simply not in accord with the facts. We are astonished that one who has the acquaintance with the literature that

STOUT has shown, should quote it with approval. Every hybridist of experience from KÖLREUTER (1760) to the present day has cited so many data diametrically opposed to it that the matter is no more worthy of discussion than is a denial that the earth is round.

Of course as to the interpretation of the facts one may hold a difference of opinion. The hypothesis of heterosis advanced independently by SHULL and EAST has, we think, served a useful purpose. The last word has not been said, however, and data accumulated by H. K. HAYES and D. F. JONES in their continuation of the experiments reported by EAST and HAYES (1912) have led the senior author to modify his views on several of the points there discussed, though not on the main conclusions. But in the meantime it is disconcerting to have our published statements misunderstood and misinterpreted. For example STOUT says (p. 419) "EAST and HAYES believe that heterozygosity gives an increase of both vigor and fertility in proportion to the number of heterozygous factors in the organism." There are two errors in this statement. Neither SHULL nor EAST has maintained that crossing increases fertility. The number of flowers and fruit is often increased, but no data have appeared which indicate a decreased percentage of nonfunctional gametes. Second, EAST and HAYES used the words "roughly proportional to the number of heterozygous factors." Leaving out the word "roughly" and taking the statement from its context, conveys a very wrong impression for it was *not* assumed that *every* germinal factor affected vigor and it was expressly stated that one could *not* assume equal effects for different factors. Again STOUT achieves a remarkable misinterpretation of the results reported in table 5 of this same paper. Here 42 inter-specific crosses are reported, of which 14 show decreased vigor (this figure should be 13 instead of 14 owing to a typographical error in reporting the first cross, *N. alata* × *Forgetiana,* which was 125 percent of the parental average in height, instead of 25 percent). STOUT leads his readers to infer that this table is the sole basis of the conclusions regarding heterozygosis, and that the conclusions are incorrect because, as he states: "There was increased vigor in only 17 cases, but there is no apparent reason why, if it is simply heterozygosity that increases vigor, more of the combinations should not show increased vigor."

Now what are the facts. The statements on the previous page (p. 27) of the paper make it clear that many varietal crosses were made (over 100 in Nicotiana alone to that date), *which showed vigor equal to, or greater than the parental average.* While not expressly stated, it may be inferred that none was found with decreased vigor. If it had been otherwise it would have been stated. Multiplication of such data was thought unnecessary in view of the exceedingly numerous results of KÖLREUTER, KNIGHT, GÄRTNER, NAUDIN, FOCKE, DARWIN and others, on the increased vigor of such hybrids. This table then, *as is shown on pages 29 and 30*, was submitted for the particular purpose of trying to establish a wholly different thesis, viz., that as germ plasms become more and more unlike, there comes a time when hybrids show (1) an inability to form germ cells (sterility), and (2) difficulty in somatic cell division. Our typographical error was unfortunate, but in view of the text given the statement made by STOUT is an inexcusable perversion of our work.

We have mentioned but two out of a goodly number of misconstructions of work with which we have been concerned. We have done this because we believe that they are paralleled in the author's criticism of most of the writers mentioned above, and because we realize that if we undertook to point out these misunderstandings in the case of other writers, the answer would be that it was merely a difference of opinion.

On the other hand, STOUT has given us a classification of types of sterility, and has reported a really immense amount of data. We hope that he will give a more constructive analysis of them later.

THE MATERIAL USED AND THE GENERAL PLAN OF THE PRESENT INVESTIGATIONS

The investigations described in this paper may be said to have been begun in 1910, when, in connection with some genetic studies on size in the genus Nicotiana, the two species *Nicotiana Forgetiana* (Hort.) Sand. and *Nicotiana alata* Lk. and Otto var. *grandiflora*[9] Comes were found to be self-sterile. These two species have been made the basis of our experiments, though later some work was done upon *Nicotiana angustifolia* R. and *P.* var. *crispa*[9] Cav., *N. commutata* Fisch. & Meyer, and *N. glutinosa* L., in which self-sterility had been discovered.

The characters of these species and of *Nicotiana Langsdorffii* L., a self-fertile species used, are described in COMES (1899), SETCHELL (1912), and EAST (1913, 1916).

From the technical standpoint the material has been ideal. Any combination of the three species *N. Forgetiana*, *N. alata*, and *N. Langsdorffii* can be made, the F_1 hybrids being completely fertile (in proper cross-fertile combinations). *N. glutinosa* and *N. angustifolia*, however, can

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[9] Hereafter *N. alata grandiflora* will be known as *N. alata* and *N. angustifolia crispa* as *N. angustifolia*.

neither be crossed together nor with the other species. The plants of each race grow rapidly and vigorously, and are not easily affected adversely by sudden changes in environmental conditions. They are not subject to serious parasites. Cuttings root well, and with care old roots will live through a second and occasionally even a third season. Emasculation and pollination are easy to perform, and seed production in fertile crosses is high.

N. Forgetiana, N. alata and *N. angustifolia* belong to the subgenus Petunioides, a fact worthy of note because nearly all of the species of this section have both showy flowers and abundant nectar which attract insects and thus promote cross-pollination. Even *N. glutinosa* has rather conspicuous blossoms, though belonging to the subgenus Rustica in which most of the species have small and unattractive flowers that are self-pollinated naturally. In other words all four of these species probably had evolved structural modifications which aided cross-fertilization long before the development of their self-sterility. We are dealing, therefore, with plants desirable both from the viewpoint of the experimentalist and of the student of evolution, a most unusual combination.

The general problem presented by this material obviously was to discover the facts regarding self-sterility, and to determine whether these facts might be fitted by a simple mathematical or chemico-mathematical description. It has been attacked along three distinct lines: (1) pedigree cultures; (2) histological studies of pollen tubes in crossed and in selfed pistils, and in inter-specific and inter-generic crosses; and (3) physiological studies of pollen tubes cultivated on artificial media.

Work along this general plan has been carried on at the Bussey Institution of HARVARD UNIVERSITY continuously since 1910, though it has not been our sole interest. It was our good fortune to have the very efficient aid of Dr. O. E. WHITE, then a graduate student and assistant at HARVARD UNIVERSITY, during the winter of 1911-12. The junior author's connection with the work began in February 1914, and has continued until the present time. In addition, Miss GRACE SHEERIN and Miss BERTHA KAPLAN have assisted in the pollination work for limited periods of time.

It being impracticable to present and to examine these various data within the limits of a single article, we propose to take up only a portion of the pedigree culture work in this paper, leaving the remaining questions to be treated later. The pedigree culture investigations have thus far involved four studies: (a) the effect of environment on self-sterility;

(b) the relations existing between self-sterile plants in intra-specific and inter-specific crosses; (c) the relations between self-sterile and self-fertile plants; (d) selective fertilization. The first two studies will be discussed here.

The usual precautions used by plant geneticists have been carefully observed, including castration of all flowers on self-sterile plants used as pistillate parents. This safeguard would not be worthy of especial mention except for the fact that it is wholly disregarded in STOUT'S recent paper (1916). We shall show in a later paper that effective pollen mixed with "own" pollen causes scarcely any acceleration of "own" pollen tubes in Nicotiana. But we cannot find that STOUT determined this for chicory, and to take for granted that there is no such effect seems to us a laxity in a scientific work.

Every important fact described has been confirmed independently by each of us, and certain of the data that have been remarkably orderly (for example, table 11) have been collected by several persons in such a manner that personal equations were largely eliminated.

It may be noted here that a preliminary report of some of the work which we now report in detail was published in 1915 (see EAST 1915). With more data in hand more definite ideas on the subject have been possible, hence several differences will be noted between the statements made then and now. It is scarcely necessary, however, to point out every difference in the interpretations, as we shall endeavor to give in full our reasons for the present conclusions.

THE EFFECT OF THE ENVIRONMENT ON SELF-STERILITY

In beginning the description of our experiments with a section on the effect of environmental changes on self-sterility a chronological inversion is made which needs explanation, particularly as carefully planned experiments designed to show the effect of individual environmental factors when all others are controlled have not been carried out. Work on the relation between self-sterile plants was started with the idea, that even though DARWIN were correct in supposing that self-sterility is seriously affected by changes in the environment, conditions might be kept so constant that no difficulties would be encountered. Indeed, this is probably the case, since no particular difficulties were experienced during several years in spite of *certain* environmental factors being constantly varied. There came a time, however, when troubles arose which were puzzling for a considerable period. Our inquiries regarding the effect of the environment on self-sterility have finally removed the stumbling-block and have made a clear and reasonable analysis of the pedigree culture work possible.

In brief these conclusions are as follows:

1. Self-sterility is a condition determined by the inheritance received, but can develop to its full perfection only under a favorable environment. This is not a strange conclusion, for perhaps particular environmental combinations are necessary for the full development of all positive somatic characters. But certain characters are much more seriously affected than others by the environmental variations likely to be met under ordinary conditions. For example, BAUR (1911) showed that *Primula sinensis rubra* produces red flowers when grown at a temperature of 20° C. and white flowers at a temperature of 30° C.; EAST and HAYES (1911) found that the red pericarp characteristic of a certain maize variety developed in sunlight but not in shade; Miss HOGE (MORGAN et al., 1915) discovered that in a Drosophila mutant with supernumerary legs the character was only called out when the animals were kept at 10° C. Self-sterility is such a character. It develops fully only under conditions which promote a normal healthy vegetative growth, and during the active part of a flowering period.

2. At the end of a flowering period and under conditions adverse to vegetative growth, self-sterility declines until a few seeds may sometimes be obtained after self-pollination. Occasionally even a full capsule is produced. The immediate cause of this partial return to a pseudo-fertility is the acceleration of pollen-tube growth that obtains under these conditions. Since we have reason to believe that the difference between a fertile and a sterile combination in these plants is the ability of the pollen grain through something inherent in its constitution to call forth in the tissue of the style in the former and not in the latter case a secretion which accelerates pollen-tube growth, it follows that in weakened style tissue some change has occurred that renders this secretion more easily produced.

3. Self-sterility can be restored in weakened plants by allowing them to go through a period of rest and then, by proper treatment, bringing them into flower anew as vigorous plants. Truly self-fertile plants cannot be forced into self-sterility by any treatment. This last conclusion is of course largely a conclusion by analogy and is not subject to rigorous proof.

4. Self-sterile races differ in their norms for self-sterility. Thus in

N. Forgetiana and in *N. angustifolia* the character is much more stable than in *N. alata* and *N. glutinosa*. In many ways this behavior indicates the existence of multiple allelomorphs for self-sterility.

The basis for these conclusions is the whole of our experience with self-sterile plants, which, it is scarcely necessary to say, cannot be cited statistically in this place. But the following facts will show, we hope, that they are well founded.

Cross No. 1 between *N. Forgetiana* and *N. alata* was made in 1909 using *N. Forgetiana* as the female. At that time both of the parents were thought to be self-fertile because a carefully bagged inflorescence of each species had yielded seed; but when the plants of the F_1 generation turned out to be self-sterile, the status of the parents was investigated more carefully. Over two hundred plants of *N. Forgetiana* have been tested under various conditions. Plants growing out of doors both on good soil and on poor soil have been tested throughout the growing season. Greenhouse-grown plants have been tested not only throughout a normal flowering period (about 3 months), but have been forced through an abnormally long flowering period during the test. Plants well nourished have been compared with plants poorly nourished, and plants well watered with plants under conditions of drouth. Both old roots and cuttings brought into a second flowering period in fine condition have been compared with much pruned old roots and cuttings in poor condition.

Only 3 cases of seed production have been observed. 2 plants at the end of their flowering period under conditions adverse to vegetative growth produced 1 and 2 capsules respectively having about 50 seed each (the normal is *ca.* 300) out of 14 tests. The third plant was not tested until near the end of its flowering period. At that time it was noted that it seemed to be self-fertile. Under test it did indeed produce several fine seed capsules after self-pollination and would undoubtedly be called a self-fertile plant were there not the following reasons for considering it an unstable self-sterile (see description of *N. alata*).

1. The plant when first tested was in a late flowering stage, yet produced capsules only in about half the tests.

2. After pruning and resting for a time the plant was brought into vigorous flower a second time. The tests during the first two weeks of this period (about 20 flowers) were all negative. The plant seemed to be perfectly self-sterile. Gradually, however, self-fertility returned as the flowering period waned.

3. Twenty-four plants grown from selfed seed of this individual, tested during the height of their flowering period, all proved self-sterile.

We are therefore forced to concede the probability that an error of manipulation or of record was made in 1909, although we may have happened upon a plant like the one just described since the original selfing was done at the end of the flowering season. Be that as it may, the conclusion is inevitable that *N. Forgetiana* (and *N. angustifolia* has yielded similar results) is a self-sterile species of remarkable stability, which only occasionally (1 in 300?) produces a plant that shows some self-fertility under adverse conditions.

TABLE 1

Progeny of pseudo self-fertile N. alata plant used in cross No. 2. Grand-progeny of original pseudo self-fertile plant. Subscripts show number of pollinations made.

Ped. No. ♀	No. selfings sterile	No. selfings giving capsules			Plants with which cross-fertile ♂	Plants with which cross-sterile ♂
		1-10 seeds	10-50 seeds	250-300 seeds		
53	6		1		57	54_2
54	5					53_2, 57_2, 58_2
56				3		53, 57, 58, 59
57	3					58
58	1				53, 59	314
59	2					314, 53_4 54, 56_3, 57,
61	3					
62	4	4		2		58, 79
64	3	1				58
65	6					79
66	12	1				58, 62, 71, 79
67	3					
68	4					
70	5	1	1			
71	3		1	1		314, 58
72	3					
73	4					
74	1					
75	8					
76	2	1		2		66
77	2					
78	9					66
79	3	1				58, 66

1 pollination 53 × 54 and 2 pollinations 59 × 53 produced 1-10 seeds each.
314 = *N. Forgetiana.*

N. alata, on the other hand, has proved to be more unstable[10] in its self-sterility; or better, it has proved to have a norm more nearly inter-

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[10] *N. glutinosa* appears to behave like *N. alata*, but has not been tested very thoroughly.

mediate between the extremes complete self-sterility and perfect self-fertility. But fundamentally it is a self-sterile species like *N. Forgetiana.*

Numerous *N. alata* plants have been tested for self-sterility under the same conditions as described above for *N. Forgetiana.* The results have been similar in that the plants were practically always completely self-sterile during the early part of a vigorous flowering season. But under adverse conditions during the latter part of the flowering period, rather a high percentage of the plants produced capsules with from 1 to 50 seeds each. Only 2 plants have been found, however, that appeared to be almost completely fertile from the middle of the flowering period onward under normal conditions. Of these plants more is to be said.

Assuming that no mistake was made in 1909 and that selfed seed was actually obtained from a field-grown plant of *N. alata,* we have records of its progeny for three generations.

Twenty-five seedlings from this seed were grown in 1914. These plants were tested for self-sterility as field-grown plants, though not as thoroughly as might be desired. 2 plants showed some self-fertility,—no tests having been made until the latter part of August. From 1 of them selfed seed was obtained and a second generation grown. 23 of these plants were tested in the greenhouse with the results shown in table 1.

Fourteen of these plants produced no seed when selfed; 9 showed some degree of self-fertility. This fertility apparently occurred only

TABLE 2

Progeny of pseudo self-fertile N. alata plant No. 56. Great-grand-progeny of original pseudo self-fertile plant.

Ped. No.	No. selfings sterile	No. selfings giving capsules		
		1-10 seeds	10-50 seeds	250-300 seeds
80	9	2	2	
81	1			
83	7			
84	3			
85	10	2	1	1
86	1			
87	5			
89	7			
90	8			
91	8		1	
92	1	1		
93	8		2	
94	4			
95	3			

when the flowering season was waning and the plants were under adverse conditions, as was stated before; but it cannot be proved that this was always the case, for one cannot draw a definite line between vigorous and weakened plants. 3 plants, excluding No. 56, produced some full capsules, but in these cases the remaining self-pollinations and sterile cross-pollinations show that the plants were not truly self-fertile. Plant No. 56, however, showed no direct indications of self-sterility in connection with the 3 self-pollinations tried. More pollinations should have been made on this plant at the beginning of a second flowering period. Unfortunately, it was discarded. The evidence of self-sterility, therefore, is wholly circumstantial. It is, that though having functional ovules No. 56 was sterile to the pollen of plants 53, 57, 58 and 59, and though having functional pollen it was sterile crossed on plant 59.

A small population was grown from the selfed seed of this plant. It is shown in table 2.

Although 5 of the plants produced some seed, if one considers the date of manipulation and the state of the plants, the evidence is all in favor of the idea that this was an effect of external conditions. There is no reason whatever for believing that any of the plants were truly self-fertile.

All told then, we have three generations of *N. alata* plants, each generation being grown from selfed seed produced from plants apparently weakened at the time of seed production, without the occurrence of a single plant which behaved in every way like a truly self-fertile individual. It seems to us, therefore, that this selfed seed might be thought of as having been produced artificially.

If this be the correct view of the matter, it is clear that there is no reason why fusion between gametes produced by a self-sterile plant cannot occur provided the male generative nucleus enters the embryo sac. Such unions may take place without affecting the self-sterility of the progeny. Even by the selection of apparently self-fertile plants for three generations no tendency toward the formation of a self-fertile race is indicated. Just how broadly one may generalize from these data is still problematical, but the two following conclusions are certainly more than guesses.

(1) Unless a male gamete complementary to every female gamete is formed, there is no selective fertilization, for *full* capsules have been found on plants that in the early part of the season and in crosses showed they were really self-sterile. Other evidence militating against selective

TABLE 3

Progeny of N. alata No. 58 × N. alata No. 56.

Ped. No.	No. selfings sterile	No. selfings giving capsules		
		1-10 seeds	10-50 seeds	250-300 seeds
96	5	1	3	
97	5	1		
98	11	1		
99	4			
101	4			
102	4			
103	8			
105	8		2	
106	7			
107	3			
108	4			
109	1	3	2	
110	3			
111		3		
113	1			
116	4			
117	6			
118	7			
119	3			
120	5	1		
121	2			
122	7			
123	5	1		
124	5			
126	1			
127	8			
128	4			
133	1	3		
135	3			
136	3	2		
137	3			
139	9	3	1	
140	4			
141	2			
144	4			
146	6			

fertilization which will be presented in a later paper has also been obtained by a different method of attack.

(2) It follows therefore that self-sterility behaves as a sporophytic character and is not the result of incompatibility between gametes.

One other bit of evidence regarding *N. alata* should be presented here. It is the behavior of the progeny of a cross between the self-sterile plant No. 58 and the apparently self-fertile plant No. 56. These data are reported in table 3.

Here again we find a considerable percentage of plants, a third to be exact, giving a few capsules having from 1 to 50 seeds each. Here again it was the plants near the end of their flowering season, the plants that had been cut back strongly, the plants that were producing flowers on one or two weakened branches, that gave the seeds. To be sure, as in other families, one or two plants apparently vigorous behaved in the same way near the end of the flowering season. But the correlation between *weakened failing* branches at the end of their flowering period and *tendency toward self-fertility* was very high even when judged only by external appearances.

The remaining data on this subject cannot be discussed in this place without repetition, since they include nearly all our pedigree culture work. And at any rate they are important only as corroborative evidence, for in our regular experiments extremely weak and old flowering branches were seldom used. For this reason we rarely had to contend with any approach toward self-fertility in self-sterile plants. But the phenomenon when met lent support to our hypothesis. Furthermore, *cross-sterile combinations behaved in the same way.*

These conclusions have been a great aid to us in analyzing our pedigree culture facts. Without them the data from two or three of our populations, where pollinations were carried on up to the end of the flowering season, would have been somewhat chaotic. They reveal, for example, that *N. alata* is just as much of a self-sterile species as *N. Forgetiana* though the expression of the character is affected more easily by external conditions. They show clearly why selection for three years accomplished nothing. The selected extreme was a non-inherited fluctuation. It is clear also why crosses between these apparently self-fertile plants and plants unquestionably self-sterile, yielded no truly self-fertile offspring in either the F_1 or F_2 generations. The plants were really self-sterile; they were pseudo-fertile, and will be so called.

In this connection it may be recalled that DARWIN (1876) found that self-sterile plants of *Abutilon Darwinii* became partly self-fertile *at the end of their flowering season.*

Keeping these things in mind, one is able to classify the pedigree culture results with great accuracy, though there are five possibilities of error.

1. There may be error of record. This we believe to be slight, owing to our various methods of checking results.

2. A true sterility either partial or complete may exist. This usually can be discovered by a microscopical examination of the pollen, and may be tested by reciprocal crosses. The reciprocal cross test has never

brought to light a case of ovule sterility and pollen fertility, but the converse is sometimes true.

3. Combinations made but once and failing must be reported as sterile; but this is an error about 4 times per hundred, since this is the ratio of failure found in combinations known to be fertile, by reason of an imperfect technique or other unknown causes. We cannot correct accurately for this error, but it must be considered when discussing exceptions to a general scheme which other data fit.

4. Combinations may fail once and succeed once in two trials, or in very rare cases fail twice and succeed twice in four trials. Experience has shown that if the capsules are normal in size and full of seed, the combination is fertile. Fertile combinations *always* give full capsules. There is no partial fertility in fertile combinations except as *true* sterility exists in some degree (see error 2). Conversely, it is possible of course to meet with a pseudo self-fertile plant like *N. alata* plant No. 56, which under adverse conditions might give full capsules of normal size after a "sterile" combination had been made. But under the environmental conditions that usually obtained during our work, this would be extremely rare,—to the best of our knowledge and belief not over 1 per 200 plants.

5. Combinations may give capsules with from 1 to 50 seeds as well as failures. These are sterile combinations. They probably occurred in only three families, because only in these families were the plants utilized during the *whole of their flowering period.* Unfortunately it must be admitted that a few errors of record may have been made with these cases. A small number of apparently successful matings were not recorded until the capsules had opened. Since the capsules were of normal size and each had contained a number of seeds, these combinations were recorded fertile, but the matter is not certain.

It is not believed that these errors are serious even when taken together but some allowance must be made for them in considering the few exceptions noted in the analyses we have made of the tables that follow.

INTER-SPECIFIC PEDIGREE CULTURE EXPERIMENTS

All of the crosses reported in this paper are between species or varieties believed to be self-sterile for the reasons set forth in the foregoing section. But because certain plants were used which under the peculiar conditions at the time of the test for self-sterility yielded some selfed seed, these plants are distinguished by the term "pseudo self-fertile." Their behavior in these crosses is further evidence that the term is justified.

Cross No. 1. N. Forgetiana × *N. alata* (*self-sterile* × *self-sterile*)

The cross to be described first is that mentioned previously in connection with the discovery of self-sterility in the genus Nicotiana. It was made in 1909, using *N. Forgetiana* as the female and *N. alata* as the male.

The F_1 generation

The F_1 population consisted of vigorous plants twenty-five[11] percent *taller* than the average of the two parents and was extremely uniform in size and in color of flowers, though the latter were not so dark a red as those of the male parent. A few individuals tested for fertility in 1910 and others from the same original cross again tested in 1912, all proved to be self-sterile. The actual tests made, some 20 plants altogether, were too few to claim self-sterility for every individual, but careful observation of about 50 other plants in the field indicated this to be the case. These observations were made by estimating the number of capsules which developed naturally on each plant, it having been determined that on self-fertile plants of an allied species, *N. Langsdorffii,* from 10 to 20 times as many capsules develop as on self-sterile plants of *N. alata,* though the ratio of flowers formed on the two species is only about 3 to 1.

No extended experiments were carried out to test the fertility of these plants in intercrosses. 6 intercrosses between sister plants were made and each was successful, but whether some cross-sterility existed or not is unknown. The pollen, however, was good in every plant examined (about 30).

The F_2 generation

From these 6 intercrosses between pairs of F_1 plants almost a thousand individuals were grown. They showed a most remarkable variation in all their characters, the range including the modal values of both grandparents. The frequency distributions for length and for breadth of corolla have been discussed in another paper (EAST 1913), and it will suffice to note here that while the coefficient of variation for length of corolla in the F_1 generation was $8.28 \pm .38$ percent, in the F_2 generation it was $22.57 \pm .39$ percent.

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[11] By a typographical error the height of this cross is made 25 percent instead of 125 percent in table V, EAST and HAYES 1912.

There was also a great range in color of corolla, which even with the considerable number of subsequent generations grown, has not been analyzed to our complete satisfaction. 4 Mendelian factors appear to describe the breeding results best, giving the 7 forms, red, magenta, light red, light magenta, light red on exterior of corolla only, light magenta on exterior of corolla only, and white. Red is epistatic to magenta, and the darker colors are epistatic to the lighter ones.

These details are given in order to emphasize the fact that here we have two races sufficiently distinct from each other to be designated as separate species, which cross easily and give a fertile F_1 generation and a wide range of forms in the F_2 generation. The fertility of the F_1

TABLE 4

Result of matings on 20 plants of the F_2 generation of cross No. 1 N. Forgetiana × N. alata.

Plants used as males

Plants used as females	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	S		F					F			F	F								
2		S									F	F	F							F
3	F		S			F		F			F	F			F		F			
4				S				F			F	F								
5	F				S		F	F	F	F	F	F	F	F	F	F	F	F	F	F
6				F		S		F			F		F	F			F			
7			F				S				F				F		F			
8	F		F	F		F	F	S		F	F	F	F	F	F	F	F			
9				F				F	S			F		F						
10					F			F		S	F	F	S	F	F	F	F			
11					?	F		F		F	S	S	F	F			F			
12			F		F					F	S	S	F	F	F	F	F	F	F	F
13								F			F	F	S		F		F			
14			F		?			F	F			F		S			F			
15					F			F			F	F			S					
16					F					F	F	F		F		S		F	F	
17					F					F	F	F	F		F		S			
18					F		F	F			F	F						S	F	
19			F		F			F						F			F	F	S	
20					?			F					F							S

generation indicates absence of any selective elimination of gametes or zygotes in its daughters, and the variation exhibited by these daughters shows conclusively that the original parents really did differ by a considerable number of hereditary factors. These matters are important in connection with the inbreeding experiment that followed.

About 40 plants from the F_2 generation were crossed and selfed on a rather large scale. One of these experiments in which 20 plants coming from 2 intercrosses between F_1 plants were used, is shown in table 4. The vertical columns give the number of the plants when used as males; the horizontal rows are the same plants when used as females. The result of each mating made is denoted by the letters F for fertile and S for sterile.

It was planned to make all possible combinations of these plants; but this proved to be impracticable, and only 154[12] were accomplished. The pollinations on the plants of this generation as well as those on the succeeding generations included in this experiment were made under various conditions of sunshine, temperature, moisture, food supply and age, but these variables appeared to have no influence on fertility. The results always checked. A small number of matings were made in the open field in August and September, 1911. The remainder were performed in the greenhouse. A part of these were made upon some of the old plants that had been transplanted during the late fall, and the others upon cuttings from the plants in the field which were again ready for operation in April, 1912. *But in all the work on the 20 plants tabled it should be noted that pollinations were made during the height of the flowering period when the plants were in good condition. Nevertheless, there may have been errors. If such did occur, cross-fertility would have been favored; since at the time the work was done upon the F_2, F_3, and F_4 generations of this cross, pseudo self-fertility was not suspected.*

The plants were each selfed from 2 to 10 times, an average of 4 times per plant, without a single seed being obtained.

Of intercrosses, 132 were made. 3 of these are indicated by question marks on the table. This is because plant 5 had defective pollen, it being the only one of the twenty in which the pollen did not show from 90 to 100 percent of morphologically perfect grains. None of the crosses where this plant was used as the male gave capsules over half-filled with

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[12] A few of the figures given here differ from those given in the preliminary report on this work (EAST 1915). This is due to rechecking the results and to the addition of a few more data. There have been no essential changes and the present figures are believed to be correct.

seeds, but since 7 matings had from 30 to 100 seeds per capsule, and since the reciprocal matings were all successful we have classed them as fertile. The matings questioned, 11 × 5, 14 × 5, and 20 × 5, ought also to be classed as fertile, since the reciprocals were fertile, but as they yielded only 2 to 10 seeds per capsule, they have been omitted from these next calculations.

Of the remaining 129 intercrosses, 126 were successful; 4 of them produced capsules having less than 50 percent of the ovules fertilized (2 pollinations each being made), the remainder produced full capsules. There were few failures among these intercrosses, though from 2 to 12 repetitions of the matings were made in almost every case. It seemed as though an intercross possible at one time could be made at any other time at the first attempt. In other words, there seemed to be no variability in ease of cross-fertilization. The failures in the fertile intercrosses were less than 4 percent, and these were complete failures which may be attributed to the technique used.

Twenty-eight intercrosses between these plants and other plants of the F_2 generation were also made with 28 successes. In addition, 92 other combinations were made between plants not shown in the table. They are not reported in detail because only a few matings per plant were made; but the gross results were 89 successes and 3 failures.

Altogether among these matings there were 54 pairs of reciprocals each of which gave the same result.

The failures in the intercrosses remain to be considered. The table shows 3 cases; of which 10 × 13 was tried 2 times; 11 × 12, 12 times; and 12 × 11, 6 times. The last pair are reciprocals, but we shall treat reciprocals separately for the present. Of the other 3 cases, 2 of them were tried 3 times, but the third was made only once, which of course does not settle the matter. Thus there were 4 definite cases, 1 probable case, and 1 questionable case of cross-sterility, a matter of 2.4 percent (6 out of 249).

The F_3 generation

Out of the many fruitful combinations of F_2 plants, 29 F_3 families were grown,—50 to 150 individuals of each being transplanted from the greenhouse to the field with due care that random samples were obtained. Field examinations as described above, indicated a total absence of self-fertile plants, and from 3 to 6 attempts to self individuals of each family resulted in failures.

The progeny of 2 red-flowered plants of the F_2 generation furnished

TABLE 5

Result of matings on 12 plants of the F_3 generation of cross No. 1, N. Forgetiana × N. alata.

Plants used as males

Plants used as females	1	2	3	4	5	6	7	8	9	10	11	12
1	S		F	F	F		F		F		F	
2		S		F	F		F		F	F	F	F
3	F		S		F		F			F	F	
4	F	F		S		S		F	F	F		
5			F		S			F	F		F	F
6	F		F	S	F	S		F	F	S		F
7	F	F	F		F	F	S	F	F		F	S
8		F	F	F		F		S		F	F	F
9	F		F		F	F			S		F	F
10			F	F	S	S	F	F		S	F	
11	F		F	F			F	F		F	S	F
12							F			F		S

the material for the continuation of our intercrossing experiment. Most of the work was done on 12 plants as set forth in table 5. Fruitless self-pollinations averaging over 3 per plant proved they were self-sterile. 102 cross-pollinations were made: 75 are shown in the table; 27 were made in a less systematic manner with 11 other plants of the same family. These resulted in 95 successes and 7 failures. Again the "possible" combinations were almost always successful. The unsuccessful matings were 4 × 6, 6 × 4, 6 × 10, 10 × 6, 7 × 12, and 10 × 5. Combination 6 × 4 was made twice and combination 7 × 12 once, the remainder were made three or more times. The first 4 matings consist of 2 pairs of reciprocals. The reciprocal of 7 × 12 was also made, but proved to be fertile. This is evidence that with further trials 7 × 12 would also have been successful, for we have *invariably* found reciprorocals to behave alike when a number of pollinations sufficient to determine definitely the status of the cross has been made. In fact 26 reciprocals gave the same result in this population. The remaining combination showing cross-sterility was between plants 21 and 27. Eliminating combination 7 × 12, therefore, 6 percent gross of cross-sterility is shown in the F_3 generation.

TABLE 6

Result of matings on 10 plants of the F_4 generation of cross No. 1, N. Forgetiana × N. alata.

Plants used as males

Plants used as females	1	2	3	4	5	6	7	8	9	10
1	S		F	F	F		F		F	F
2	F	S	F	F		F	F	S	F	
3			S		F				F	F
4	F			S	F	F	F		F	
5		S			S	F		S	F	F
6	F		F			S	F			F
7	F	F		F	F	F	S			F
8	F		F		S		F	S		F
9	F	F	F		F		F		S	
10	F		F		F	F	F		F	S

TABLE 7

Result of matings on 20 plants of the F_5 generation on cross No. 1, N. Forgetiana × N. alata.

Plants used as males

Plants used as females	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	S		F	S	F			F	F	F	F	F	F	F	F	F	F			
2	F	S	F	S	S				F		F	F	F	F	F	S	S	F	F	S
3			S	F	F			F	F	S	S									
4																				
5													F							
6	F					S	F	F	F	F	F	F	F							F
7	F	F	S	S	F		S	F	S	S	S									
8					F			S	F	F										
9		F			S				S	F	F	F	F	F						
10			S		S					S										
11											S									
12			F		F		F	S	F	F	F	S	F							
13			S										S							
14			F		F	S	F	F	F	F	F	F	F	S						
15			F		F	S	F	F	F	F	F	F	F	S	S	F				
16			F		F	F	F	F	S	F	F	F	F	F	F	S	F	F	F	S
17			F		F	F	F	S		F	S	S	F	F	F	F	S			
18		F	S		F	F	S	F	F	S	S	F	S	F	F		F	S	F	
19			F		S	F	F	F		F	F	F	F	F	F		F		S	S
20																				

The F_4 generation

Only 2 of the F_3 combinations were grown during the next season and the pressure of investigations along other lines was such that but little work was done upon them. Field examination and tests on 21 plants, however, showed us no self-fertility. 10 of the progeny of 2 red-flowered F_3 plants, had 52 matings made upon them, 15 being reciprocals giving duplicate results. In addition 6 random matings with other plants of the family were tried with 1 failure. There were 48 successful and 4 unsuccessful matings on the 10 plants shown in table 6. The fertile matings yielded good capsules as usual with 3 exceptions, there being but 7 complete failures out of over 200 pollinations. Of the unsuccessful combinations, pollinations were made as follows: 2 × 8, 4 trials; 5 × 2, 6 trials; 5 × 8, 4 trials; 8 × 5, 5 trials; and 8 × 12, (not shown in the table) 4 trials. Each of these cases is fairly certain, therefore, and gives us a gross cross-sterility ratio of nearly 9 percent.

The F_5 generation

Only 1 F_5 family was studied, but as it was planned to discontinue this particular experiment, considerable attention was given to it. As was also true of the F_3 and F_4 generations, the work was carried on under field conditions. Similarly again, it was produced by mating two red-flowered sibs.

A random sample of 20 plants was marked for work, and 439 pollinations made (table 7). Of these pollinations 92 were wholly unsuccessful attempts to secure selfed seed made on 17 plants, an average of 5.5 pollinations per plant. Thus there is no question about the self-sterility of each plant tested. Plants 4, 5 and 20 were not tested. Plant 4 had such bad pollen that results with it are valueless, and plants 5 and 20 were somewhat sickly. Plant 9 also had such poor pollen that the seed capsules were not full, but a classification of the matings where it was used could be made without any serious chance of error. 274 pollinations were made on the 119 intercrosses that proved fertile. Only 12 of these attempts failed, and 5 of them were on crosses between No. 9 and No. 3. Thus only 4 attempts per hundred failed in the intercrosses that were classed as fertile from records of other pollinations, showing conclusively, we think, that inbreeding had produced no quantitative diminution in fertility among "possible" combinations, the percentage of failures in fertile crosses in the F_2 generation being about the same.

The remaining 73 pollinations were unsuccessful attempts to obtain seed in 33 intercrosses. The details are shown in table 8.

TABLE 8

Record of unsuccessful cross-pollinations made on the F_5 generation of cross No. 1, Nicotiona Forgetiana × N. alata.

Mating	Pollinations	Mating	Pollinations	Mating	Pollinations
1 × 4	1	7 × 9	1	16 × 20	3
2 × 4	1	7 × 10	1	17 × 8	2
2 × 5	1	7 × 11	1	17 × 11	2
2 × 16	2	9 × 5	3	17 × 12	3
2 × 17	2	10 × 5	1	18 × 3	3
2 × 20	3	12 × 8	3	18 × 7	3
3 × 10	2	13 × 3	2	18 × 10	3
10 × 3	2	14 × 6	3	18 × 11	3
3 × 11	2	15 × 6	3	18 × 13	2
7 × 3	3	15 × 14	2	19 × 5	2
7 × 4	3	16 × 9	1	19 × 20	4

It will be seen that only 1 reciprocal cross was made on these plants and this was by accident. A large number of reciprocals had been made on other crosses always with the same results when tried a sufficient number of times to make classification conclusive. It was decided therefore, to make as many distinct matings as possible in order to make a thorough test of the mating proclivities of the plants under observation. The result is that the percentage of cross-sterility found in the F_5 generation is not strictly comparable with the percentages found for the earlier generations where matings were made at random and each mating counted. To be sure a few reciprocal matings[13] were made in F_5, but the percentage is very much less than in the preceding generations. The gross cross-sterility found in F_5 was 21.7 percent, if the 8 crosses where only 1 pollination was made be counted. By the theory of error 1 of these cases might be excluded, while for certain other reasons (see table 9) error is suspected in another case, but since this correction would reduce the cross-sterility percentage by only 1.2, the figures 21.7 will be allowed to stand.

Eight other intercrosses between other plants of this same population were also made. We have not thought it necesssary to include them in the table because the attempts at crossing were so sporadic, but the percentage of cross-sterility would scarcely be changed, for 7 out of 8 intercrosses were fertile.

A number of other facts appear in the data shown in tables 7 and 8, which are not apparent without careful study. In accordance with their behavior in intercrosses, the plants may be grouped into 5 classes in

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[13] Seven reciprocals were made altogether in this family with like results.

which there is intra-class sterility and inter-class fertility, with very few exceptions. This grouping is shown in table 9. The two columns at the left show the division into groups, and the pedigree numbers of the plants within each group. The next 5 columns show the number of individual cases of cross-fertility within each group. For example, plant No. 3 was fertile with 5 plants of group B, with 2 plants of group C, with 3 plants of group D, and with the single plant comprising group E. The last 5 columns show the number of individual cases of cross-

TABLE 9

Plants of F_6 generation of cross No. 1, N. Forgetiana × N. alata, grouped in accordance with their behavior in intercrosses.

Group	Ped. No.	Number cases fertile within group					Number cases sterile within group				
		A	B	C	D	E	A	B	C	D	E
A	3	0	5	2	3	1	5	0	0	0	0
	7	0	4	3	3	1	4	**1?**	0	0	0
	10	0	3	3	3	1	3	**1?**	0	0	0
	11	0	4	3	1	1	3	**1**	0	0	0
	13	0	5	3	2	1	2	0	0	0	0
	18	0	5	3	3	–	5	0	0	0	–
B	2	5	**2**	2	1	1	0	3	0	**1**	0
	5	4	**1**	2	3	1	**1?**	3	0	0	0
	9	5	**1**	3	2	1	**1?**	2	0	0	0
	16	6	**2**	3	3	1	0	3	0	0	0
	19	6	**2**	3	3	–	0	2	0	0	–
	20	–	0	1	–	–	–	3	0	–	–
C	6	5	4	0	3	1	0	0	2	0	0
	14	6	5	0	3	1	0	0	2	0	0
	15	6	5	0	3	1	0	0	2	0	0
D	8	4	4	3	0	1	0	0	0	2	0
	12	6	5	3	0	1	0	0	0	2	0
	17	4	3	3	0	1	**1**	**1**	0	2	0
E	1	5	4	3	3	0	0	0	0	0	0

sterility within each group. The exceptional cases where there is inter-class sterility or intra-class fertility are printed in bold-face type. By utilizing the mating record of a plant either when used as a male or female in making the classification, all of the plants could be grouped excepting number 4 which had very bad pollen. It is excluded on this account.

The number of exceptions appear at first sight to be rather large but it must be remembered that one exceptional mating makes two irregularities appear in the table. If 7 is sterile with 9, 9 is sterile with 7, for example, and both exceptions are noted.

Number 2 and number 17 are anomalous plants; the remainder behave very regularly. 2 is sterile with 17 where one would expect to find fertility: this is also true of the mating 17 × 11. Both of these matings were made twice, which establishes the sterility rather definitely. In addition 2 is fertile with both 9 (thrice) and 19 (twice) of the same group, though it properly belongs in group B from its sterility with 5, 16 and 20, and its fertility with at least 1 plant of each of the other groups. The mating between 2 and 9 was fertile only 3 times in 6 trials, however, and may indicate a *pseudo-fertility due to external conditions.* The cross was made reciprocally; 2×9 was fertile in both trials, but 9×2 was fertile but once out of 4 trials.

Eliminating plants 2 and 17 from consideration, there are left only 4 unconformable matings. There are 2 cases of inter-class sterility, 9 with 7 and 10 with 5. Each of these matings was made but once, however, and their sterility is questionable because 4 times per 100 one obtains no seed in matings that otherwise prove fertile. The exceptional fertile matings, 5 with 16 (thrice) and 16 with 19 (twice), on the other hand, appear to be definitely established.

If one admits the possible fertility of combinations 9 × 7 and 10 × 5, then, 16 plants allow themselves to be grouped into five classes A, B, C, D, and E, with no anomalous behavior whatever. Each is cross-sterile with every plant of its own class and cross-fertile with every plant of every other class with which it is tested. True, 3 anomalies remain, plants 2, 16 and 17. Number 17 of class D shows a perfectly regular behavior except with plant 11 of class A and plant 2 of class B. Plants 2 and 16 show their irregularities only within their own class except in the cross between 2 and 17, which leads us to suspect pseudo-fertility.

The conclusion seems just, therefore, that this grouping is real and significant, since the great majority of these plants (in this sample of the population, 84 percent) shows an absolutely regular behavior and the small minority of exceptional plants presents but a few irregularities.

If one admits the justice of this classification there comes the question of the number and composition of such groups in the F_5 generation of this cross. 19 plants form a very small sample of such a population. What is the composition of the whole population? The first thing to be noted is the varied size of the groups. The number of individuals in each class is 6, 6, 3, 3, and 1, respectively. Even with a due allowance for the smallness of the sample, it is clear that there is little probability of the plants being distributed in equal-sized classes. It is hardly more probable that the distribution will fit a Mendelian $(3/4 + 1/4)^n$ expansion. It is reminiscent, however, of a normal binomial expansion $(1/2 + 1/2)^n$. The resemblance is possibly illusory, but 0, 3, 6, 6, 3, 1 is too much like 1, 5, 10, 10, 5, 1 to escape notice, particularly as on the theory of random sampling it is possible for the whole population to contain from 1 to 3 more classes. Be that as it may, we can certainly conclude that the F_5 generation of this particular cross contains *no more* than from 6 to 8 groups—the chances are practically negligible that there might be 10—which are intra-class sterile and inter-class fertile, and within which the distribution of individuals bears some similarity to that of a normal frequency distribution.

Let us now consider whether a possible meaning can be attached to the results obtained in this experiment.

Argument on cross No. 1

We early assumed a working hypothesis in part similar to and in part different from that of MORGAN, viz.; first, self-sterility is heritable; second, as regards that part of the constitution of pollen grains which affects the behavior of self-sterile plants all pollen grains produced by each plant are alike, i.e., with reference to self-sterility pollen grains behave as if they were sporophytic; third, under normal conditions the pollen tubes produced by pollen from any self-sterile plant will not grow in styles of that plant with a rapidity sufficient to reach the ovules during the "life" of the flower, on account of this "likeness" of constitution; fourth, pollen tubes will grow with a rapidity sufficient to allow fertilization to occur if the constitutions of the two proposed parental plants *differ* from each other in any of these essential factors, by reason of a stimulus possibly analogous to that which makes growth more vigorous in first generation hybrids.

The first assumption has been demonstrated more or less clearly by all who have worked upon self-sterile plants. It is proved for self-sterile Nicotiana species both by the experiments reported here, and by those to be published later on the relation between self-fertile and self-sterile plants.

The second assumption is proved circumstantially by the fact that reciprocal matings always duplicate each other. Direct experiments showing that selective fertilization does not occur have also been made, and will be the subject matter of another paper.

If there be any justification for the third and fourth assumptions, a cross between two self-sterile species differing by a large number of hereditary factors (expecting some of the differences to be effective) should show a high degree of cross-fertility in the F_1 and F_2 generations, followed by an increasing percentage of cross-sterility in later generations produced by the closest possible inbreeding. The reason for such a belief is, of course, the well-known fact that inbreeding increases homozygosis. Such being the case, plants ought to appear with "like" constitution as far as the factors affecting cross-fertility are concerned, and these should be cross-sterile to each other. If the factors affecting cross-fertility are relatively few in number, a small number of intra-sterile, inter-fertile groups should be found after a comparatively limited amount of inbreeding. This, broadly speaking, we believe to be a plausible interpretation of the facts found. A detailed interpretation is given later.

In general, the F_2 generation of such a cross—between species—might be expected to show an approach to the maximum limit of cross-fertility, since the F_2 generation usually shows greater variability than succeeding generations. But in the case of self-sterility where the self-sterile plants must be supposed to differ in constitution among themselves, this is probably not strictly true. If one could test a large series of F_2 populations from various original and F_1 matings, he ought to find a variable degree of cross-fertility, with the maximum reached only in certain cases.

In this instance, no claim can be made that we are dealing with the maximum. We can only report the results for this case, pointing out that in crosses No. 2 and No. 3, the cross-fertility is much lower.

One of the best systems of inbreeding in the case of self-sterile plants is to mate sister plants in successive generations, for such crossing, after an original mating $Aa \times Aa$, by Mendelian recombination ultimately gives a population in which AA and aa each approach $\frac{1}{2}$ and Aa approaches 0. Expectation of homozygosis in successive matings is $\frac{1}{2}$, $\frac{5}{8}$, $\frac{11}{16}$, $\frac{24}{32}$ - - - - 1 (JENNINGS 1916). This system seemed to suit our purpose better than any scheme of mating parent with offspring, because of the difficulty of keeping plants alive for several years.

It is regretted that so little is known about the cross-fertility of the F_1 generation, but this bit of ignorance does not affect our test seriously. This really begins with the inter-cross of two self-sterile F_1 plants, which were similar in appearance, but were producing numerous different types of gametes, as is proved by the extremely variable F_2 generation.

The cross-sterility[14] of the F_2 generation was 2.4 percent, if the sixth case of sterility where only one pollination was made, be included. The result on the 20 plants tested rather thoroughly was 3 cases of cross-sterility out of 131 matings. The result on the other twenty-odd plants tested less thoroughly was 3 cases of cross-sterility out of 120 matings. And this percentage of cross-sterility may have been too low, as was mentioned before, because of our failing to suspect pseudo cross-sterility at this time. But taking this low estimate of cross-sterility at its face value, it is clear that no hypothesis of Individualstoffe (Cf. JOST 1905) is necessary to account for the results. The presence of even 6 cases of cross-sterility in 251 matings eliminates this requirement definitely.

The number of classes which would be necessary to give such an amount of cross-sterility, on the assumption of inter-class fertility and intra-class sterility, depends upon what is presupposed as to the frequencies within the classes.

As we shall have a number of such estimations to make, let us consider the matter here. It is always dangerous to calculate *a posteriori* probabilities. But because this danger is realized, and the probabilities calculated must be used with caution, it seems best to use as simple an approximation as possible. Therefore, we have assumed that if S and F represent the total number of sterile and of fertile matings found, the probable error of the determination $\frac{S}{S+F}$ is $\pm \frac{.6745}{S+F}\sqrt{\frac{(S)\,(F)}{S+F}}$. In obtaining this figure, self × self is added to the cross-sterility of course, S representing the total of sterile combinations. The fraction $\frac{S}{S+F}$ then gives us a measure of the probable number of classes for

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[14] In our preliminary paper (EAST 1915) judgment was withheld as to the validity of the apparent cases of cross-sterility in the F_2 generation of this cross. The recovery of a misplaced data card with records of duplicate cross-pollinations made on the combinations that had shown apparent cross-sterility, by Dr. WHITE, gives us the grounds for our present conclusions.

$\frac{S}{S+F}=\frac{\Sigma(C_a^2+C_b^2+\ldots\ldots\ldots C_r^2)}{\Sigma(C_a+C_b+\ldots\ldots\ldots C_r)^2}$, where r is the number of classes, and C_a, C_b, etc., are the number of individuals within each class.

If the classes are of equal size, the ratio of sterility to total number of combinations is $\frac{1}{n}$ where n is the number of classes; for if there are x individuals in each class the sterility is $\frac{nx^2}{n^2x^2}=\frac{1}{n}$. If on the other hand, the distribution of individuals within the classes is that of the coefficients of the point binomial, these coefficients must be substituted.

With these two assumptions as to distribution, the following percentages of sterility to total number of matings is found:

Number of classes	Equal size	Point binomial	Number of classes	Equal size	Point binomial
4	25.0	31.3	15	6.6	15.0
5	20.0	27.3	16	6.2	14.5
6	16.7	24.6	17	5.9	14.0
7	14.3	22.6	18	5.6	13.6
8	12.5	20.9	19	5.3	13.2
9	11.1	19.6	20	5.0	12.8
10	10.0	18.5	21	4.8	12.5
11	9.0	17.6	22	4.5	12.2
12	8.3	16.8	23	4.3	11.9
13	7.7	16.1	24	4.2	11.7
14	7.1	15.5	40	2.5	9.0

Should one wish to make the calculation from cross-sterility only on account of the self-sterility determinations being selected values the formula becomes

$$\frac{S_c}{S_c+F}=\frac{\Sigma(C_a^2+C_b^2+\ldots\ldots\ldots C_r^2)-\Sigma(C_a+C_b+\ldots\ldots\ldots C_r)}{\Sigma(C_a+C_b+\ldots\ldots\ldots C_r)^2-\Sigma(C_a+C_b+\ldots\ldots\ldots C_r)}$$

but this correction is unnecessary under most circumstances.

Taking now the gross returns on the F_2 generation at their face value, 2.4 percent cross-sterility, or 15.8 percent total sterility on the 40 plants used, the number of classes of approximately equal size necessary to account for the results is between 8 and 14. But the groups which were afterward found in the F_5 generation, were not of equal size. Their frequencies resembled rather those of a point binomial. Assuming such a distribution within the classes of F_2, the number of classes would lie between 12 and 25.

These class number determinations have been made roughly on purpose. There are three reasons for doing this.

In the first place, there is reason to believe that the proper percentage of cross-sterility was not obtained. Our calculations were made by including with the matings listed in table 4, 28 matings of plants shown in table 4 with other plants, and 92 matings made rather unsystematically between about 20 plants not shown in that table. Data as to the age, condition, flowering period, etc., of these plants were not recorded. Furthermore, fertility and sterility were usually recorded merely as *F* and *S* without data as regards the percentage of seeds in the capsules. For our present purpose, therefore, they have *not* the value of the data recorded in table 4.

Probably the correct way to treat the data of table 4 would be as follows. Consider every mating as if it were made reciprocally whether actually accomplished or not. For example, 5 × 1 is fertile; then assume 1 × 5 to have been fertile even though that mating was not attempted, since reciprocals always have given the same results. If this be done the records show 184 cases of cross-fertility, 4 cases of cross-sterility and 20 cases of self-sterility. Another question then arises. These plants supposedly were *all* in good condition and in general were mated only at the height of the flowering season. But we did not at that time suspect pseudo cross-fertility, and made no particular attempt to clear up doubtful cases, as was done later on crosses No. 2 and 3. Now crosses 3 × 17, 5 × 15, 6 × 8 and 19 × 14, although made twice each, showed less than 50 percent of the ovules fertilized. The reciprocal of 6 × 8 was clearly fertile, so this mating remains in the "fertile" column. But there is good reason from analogous results in the other families for considering the other 3 matings as sterile. The mating 5 × 15 may be questionable, but as 5 had such bad pollen we cannot be certain of the placing of mating 15 × 5, as was stated earlier. If then we remove these matings from the fertiles to the steriles, which seems the logical thing to do, there are 178 cases of cross-fertility, 10 cases of cross-sterility and 20 cases of self-sterility. The total percentage of sterility is 14.4 (30:208), with very little selective advantage to sterility on account of self-fertilizations.

With these facts in view, we believe it reasonable to assume that between 8 and 14 approximately equal-sized intra-sterile classes or between 12 and 25 intra-sterile classes with the individuals distributed according to the point binomial coefficients, are represented in the F_2 generation,—these being taken as distributional extremes.

The second reason for approximating the number of classes is because the number of individuals investigated is comparatively small, and the probability that they are not a fair sample of the population correspondingly large.

The third reason is that the probable upper limit of the number of classes is all that is essential to our purpose. *The point is, that should the answer lie between 27 and 81 classes, the difference could be accounted for by 1 additional Mendelian factor pair.* The number of actual classes in the F_2 generation of a Mendelian population is 3^n where n represents the number of allelomorphic pairs; and 3^3 is 27, while 3^4 is 81.

Thus it is clear that with the assumptions made previously regarding the cause of self-sterility, our probable maximum cross-fertility can be interpreted by 3 (possibly 4) effective allelomorphic pairs.

For the same reasons for which it was thought best to *correct* the gross percentage of cross-sterility found in the F_2 generation, the later generations of this cross ought to be revised.

Considering then only the matings of the F_3 generation shown in table 5, if one counts reciprocals fertile or sterile as the case may be with the mating made, there are 98 fertile combinations and 6 sterile combinations. But mating 1 × 5, made twice, yielded capsules only 30 and 35 percent full, respectively; and mating 9 × 3, made thrice, yielded capsules only from 20 to 30 percent full. If, as seems probable, these are really sterile matings, the ratio of cross-sterility to the total number of cross-combinations becomes 10 to 104 or 10 percent, and the ratio of total sterility to total number of combinations becomes 22 to 116 or 19.1 percent.

Similarly correcting the results listed in table 6 for the F_4 generation, we find 16.2 percent of cross-sterility in the cross-combinations and 26.2 percent of total sterility in all combinations, with indications that plants 2, 5 and 8 belong in one class, plants 6 and 7 in a second class, and plants 9 and 10 in a third class. This result is obtained thus: there are listed 68 fertile and 6 sterile combinations, but matings 10 × 9 (made twice), 6 × 7 and 7 × 6 are now classed as sterile because they uniformly gave capsules less than 40 percent full.

The cross-sterility of the F_5 generation has already been analyzed sufficiently carefully in explaining table 9. Measured as above it is 22.2 percent.

Unquestionably the samples of the populations from which these results were obtained were so small and the number of matings so few, *that the probable errors are large; but rough as the determinations may be, we think that no one can question the general conclusion that in these three generations from repeated sib matings cross-sterility has increased immensely.*

The cross-fertility of F_2 in this cross, as compared with the cross-fertility in those to be described next, is high. It may not be the maximum cross-fertility possible in a population from one original mating, but it is the highest found in 16 families that we have studied rather thoroughly. High as it is, nevertheless, the probable maximum number of inter-fertile, intra-sterile classes which it contains is *less than 25*, and this number may be interpreted by the permutations of 3 Mendelian allelomorphic pairs. Further the probable number of these classes in the F_5 generation can hardly be more than 8, a figure which may be interpreted by only 2 effective allelomorphic pairs. We were decidedly in error, therefore, when in 1915 we said (EAST 1915): "This is a straight mathematical problem and it is hardly necessary to say that it is insoluble by a strict Mendelian notation such as CORRENS sought to give." In justice it should be said, however, that at that time, the existence of cross-sterility in the F_2 generation was uncertain through a supposed lack of confirmatory data which was really in our possession and had been overlooked.

Cross 2. N. alata × N. Forgetiana (pseudo self-fertile × self-sterile) and cross 3. N. Forgetiana × N. alata (self-sterile × pseudo self-fertile)

The two crosses to be described next are reciprocals made with the same two individuals. It was our intention to repeat the cross just described together with its reciprocal, and to make a more thorough study of the first hybrid generation. At the same time we intended to study the relation between self-sterility and self-fertility by crossing *N. Forgetiana* with a fertile plant of *N. alata,* since *N. alata* was then supposed to be a mixed population consisting of self-sterile and self-fertile plants. Both of these crosses were made. In crosses No. 2 and No. 3 the "self-fertile" daughter of the original supposedly self-fertile plant described on page 534 was used as the *N. alata* parent. Soon after work was started on these plants, our evidence was so conclusive that *N. alata* was always self-sterile and that this particular individual showed only pseudo-fertility caused by external conditions, that we decided to use *N. Langsdorffii* as the self-fertile strain in a series of crosses and to continue this work as a repetition of cross No. 1.

TABLE 10

Result of matings on F_1 plants 0 to 39 of cross No. 2, N. alata × N. Forgetiana and on plants 40 to 52 of cross No. 3, N. Forgetiana × N. alata. Number of pollinations shown by subscripts.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
0	44, 46_3	22_2, 34, 38_3, 49
1	2, 3, 4, 6, 41	8_2
2	4, 18, 41, 44, 52	9, 22, 23_2
3	2, 9, 14, 23, 29	4_4, 6_2, 18_3, 41_3, 46
4	2_2, 9, 10, 44_2	18
5	2, 3, 6, 9, 10_2, 18_2, 46	8_2, 44_2
6	5, 10_2, 43, 44	3_2, 4_3, 18_2, 40_2
7	2, 13, 22, 44	18_3, 46
8	6, 9, 10, 39, 40, 46_3	5_3, 44_4
9	3, 18, 44, 52_2	2_4, 10, 23_2, 37, 48
10	4, 6, 18, 40, 44	2_2, 23, 24_2, 27_3, 34_3, 48_2
11	2, 8, 12, 15, 34, 44, 46	
12	9, 16, 22, 43	6_3, 18_3, 46_3, 52
13	3, 8, 18_2, 44, 46	2, 9_2, 15_3, 21_2, 34_7
14	18_2, 20, 43	10, 34_1
15	1, 3, 16_2, 17, 18, 20	9_5, 13_2, 14, 23_7, 44
16	13, 14, 18, 25, 43_3, 46	17_2, 29_3
17	14, 18, 19, 20, 22, 30	16, 26_5, 44_3
18	2_2, 9_2, 21_2, 23, 28, 34, 36, 44	3, 46_3
19	17, 22, 28, 34, 44	18_2
20	2, 8_2, 9, 16, 18, 20_2, 21, 22_2, 26, 36, 40, 44	43_2
21	4, 12, 16, 18, 46	2, 9_4, 22_2, 25, 27_2, 37
22	12, 42, 44	14, 23_4, 24, 36, 48_3
23	41	9_2, 10_3, 37, 48_2
24	3_2, 6, 20, 26, 28, 44	10_2, 22_2, 23, 30, 37
25	8, 33, 44_2, 46_3	2_2, 9, 23_3, 27
26	9, 18, 22, 23_2, 25, 40, 48	28, 29_3, 44_3
27	3_5, 18, 32, 44, 46	2, 9_2, 30_3, 34_3, 48
28	2_2, 3, 23_2, 27, 39, 46_2	8, 26_2, 29_3, 44_3
29	2, 14_3, 18_2, 22, 23, 24, 25, 30, 34, 37, 41, 46	5_2, 26, 28, 31_4, 44_3
30	8_3, 29, 33, 44_2, 45, 46	9, 21_4, 22_3, 27
31	22, 32, 52	8_2, 29, 36, 44
32	9, 21, 23, 29, 30, 34, 43, 44	18, 33, 46_2
33	8, 16, 23, 31, 46	18_5, 32_2
34	28, 41, 44, 46	10_2, 23_2, 24, 37_2
35	3, 9, 18, 21, 27, 30, 34, 37, 42	8_3
36	8_2, 33, 44_2, 46	10_3, 23
37	39, 42_2, 43, 44, 46_2	9_2, 10_3, 22, 23_4, 34_3, 38
38	28, 35, 39, 42, 43, 46_2	34_3, 37_3, 47
39	9, 44	18, 40_2, 42_3
40	22, 43, 44, 47, 49	6, 33_5, 46_2
41	10, 37, 44, 48	33_2, 40, 46_4
42	20, 44	39_3, 41, 45_2
43	5, 27, 33, 38, 39, 40_2, 42, 44, 46, 51	
44	10, 14, 23, 34, 45	
45	18, 44, 48	46_2, 52
46	10, 22, 37, 44, 51	52_4
47	20, 42, 44, 45, 46, 51, 52	38_2
48	40, 41, 43, 46	10, 23_2, 24_2, 27_2, 34
49	42, 44, 45	0, 9, 27, 34_3, 47
50	18, 39, 51, 52	9, 27_5, 37_2
51	9, 18, 23, 39, 45, 46, 50	8, 29
52	10, 23, 29, 37, 51	3_2, 4_2, 6, 18_2, 41_2, 45_3, 46_2

It is reasonable to consider these crosses in a sense to be repetitions of cross No. 1, but one must not assume that they are duplicates of cross No. 1. Both *N. alata* and *N. Forgetiana* must consist of plants which differ among themselves in the factors that affect self-sterility, hence only by following through a number of F_1 generations where these species are involved could one expect to find results duplicating those of cross No. 1. The data are none the less interesting, however, because the crosses are only similar and not identical.

The F_1 generation

All of the individuals resulting from this cross were grown in a greenhouse as potted plants. The F_1 generation came into blossom during the latter part of the winter. Conditions were extraordinarily favorable for growth and the pollinations were all made while the plants were vigorous, hence scarcely any trouble arose over classification of the results.

Our study was made on a population of 53 plants. Pedigree numbers from 0 to 39 inclusive represent cross No. 2, *N. alata* × *N. Forgetiana;* pedigree numbers 40 to 52 inclusive represent cross No. 3, *N. Forgetiana* × *N. alata.*

Each plant was selfed one or more times, and all proved absolutely self-sterile. Further *each plant* was *back-crossed* with pollen from a single plant of each of the parent species *with complete success in every instance.* The plants used in this work were not the individuals that entered into the cross under discussion, however, for unfortunately these were not available.

The numerous cross-pollinations made are shown in table 10. There were 103 reciprocal matings. Of these 100 gave duplicate results, 39 pairs being fertile and 61 pairs sterile. The three which did not check are:

2 × 3,	sterile,	1 pollination	classed as fertile
3 × 2,	fertile,	1 pollination	
6 × 52,	fertile,	1 pollination	classed as sterile
52 × 6,	sterile,	1 pollination	
37 × 21,	fertile,	1 pollination	classed as sterile
21 × 37,	sterile,	1 pollination	

Since but one pollination was made in each of these cases we have made our decision as to fertility or sterility by a consideration of the circumstantial evidence. The behavior of these plants in other crosses shows conclusively that 3 should be fertile with 2, 6 sterile with 52, and 21 sterile with 37. They have been classed accordingly. That this grouping is correct is further shown by the fact that the mating 3 × 2 (classed as fertile) was made at the height of the flowering season, while the matings 6 × 52 and 37 × 21 (classed as sterile) were respectively the last and next to the last matings made on those plants.

In spite of the fact that plants 0-39 are from cross No. 2, *N. alata* × *N. Forgetiana,* and plants 40-52 are from cross No. 3, *N. Forgetiana* × *N. alata,* they behave as one family in inter-crosses. The entire population can be grouped into 6 classes in which there is inter-class fertility and intra-class sterility (table 11). The following explanation may be necessary to make it clear just how table 11 was obtained from table 10. Table 10 shows all of the matings; but in the form given it is not easy to see at a glance every combination in which a particular plant was used, both as male and as female. It was necessary, therefore, to make a new table in which the pedigree numbers in the column at the left were tabled as males, and the pedigree numbers in the columns headed "Fertile matings" and "Sterile matings" were tabled as females. Thus plant 2 used as a female was fertile with pollen from plants 4, 18, 41, 44, and 52, and sterile with plants 9, 22 and 23; but pollen from plant 2 was fertile on plants 1, 3, 4, 5, 7, 11, 18, 20, 28 and 29, and sterile on plants 9, 10, 13, 21, 25 and 27. It is clear, therefore, that instead of the 8 matings on plant 2 that table 10 appears to show, there are really 21, the 3 reciprocals of course being counted but once.

These tables were combined for analysis. In the interest of economy only one is shown, however, since the second can easily be made from the first.

The four exceptions in this huge set of matings are in reality negligible though they are emphasized in the table by bold-faced type. Matings 15 × 44 and 31 × 36 were sterile, though they do not belong to the same class. Plant 15 was sterile to 4 plants of class A and fertile to 2 plants of class B, 3 plants of class C, and to the isolated individuals forming classes D and F. It is unquestionably a member of class A. Plant 44 was sterile to 7 individuals in class C and fertile to 17 plants of class A, 12 plants of class B and to the singletons forming classes D, E and F. This evidence places it unmistakably as a member of class C. Plant 31 is also a member of class C as evidenced by 3 sterile matings within that class and by fertile matings with 1 plant of class A and 3 plants of class B. Plant 36 is like plant 15 thrown into class A by its sterility with 3 others of that class, and by its fertility with 3 individuals

TABLE II

Plants of F_1 generation of reciprocal cross between N. Forgetiana and N. alata, grouped in accordance with their behavior in inter-crosses. Plants 0-39 are products of cross No. 2; plants 40-52 are products of reciprocal cross No. 3.

Group	Ped. No.	Number cases fertile within group						Number cases sterile within group					
		A	B	C	D	E	F	A	B	C	D	E	F
A	0	0	1	1	—	—	—	4	0	0	—	—	—
	2	0	6	5	1	—	1	8	0	0	0	—	0
	9	0	7	6	1	—	—	13	0	0	0	—	—
	10	0	7	3	—	—	—	10	0	0	—	—	—
	13	0	4	3	—	—	—	5	0	0	—	—	—
	14	0	2	4	1	1	—	4	0	0	0	0	—
	15	0	2	3	1	—	1	4	0	**1**	0	—	0
	21	0	5	2	1	—	—	8	0	0	0	—	—
	22	0	6	5	1	—	—	9	0	0	0	—	—
	23	0	6	5	—	—	—	11	0	0	—	—	—
	24	0	2	4	1	—	—	7	0	0	0	—	—
	25	0	2	5	—	—	—	5	0	0	—	—	—
	27	0	4	3	—	1	—	10	0	0	—	0	—
	30	0	4	5	—	—	—	5	0	0	—	—	—
	34	0	5	4	—	—	1	11	0	0	—	—	0
	36	0	3	2	1	—	—	3	0	**1**	0	—	—
	37	0	5	3	—	1	—	9	0	0	—	0	—
	38	0	3	2	—	1	—	4	0	0	—	0	—
	47	0	5	2	1	—	—	2	0	0	0	—	—
	48	0	4	1	—	1	—	7	0	0	—	0	—
	49	0	3	1	—	—	—	5	0	0	—	—	—
	50	0	3	1	—	—	—	3	0	0	—	—	—
B	3	8	0	5	—	—	—	0	6	0	—	—	—
	4	4	0	2	—	—	—	0	4	0	—	—	—
	6	2	0	4	—	1	—	0	6	0	—	0	—
	7	3	0	1	—	—	—	0	2	0	—	—	—
	12	3	0	1	—	1	1	0	4	0	—	0	0
	18	12	**1**	9	1	—	—	0	11	0	0	—	—
	19	2	0	3	—	—	—	0	1	0	—	—	—
	32	6	0	3	—	1	—	0	3	0	—	0	—
	33	4	**1**	3	0	1	—	0	4	0	—	0	—
	39	4	0	4	—	1	—	0	3	0	—	0	—
	40	5	0	3	1	1	—	0	5	0	0	0	—
	41	6	0	3	—	—	—	0	6	0	—	—	—
	42	5	0	2	1	1	—	0	3	0	0	0	—
	45	4	**1**	2	—	—	—	0	3	0	—	—	—
	46	14	**1**	7	—	1	1	0	9	0	—	0	0
	52	7	0	3	—	—	—	0	8	0	—	—	—
C	1	2	4	0	—	—	—	0	0	1	—	—	—
	5	3	4	0	—	1	—	0	0	3	—	0	—
	8	6	5	0	1	—	1	0	0	7	0	—	0
	16	5	4	0	1	1	—	0	0	2	0	0	—
	17	4	2	0	1	—	—	0	0	3	0	—	—
	26	6	2	0	1	—	—	0	0	4	0	—	—
	28	6	5	0	—	—	—	0	0	4	—	—	—
	29	9	6	0	—	—	—	0	0	7	—	—	—
	31	1	3	0	—	—	—	**1**	0	3	—	—	—
	35	7	3	0	—	—	—	0	0	1	—	—	—
	44	17	12	0	1	1	1	**1**	0	7	0	0	0
	51	4	5	0	—	1	—	0	0	2	—	0	—
D	20	9	3	5	—	1	—	0	0	0	—	0	—
E	43	5	8	4	1	—	—	0	0	0	0	—	—
F	11	3	2	2	—	—	—	0	0	0	—	—	—

of class B, with 2 of class C, and with the lone plant of class D. In view of this evidence and the fact that in these two matings but one pollination was made in each case, they are much more likely to be errors of record or of technique than true exceptions to our classification.

The other two exceptions, matings 45 × 18 and 33 × 46, were fertile where from the evidence of numerous other matings they should have been sterile. Here again but one pollination was made in each case; and, coincidence though it may be, *each pollination was the last mating made on that particular plant.* What is more probable than that this is a pseudo fertility appearing during the wane of the flowering season of the two mother plants, No. 45 and No. 33?

Six groups appear in table 11, but there is proof of the existence of only five. Groups A, B, C, D and E are definitely established. Plant 11, on the other hand, is an isolated individual rather than a class. It does not belong to groups A, B, or C; but unfortunately it was not crossed either with class D (plant 20) or with class E (plant 43), hence one cannot say that it does not fall into one or the other of these two classes.

In the three large groups the distribution of individuals is 22, 16 and 12. About all that can be said about the type of this distribution is that the classes appear not to be of equal size. On the other hand, it is interesting to note that the plants of both cross No. 2 and cross No. 3 fell into the three groups as if they were samples of the same population. There were 40 plants of cross No. 1, and 12 plants of the reciprocal cross No. 2. In the classes A, B and C the proportions were 18, 10, 10 and 4, 6, 2, respectively. This similar behavior of the progeny of reciprocals seems to us strong corroboratory evidence in favor of the conclusion that reciprocal crosses always behave in like manner as regards self-sterility.

It is interesting here to check our *a posteriori* probabilities with the facts. There were 278 fertile matings made in this family, of which 39 were reciprocals, making 478 (278 × 2—78) fertile combinations altogether. There were 167 sterile matings, of which 61 were reciprocals, making a total of 212 (167 × 2—122) cross-sterile combinations. If to the cross-sterile combinations, the 53 self-sterile combinations be added, there is a total of 265 sterile combinations out of 743,—a percentage of 35.6 ± 1.2. Assuming a point binomial distribution of individuals we should expect 4 intra-sterile classes for this percentage of sterility; but since we must discount the selection of self-combinations a little, perhaps 5 classes may be taken as the probable expectancy.

It was planned to continue the study of this family—considering it as a single cross—on populations obtained by back-crossing a representative of each of the large classes A, B, and C with both parents, and by intercrossing the same three individuals among themselves. This rather Herculean task has not been finished. The progeny of a part of these matings was investigated as thoroughly as time permitted in 1915-16, but much remains to be done. These families came from the following combinations:

Family D, *N. alata* plant 53 × plant 44 of class C
Family E, *N. alata* plant 58 × plant 44 of class C
Family F, plant 34 of class A × *N. Forgetiana*
Family G, plant 44 of class C × *N. Forgetiana*
Family H, plant 44 of class C × plant 10 of class A
Family I, plant 44 of class C × plant 34 of class A
Family J, plant 52 of class B × plant 23 of class A
Family K, plant 52 of class B × plant 44 of class C

In families D and E we have two *N. alata* plants 53 and 58 crossed with the same plant of cross 3 (table 11), No. 44 a member of class C. Families F and G were produced by crossing individuals of classes A (34) and C (44) with the same plant of the other parent species *N. Forgetiana*. The four remaining families are true F_2 generations formed by mating two F_1 plants. There is a duplicate test of plant 44 (class C) with two plants of class A, 10 and 34. Then there is a test of plant 52 (class B) with plant 23 of class A and plant 44 of class C. Thus plant 44 of class C enters into two back-crosses with *N. alata*, one back-cross with *N. Forgetiana*, and matings with two individuals belonging to class A and one individual belonging to class B.

Family D,—*N. alata* plant 53 × plant 44 of class C, cross No. 3

The first of the eight F_2 populations of crosses No. 2 and No. 3 was produced by back-crossing. Plant 53 of *N. alata* (table 1), a plant apparently[15] fertile with sister plants 57 and 58, and sterile with sister plants 54, 56 and 59, was crossed with the pollen of plant 44 of class C, cross 3. In a manner of speaking, it may be called $P_1 \times F_1$, if it be remembered that plant 53 is not the same plant of *N. alata* used in making cross No. 3.

Table 12 shows the self-pollinations made on 39 plants. They behaved in much the same manner as the *N. alata* plants recorded in tables 1-3. One-third of them produced some seed, though from 1 to 10 failures

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[15] See page 533.

TABLE 12

Family D.—Record of self-pollinations on progeny of N. alata plant 53 × plant 44 of F_1 of cross No. 3

Ped. No.	No. selfings sterile	No. of selfings giving capsules with		
		1-10 seeds	10-50 seeds	250-300 seeds
151	11			
152	15			
153	6			
154	10			
155	14			
156	10	1		
157	1			
158	4			
159	2			
160	2			1
161	1			
162	8			
163	3			
164	7			
165	3	2		6
166	10	2		
167	7			
168	1			
169	1			
170	9			
171	5		2	
172	1			
173	2			
174	10			
175	1			1
176	3			
177	10	2		
178	11		5	
179	5			
180	4			
181	5			
182	8		1	
183	5		1	
184	3			
185	1	4		
186	6		1	
187	8	4		
188	16			
189	9			

were also recorded for the same plants. The remaining plants produced no capsules. There was an extremely high correlation between this partial fertility which we have regarded as false, and the close of the reproductive period. Yet one cannot say that every plant can be made to produce seeds at this phase of the life cycle, even under adverse conditions. This may be the case, but we have been unable to demonstrate it. 4 plants in this family, however, gave a very nice demonstration of the fact that complete self-sterility returns with the return of a new flowering season. A number of these plants were selfed at various times during two flowering periods, and plants 156, 166, 177 and 178, though giving a

TABLE 13

Family D.—Record of cross-pollinations on progeny of N. alata plant 53 × plant 44 of F_1 of cross No. 3 outside of family D.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
152		204 Family E
153		204 Family E
167	201 Family E	
171	201 Family E	
174	58 *N. alata*	

TABLE 14

Family D.—Record of cross-pollinations on progeny of N. alata plant 53 × plant 44 of F_1 of cross No. 3.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
151	159	
152	160	185
153		152
154	151	153
155		154
157	154	
159		
160		174
161		162
162		163_2, 168
163	185	
168	173	
170	168	
172	175	
173	159_2	
174		
175		185
177	168, 182_2	183
179	177	
180	177	
181		183
182	160, 183	185
183		177
184	185	188
185	160, 174	
186	185	160?
187		185
188	185	

few poor capsules at the end of the first flowering season, showed complete self-sterility from the beginning to the height of the second flowering period. Then, in two cases, the slight degree of fertility shown at the end of the first flowering season returned. 3 plants produced full capsules. No. 160 and No. 175 yielded 1 each, both according to the late

TABLE 15

Family D.—Progeny of N. alata plant 53 × plant 44 of F_1 of cross No. 3 grouped in accordance with their behavior in inter-crosses.

Group	No. Ped.	No. cases fertile within group						No. cases sterile within group					
		A	B	C	D	E	Ind.	A	B	C	D	E	Ind.
A	152	0	1	—	—	—	—	2	0	—	—	—	—
	153	0	—	—	—	—	—	2	—	—	—	—	—
	154	0	—	—	—	—	2	2	—	—	—	—	0
	155	0	—	—	—	—	—	1	—	—	—	—	—
	175	0	—	—	—	—	1	1	—	—	—	—	0
	182	0	1	—	2	—	—	1	0	—	0	—	—
	185	0	3	1	—	2	—	4	0	0	—	0	—
	187	0	—	—	—	—	—	1	—	—	—	—	—
B	160	3	0	—	—	—	—	0	2	—	—	—	—
	174	1	0	—	—	—	—	0	1	—	—	—	—
	186	1	0	—	—	—	—	0	1	—	—	—	—
C	161	—	—	0	—	—	—	—	—	1	—	—	—
	162	—	—	0	—	—	—	—	—	3	—	—	—
	163	1	—	0	—	—	—	0	—	1	—	—	—
	168	—	—	0	1	—	2	—	—	1	0	—	—
D	177	1	—	1	0	—	2	0	—	0	1	—	0
	181	—	—	—	0	—	—	—	—	—	1	—	—
	183	1	—	—	0	—	—	0	—	—	2	—	—
E	184	1	—	—	—	0	—	0	—	—	—	1	—
	188	1	—	—	—	0	—	0	—	—	—	1	—

TABLE 16

Family E.—Record of cross-pollinations on progeny of N. alata plant 58 × plant 44 of F_1 of cross No. 3.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
191		197
192		191
193		44_3, 58_3
194		58_2
195		193, 204
197		199
199		195
200		204
202		197
204		152 Family D
205		204

season expectation. No. 165, on the other hand, was somewhat of an exception to the usual rule, in that it produced 6 full capsules out of 11 pollinations. There were 2 failures and 2 small capsules with from 1-10 seeds each from pollinations made during the height of the flowering season. Toward the end of the flowering period the plant was tested again and yielded 6 good capsules out of 7 flowers selfed.

Five plants of family D were crossed with individuals outside of that group, as is shown in table 13. 2 plants, 167 and 171, were fertile with the pollen of plant 201 of family E, while 2 other plants, 152 and 153, were sterile to the pollen of plant 204 of family E. Plant 174 was fertile with *N. alata* plant 58.

Only 36 cross-matings were made between plants of this family (table 14). Of these, 16 were failures. In spite of this small number of intercrosses, 20 out of 28 plants can be shown to belong to not over 5 classes wherein the plants are intra-class sterile and inter-class fertile (table 15). The other 8 plants show only 1 or 2 cases of cross-fertility and no cross-sterility, and may or may not belong to separate groups. Their fertility with the other classes is shown in the column marked "Indeterminate."

There are no exceptions in table 15. Each plant in every group is wholly intra-class sterile and inter-class fertile as far as it was tested. But these five groups are not necessarily independent. A is not B, C, D, E, 151, 157, or 172; B is not A; C is not A, D, 170 or 173; and D is not A, C, 179 or 180. Therefore B may be C, etc., and the existence of only three groups is demonstrated.

An estimation of the number of classes by formula is hardly desirable on account of the small number of combinations made per plant, though the total number of combinations is larger than appears at first sight because only 1 reciprocal (sterile) was made. There are really 70 combinations of which 30 are sterile, a cross-sterility percentage of 42.8.

Family E.—*N. alata* plant 58 × plant 44 of class C of cross No. 3

Family E resulted from a cross between *N. alata* plant 58 and plant 44 of class C, cross No. 3. The interesting thing about the family is its lack of fertility not only when selfed but also in crosses. 10 plants were mated together in such a manner that the chain of evidence was not broken, as can be seen by studying table 16, with no evidence whatever of any fertility between them. *They all belong to one class showing perfect intra-class sterility.* In addition, if one may assume that all of the individuals would have behaved as plants 193 and 194, the group was sterile to the 2 parents. Plant 204 was also sterile reciprocally with plant

152 of family D, and as a male with plant 153 of family D. The only sign of cross-fertility shown was when pollen from plant 201 (which also belonged to family E) was used on plants 167 and 171 of family D, yet in appearance the pollen of these plants was perfectly good.

It is unfortunate that the behavior of more plants of this family was not investigated, but a good many plants needed attention at the same time during the period these were in flower, and the importance of establishing definitely whether the entire family belonged to one class was overlooked until too late. It is clear, however, that if other classes existed, they must have contained relatively fewer individuals than the one found.

Judged by its parents family E appears to be a duplicate of family D. *N. alata* plant 58 was apparently fertile to its sister plants 53 and 59, and sterile to its sister plants 54, 56, 57, 62, 64, 66, 71 and 79; plant 53, the female parent of family D, was apparently fertile to plants 57 and 58,

TABLE 17

Family F.—Record of self-pollinations on progeny of plant 34 of F_1 *of cross No. 2 × plant AA of N. Forgetiana.*

Ped. No.	No. selfings sterile	No. of selfings giving capsules with		
		1-10 seeds	10-50 seeds	250-300 seeds
207	9			
211	8			
212	4			
214	11			
215	1			
216	22			
217	12			
218	8			
219	5			
225	11	1		
227	3			
228	12			
229	1			
230	10			
231	10			
232	9			
233	1			
234	1			
235	5			
236	6	1		
237	6			
239	9			
240	11			
241	1			
242	12		1	
243	18			
244	1			

and sterile to plants 54, 56 and 59 of the same family. But considering the behavior of *N. alata* plants 53-79 of table 1 as a whole there is good reason to believe that they all belong to 1 intra-sterile class and that the fertility of matings 53 × 57, 58 × 53 and 58 × 59 is pseudo-fertility. For this reason one might expect family D and family E to behave similarly; but unless one assumes the existence of other classes of low frequency in family E, their behavior was different.

Family F.—Plant 34 of class A × plant AA of *N. Forgetiana*

Family F resulted from crossing plant 34 of class A, cross No. 2, with a plant of *N. Forgetiana;* but, as in families D and E, it was not a true back-cross, since the plant of *N. Forgetiana* used was not the individual that participated in the original mating.

Selfings were made on 27 hothouse-grown plants with the results shown in table 17. It will be noticed that only 3 individuals produced any seeds at all. No. 225 yielded 1 capsule containing 8 seeds in 12 tests; No. 236 produced 1 capsule containing 7 seeds in 7 trials; and No. 241 finally produced a single capsule having about 30 seeds after 12 pollinations. This is a considerably smaller seed production than was recorded for family D, and we believe it to be due to the fact that family F came into blossom somewhat later than family D, thus making it practicable to conclude the pollinations during the height of the flowering season.

A few pollinations were made between plants of this family and plants of family G, the results of which are set forth in table 18. They will be discussed when describing that family.

We were able to make 151 cross-matings on this family, with the results shown in table 19. Some of these matings, unlike the self-pollinations were made rather late in the flowering season. These made trouble in some cases, and had to be repeated several times before a proper decision as to fertility or sterility could be made. In all there were 17 matings that gave seeds in some tests and none in other trials. If the capsules were full and the majority of pollinations succeeded, the mating was called fertile; if the capsules were small and poorly filled, and the majority of the pollinations failed completely, the mating was called sterile.

These 17 matings, we believe, are listed correctly, but there are a few matings made but once during the latter part of the season which may be recorded erroneously.

In addition, plant No. 225 had poor pollen and decision as to the

TABLE 18

Family F.—Record of cross-pollinations on progeny of plant 34 of F_1 of cross No. 2 × plant AA of N. Forgetiana outside of family F.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
216	278 fam. G.	247 fam. G.
219		250 fam. G.
239		247 fam. G.
241	250 fam. G.	
243		247 fam. G.
244	247 fam. G.	

TABLE 19

Family F.—Record of cross-pollinations on progeny of plant 34 of F_1 of cross No. 2 × plant AA of N. Forgetiana.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
207	211, 216, 225	
209	212, 216, 231	
211	209_3, 214, 216	212_2, 215
212	214_2, 216_4, 231	
214	209, 211, 216_2, 217, 219, 228	
215	214, 217_2, 219, 222	
216	212, 217_3, 219_2, 223	239
217	216_2, 218, 219_2	212
218	217, 219	
219	214_2, 215, 216, 217_4, 218_2, 227	228
221	227	
222	217_2, 218, 219_2	
223	216, 225_3, 227, 228, 230, 236	
224	217, 219, 223, 225_2	
225	216, 217_2,, 219, 221, 223, 228, 230_2, 234_2, 235	
226	227_2, 228, 230, 234_3	223
227	219_2, 224, 225_3	
228	222, 223_2, 225_2, 227, 230	219
229	209, 214, 216, 219, 231_4	
230	223, 225_2, 227, 234, 236	
231	212, 214, 219, 229_5	
232	236, 239_2, 243	219, 234
233	223, 234, 239	
234	225, 226, 230_3, 239_3	219, 228, 232_2
235	236_2, 239	232
236	232, 234_2, 239_2, 243	233
237		235_2
238	239, 243_2 244_2	
239	219, 232, 235, 236, 240	243_2
240	234, 236, 239, 243	
241	234_2, 236, 244_3	239, 243_3
242		239, 243_2
243	234, 244_2	239_4, 241, 242
244	236, 238_2, 239_2, 241_2, 243_2	
245	238_2, 241_2, 243, 244	

character of three matings (with 219, 227 and 230) was made on the basis of the successes obtained when No. 225 was used as female.

There were 23 unsuccessful and 128 successful cross-matings in this family. Of these combinations, 55 were reciprocals fertile in both matings and 10 were reciprocals sterile both ways.

Eighteen of the plants can be grouped into 6 inter-class fertile, intra-class sterile groups of 2 or more plants each (table 20), but these groups are not necessarily independent. A is not B, C, D or F; B is not A, C, D, E or F; C is not A or B; B is not A, B or F; E is not B or F; and F is not A, B, D or E. Therefore, C may be D, E or F; D may be C or E; E may be C or D; and F may be C. But since 2 of these alternatives are mutually exclusive, it is definitely established that at least 4 of these groups are independent of each other.

This matter is shown more clearly in table 21, where the 17 other plants which exhibited no cross-sterility are also listed. From this table by the process of elimination cited above it can be shown that 5 separate inter-class fertile, intra-class sterile groups must exist. Since there are 16 plants unplaced because they have had only a few cross-matings made upon them, however, it may be well to compare the number of classes proved with the number to be expected from the percentage of sterility

TABLE 20

Family F.—Progeny of plant 34 of F_1 of cross No. 2 × plant AA of N. Forgetiana grouped in accordance with their behavior in inter-crosses.

Group	Ped. No.	No. cases fertile within group						No. cases sterile within group					
		A	B	C	D	E	F	A	B	C	D	E	F
A	219	0	2	1	—	—	—	3	0	0	—	—	—
	228	0	—	—	2	—	—	2	—	—	0	—	—
	232	0	2	—	—	0	1	2	0	—	—	1	0
	234	0	3	—	1	—	2	3	0	—	0	—	0
B	216	1	0	2	1	—	—	0	1	0	0	—	—
	239	3	0	—	—	1	2	0	4	—	—	0	0
	241	1	0	—	—	—	1	0	2	—	—	—	0
	242	—	0	—	—	—	—	—	2	—	—	—	—
	243	2	0	—	—	—	1	0	3	—	—	—	0
C	211	—	1	0	—	—	—	—	0	2	—	—	—
	212	—	1	0	—	—	—	—	0	1	—	—	—
	215	1	1	0	—	—	—	0	0	1	—	—	—
D	223	1	1	—	0	—	2	0	0	—	1	—	0
	226	2	—	—	0	—	—	0	—	—	1	—	—
E	235	0	1	—	—	0	1	1	0	—	—	1	0
	237	—	—	—	—	0	—	—	—	—	—	1	—
F	233	1	1	—	1	—	0	0	0	—	0	—	1
	236	2	3	—	1	1	0	0	0	—	0	0	1

TABLE 21

Family F.—Progeny of plant 34 of F_1 of cross No. 2 × plant AA of N. Forgetiana grouped to show inter-class fertility.

	A	B	C	D	E	F	207	209	214	217	218	221	222	224	225	227	229	230	231	238	240	244	245
A		7	1	3		3			2	1	1		2	1	3	2	1	2	1		1		
B	7		2	1	1	4	1	1	1	1					1		1			2	2	3	2
C	1	3					1	2	3	1			1						1				
D	3	1				2								1	1	2		2					
E		1				1									1								
F	3	4		2	1													1			1	1	
207		1	1												1								
209		1	2						1								1		1				
214	2	1	3					1		1							1		1				
217	1	1	1						1		1		1	1	1								
218	1									1			1										
221															1	1							
222	2		1							1	1												
224	1			1						1					1	1							
225	3	1		1	1		1			1		1		1		1		1					
227	2			2								1		1	1			1					
229	1	1						1	1										1				
230	2			2		1									1	1							
231	1		1					1	1								1						
238		2																				1	1
240	1	2				1																	
244		3				1														1			1
245		2																		1		1	

found, on the theory of a distribution of individuals corresponding to the frequencies of the coefficients of the binomial expansion. In family F there are 128 fertile matings, of which 55 are reciprocals, a total of 146 (128×2—110) fertile combinations. Likewise there are 23 sterile matings, of which 10 are reciprocals, a total of 26 (23 × 2—20) sterile combinations. This amounts to a cross-sterility of 15.1 percent. Adding the 35 self-combinations to the steriles, gives 61 cases of sterility out of 207 combinations,—a percentage of 29.4. We should expect only about 5 intra-sterile classes in this population, therefore, unless a very broad allowance is made for *selection* of matings that were sterile.

TABLE 22

Family G.—Record of cross-pollinations on progeny of plant 44 of F_1 of cross No. 3 × plant AA of N. Forgetiana outside of family G.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
247	44 ♀ parent, F_1 plant	
249	44 ♀ parent, 351_3, fam. H, 467_2, fam. I	
258	34_2 F_1 plant	
278	219 fam. F, 374 fam. H, 467 fam. I	
281	405_2 fam. I	
293	44 ♀ parent, F_1 plant	
308	34_2 F_1 plant	

Family G.—Plant 44 of class C, cross No. 3 × plant AA of *N. Forgetiana*

Family G was produced by mating plant 44 of class C, cross No. 3, with the same plant of *N. Forgetiana* used in producing family F. In all, 53 hothouse-grown plants had some work done upon them, although in a few cases only one mating was made. These plants were studied during a complete flowering season, but nearly all of the work was completed before the period of decline in reproductive vigor so that only a few cases of pseudo-fertility were found. 31 of the plants were selfed from 1 to 19 times with the production of a few seeds in one attempt at selfing only (308). In 12 other matings there was some conflict in the results. These were classified, as before, by recording as fertile those that gave full capsules in two or more trials even though one trial failed, or by recording as sterile those in which a majority of the trials failed even though a portion of the pollinations did produce a few seeds (less than 15 percent of normal).

Table 22 records the crosses made when plants outside of family G

TABLE 23

Family G.—Record of cross-pollinations on progeny of plant 44 of F_1 of cross No. 3 × plant AA of N. Forgetiana.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
247	248_7, 250_4, 253_4, 256, 263, 276	
248	247	
249	247_2, 250_2, 256	
250	247_3, 249	253
251	270	
252	247_2, 249, 256, 257	250, 255
253	247_3, 256, 262	255
255	249, 262	252
256	253	
257	255, 256_2	
258	247, 257, 269	270_2
259	258_3, 262	
260	251_2, 255, 262	
262	266, 271	
263	250, 253	258_2
265	263, 266, 269, 276_2, 278	
266	263, 265, 270_2, 281	
267	251	
268	284	289
269	270, 281	
270		260
271	274	
272	255, 256, 270, 281	
273	275, 276, 281	
274	269, 275_2	270_2
275	258, 270_2, 274, 278	
276	270, 275, 281	
278	269, 274, 285, 289	281_2, 284_2
279	289	281
281	276_3, 284	275, 278
283	270	276
284	281, 285, 289	
285	289, 293, 306, 309	304_3
286	278, 284	289
288		289
289	265, 269, 284, 293_2	286, 306
290	258, 278, 289	
291		284
293	274, 285_2, 289, 290	284
295	310	
297	289	
298	284_2	
303	285_2, 304, 306_2	
304	293, 306_2, 309, 310	307
305	312	311
306	284, 293, 304_3, 309, 310	289
307	310	304
308	312	
309	304_4, 310_3, 311	308
310	304, 308, 309_2, 311, 312	
311	308_4, 309_2, 310_2	
312	309, 310_2	311_3

were used as pollen parents. The 11 matings tried were all successful. 3 back-crosses were made with plant 44, 2 with plant 34 of F_1, 2 with plants of family H and 3 with plants of family I. It should be noted, however, that of 7 crosses of plants of family F with pollen from individuals of family G, 4 were failures. On the other hand, G family pollen was fertile on 3 plants of family H (table 27) and on 1 plant of family I (table 30).

Table 23 shows the cross-matings made within family G. There were 126 successful matings,—19 being pairs of reciprocals,—making 214 successful combinations. 29 matings were sterile, including 5 pairs of reciprocals,—48 combinations in all. 314 combinations have been made, therefore, 100 being sterile (52 selfs + 48 crosses) and 214 fertile. The probable sterility is thus 31.2 percent ± 1.8 percent.

Table 24 shows 27 plants of this family grouped in accordance with

TABLE 24

Family G.—Progeny of plant 44 of cross No. 3 × plant AA of N. Forgetiana grouped in accordance with their behavior in inter-crosses.

Group	Ped. No.	No. cases fertile within group						No. cases sterile within group					
		A	B	C	D	E	F	A	B	C	D	E	F
A	250	0	1	—	—	—	—	2	0	—	—	—	—
	252	0	—	—	—	—	—	2	—	—	—	—	—
	253	0	1	—	—	—	—	2	0	—	—	—	—
	255	0	1	—	—	—	—	2	0	—	—	—	—
B	258	—	0	1	—	—	—	—	2	0	—	—	—
	260	1	0	—	—	—	—	0	1	—	—	—	—
	263	2	0	—	—	—	—	0	1	—	—	—	—
	270	—	0	1	—	—	—	—	3	0	—	—	—
	274	—	0	3	—	—	—	—	1	0	—	—	—
C	275	—	3	**1**	—	—	—	—	0	1	—	—	—
	278	—	1	**1**	2	1	—	—	0	2	0	0	—
	279	—	—	0	1	—	—	—	—	1	0	—	—
	281	—	—	**1**	—	—	—	—	—	3	—	—	—
	284	—	—	**1**	4	—	—	—	—	3	0	—	—
	291	—	—	0	—	—	—	—	—	1	—	—	—
	293	—	1	0	2	2	—	—	0	1	0	0	—
D	268	—	—	1	0	—	—	—	—	0	1	—	—
	286	—	—	2	0	—	—	—	—	0	1	—	—
	288	—	—	—	0	—	—	—	—	—	1	—	—
	289	—	—	4	0	1	—	—	—	0	4	0	—
	306	—	—	2	0	2	—	—	—	0	1	0	—
E	285	—	—	3	2	0	—	—	—	0	0	1	—
	304	—	—	1	1	0	—	—	—	0	0	2	—
	307	—	—	—	—	0	—	—	—	—	—	1	—
F	305	—	—	—	—	—	**1**	—	—	—	—	—	1
	311	—	—	—	—	—	0	—	—	—	—	—	2
	312	—	—	—	—	—	**1**	—	—	—	—	—	1

TABLE 25

Family G.—Progeny of plant 44 of cross No. 3 × plant AA of N. Forgetiana grouped to show inter-class fertility.

	A	B	C	D	E	F	247	248	249	251	256	257	259	262	265	266	267	269	271	272	273	276	283	290	295	297	298	303	308	309	310
A		3					3		3		2	2		2						1											
B	3		5				2			2		1	1	1	1	2		3	1	1		1	1	1							
C		4		9	3										1	1		2		1	2	2		2			1				
D			9		3										1			1						1		1		1		1	1
E			4	3																								2		2	2
F																													2	2	2
247	3	2						1	1		1											1									
248							1																								
249	3						1				1																				
251		2															1														
256	2						1		1			1								1											
259		1									1																				
257	2	1												1																	
262	2	1											1			1			1												
265		1	1	1												1		1				1									
266		2	1											1	1																
267										1																					
269		3	2	1											1																
271		1												1																	
272	1	1	1								1																				
273			2																			1									
276		1	2				1								1						1										
283		1																													
290		1	2	1																											
295																															1
297				1																											
298			1																												
303				1	2																										
308						1																									1
309				1	2	2																									1
310				1	2	2																			1				1	1	

their behavior in inter-crosses. There are 6 classes as tabled with a frequency of 3, 5, 7, 5, 4, 3. There are 3 exceptions among the fertile matings, 275 × 278, 281 × 284 and 305 × 312. Only one pollination each was made on the first and third of these combinations, but the second was made reciprocally—*the last of the flowering season*—one pollination each way. *There were no sterile exceptions.*

Though 6 intra-sterile groups are tabled, there is definite proof of the existence of only 3 classes. This is easily seen by referring to the table. Classes C, D and E must be different, but the other 3 groups might have proved to fall in with them had the proper crosses been made. Nor can the existence of more than 4 intra-class sterile groups be proved even by the complete table of inter-class fertility shown as table 25. By our probability formula also the presumption is that there are but 4 or 5 classes, whether the distribution of individuals be according to the coefficients of the binomial expansion or into classes of equal size.

Family H.—Plant 44 of class C, cross No. 3 × plant 10 of class A, cross No. 2.

Family H was one of the 30 true F_2 populations possible from combinations of the 6 different F_1 classes. It was produced by crossing plant 44 of class C, cross No. 3 with pollen from plant 10 of class A, cross No. 2. 70 plants were grown in the greenhouse. Self-pollinations were made on 33 of these individuals with the results listed in table 26. In view of previous results it seemed hardly necessary to self every member of the population. If this had been done a truly self-fertile plant might have been discovered, of course, but it is exceedingly improbable. Of those selfed, 5 did produce some seed,—the amounts being shown in the table. These capsules were all produced at the very end of the flowering season, except 1 with 8 seeds in it on plant 316. There is a chance that these seeds were produced by foreign pollen, though it is hardly necessary to "explain" such a rare exception to the general rule.

This family was studied through a long flowering season. Many matings were made, and the work completed before we were certain of the effects of environment on self-sterility. For this reason some of the matings made toward the end of the season were not tested as thoroughly as should have been done. Further, no records of the number of seeds were taken in the case of several capsules that were not full. Thus it is altogether likely that several matings marked fertile were in reality sterile. The maximum number of such errors, we should judge from a careful examination of our records ought not to be over 10.

It is also probable that the usual experimental error of 4 failures per hundred in actually fertile matings obtains in cases where a mating was made but once and proved sterile. There were 63 such matings in the intra-family crosses, thereby making 3 such errors probable. The remaining combinations were judged by several matings and by reciprocal crosses, and are likely to be correct.

It is clear that the errors mentioned above are largely compensatory when figuring the percentages of fertility or sterility in the matings made, but they will stand revealed when endeavoring to group the individuals in intra-sterile classes.

The record of back-crosses and crosses made with plants outside of

TABLE 26

Family H.—Record of self-pollinations on progeny of plant 44 (F_1, cross No. 3, class C) × plant 10 (F_1, cross No. 2, class A).

Ped. No.	No. selfings sterile	No. of selfings giving capsules with			
		1-10 seeds	10-50 seeds	50-150 seeds	250-300 seeds
314	1				
315	4		2	2	
316	9	1	3		
317	4				
318	3				
321	1				
324	1				
330	1				
331	2				
332	6				
333	1				
334	1				
335	3				2
336	1				
340	1				
342	3				
347	1				
350	3		1		
351	1				
353	1				
354	10				
358	9		1		
362	4				
363	7				
368	4				
370	1				
371	3				
373	4				
374	2				
378	1				
381	2				
382	2				
385	1				

TABLE 27

Family H.—Record of cross-pollinations on progeny of plant 44 (F_1, cross No. 2, class C) × plant 10 (F_1, cross No. 2, class A) outside family H.

Ped. No. ♀	Fertile with parents ♂	Sterile with parents ♂	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
314	44, 10_2			
315	44_2			
317	44_2	10_4	311 fam. G	34_3 F_1
318		10		
319		10_2	311 fam. G	
320	10_2			
321		10_4	311 fam. G	
322		10_3		
324		10		
327	44_3	10_2		
328	44_2			
329	44_3			
330		10_4		
331	44_6	10		
334		10		
335	44_2, 10	10		
337		10	477 fam. J, 524 fam K	
339				18 F_1
340		10		
342	44_2, 10 poor	10_3		467 fam. I
349		10		
350	44	10_2		
351		10		
354	44_2	10_4	467 fam. I	34_4 F_1, 401 fam. I
362	10, 8 seeds	10_3		34_2 F_1
363	44	10_4		
365	44			
366		44		
367	10	10		
368	10_2		467 fam. I	
371	44		467 fam. I	
373	44_1, 10			
374	44_3			401 fam. I, 467_2 fam. I
378	44			
379	44_4	10_2		
381	44, 10		405 fam. I, 415 fam. I	
382	44_2, 10_2			
384	44			
385	44_2	10		

family H, are shown in table 27, but they can be discussed best after dealing with the intra-family matings.

Excluding selfings, 312 intra-family matings were made on 56 plants. If we take all of these plants to be self-sterile,—a reasonable assumption even though a few of them were not selfed—448 combinations out of a possible 3136 were attempted. The figure 448 is the sum 153 × 2 = 306 fertile matings, minus 100, the number of fertile reciprocals, plus 159 × 2 = 318 sterile matings, minus 132, the number of

TABLE 28

Family H.—Record of cross-pollinations on progeny of plant 44 (F_1, cross No. 2, class C) × plant 10 (F_1, cross No. 2, class A).

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
315		316, 317, 318_2
316	320, 324	317_2, 318, 321, 331
317	320	315_2, 318, 321, 327_2, 328_3
318	320_2, 328	315_3, 316, 317_5, 321_2, 324
319	347	315, 316, 317_5, 354_2, 381
320	317, 318, 321, 322, 324_2, 328_2, 354_2, 381	342, 351
321	320_2, 335_2, 381	315_2, 316_2, 322_2, 328_2
322	320_2, 325, 381_2	321, 324, 328, 329
324	320_3, 325, 342, 367_2, 379_2	322, 327, 328, 331, 354_2
325	317, 322_2, 324, 329	
327	351	330, 336_3, 337_2, 340_2, 345
328	335, 337	317, 324, 327, 329, 342
329	325, 347	324_3, 327, 328
330	335	327, 334, 374
331	335_3	316_3, 327, 328_2, 329, 330, 336
333	336_2	
334	335	331_2, 337, 374
335	321, 324, 327, 328, 329, 331	320, 336, 381_2
336	328, 351_2	327_2, 331, 337_2, 342, 345
337	339	327, 336, 340_3
338	327, 337, 341, 342	
339	318, 327, 336, 337_2, 340, 342_2	338
340		327, 337_2, 342_3, 345, 346
341	327_2, 331, 337_2, 340_4, 342_4	
342	347_2, 351, 373_2, 381	336, 340, 345, 354_2, 371, 374
345		327, 337_2, 342
347	337_3, 340, 342, 349_2, 354	
348	342, 347	351
349	351_2	342_2
350	381_2	334, 337, 340, 349, 354_2, 359, 363
351	349_2, 350, 353, 354_2, 362	320, 368, 381_2
352	327, 342, 349_3	348
353	351_2	354_2, 362_3
354	351_2, 371	317_6, 337, 350, 363_2, 374_3
355	342_2, 351, 354_2, 381	
358		354_4, 362
359	347, 351, 355_2, 366_2	342, 354_2, 362, 371_2
360		362, 363_3
362	368, 381_2	340, 354, 358, 363, 365
363	351, 366, 368_2	350_2, 354, 365
365	355, 368	354, 359, 362, 363, 374
366	351, 354_2, 360, 363, 365, 368_2	
367	354_2, 370_3, 371	378
368	354, 363, 371, 374	320_2, 367, 381_6
370	367_2, 368_3, 373, 378	371, 372, 374
371	366, 368, 381_2	365_2, 374_7
372	367_3, 368, 381	371_2, 374
373	354_2, 367, 370_2, 374_3, 385, 371_4	320_2, 368, 381
374	373, 378, 381	371_6
378	373, 381_7, 383	379_2
379	374_4, 381_2, 383	354_2, 373_8
381	317_3, 340, 341, 342_2, 354_4, 374_2, 378	351, 367, 368_2, 373
382	354_2, 374, 379	367, 368, 373_2, 381
383	378_4, 384	367
384	381	378_2
385	381_2	378

TABLE 29

Family H_1—Progeny of plant 44 (F_1, cross No. 2, class C) × plant 10 (F_1, cross No. 2, class A) grouped in accordance with their behavior in inter-crosses.

Group	Ped. No.	No. cases fertile within group					No. cases sterile within group				
		A	B	C	D	Ind.	A	B	C	D	Ind.
A	315	0	—	—	—	—	5	—	—	—	—
	316	**1**	1	—	—	—	6	0	—	—	—
	317	0	2	—	—	1	8	0	—	—	0
	318	**1**	1	—	1	—	5	0	—	0	—
	319	0	0	—	—	1	4	**1**	—	—	0
	321	0	3	—	—	—	6	0	—	—	—
	322	0	2	—	—	1	4	0	—	—	0
	324	**2**	3	1	—	1	7	0	0	—	0
	327	0	3	—	2	1	10	0	—	0	0
	328	**3**	2	—	—	—	8	0	—	—	—
	329	0	1	—	—	2	5	0	—	—	0
	330	0	1	—	—	—	4	0	—	—	—
	331	0	1	—	—	1	8	0	—	—	0
	334	0	1	—	—	—	5	0	—	—	—
	336	**1**	1	—	1	1	5	**1**	—	0	0
	337	**1**	—	—	2	2	7	—	—	0	0
	340	0	1	—	1	2	7	0	—	0	0
	342	**1**	6	—	2	3	9	**1**	—	0	0
	345	0	—	—	—	—	5	—	—	—	—
	346	0	—	—	—	—	1	—	—	—	—
	349	0	2	—	—	1	2	0	—	—	0
	350	0	2	—	—	—	7	0	—	—	—
	353	0	1	—	—	—	2	0	—	—	—
	354	**1**	7	0	—	3	13	0	**1**	—	0
	358	0	—	—	—	—	2	—	—	—	—
	359	0	1	—	—	3	6	0	—	—	0
	360	0	—	—	—	1	2	—	—	—	0
	362	0	3	—	—	—	8	0	—	—	—
	363	0	2	—	—	1	5	0	—	—	0
	365	0	1	—	—	2	6	0	—	—	0
	370	0	3	1	—	—	3	0	0	—	—
	371	**1**	4	—	—	1	6	0	—	—	0
	372	0	3	—	—	—	3	0	—	—	—
	374	0	4	2	—	—	8	0	0	—	—
B	320	8	**1**	—	—	—	**1**	4	—	—	—
	335	8	0	—	—	—	**1**	2	—	—	—
	348	1	0	—	—	1	0	2	—	—	0
	351	9	0	—	—	2	0	4	—	—	0
	352	3	0	—	—	—	0	1	—	—	—
	367	5	**1**	0	—	—	0	4	**1**	—	—
	368	8	0	—	—	1	0	6	—	—	0
	373	5	**1**	2	—	—	0	4	**1**	—	—
	381	11	**1**	4	—	2	**1**	6	0	—	0
	382	2	0	1	—	—	0	4	0	—	—
	383	—	0	3	—	—	—	1	0	—	—
C	378	2	3	0	—	—	0	**1**	3	—	—
	379*	2	3	0	—	—	**1**	**1**	1	—	—
	384	—	2	0	—	—	—	0	1	—	—
	385	—	2	0	—	—	—	0	1	—	—
D	338	3	—	—	0	1	0	—	—	1	0
	339	6	—	—	0	—	0	—	—	1	—
Ind.	325	4	—	—	—	—	0	—	—	—	—
	333	1	—	—	—	—	0	—	—	—	—
	341	5	—	—	—	—	0	—	—	—	—
	347	8	1	—	—	—	0	0	—	—	—
	355	4	2	—	—	—	0	0	—	—	—
	366	6	2	—	—	—	0	0	—	—	—

* Probably not really a member of group C.

sterile reciprocal, plus the 56 self-combinations. The probable total sterility in the population is 54.0 percent ± 1.4 percent, therefore, which makes it unlikely that more than 3 or 4 intra-sterile classes are present. These matings are shown in table 28.

The individuals are grouped with reference to their behavior in intercrosses in table 29. This table appears to reveal 4 classes containing 34, 11, 4 and 2 plants, respectively, in addition to 6 indeterminate individuals. Let us see what it really shows.

In the first place, there are 8 exceptions—fertility where there should be sterility—in the fertility columns. They are as follows, each mating being made but *once.*

Class A 316 × 324
“ “ 318 × 328
“ “ 324 × 342
“ “ 328 × 337
“ “ 336 × 328
“ “ 354 × 371
Class B 320 × 381
“ “ 373 × 367

There are also 6 exceptions—sterility where there should be fertility—in the sterile columns, and here one mating (No. 4) was made twice and one mating (No. 6) three times. These exceptions are as follows:

1. B × A 319 × 381
2. B × A 320 × 342
3. B × A 335 × 336
4. C × A 379 × 354
5. B × C 367 × 378
6. C × B 379 × 373

These exceptions are no more than were to have been expected from the predictions made above from *a priori* calculations. Of the fertile exceptions, at least 5 were made at the last of the season. No data regarding percentage of seed obtained to seed expected in full capsules were recorded, unfortunately, but it is probable from our other experiences that the majority of them produced only partly filled capsules, and would have proved sterile had they been made earlier. The sterile exceptions 379 × 354 and 379 × 373, made twice and thrice respectively are of little consequence because 379 falls into class C only through the single sterile mating 378 × 379 (made twice). Thus we could just as reasonably call 379 an indeterminate,—that is a plant fertile in all combinations tried,—and have but the sterile exception 378 × 379 for which to account. It could not go into groups A or B, though sterile with one plant of each of those groups because it also was fertile with 2 members of group A and with 3 members of group B.

This interpretation may be made either way without affecting the chief point the table was designed to show. No indeterminate individual and neither plant of the very uncertain class D, which was based on the single case of sterility 339 × 338, were crossed with plants of class C. Therefore the 3 classes A, B and C are the only ones for which we can claim independence.

A meaning can now be given to the results of the back-crosses which were listed in table 27. 38 plants were crossed with pollen from one or both parents. Out of the 23 plants crossed with No. 44 just 1 was sterile,—a single pollination of 366 × 44. It is possible that this mating also might have shown fertility if tested further, but it may show that 366 is the only plant among those tested that belongs to the same intra-sterile class as 44.

Plant 10 was used as pollen parent with 29 plants, of which 10 produced some seed. Plant 342 produced a few seeds which seemed to be parthenocarpic out of 4 tests, and plant 362 yielded 8 seeds in 1 of the 4 tests made. Therefore we have no hesitancy in classifying them as sterile. Plant 314, which was fertile to plant 10 pollen, was discarded early and is not classified in table 29. For this reason it may be left out of consideration. Plants 335 and 367 were fertile in one pollination each, and sterile in one pollination each. Since they gave full capsules in each of the successful pollinations, however, let us record them as fertile. Now what is the result? *Out of 20 sterile matings 18 are with plants belonging to class A.* The first exception is with the plant 379 which behaved so irregularly—as shown by table 29—that it is just as likely to be a member of class A as class C. The second exception is a single pollination with plant 385 of class C. *Fertility is shown in 7 cases, all of which are with class B.* Furthermore, the 3 sterile matings made with pollen from plant 34, a member of the same F_1 class as plant 10, are with plants of class A of family H. And the 1 sterile mating made with plant 18, a plant of F_1 class B, is with plant 339, a member of class D of family H. Therefore, it seems unquestionable that Plant 44 (and thus class A of F_1) belongs to the class A of family H.

TABLE 30

Family I.—Record of cross-pollinations on progeny of plant 44 (F_1, cross No. 3, class C) × plant 34 (F_1, cross No. 2, class A) outside family I.

Ped. No. ♀	Fertile with parents ♂	Sterile with parents ♂	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
387	44			
391	44	34_4		
392				372_6 fam. H
394	44_2			
395	44			
396	44			315 fam. H, 317 fam. H
398	44, 34_3			
400	44	34_4		
401	44	34_6		10_3 F_1, 354 fam. H, 374 fam. H
405	44_2		351 fam. H	354 fam. H, 374 fam. H
408	44		337 fam. H, 477 fam. J, 524 fam. K	
409	44	34_4		
412	44, 34_2 late	34_2		
413	44		374 fam. H	
415	44	34_4		354 fam. H, 374 fam. H
421			490 fam. J	
425	34			
426	44_2	34_2		
430	44	34_5		
431	44, 34_2			377 fam. H
432			377_3 fam. H	
433	44			
440	44_2		381 fam. H	
442	44			
444	44_2	34		
446	44			
448			474 fam. J, 475 fam. J	
451	44_2	34_2		
455	44_4	34_2		
456	44_2	34_6		
457	44			
458		34_2	320 fam. H, 381 fam H	
460				380 fam. H
463	44			
465	44			
467	44		278_4, fam. G, 320 fam. H, 381 fam. H, 489 fam. J	374_3 fam. H
468		34_2		
470		34_4		

TABLE 31

Family I.—Record of cross-pollinations on progeny of plant 44 (F_1, cross No. 3, class C) × plant 34 (F_1, cross No. 2, class A).

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
387	395, 396	
390		396_2
391		396_2
392		395, 444_3, 468_2
394	390, 396_2, 398, 400, 405	
395		400, 405_2
396	413	400_4, 401_4, 405_2, 415
398	396, 400	413
400	401	396_2
401		405_2, 415_2, 426, 467
405	398_2, 413_2	401_3, 408, 415, 467_2
408	413	405_2, 409, 415, 426
409		408_3
412	420	
413	396, 401, 405_5, 408, 415, 418, 420	
414	425_2	415_2
415	413, 425	401_2, 405_2, 412, 414, 420_2, 426, 458
418	431	412_2
420	431, 425	401_2, 405, 415_2, 425_2, 426_3, 458
421		467
425		431
426	425_3, 433	405, 420_3, 440_2, 445, 458, 464_2
430	426_2, 433_3	431, 458
431	401_2, 426_4, 433, 439, 455	430, 440
432	431_3	
433	405, 426, 431, 439, 440_3, 451, 458	
439	433	440, 444
440	433	415, 426_2, 439_2, 444_2, 451_2, 457
442	439, 446	444_2, 451
444	431, 456, 458_2	440_3, 451
445		
446	442	430_2, 431
448		421_2
451		442, 444_3, 455_2, 467
455		415, 426_2, 440, 451_4, 456_2
456	444	455_2, 457_5, 458_2, 467_2
457	463_2	455, 456_4, 458, 467
458	413	405, 420, 451, 455_4, 456_2, 467
460		392_4, 468_3
463	456_2, 457_2, 465	
464		
465	456, 463	457_2
467		455, 456_2
468		
470		465_4

Family I.—Plant 44 of class C, cross No. 3 × plant 34 of class A, cross No. 2

Family I was produced from seed obtained by pollinating plant 44 of class C, cross No. 3, with pollen of plant 34 of class A, cross No. 2. It is therefore a test of the similarity of constitution of plants of class A of F_1, since plant 44 was crossed first with plant 10 of class A to produce family H and then with plant 34 of class A to produce family I.

83 greenhouse plants were grown; but the task of manipulating that number proved too great and very nearly one-half of them were discarded after several weeks of work, permitting our efforts to be more concentrated. We have not thought it necessary to report any of the pollinations made on the rejects.

Of the plants remaining, 25 were selfed from 1 to 6 times between the first and the middle of the reproductive period without obtaining a single seed. Somewhat contrary to what might have been expected, 6 of these same plants were again selfed several times during the latter part of the season with the same result. This does not prove that no seed could have been obtained at that time if further pollinations had been made, however, as a few seeds were produced in a part of the pollinations of 22 cross-matings made during the waning of the flowering period, where continued pollinations made before had left no doubt as to the sterility of the combination. In 9 other matings, 1 pollination each produced no capsule, but in each case other matings—usually several—giving full capsules, proved them to be fertile. They were therefore so recorded.

Table 30 shows the record of back-crosses with pollen of the parents, and also the crosses made with plants outside of the family. It will be discussed after making the usual classification.

The inter-crosses in this family are shown in table 31. About one-sixth of the 2025 different combinations possible with 45 plants were accomplished. The table shows 61 fertile and 97 sterile matings, including 13 pairs of fertile reciprocals and 20 pairs of sterile reciprocals. The total number of different cross-combinations, therefore, is 250, made up of 96 fertile and 154 sterile combinations. Adding the 45 self-combinations, we have 199 steriles out of a total of 295 combinations. The probable sterility in the population is thus 67.5 percent ± 1.8 percent, and we should scarcely expect more than 3 or at most 4 intra-sterile classes even if a Mendelian dominant type $(3 + 1)$ of distribution in the classes be assumed.

The grouping actually obtained is set forth in table 32. Three classes containing 34, 4 and 2 individuals, respectively, and 5 unplaced plants, appear. There are 6 fertile exceptions:

400 × 401
412 × 420
442 × 439
444 × 456
444 × 458
465 × 456

TABLE 32

Family I.—Progeny of plant 44 (F_1, cross No. 3, class C) × plant 34 (F_1, cross No. 2, class A) grouped in accordance with their behavior in inter-crosses.

Group	Ped. No.	No. cases fertile within group				No. cases sterile within group			
		A	B	C	Ind.	A	B	C	Ind.
A	390	0	—	—	1	1	—	—	0
	391	0	—	—	—	1	—	—	—
	392	0	—	—	—	4	—	—	—
	395	0	—	—	1	3	—	—	0
	396	0	—	2	2	6	—	0	0
	400	**1**	—	1	1	2	—	0	0
	401	**1**	1	1	—	6	0	0	—
	405	0	—	2	2	10	—	0	0
	408	0	—	1	—	4	—	0	—
	409	0	—	—	—	1	—	—	—
	412	**1**	—	—	—	2	—	—	—
	414	0	1	—	—	1	0	—	—
	415	0	1	1	—	10	0	0	—
	418	0	1	1	—	1	0	0	—
	420	**1**	1	—	—	5	0	—	—
	421	0	—	—	—	2	—	—	—
	426	0	3	—	1	10	0	—	0
	439	**1**	1	—	1	2	0	—	0
	440	0	0	—	1	7	**1**	—	0
	442	**1**	1	—	—	2	0	—	—
	444	**2**	2	—	—	5	0	—	—
	445	0	—	—	—	1	—	—	—
	448	0	—	—	—	1	—	—	—
	451	0	—	—	1	6	—	—	0
	455	0	1	—	—	7	0	—	—
	456	**2**	—	—	1	4	—	—	0
	457	0	—	—	1	6	—	—	0
	458	**1**	0	1	1	9	**1**	0	0
	460	0	—	—	—	2	—	—	—
	464	0	—	—	—	1	—	—	—
	465	**1**	—	—	1	2	—	—	0
	467	0	—	—	—	8	—	—	—
	468	0	—	—	—	2	—	—	—
	470	0	—	—	—	1	—	—	—
B	425	4	0	—	—	0	1	—	—
	430	1	0	—	1	**1**	2	—	0
	431	6	0	—	2	**1**	3	—	0
	446	2	0	—	—	0	2	—	—
C	398	3	—	0	1	0	—	1	0
	413	8	—	0	—	0	—	1	—
Ind.	387	2	—	—	—	0	—	—	—
	394	4	—	1	—	0	—	0	—
	432	—	1	—	—	—	0	—	—
	433	6	2	—	—	0	0	—	—
	463	3	—	—	—	0	—	—	—

TABLE 33

The behavior of individuals of the various groups of family I toward individuals of the various groups of family H.

Class of fam. I	Ped. No. of fam. I	Fertility with individuals of family H				Sterility with individuals of family H			
		Class A	Class B	Class C	Ind.	Class A	Class B	Class C	Ind.
A	392					372			
	396					315, 317			
	401					354 recip., 374 recip.			
	405		351, 381			354, 374			
	408	337							
	415		381			354, 374			
	440		381						
	458		320, 381						
	460					342			380
	467	337, 371	320, 368, 381			374 reciprocal			
B	431								377
C	413	374							
Ind.	432				377				

Four of these matings were made but once, 1 was made twice and 1 was made reciprocally. The last 2 and 1 other were end-season matings, the others were mid-season matings. There are 2 sterile exceptions, 431 × 440 and 430 × 458, each tried but once. The number of combinations that form the basis of our grouping is so large, that there is little danger in accepting the classification as given, however, since these errors might have crept in in various other ways, as has been shown before. But it should be mentioned that plant 430 falls just as readily into group A as it does into group B.

The evidence in this table does not support the idea of more than 3 classes. A and B are well established. But C may be B, since neither members of the class were crossed with any B individuals. Of the indeterminates, 387, 394 and 463 may be B and 432 may be A. The sole positive evidence of a third class, therefore, rests upon plant 433, which is not A (6 matings in evidence) nor B (2 matings in evidence).

Let us now consider the back-crosses shown in table 30. Every cross made with the pollen of plant 44, 29 in number, was fertile. On the other hand 15 back-crosses with pollen from plant 34 were sterile, though an average of over 3 pollinations per plant was made. Seed was obtained in only 1 instance: 4 pollinations were made on plant 412, and 2 made late in the season gave some seeds. The interesting feature in these 15 sterile matings is that 14 of them were made on plants of class A, and the fifteenth on plant 430, which, though tabled in class B may just as readily be placed in class A.

But 3 plants were fertile to pollen of plant 34,—plants 425 and 431 of class B and plant 398 of class C.

A single mating of plant 10 on plant 401 of class A was sterile. Since plant 10 and plant 34 belong to the same class of the F_1 generation, this mating may be compared with the 3 sterile matings of class A plants of family H with pollen from plant 34.

Note then the similarity between families H and I. Each has 3 independent inter-fertile, intra-sterile groups with almost the same distribution of individuals within the classes; each behaves similarly in back-crosses. With the exception of a single unclassified plant of family H, all of the plants tested of both families were fertile with plant 44 of class C of the F_1 generation, the female parent of both. With regard to plants 10 and 34, the male parents of families H and I respectively, both of which belonged to class A of the F_1 generation, each was sterile with class A plants of both families and each fertile with other plants of their respective families. The conclusion is unavoidable, therefore, that class

A of the F_1 generation, class A of family H, and class A of family I, are identical.

This is not the only evidence that can be brought forward in favor of the similarity of these two families. A sufficient number of crosses (table 33) was made between the two populations to prove that class A of family H and class A of family I are the same. Ten members of class A of family I were crossed with plants from family H. Three pairs of reciprocals were made with like results for each pair. Counting these pairs as but 1 mating each, members of class A of family I were crossed 14 different ways with members of class A of family H. Of these matings 11 were sterile, and 3 fertile. But of the fertile matings, 2 were with 337 and did not give full capsules. These same class A plants of family I were also mated 9 times with members of class B of family H, and all matings were fertile. Bearing these results in mind, the single sterile mating of 460,—family I, class A,—with 380,—unplaced member of family H,—is pretty good evidence for placing 380 in class A of family H. Likewise, the sterility between 431 and 377 is evidence that 377 of family H is not a member of that family's class A, a conclusion supported by its fertility with unplaced 432 of family I. The remaining cross, plant 413 of class C of family I with plant 374 of class A of family H, was fertile.

We do not believe it rash to assert that this makes a complete case. There can be no doubt that families H and I are practically duplicates of each other. *In this instance, then, two plants belonging to a single class in which all of the individuals were cross-sterile with each other, when crossed with the same individual have produced populations as similar to each other in their behavior in crossing as if they were samples of the same population.*

This does not prove that all members of an intra-sterile class crossed with the same individual would produce identical populations. No such claim is made. It does indicate very strongly, however, that in this particular case, these 2 plants of the F_1 class A (10 and 34) are identical in that part of their constitution which affects self- and cross-sterility. The criticism may be offered that these results show merely a kind of dominance exhibited by plant 44, but if this be true, it is a dominance of a strikingly perfect kind.

Family J.—Plant 52 of class B, cross No. 3 × plant 23 of class A, cross No. 2

As has just been shown, F_1 plants of class C when crossed with their

TABLE 34

Family J.—Record of cross-pollinations on progeny of plant 52 (F_1, *cross No. 3, class B*) × *plant 23* (F_1, *cross No. 2, class A*) *outside family J.*

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
475	524 fam. K	
477	377 fam. H, 467 fam. I	
487	512 fam. K	
489	467_2 fam. I	
490	421 fam. I	
495	18 F_1	
499	18_2 F_1	
502	18 F_1, 512 fam. K	

sisters of class A give populations having a high percentage of cross-sterility and by the same token a small number—2 or 3—of intra-sterile groups. Family J tests the behavior of an F_1 plant of class B with a class A sister.

30 plants of this family were grown in the greenhouse, 6 dying or being discarded. They were all selfed from 1 to 12 times with no production of seed except on plants 473 and 489. These 2 individuals produced seed the latter part of the flowering season. No. 473 was selfed 7 times at various periods. The first 2 pollinations yielded no seed, the third and fourth a few seeds, and the last 3 half-filled capsules. No. 489 was selfed 9 times. The first 3 were failures; the remainder induced capsules, the last 3 pollinations producing a full quota of seed.

Only 1 back-cross was made. No. 474 was fertile with No. 52.

The few other crosses made with plants outside the family are recorded in table 34. All were successful. It should be noted that 3 of these successes were with plant 18, another member of class B of the F_1 generation.

As usual only a comparatively few of the 576 combinations possible between 24 plants were made. The record of cross-pollinations listed in table 35 are sufficient, however, to show the striking difference in percentage of cross-sterility between this family and the 2 families just described. There are 65 fertile matings including 14 pairs of fertile reciprocals, making 102 fertile combinations in all. Since there are no sterile reciprocals, the 13 sterile matings are equivalent to 26 sterile combinations. Adding the 24 self-combinations, gives a ratio of sterility to total combinations of 50 : 152. The probable sterility in this family is therefore 32.9 percent $\pm$ 2.6 percent, which leads us to expect about 5 intra-sterile groups.

The grouping made possible by the sterile matings is shown in table 36. There are no exceptions. Each individual in every group shows perfect inter-class fertility and intra-class sterility as far as they were tested. Apparently there are 4 classes containing 7, 4, 2 and 2 individuals, respectively, together with 9 plants which showed no cross-sterility and are unplaced.

TABLE 35

Family J.—Record of cross-pollinations on progeny of plant 52 (F_1, cross No. 3, class B) × plant 23 (F_1, cross No. 2, class A).

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
473	474, 475_6, 485_3	480
474	475_3, 480_2, 482, 485	
475	474, 477, 480, 482, 485_4	
477	473, 475_3, 482, 485_6	
478	484, 485	
480	474, 475_3, 482, 486, 487_2, 491	
482	474, 484, 485_2	
484	474, 480, 482, 487_2	485_3
485	474, 475, 482_5, 492	484
486	485, 492_3	474, 495
487	474, 482, 484, 486, 492, 499	
488	482, 487,	484
489	477, 492	486
490	489	
491	480	484
492	484, 487_2, 493, 495_2	
493		502
494		486, 502
495	499	
496		492
499	502_4, 503	
500	486, 493, 499, 502, 503	
502	499	495
503	499_2, 500, 502_2	

Table 37 shows the evidence for independence between these groups more clearly. A, B and C or D must be independent, but C and D may belong to one class since they were not crossed together. In addition 475, 477 and 482 are independent of each other and of A, B and C. Thus there are apparently 6 independent classes with frequencies of 7, 4, 2, 1, 1 and 1, these frequencies being subject to change of course given the data necessary to fit the remaining individuals into their proper niches. Before accepting this classification at its face value, however, we ought

TABLE 36

Family J.—Progeny of plant 52 (F_1, cross No. 3, class B) × plant 23 (F_1, cross No. 2, class A) grouped in accordance with their behavior in inter-crosses.

Group	Ped. No.	No. cases fertile within group					No. cases sterile within group				
		A	B	C	D	Ind.	A	B	C	D	Ind.
A	474	0	2	2	—	3	1	0	0	—	0
	486	0	1	1	1	2	4	0	0	0	0
	489	0	—	—	1	2	1	—	—	0	0
	493	0	—	—	1	1	1	—	—	0	0
	494	0	—	—	—	—	2	—	—	—	—
	495	0	—	—	1	1	2	—	—	0	0
	502	0	—	—	—	3	3	—	—	—	0
B	484	1	0	1	1	3	0	3	0	0	0
	485	1	0	1	1	4	0	1	0	0	0
	488	—	0	—	—	2	—	1	—	—	0
	491	—	0	1	—	—	—	1	0	—	—
C	473	1	1	0	—	2	0	0	1	—	0
	480	2	2	0	—	3	0	0	1	—	0
D	492	4	2	—	0	1	0	0	—	1	0
	496	—	—	—	0	—	—	—	—	1	—
Ind.	475	1	1	2	—	2	0	0	0	—	0
	477	1	1	1	—	2	0	0	0	—	0
	478	—	2	—	—	—	—	0	—	—	—
	482	1	3	1	—	3	0	0	0	—	0
	487	2	2	1	1	2	0	0	0	0	0
	490	1	—	—	—	—	0	—	—	—	—
	499	2	—	—	—	3	0	—	—	—	0
	500	3	—	—	—	2	0	—	—	—	0
	503	1	—	—	—	2	0	—	—	—	0

TABLE 37

Family J.—Progeny of plant 52 (F_1, cross No. 3, class B) × plant 23 (F_1, cross No. 2, class A) grouped to show inter-class fertility.

	A	B	C	D	475	477	478	482	487	490	499	500	503
A		3	3	4	1	1		1	2	1	2	3	1
B	2		3	2	1	1	2	3	2				
C	3	3			2	1		1	1				
D	4	2							1				
475	1	1	2			1		1					
477	1	1	1		1			1					
478		2											
482	1	3	1		1	1			1				
487	2	2	1	1				1			1		
490	1												
499	2								1			1	1
500	3										1		1
503	1										1	1	

to see whether the independence of any of the 3 single plants is based upon a single pollination. Plants 475 and 477 were fertile reciprocally, 4 pollinations being made in all, but plants 475 and 482, and plants 477 and 482 were crossed but once. This is also true of the basis of independence between 477 and A, 477 and C, and 482 and C. It depends on 1 pollination in each case.

For these reasons it is hardly likely that more than 6 independent classes exist in this population, and the chances are perhaps even that there are only 5. Nevertheless, family J unquestionably contains 2 or 3 more intra-sterile classes than family H or family I.

TABLE 38

Family K.—Record of cross-pollinations on progeny of plant 52 (F_1, cross No. 3, class B) × plant 44 (F_1, cross No. 3, class C).

Ped. No. ♀	Fertile with parents ♂	Fertile with Ped. No. outside family ♂	Fertile with Ped. No. within family ♂	Sterile with parents ♂	Sterile with Ped. No. outside family ♂	Sterile with Ped. No. within family ♂
505						508
507			515			
508						505
509						
511	44, 52_2		508, 509			512_3
512	52			44		520
515			524	44		
517				44		524
520	52			44_3		512_2
521	44, 52		512			
524	44, 52	58 *N. alata*				
525			520_2			
527			505_2, 509			
528	52_3		58_2 *N. alata*			

Family K.—Plant 52 of class B, cross No. 3 × plant 44 of class C, cross No. 3

Very little was done upon family K, as table 38 shows, though this family resulting from crossing a plant of class B (52) with our much used plant 44 of class C, might have proved very interesting. The plants would possibly all have shown fertility in back-crosses with 52, while only a part would have proved fertile with the other parent. This is the indication of the few matings made. There were 6 cases of fertility and none of sterility with No. 52, and 3 cases of fertility and 4 of sterility with 44.

2 plants were crossed with *N. alata* plant No. 58; both were successful. These were the only crosses made outside of the family with K plants

used as females. But K pollen was fertile on several plants of other families; viz., 524 on 337 of family H, on 408 of family I, and on 475 of family J; 512 on 487 and 502 of family J.

The 14 matings made within the family, including as they do 2 pairs of sterile reciprocals, are hardly a sufficient basis for even a guess as to the amount of cross-sterility present potentially. We can only say that the number of intra-sterile classes would not have been large, the percentage of sterility probably lying between 35 and 50.

Argument on cross No. 2 and cross No. 3

If further evidence of the beautiful regularity with which plants belonging to the same intra-sterile class behave in crosses be desired, it is found in the crosses between families cited in tables 13, 18, 22, 27, 30, 33 and 34.

Plants 152 and 153 of class A, family D, were both sterile with family E pollen which is presumably of one kind. The mating 152 D × 204 E was even made reciprocally. Plants 167 and 171 of family D, which were discarded after a few matings had been made and were therefore undetermined as to class, were fertile to pollen of family E.

In family F, plants 216, 239 and 243, all of class B were each sterile with the pollen from the unplaced plant 247 of family G. Plant 244, an unplaced plant of family F was fertile with the pollen of 247, however. On the other hand, plants 216 and 241 of family F, class B were fertile with the pollen of plants 278 of class C, family G and 250 of class A, family G, respectively. Plant 278 of class C, family G, was also fertile with the pollen of plant 219 of class A, family F, although plant 219 was sterile with the pollen of plant 250 of class A, family G.

If we may say that sterility shows likeness of constitution and fertility unlikeness of constitution, these results show: (1) that class A of family F and class A of family G are alike; (2) that class A of family F and class C of family G are unlike; (3) that class B of family F and classes A and C of family G are unlike, as they should be since classes A of both families are alike; and (4) that the unplaced plant 247 of family G belongs in with class B of family F, as might very well be the case.

In the remaining matings between plants belonging to different families there was no sterility, except among those matings between families H and I already discussed. They are none the less interesting, however, because they show that once fertility has been found between classes belonging to different families, all matings between plants belonging to these classes will prove fertile barring experimental error.

In family G, unplaced plant 249 was fertile with plant 351 of class B, family H and with plant 467 of class A, family I. Plant 278 of class C was fertile with pollen from plant 374 of class A, family H. Plants 278 and 281, both members of class A, were also fertile with plants 467 and 405 of class A, family I, respectively. Thus 2 combinations between the classes A of families H and I proved to be fertile.

Likewise, 3 plants of class A, family H, 317, 319 and 321, proved to be fertile with the pollen of plant 311 of class F, family G. Another plant of class A, 337, also proved to be fertile with the unplaced plants 477 of family J and 524 of family K.

Fertile matings were made as follows between 4 plants of class A, family I, and plants of families G and J; 408 with 477, of family J unplaced; 421 with 490, of family J unplaced; 448 with 474 of family J, class A, and with 475 of family J unplaced; 467 with 278 of family G, class C, and with 489 family J, class A.

Fertile matings were also made with the pollen of 3 family I, class A plants on plants of family J. Pollen of 467 was fertile on 477 unplaced and on 489, class A of family J, and pollen of 421 was fertile on 490 unplaced of family J.

Thus plants of class A of family I were fertile once with a plant of class C, family G, 4 times including a reciprocal with unplaced plants of family J, and 3 times including a reciprocal with plants of class A, family J.

In these matings between families, then, not a single one militates against our conception of inter-fertile, intra-sterile groups. We believe, therefore, that the fundamental basis of this grouping is established beyond doubt, and that the actual groups as submitted in the foregoing pages are sufficiently exact to be made the foundation of a theoretical interpretation of the behavior of self-sterile plants among themselves.

Undoubtedly there will come the critic who will say we have been at some pains to make out a case for the presence of inter-fertile, intra-sterile classes in this family. He will point out that some of the exceptions among the matings may not have been due to experimental errors and hence must have subtle meanings other than those given, that our phrase "pseudo-fertility due to environment" veils the real facts. Let us forestall him.

Of course *some* of the matings which form exceptions to the rule of inter-fertile, intra-sterile classes may be the effect of an unknown biological cause; certainly factors other than environmental may be the basis of a portion of the change from sterility to partial fertility in certain matings as the flowering season wanes.

The first thing to establish, however, was a broad general rule for the behavior of self-sterile populations. This has been done by the work on these 2 crosses. *The members of any population of the self-sterile species under consideration fall naturally into a relatively small number of groups, each individual being cross-sterile reciprocally with every member of the same group and cross-fertile reciprocally with every other individual.* The sum total of the exceptions to this rule is well within the limits of experimental error, even though the question is one in which every bit of evidence, like pieces of a jig-saw puzzle, must fit, if a solution is to be obtained. The exceptions to the rule, in fact are of another order of magnitude than the confirmations. If, therefore, true exceptions do occur, they are so rare that the usefulness of the rule is not in the least impaired. Other general matters must be settled before it is even desirable to endeavor to inquire into them.

Lest there be some difficulty in carrying in mind the essential facts regarding the grouping of the plants of this series, let us summarize them here.

The two self-sterile species *N. Forgetiana* and *N. alata* were crossed reciprocally. The progeny of these two crosses behaved so similarly that collectively the 53 individuals studied could be placed in 6 intra-sterile classes 5 of which were proved to be independent. The remaining questionable group consisted of one plant.

From this population 8 families were raised which were characterized as follows:

D = *N. alata* plant 53 × plant 44, class C; probably consisted of 4-6 classes, 3 being established.
E = *N. alata* plant 58 × plant 44, class C; probably consisted of 1 class.
F = plant 34, class A × plant AA, *N. Forgetiana;* probably consisted of 5-6 classes, 4 being established.
G = plant 44, class C × plant AA, *N. Forgetiana;* probably consisted of 4-6 classes, 3 being established.
H = plant 44, class C × plant 10, class A; probably consisted of 3 classes, 3 being established.
I = plant 44, class C × plant 34, class A; probably consisted of 3 classes, 3 being established.
J = plant 52, class B × plant 23, class A; probably consisted of 5-6 classes, 5-6 being established.

K = plant 52, class B × plant 44, class A; probably consisted of 4-6 classes.

It was also determined that class A of the F_1 generation, class A of family H, and class A of family I are identical.

Cross No. 4. N. commutata × N. Forgetiana (self-sterile × self-sterile)

The race used here with the pollen of *N. Forgetiana* was received from Italy under the name *N. commutata* Fisch. and Meyer. It is the plant called *N. Langsdorffii* Weinm. variety *grandiflora* by COMES (1899). Of it he says: "Elle est connue depuis 1835 dans les jardins européens, mais on en ignorait la patrie." It has been duplicated in our experiments by crosses between *N. alata* and *N. Langsdorffii*. It is an additional argument in favor of such an origin, that it is self-sterile, since *N. Langsdorffii* is always self-fertile. When crossed with *N. Langsdorffii* the F_1 plants are self-fertile. The behavior of this race when crossed with *N. Forgetiana* is interesting, therefore, whether it be a true wild species or was produced by hybridization. In the first case, a new species cross is reported, in the second case, a self-sterile race extracted from a cross between a truly self-fertile species and a self-sterile species, is crossed again with a different self-sterile species.

The F_1 plants were highly fertile, in the sense that 90-100 percent of the pollen was normal in nearly every plant, and that "proper" combinations yielded full capsules.

A rather small number, 12, field-grown F_1 plants were used in our experiments. These were selfed from 3-10 times, an average of over 4 pollinations per plant. 11 were completely self-sterile, yielding not a single seed. Plant No. 3, however, produced 4 good capsules out of 4 pollinations. This plant behaved like a real self-fertile. Crossed as a female with each of the other 11 individuals it was fertile; crossed as a male with all but plants 5 and 11, it was also fertile. Further, it was fertile as a female with *N. Forgetiana*. The meaning of this behavior has not been determined conclusively. Two interpretations are possible. Owing either to its hybrid origin (self-fertile × self-sterile) or to a recent introduction of *N. Langsdorffii* "blood," the race is a mixture of self-fertile and self-sterile plants; or, by reason of its having been grown near *N. Langsdorffii* the preceding generation, the seed from which this plant came was produced by a stray pollen grain of that species. The second interpretation seems more probable, since we have corroborated

TABLE 39

Result of matings on F_1 plants of cross No. 4, N. commutata × N. Forgetiana.

Ped. No. ♀	Fertile with Ped. No. ♂	Sterile with Ped. No. ♂
1	3_3	2_9, 4_5,
2	3_2	1_3, 4_4
3	1_3, 2_5, 4_3, 5_5, 6_3, 7_3, 8_5, 9_4, 10_4, 11_2, 12_3	
4	3_2, 5_2, 6_3	
5	8_2, 10_4	6_4, 7_5
6	1_4, 3, 4_4, 8_4, 10_4, 12_4	5_5, 7_4, 9_4, 11_5
7	3_4, 4_5, 12_4	5_2, 11_3
8	1_4, 2_4, 3_5, 4_3	9_4, 10_2
9	1_4, 2_2, 3, 4_4, 12_4	7_2, 8_3
10	1_3, 2_4, 3_3, 4_3, 12_4	7_2
11	1_4, 2_4, 8_5, 10_6	5_5, 9_5
12	3_3, 7_3, 11_3	1_3, 2_2, 4_3

TABLE 40

Plants of F_1 generation, cross No. 4, grouped in accordance with their behavior in inter-crosses.

Group	Ped. No.	No. cases fertile within group			No. cases sterile within group		
		A	B	C	A	B	C
A	1	0	3	2	3	0	0
	2	0	2	2	3	0	0
	4	0	4	2	3	0	0
	12	0	4	1	3	0	0
B	5	1	0	2	0	3	0
	6	3	0	2	0	4	0
	7	2	0	0	0	4	1
	9	4	0	0	0	3	1
	11	3	0	2	0	4	0
C	8	3	3	0	0	1	1
	10	4	3	0	0	1	1

COMPTON's conclusion that true self-fertility is completely dominant over self-sterility.[16]

In this family 70 cross-matings were made, of which 48 were fertile and 22 sterile. These matings were each made more than once, as is shown by the subscripts in table 39. There were 22 pairs of fertile reciprocals and 4 pairs of sterile reciprocals. By multiplying the sterile and the fertile matings each by 2 and subtracting in each case the proper

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[16] The relation between self-fertile and self-sterile plants is to be made the subject of a later paper.

TABLE 41

Intercrosses between progeny of pseudo self-fertile N. alata plant used in cross No. 2. Compare with table 1.

Ped. No.	Plants with which fertile		Plants with which sterile	
	as ♂	as ♀	as ♂	as ♀
53	57	58	54_2	54_2, 56, 59_4
54			53_2 57_2, 58_2	53_2, 59
56			53, 57, 58, 59	59_3
57		53	58	54_2, 56, 59_3
58	53, 59			54_2, 56, 57, 62, 64, 66, 71, 79
59		58	53_4, 54, 56_3, 57_3	56
62			58, 79	66
64			58	
65			79	
66			58, 62, 71, 79	76, 78, 79
71			58	66
76			66	
78			66	
79			58, 66	62, 65, 66

number to allow for the reciprocals, we find that there were 52 fertile combinations and 36 sterile combinations.

If the self-fertile plant is omitted, there are 66 cross-combinations, each well established by more than 1 pollination through which one may group the remaining 11 individuals in intra-sterile classes. This grouping is shown in table 40. The 11 plants fall into 3 classes consisting of 5, 4 and 2 individuals. There is not a single case of intra-class fertility and but 2 instances of inter-class sterility. Matings 10 × 7 and 9 × 8 show sterility where fertility is to be expected.

Argument on cross No. 4

Outside of the fact that a plant which seems to be a true self-fertile appeared in this family and was tested with 11 self-sterile plants, no new phenomena are found in cross No. 4. The same cross-sterility, the same small number of inter-fertile, intra-sterile classes is found here that is found in crosses No. 2 and No. 3. Cross No. 4 merely furnishes corroboratory evidence of facts discussed earlier in the paper. It does show, however, that the facts discovered in crosses 1, 2 and 3, are not peculiar to a single hybrid.

INTRA-SPECIFIC PEDIGREE CULTURE EXPERIMENTS

Our experiments within each of these species can be described very briefly for they have been confined largely to self-sterility tests. Not a single thorough inquiry into the cross-mating proclivities of the plants of a pure (?) species has been made. This may seem very odd when so much time has been spent on inter-specific crosses. But our resolution to favor the wider crosses is not without reason. We have satisfied ourselves that the crosses within a species behave in a manner similar to that of the crosses already described. It seems probable, therefore, that intra-specific crosses would provide no data that could not be obtained from inter-specific crosses, although the converse might not be true.

N. Forgetiana. Between 200 and 300 plants of *N. Forgetiana* have been selfed under various environmental conditions, with pseudo-fertility in only 3 instances, as has already been described. *N. Forgetiana* is therefore a species on which environmental variations have little effect. It is a species in which, if one could measure accurately the intensity of the particular environmental factors that affect the full production of self-sterility, either the norm for a standard average environment would stand markedly toward the *sterile* end of the scale, or the dispersion coefficient would be small. The environmental complex that tends towards the *greatest* amount of pseudo self-fertility is necessary for any visible effect on the plants.

A small number of intra-sterile classes has been shown to exist in *N. Forgetiana.* Judging from cross-sterility percentages, the probable maximum is between 5 and 8 groups, but no accurate classification has been made.

N. angustifolia. Between 80 and 100 plants of *N. angustifolia* have been tested for self-sterility without the production of a single seed. This work was done during three summer seasons on field-grown plants. A certain environmental variation obtained of course, but since no pollinations were made at the extreme end of a flowering season, one cannot maintain that no pseudo-fertility exists. We are only justified in stating that *N. angustifolia* is similar to *N. Forgetiana* in being difficult to influence by environmental changes.

Intra-sterile groups have also been demonstrated in this species. Their number has not been determined but is probably no greater than in *N. Forgetiana.*

N. alata. We have shown earlier that *N. alata* is a self-sterile species in which a considerable amount of pseudo self-fertility appears at the end of the flowering season under adverse conditions. In other words if the environmental factors affecting self-sterility could be measured as suggested in the case of *N. Forgetiana,* either the norm for a standard average environment would be further toward the *fertile* end of the scale than in the latter species, or the dispersion coefficient would be larger.

As in the other two species, intra-sterile classes have been proved to exist, the maximum number probably being smaller than in *N. Forgetiana* or *N. angustifolia.*

The most important new fact discovered in *N. alata* is the probability that a population may exist consisting of only one intra-sterile class (compare family E). Recall that self-sterility is a sporophytic character, that inbreeding decreases the number of intra-sterile classes, and that there is no physiological or morphological obstacle to the fusion of any two complemental gametes provided they meet. All of these facts favor the idea that the behavior of self-sterile plants among themselves,—given the presence of the character self-sterility through the presence of a homozygous factor *X*,—is due to underlying causes which may be pictured as follows. A certain number of factors which affect self-sterility exist. The action of these factors is not cumulative. Mating is possible normally only to plants which differ in at least one of these factors.

If these premises be correct, after a very few generations of self-sterile plants raised from selfed seed by taking advantage of the phenomenon of pseudo self-fertility, one should find a population resulting from a single capsule which is homozygous for these effective factors and which is therefore wholly cross-sterile under normal conditions.

These conditions are very nearly met by the behavior of the grand-progeny of the original pseudo self-fertile *N. alata* plant that is recorded in table 1. Table 41 is made up from table 1 by tabling the cross-matings both ways when only made one way because of our belief that reciprocal crosses are always identical. By this table it appears that the 3 matings 53 × 57, 58 × 53, and 58 × 59 are fertile. Tabled both ways there are 6 fertile combinations. But let it be recalled that these matings were made during a long flowering season, and that during its wane several of the self-pollinations produced seed. What is more likely than that some sterile cross-matings should show pseudo-fertility at the same time? Our evidence is this. Of these matings 1 was made the middle of the season and did not give a full capsule, the other two were made at the end of the season. But this is not all. Our demonstration that every member of an intra-sterile class should be sterile with every other member is the result of an experience with nearly 10,000 cross-pollinations. The exceptions which have been met are very infrequent and are well within the expected experimental error. Now if table 41 be examined carefully, it is seen that there is every indication that all of the 14 plants listed belong to *one class* and that the 3 apparently fertile matings are due to pseudo cross-fertility.

N. glutinosa. Not over a dozen plants of *N. glutinosa* have been tested for self-sterility. It appears to behave like *N. alata.* Cross-fertility has been demonstrated, but the number of cross-matings made is not sufficient to prove the existence of intra-sterile groups. The above statement also holds for the race described as *N. commutata.*

SUMMARY AND INTERPRETATION OF THE RESULTS

The experiments on the self-sterile species *Nicotiana Forgetiana, N. alata, N. glutinosa* and *N. angustifolia* described in the foregoing pages, concern only the behavior of self-sterile plants when bred *inter se.* All questions connected with the relation between true self-fertility and self-sterility have been omitted designedly as pertaining to a distinct problem. The inquiry thus limited is believed to have established the following points:

1. Self-sterility is inherited.

2. The four species *N. Forgetiana, N. alata, N. glutinosa* and *N. angustifolia* breed true to the tendency toward self-sterility.

3. Self-sterility is fully expressed in these species from the beginning to the middle of the flowering season. Toward the close of the flowering season, especially in plants exhibiting the effect of adverse environmental conditions, some self-fertility may be shown. That this phenomenon is simply a non-inherited fluctuation is confirmed in four ways: (a) the graduated character of the increased fertility as the flowering season wanes, (b) the return to complete self-sterility at the beginning of a second flowering season, (c) the sterility of all progeny raised from selfed seed, and (d) the failure to obtain an increased tendency toward self fertility after three successive generations had been raised from selfed seed of the most extreme variants. It has been called pseudo self-fertility.

This fact naturally shows that self-sterility, whatever its nature, is only a physiological impediment to self-fertilization.

4. Other environmental factors appear to have little or no influence on self-fertility.

5. The waning of the reproductive period affects *N. alata* and *N. glutinosa* more markedly than it does *N. Forgetiana* or *N. angustifolia.* This indicates multiple allelomorphism in a fundamental factor the presence of which is necessary for the development of self-sterility.

(N.B. This factor should not be confused with any of those assumed in the interpretation of the behavior of self-sterile plants among themselves).

6. Cross-sterility in its nature identical with self-sterility was found in every population of self-sterile plants tested. The percentage of cross-sterility in different populations, based in each case on numerous cross-matings, varied from 2.4 percent to 100 percent.

7. Omitting fluctuations toward self-fertility correlated with a waning flowering period and a few cases of true sterility as indicated by microscopical examinations of the pollen, no variability in fruitfulness was noticed in "fertile" combinations. Fertile matings always resulted in full capsules.

8. Self-sterility behaves as a sporophytic character. This is demonstrated by the behavior of reciprocal matings,—pairs of reciprocals always giving like results either when fertile or sterile. It follows from this fact that no selective fertilization occurs.

9. The F_2 generation of a cross between *N. Forgetiana* and *N. alata* showed a low percentage of cross-sterility, 2.4 percent. This cross was followed to the F_5 generation by means of successive sib matings. The F_5 generation showed 21.6 percent cross-sterility.

In a repetition of this cross made with different plants, several F_2 populations studied each showed much higher percentages of cross-sterility.

10. All of the individuals of a family arising from one mating may be fertile with both parents, but a part of the individuals may be sterile with one or with both parents.

11. Cross-sterility exhibits a regularity of behavior such that if A is sterile with B and with C, it may be predicted that B will be sterile with C. On the basis of this cross-sterility the plants in each family may be divided into a relatively small number of groups in which each member of a class is sterile with every other member of that class and fertile with every member of every other class.

12. The distribution of the individuals within each class in several of the families studied was such that the classes may not be assumed to be of the same size. In certain cases this distribution rather resembled that of the coefficients of a point binomial.

13 Assuming a point binomial distribution of individuals within the classes as a limiting type, the number of intra-sterile classes necessary to account for the highest percentage of cross-fertility found is estimated to be less than 25. In most of the families tested the number of intra-

sterile classes varied from 1 to 6. In a cross between *N. alata* and *N. Forgetiana* in which 53 F_1 plants were tested rather thoroughly, 5 (or 6) such classes were found.

14. In those instances where a part of the individuals of a family were sterile to one or to both parents, only the members of a single class behaved in that manner.

15. Individuals belonging to different families as well as to different generations may belong to a single intra-sterile class.

16. Individuals belonging to different intra-sterile classes of the F_1 generation when mated with the same individual, produced populations varying in the number of intra-sterile classes.

17. Individuals belonging to a single intra-sterile F_1 class when mated with the same individual, sometimes produced populations having the same number of intra-sterile classes, a similar distribution of individuals within the classes, and possibly the same classes (see families H and I). It is not established that this behavior is universal, however. In the one case where the status of both the parents and the progeny as regards cross-sterility was established very definitely (families H and I), the two populations behaved in this manner; but in a case where the status of neither the parents nor their progenies (families D and E) was quite so clear, the two populations appeared to behave differently.

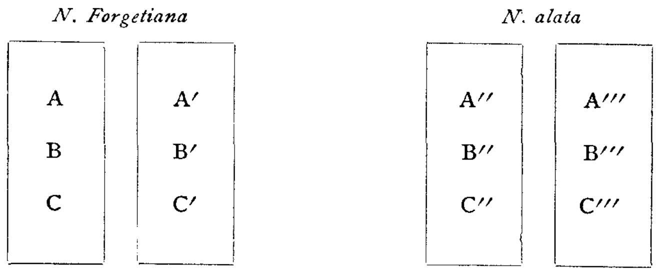
This rather varied series of facts can be given a very simple interpretation in keeping with recent interpretations of other inheritance phenomena provided judgment be suspended on one or two obscure points.

Let us assume first that a self-sterile species is self-sterile because it is homozygous for a fundamental self-sterility factor. Second, let us assume that a series of partially coupled factors affect the behavior of self-sterile plants among themselves. The action of these factors is on the sporophyte, and the nature of this action is such that two plants are not fertile together unless they differ by at least one of these factors.

It is not necessary to define the action of these factors more specifically, although this will be attempted in a subsequent publication. It may make matters somewhat clearer, however, to state that the immediate difference between a fertile and a sterile combination is in the rate of pollen-tube growth. If at the height of the season a series of self-pollinations and a series of cross-pollinations are made on a single plant and the pistils fixed, sectioned and stained at intervals of 12 hours, it is found by plotting the average length of the pollen tubes in each pistil against time in 12 hour periods that the growth curve of selfed pollen tubes is a straight line which reaches less than half the distance to the ovary during the life of the flower, while the curve of crossed pollen tubes resembles that of an autocatalysis and reaches the ovary in less than 96 hours. Further, it is unnecessary to know why gametes, which themselves bear various factors effective on the behavior of self-sterile plants, should act during the process preliminary to fertilization as if each bore the factors characteristic of the plant on which they were produced. Attention is called, however, to the fact that modern discoveries tend more and more to show that the sole function of the gametophytes of the Angiosperms is to produce sporophytes. The characters which they possess appear to be wholly sporophytic, the factors which they carry functioning only *after* fertilization. In other words, the hereditary genes carried by pollen grains—and probably by eggcells—may be thought of as being dormant until the appropriate time comes for them to play their proper parts.

It may be helpful to draw a picture of what may be expected to happen under the assumptions which have been made and to see how closely the actual facts are paralleled. First, it should be stated that no interpretation of the fact that within a family the intra-sterile classes are often of unequal size can be made without assuming linkage except by a number of awkward subsidiary assumptions. Second, our picture is as simple as possible in view of the facts at hand, but it may be extended *ad libitum* as far as number of factors is concerned. Third, since all of the facts of Mendelism are merely those to be expected from the known behavior of the chromosomes as carrying bodies for our hypothetical genes, chromosome diagrams are used without apology.

Assume first then that a plant of *N. Forgetiana* is heterozygous for 3 linked factors effective on the behavior of self-sterile plants, and that the homologous chromosomes of an *N. alata* plant are heterozygous for different multiple allelomorphs of the same factors. The two plants may be represented thus.



These plants cannot be self-fertilized because all of their gametes are influenced by their sporophytic constitution ABC.A′B′C′ and A″B″C″. A‴B‴C‴, respectively, nor can either be fertilized by gametes borne on a plant of like constitution.

Now each of these plants of *N. Forgetiana* and of *N. alata* produces 8 types of gametes. *N. Forgetiana,* for example, produces great numbers of ABC and A′B′C′, medium numbers of A′BC, AB′C′, ABC′ and A′B′C by one crossover or linkage break, and small numbers of AB′C and A′BC′ by double crossing over. *N. alata* behaves in a similar manner. Thus the progeny of this cross will consist of $8^2 = 64$ intra-sterile, inter-fertile groups of individuals, the groups being of various sizes. Further, since no individuals with constitutions ABC.A′B′C′ or A″B″C″. A‴B‴C‴ are produced in the F_1 generation, every F_1 class will be fertile with both of its parents.

Since by hypothesis two plants need differ by but one effective factor in order to be fertile in inter-crosses, it is clear that matings may occur in which certain of these factors are homozygous. To illustrate, it is possible to obtain two plants of constitutions ABC.A′B′C and A″B″C. A‴B‴C among the grandchildren of this generation. The factor C is homozygous and can be left out of consideration since the two plants form only 4 different types of gametes each. The first forms gametes AB and A′B′ in large numbers, and A′B and AB′ in small numbers; likewise the second forms gametes A″B″ and A‴B‴ in large numbers, and A″B‴ and A‴B″ in small numbers. Even with the elimination of the C allelomorphs as effective differences, therefore, it is possible to obtain a family having 16 intra-sterile classes by crossing two such plants. Of these classes 4 will be large, 8 medium and 4 small.

It is not unlikely that 16 classes is the maximum that need be considered, but what of the smaller number of groups usually found? The answer is that simplification can go on and on until very few intra-sterile classes are formed.

Suppose, for example, that AB.AB′ is crossed with AB.A′B; 4 classes will be formed AB.AB, AB.A′B, AB′.AB and AB′.A′B, of which the third class will be sterile with the female parent and the second class sterile with the male parent. Or, suppose that AA′ is crossed with AA″. Again 4 classes will be formed, AA, AA′, AA″ and A′A″. AA may then be crossed with AA′, and only 2 intra-sterile classes formed.

This may be assumed to be the simplest form in which a natural population of self-sterile plants may exist, but theoretically it is possible by taking advantage of the phenomenon of pseudo self-fertility or pseudo cross-fertility to obtain a family consisting of but 1 group. In such a family every plant would be sterile with every other plant. It is possible that the two families met in the course of our experiments in which cross-sterility appeared to be universal, were of this kind.

This hypothesis fits perfectly what to us seem the important experimental facts. One may have F_1 generations of various types of complexity, with an increasing simplicity in succeeding generations through inbreeding; or, the F_1 generation may be less complex than the F_2 generation,—the effect of inbreeding first becoming apparent in the F_3 generation. Cross-sterility with resultant intra-sterile classes in single or in different families is explained. Both sterility and fertility in back-crosses is clear. The similar behavior of reciprocal crosses is reasonable. Perfect intra-sterility in the asexual progeny of a self-sterile plant is what is to be expected. The facts established by DARWIN and by CORRENS when viewed with due consideration for pseudo-fertility become orderly. And yet this is but hypothesis, to be modified, extended, restricted or superseded as becomes necessary. If it proves useful for a time it will have served its purpose. Even now there are points upon which other heredity phenomena throw no light. We will devote a concluding paragraph to their discussion.

In our experimental work the number of intra-sterile classes and the number of individuals within each class were determined as definitely as possible. But these experiments have been too much of the pioneer type not to be rough in many ways. With our present experience the same facts could be determined more accurately and on much larger populations with less work than the original determinations demanded, and this appears to be a requisite for further advance. According to our hypothesis, accepting it without subsidiary refinements, the number of classes should always be even, and the classes should be equal in size when only 2 or 4 make up the population. Furthermore there should always be pairs of classes containing the same number of individuals. Now in making some of our calculations we have assumed that the individuals are distributed within the classes in numbers corresponding to the frequencies of the point binomial. Such a distribution was assumed only as a limiting type of unequal grouping, however, there being scarcely any evidence that such a distribution is characteristic. As a matter of fact only in the F_1 of cross No. 2 and No. 3 and its descendants, families H and I, is it possible to say that the number of individuals within the various classes may not be approximately equal. But in these cases we stumble upon an obstacle that cannot be cleared away with our present knowledge. The distributions found in these families are such that larger samples of the populations could not give us classes of equal size. For the present we must accept the conception of a small number of intra-sterile groups in certain families with all that this involves. We might explain them by subsidiary hypotheses of differential vitality or by reduplication in the sense of BATESON, but since there is no other good reason for such assumptions we prefer to leave these matters in abeyance.

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