

SPECIFIC DIFFERENCES IN *PETUNIA*

I. INCOMPATIBILITY

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1. INCOMPATIBILITY IN THE GARDEN *PETUNIA*

Incompatibility may be defined as the failure, following mating or pollination, of a male gamete and a female gamete to achieve fertilization, where each of them is capable of uniting with other gametes of the breeding group after similar mating or pollination. Two classes of incompatibility may then be distinguished:

(i) *Heteromorphic incompatibility*, which is associated with, and dependent on, variation in floral morphology. In heterostyled plants, which fall into this class, the incompatibility reaction depends immediately on the relative positions of anther and stigma, and only indirectly on the genetical constitutions of the plants themselves. Such incompatibility is also described as illegitimacy.

(ii) *Homomorphic incompatibility*, which is not dependent for its action on morphological variation. Here the incompatibility reaction depends directly on the relation existing between the genetical constitution of the zygote producing the female gamete and, in most cases, the genetical constitution of the male gamete. This may be termed *gametic incompatibility* in contradistinction to *zygotic incompatibility* where it is the genetical constitution of the zygote producing the male gamete, rather than that of the male gamete itself, which, together with the constitution of the female zygote, determines the incompatibility reaction. The fungi show a somewhat similar phenomenon, which may conveniently be designated *haploid incompatibility*, as it is manifested only between haploid individuals, the diploid phase never entering into the reaction.

Only one organism, *Capsella grandiflora* (Riley, 1936), has been shown to display zygotic incompatibility, though the sea squirt, *Ciona*, may fall into this class; but the gametic type seems to be very common (see East, 1940). The inheritance of gametic incompatibility conforms very generally to the oppositional factor scheme first devised by East & Mangelsdorf (1925) to explain the behaviour of *Nicotiana glauca* and its derivatives, and by Filzer (1926) for *Veronica syriaca*.

In these species each plant is normally completely self-incompatible, though in *Nicotiana glauca* seed can be obtained from self-pollination in the bud or under other exceptional circumstances. Elsewhere, however, complications may be introduced by two agents. One of these is the existence of so-called fertility allelomorphs, which will now be termed

compatibility allelomorphs in accordance with the present terminology. East (1929) and Anderson & de Winton (1931) have described such allelomorphs from the self-compatible *N. Langsdorffii*, and they have also been discovered in other genera.

As their name suggests, such compatibility genes are allelomorphic to the incompatibility genes of the related self-incompatible species. A different type of compatibility has, however, been discovered within the normally incompatible groups themselves. Where normally incompatible pollinations set seed, it is referred to as pseudo-fertility, or as is now more appropriate, pseudo-compatibility. Such a result may be due to end-season effects or to a special pollination technique, and indeed occasional pseudo-compatibility may be characteristic of any incompatibility system; but it may also be brought about by the action of non-allelomorphic genes which have the effect of weakening or overriding the incompatibility reaction (East, 1929; Brieger, 1930). It may be remarked that the existence of strong pseudo-compatibility, or of compatibility allelomorphs in a normally incompatible group seems to be associated with a hybrid origin of the plants in question or of their ancestors.

Incompatibility has long been known in *Petunia*, but self-compatibility has always been found in some of the plants used in each of the investigations. Correns (1912) records a cross between *P. nyctaginiflora* (*P. axillaris*) and *P. violacea*, which gave both self-compatible and self-incompatible progeny. Though, as we shall see later, such a result can be obtained by intercrossing these species, it is more likely that Correns was using horticultural varieties derived from the original species cross of 1834 (see below), for *P. violacea* at least seems to have been lost from cultivation in Europe during the latter part of the nineteenth and the early twentieth centuries. Terao (1923) intercrossed self-compatible and self-incompatible plants of *P. violacea* and obtained both self-compatible and self-incompatible progeny. Later, Terao & U (1929) distinguished several grades of incompatibility showing complicated inheritance. Some evidence was, however, obtained of the existence of a series of incompatibility allelomorphs of the *Nicotiana* type.

A fuller investigation of incompatibility in *Petunia violacea* was carried out by Harland & Atteck (1933), who showed that inheritance basically followed the oppositional factor scheme of *Nicotiana* and *Veronica*, but that self-compatibility also occurred 'probably owing to the segregation of minor factors for self-fertility'. We may, however, doubt whether either Terao's plants or those of Harland & Atteck were in fact pure *Petunia violacea*, for garden strains are commonly known by this name. Indeed, Harland & Atteck describe their plants as having white, purple, violet and mauve flowers, whereas wild *P. violacea* is magenta in colour. Wergin (1936), whose findings are much the same as those of Harland & Atteck, except that he discovered a major incompletely dominant self-compatibility gene not allelomorphic with the incompatibility factors, definitely used horticultural hybrids. This is also true of Tseng (1938), who found that selfing compatible plants gave progenies including self-incompatibles and intercrossing self-incompatibles gave some self-compatible progeny.

An attempt was made by Tjebbes (1931, 1932) to obtain the two species, *P. axillaris* and *P. violacea*, for studies of their relative fertilities; but inasmuch as neither was found to be self-compatible, while they intercrossed freely, it is to be presumed that he was successful only in obtaining garden strains approaching more or less to the parental specific types.

Thus there is reason to believe that all these investigations, which have brought to light an oppositional factor scheme complicated by non-allelomorphic compatibility genes, have been made on garden strains. Clearly the cause of this complicated inheritance must be sought in the behaviour of the parental species, which also might be expected to throw further light on the genetical control of incompatibility and its evolution.

2. THE ORIGIN OF THE GARDEN *PETUNIA*

The garden *Petunia* originated from the hybridization of the white-flowered species *P. axillaris* (often called *P. nyctaginiflora*) and the magenta-flowered *P. violacea* (also called *P. integrifolia*). The former, which was discovered by Commerson along the shores of the River Plate, was introduced into cultivation in 1823. Bailey (1896) records that it sets seed very readily and often persists for long periods in gardens by self-sowing. *P. violacea* flowered first in cultivation at the Glasgow Botanic Garden in 1831, from seed sent by John Tweedie, resident at Buenos Aires. A hybrid between the two species flowered at Glasgow in 1834, and by 1837 a number of ornamental strains had been developed. *P. violacea* must have proved difficult to keep by seed, as it was lost from cultivation in the nineteenth century.

In 1916, however, this species was reintroduced at Kew Gardens from material sent by C. E. R. Rolland, vice-consul at Montevideo. This *P. violacea* has been maintained at Kew since that time by cuttings. It has never set a full crop of seed there, but was hybridized with *P. axillaris* in 1931.

P. axillaris was reintroduced by Prof. M. C. Ferguson of Wellesley College, Massachusetts, from seed collected by Prof. L. R. Parodi from the River Plate region. Ferguson & Ottley (1932) give detailed descriptions of this *P. axillaris* and of *P. violacea* which they obtained as a cutting from the Kew stock. They record that *P. axillaris* sets an abundance of seed after self-pollination, while *P. violacea* sets little if any.

I am indebted to Prof. Ferguson for seed of her wild *P. axillaris* and to the Director of Kew Gardens for cuttings of *P. violacea*. Thus my stocks are the same as those of Ferguson & Ottley, and accord fully with their descriptions.

I am also indebted to Mr H. G. Callan for the information that both species are diploid ($n=7$), and that meiotic pairing of the chromosomes in the species hybrid is complete, the chiasma frequency being, in fact, intermediate between those of the parental species. The bad pollen produced by the hybrid must thus be attributed to unbalance of some of the gametic combinations of chromosomes.

3. THE BEHAVIOUR OF THE PARENTAL SPECIES

All plants of *P. axillaris* set an abundance of seed on being self-pollinated or intercrossed. With rare exceptions, which may reasonably be attributed to faulty management, some scores of pollinations have yielded a full capsule of seeds. No trace of incompatibility has been found.

The situation is, however, very different in *P. violacea*. Here self-pollination is rarely followed by the production of seeds. A small quantity of seed has been obtained in this way from a few flowers, but these cases may be attributed to a low degree of pseudo-compatibility, the plants being, in the broad sense, incompatible. Bud pollinations did not appear to lead to any greater pseudo-compatibility than the pollination of open flowers.

The original material of *P. violacea* consisted of two plants which had been derived ultimately as cuttings of the same individual. Hence it has been impossible to determine whether the inheritance of incompatibility depends on a series of multiple allelomorphs as in *Nicotiana*. It has, however, been possible to show that incompatibility in *Petunia violacea* resembles that of *Nicotiana* and *Veronica* in being of the gametic type.

Forty-three plants were raised from seed given by pseudo-compatible pollinations of the original plant. All of these were self-incompatible and twenty-five failed to set seed freely after pollination with their parent. Fifteen of the remainder set seed freely with parental pollen and the other three set some seed, though not in all pollinations, the failures, of course, being quite possibly due to faulty management. One of the fifteen plants which set regularly with the parent was used as a pollinator on the rest, but no seed was obtained. Later tests were made of some of these fifteen as males on to the parent but no seed resulted. Furthermore, when allowed to interpollinate freely by natural means only the plants which were compatible with the parental pollen set seed, and they set an abundance. Thus, if the parent is S_1S_2 the progeny fall into two groups, twenty-five being also S_1S_2 , and so failing to set with parental pollen, and fifteen being S_2S_2 , which set with their parent used as male, but fail when used as male on their parent. Two of the three doubtful cases are most probably of the type S_2S_2 . On the basis of observations on pollen-tube growth, made by Mr A. J. Bateman, the remaining plant was S_1S_1 , but no final compatibility tests were possible.

Such behaviour is characteristic of gametic incompatibility, and in view of the existence of an allelomorphic series of incompatibility genes in the garden *Petunia*, there can be little doubt that *P. violacea* reproduces the system found in *Nicotiana* and *Veronica*.

Neglecting the three doubtful cases, we have a segregation of 15 S_2S_2 : 25 S_1S_2 , so showing a noticeable excess of S_2S_2 homozygotes and a very marked deficiency of S_1S_1 homozygotes. A similar family was observed by Harland & Atteck, consisting of fifteen heterozygotes, ten homozygotes of one kind and four homozygotes of the other kind. In other families Harland & Atteck observed a deficiency of both homozygous types. This they attributed to reduced viability of homozygotes, to which we may perhaps also ascribe the shortage of S_1S_1 plants in the present data. It would, however, seem likely that the excess of the other type of homozygote, provided that it is not illusory, is due to S_2 pollen growing more easily and achieving fertilization more often than S_1 pollen, when placed on to an S_1S_2 stigma. The existence of such a difference in incompatibility strength between the two allelomorphs is supported by the behaviour of the first generation hybrids between the species, to which we shall now turn.

4. THE SPECIES HYBRID

Crosses have been successful between the two species only when *Petunia axillaris* has been used as the female. Out of some dozens of crosses made the other way, not one set a single seed. This is all the more remarkable when it is remembered that *P. axillaris* has a style some four or five times as long as that of *P. violacea*. Clearly success depends on the physiological relations existing between pollen and style, rather than on the length of tissue down which the pollen tubes must grow.

The frequency of successful pollinations of *P. axillaris* by *P. violacea* depends on external conditions. In 1939 no hybrids were obtained after using pollen of the S_1S_2 *P. violacea* on 101 flowers of *P. axillaris* in the greenhouse; but out of about twenty

similar crosses made under a muslin cage in the open three gave good capsules of hybrid seed. In 1940, however, thirty-five flowers of *P. axillaris* when pollinated in the greenhouse by S_2S_2 *P. violacea* gave three capsules.

The breeding behaviour of the hybrids and of their parents is shown in Table 1. Two classes were distinguishable by their behaviour following self-pollination in the first groups of hybrids, whose male parent was S_1S_2 *P. violacea*. In the first of these two classes each plant set seed freely with its own pollen, and hence the class was termed self-compatible (s.c.). The other class often failed to set seed after self-pollination, but

Table 1

♂ \ ♀	<i>P. violacea</i>		s.i. [S_1S_2]				s.c. [S_2S_1]					
	<i>P. axillaris</i> [S_2S_1]	[S_1S_2]	[S_2S_2]	1	2	3	4	1	2	3	4	5
<i>P. axillaris</i> : [S_2S_1]	Many s	Many f Few s	Many f Few s	4 s	4 s	2 s	—	—	—	2 s	2 s	2 s
<i>P. violacea</i> : [S_1S_2]	Many f	Many f Few p	Many f	8 f	6 f	1 p 12 f	—	—	—	10 f	12 f	8 f
[S_2S_2]	4 f	Many s	Many f	2 s	3 s	2 s	—	—	—	2 s	1 s 1 p	2 s
s.i. [S_1S_2]:												
1	7 f	3 s	2 s	3 s 4 p 7 f	1 s 5 p 2 f	2 p	2 p	—	2 s	2 s	2 s	2 s
2	7 f	4 s	2 s	3 p 2 f	5 s 2 p 5 f	1 s 1 p	2 f	—	2 s	4 s	2 s	2 s
3	12 f	4 s	2 s	2 f	2 f	5 p 5 f	2 s	—	2 s	3 s	2 s	2 s
4	—	—	—	2 f	2 f	4 f	2 f	—	—	2 s	2 s	2 s
s.c. [S_2S_1]:												
1	2 p	2 s	—	3 s	1 s	2 s	—	2 s	—	2 s	2 s	2 s
2	1 p 1 f	2 s	—	2 s	1 s	2 s	—	—	2 s	2 s	2 s	2 s
3	2 s 4 p 3 f	3 s	2 p	4 s	4 s	4 s	1 s	—	2 s	4 s	2 s	2 s
4	13 f	4 s	1 p 1 f	2 s	2 s	3 s	2 s	—	2 s	3 s	4 s	2 s
5	3 s 8 f	3 s	2 f	2 s	2 s	2 s	2 s	—	2 s	2 s	2 s	4 s

Many indicates more than 20 pollinations. s indicates successful pollination, p partially successful pollination and f unsuccessful pollination as judged by seed set. The number preceding the letter shows the number of flowers which gave the result. Thus 3 s, 4 p, 7 f means 3 flowers successfully pollinated, 4 flowers partially so, and 7 flowers failing to give seed.

sometimes set a small capsule and even occasionally a full capsule. Though some seed was thus obtained by self-pollination, this class was clearly much less self-compatible than the first and so was designated, for convenience, as self-incompatible (s.i.). On intercrossing it was found that plants of like class gave results similar to those following self-pollination, while all plants set seed freely with pollen from an individual of the opposite class. Two of the three families of hybrids were adequately tested and showed segregations of 5 : 9 and 9 : 3 for s.i. : s.c. respectively, one plant in each family being ambiguous. The third family was less adequately tested, but, so far as the results are trustworthy, contained three s.i. and twelve s.c. plants. This makes a total of seventeen

s.i. and twenty-four s.c., agreeing fairly well with a ratio of 1 : 1. Two further families from a similar cross were grown two years later and out of seventeen tested plants, nine were s.i. and eight s.c., again agreeing with 1 : 1.

The obvious assumption for explaining this segregation in the F_1 hybrids is to suppose that the distinction of s.i. : s.c. depends on whether the hybrid has received S_1 or S_2 from *P. violacea*. It might further be supposed that, since S_2 pollen seems to grow somewhat better than S_1 pollen in incompatible pollinations within the parent species, the s.c. hybrids would carry S_2 . This was tested by making new hybrids using S_2S_2 *P. violacea* as the male parent. Three such hybrid families, comprising forty-five plants, were grown, and each individual tested by selfing two flowers and also by crossing two more flowers with pollen from a known s.i. plant of the previous group. These pollinations should fail if the plant is s.i., but be successful if the plant is s.c. All four pollinations were successful on thirty plants, eight plants gave three sets and one failure, three gave two sets and two failures and four were imperfectly tested owing to the breakage of branches or flowers before it was possible to observe the results of the pollinations. Bearing in mind that these tests were made in the open, the only protection being afforded by waterproof bags, it is clear that all the plants may legitimately be regarded as falling into the s.c. class. Thus the s.c. plants have received S_2 , and the s.i. plants S_1 , from the *P. violacea* parent.

Tests were also made of the hybrids with the parent species, both S_1S_2 and S_2S_2 *P. violacea* being used (Table 1). All the hybrids tested, whether s.c. or s.i., gave seed when used as males on *P. axillaris*. The reciprocal cross, however, reveals a further difference between the two types of hybrid; the s.i. type fails to set seed with *P. axillaris* pollen, while four plants of the s.c. kind set a partial crop with *P. axillaris* pollen. One s.c. plant was unique in failing to set any seed following thirteen such pollinations.

All the hybrids set seed after every pollination with the heterozygous *P. violacea* (S_1S_2), but with the exception of one small capsule, they failed as pollen parents in the reciprocal cross. The homozygous (S_2S_2) *P. violacea* succeeded as pollen parent on all three s.i. hybrids with which it was tried, but in six pollinations on to s.c. hybrids, two on each of three plants, it gave three failures and three partial sets, i.e. small capsules. In the reciprocal cross, however, S_2S_2 *P. violacea* × hybrid, both types, i.e. using s.i. and s.c. as pollen parents, gave seed, with the exception that one capsule out of six was small when s.c. pollen was used.

We have seen that the s.i. hybrids are of the constitution S_1S_a and the s.c. hybrids S_2S_a , where S_a is the allelomorph which *P. axillaris* must be presumed to carry. Then either S_a or non-allelomorphic genes contributed by *P. axillaris* to the hybrid must weaken the incompatibility reaction which S_1 and S_2 control; for S_2S_a sets seed freely on self-pollination and S_1S_a sets some seed. This compatibility cannot be attributed to the S_a pollen being successful in growing down S_1S_a and S_2S_a styles for two reasons. In the first place both types of hybrid carry S_a , yet show different behaviour, so that S_1 and S_2 must take some part in the reaction. Secondly, *P. axillaris* pollen, which is S_a by definition, fails completely on S_1S_a styles and gives only poor sets on S_2S_a . The self-compatibility of the hybrids is most reasonably attributed, at least in the main, to growth of the S_1 and S_2 pollen, the action of these genes being weakened by other genes originating from *P. axillaris*.

The behaviour of the crosses s.i. × s.c. and s.c. × s.i. is in full accord with this assump-

tion. Each sets seed freely as would be expected if the behaviour of the hybrids is conditioned by the action of S_1 and S_2 , for one cross is $S_1S_a \times S_2S_a$ and the other $S_2S_a \times S_1S_a$. If, on the other hand, hybrid compatibility was due to the growth of S_a pollen, there is no reason why the cross s.i. \times s.c. should set more freely than s.i. does after self-pollination. It might, of course, be supposed that hybrid compatibility was due to the growth of S_a pollen, the difference between the s.c. and s.i. classes being due to the different action of S_1 and S_2 in inhibiting or reducing the growth of such pollen. This, however, is a complicating assumption for which there is no external evidence, and is unlikely to be true because it postulates a sharp difference between two allelomorphs, which otherwise show qualitatively similar behaviour, in their reaction to an allelomorph from a different species.

The crosses with heterozygous *P. violacea* are interpretable on the same hypothesis. The S_1S_2 styles of this *P. violacea* should reject both the S_1 pollen of the s.i. hybrids and the S_2 pollen of the s.c. hybrids, but the reciprocal should be compatible because S_1 pollen from *P. violacea* will be successful on S_2S_a styles while S_2 pollen will be successful on S_1S_a styles.

The hypothesis, as so far elaborated, implies a negative behaviour on the part of S_a except for a possible weakening effect on the action of S_1 and S_2 . This may, however, be equally well attributed to non-allelomorphic genes introduced by *P. axillaris* into the hybrid. The crosses with *P. axillaris* accord with the hypothesis. *P. axillaris* pollen is not successful on either *P. violacea*, heterozygous or homozygous, or on s.i. hybrids. It is only slightly successful on s.c. hybrids. Clearly both S_1 and S_2 , especially the former, inhibit the growth of S_a pollen. Equally, S_1 and S_2 pollen from *P. violacea* will grow down the S_aS_a style of *P. axillaris*, though, perhaps owing to the great length of or to the existence of isolating genes in the latter, it is not always successful. The crosses of pollen from the hybrids on to *P. axillaris* are less informative, as setting could be attributed to the sole action of S_a pollen from the hybrids. Pollen-growth observations, made by Mr A. J. Bateman, suggest, however, that, as might be expected, S_1 and S_2 pollen from the hybrids is also successful. Thus S_a pollen is repelled by both S_1 and S_2 in the styles, but S_aS_a styles do not repel either S_1 or S_2 pollen. (Pollination of *P. axillaris* by *P. violacea* is not always successful, but this may be attributed to isolating genes as will be shown elsewhere.) We may note too that S_2 is of weaker action than S_1 . S_2 pollen grows better down S_2S_a styles than does S_1 pollen down S_1S_a styles. This is in accordance with the observation made in the previous section that S_2 pollen is perhaps more successful than S_1 pollen in pseudo-compatible pollinations within *P. violacea*.

When, however, we turn to the crosses between the hybrids and the S_2S_2 *P. violacea* plant, we find that this simple hypothesis is not in itself sufficient. The crosses S_1S_a hybrid \times S_2S_2 *P. violacea* and reciprocal are both fully compatible, as the hypothesis would lead us to expect. Also the cross S_2S_a hybrid \times S_2S_2 *P. violacea* is only weakly compatible; but S_2S_2 *P. violacea* \times S_2S_a hybrid set seed freely in five cases out of six, the odd case being one of partial compatibility. We can make a table showing the action of S_2 in the hybrid and in *P. violacea*:

Style of <i>P. violacea</i> , S_2S_2 s.c. hybrid, S_2S_a	Pollen from	
	<i>P. violacea</i> , S_2S_2	s.c. hybrid, S_2S_a
	Fails	Takes
	Fails	Takes

It is thus the S_3 pollen from the hybrid which is successful on styles carrying S_2 rather than S_2S_a styles which are less inhibitory to S_2 pollen than S_2S_2 styles, though there is a slight weakening of the action of S_2 in S_2S_a styles. This can be accounted for if we assume that the weakened action of S_2 in the hybrids is due not to the action of S_a but to one or more non-allelomorphic genes from *P. axillaris*. S_a cannot be present in S_2 pollen from the s.c. hybrids, but non-allelomorphic genes can. Furthermore, there must either be several such modifying genes, or, if there is only one, it must be nearly recessive; for otherwise S_2S_a styles would show as weak an S_2 reaction as does S_2 pollen. If, however, there are several modifying genes, of which S_2 pollen could carry various combinations, some having a greater effect on the action of S_2 than others, or if, in the case of a single gene, it is nearly recessive, the poor compatibility of S_2 pollen from *P. violacea* on s.c. hybrid styles can be reconciled with the fully compatible behaviour of S_2 pollen from the hybrid on both S_2S_2 *P. violacea* and S_2S_a hybrid styles.

It may be observed, too, that the unique complete failure of *P. axillaris* pollen on the style of s.c. plant 4, also suggests the existence of at least one non-allelomorphic modifier of the action of S_2 , in this case one strengthening its repulsion of S_a pollen. This unique case cannot be attributed to variation in the S_a pollen from *P. axillaris* used in the crosses, because the same two *P. axillaris* plants were used to test all the s.c. hybrids.

The modified effect of S_2 pollen from the hybrids is not shown on the style of an S_1S_2 *P. violacea*. Here S_2 pollen fails whether it came from pure *P. violacea* or from an s.c. hybrid. It would thus appear that S_2 has a stronger action in S_1S_2 styles than it does in S_2S_2 or S_2S_a styles. S_1 must be supposed to have the property of making S_2 inhibit S_2 pollen more efficiently in the styler tissue, even though S_1 itself has no noticeable inhibitory effect on S_2 pollen. It is impossible to say whether S_2 similarly strengthens the action of S_1 in S_1S_2 styles, as no S_1S_1 homozygotes have been used in the experiments, and so no standard of comparison is available.

An alternative explanation of the results would be to suppose that S_2 has not the property of inhibiting the growth of S_a pollen, whereas S_1 has this property. Then S_a pollen from an s.c. hybrid would grow down an S_2S_a style but would be inhibited in an S_1S_a style. Reasons have already been given for rejecting this hypothesis, which depends on the unlike reaction of two otherwise similar allelomorphs to a third allelomorph from a different species. A further test is, however, possible. On this view, if s.c. pollen is placed on an S_2S_2 *P. violacea* style, one-half the pollen at most should grow, viz. the S_a pollen. If, on the other hand, pollen is taken from an s.i. hybrid all of it should grow, for neither the S_1 nor the S_a pollen would be inhibited by the S_2S_2 style. Mr A. J. Bateman has examined pollen growth following pollinations of these two kinds, and can find no evidence of a difference of the magnitude which would be expected on this view. Thus we are strengthened in our conclusion that it is the S_2 pollen from the s.c. hybrid which is successful on S_2S_2 *P. violacea* styles, and that S_1 has the effect of reinforcing the action of S_2 when present in the same style.

5. BEHAVIOUR IN LATER GENERATIONS

The behaviour of the F_1 species hybrids in relation to one another and to their parents is thus explicable in terms of the following four postulates:

- (i) S_1 and S_2 from *P. violacea* act as oppositional factors of the *Nicotiana* type.
- (ii) The allelomorph S_a , which *P. axillaris* must be presumed to carry, has the negative

properties of never growing efficiently down a style containing either S_1 or S_2 , or both, and of never inhibiting the growth of S_1 and S_2 pollen.

(iii) *P. axillaris* carries a gene or genes, non-allelomorphic with S , which modify the incompatibility reaction of both S_1 and, especially, S_2 in style and pollen.

(iv) S_1 strengthens the action of S_2 , in S_1S_2 styles, in inhibiting the effective growth of S_2 pollen.

Verification of these postulates, especially (iii) and (iv), was sought from the behaviour of F_2 plants and of plants obtained by backcrossing with the parental species. Of these the F_2 's are the most informative.

Four types of F_2 family can be raised, viz. from self-pollination of s.i. hybrids, from self-pollination of s.c. hybrids, and from the reciprocal crosses between s.i. and s.c. s.i. plants have the constitution S_1S_2 and, by hypothesis, should give equal numbers of S_1S_2 and S_1S_1 offspring after self-pollination. A very small proportion of S_2S_2 offspring might perhaps occur, as, even though S_2 pollen does not easily grow down S_1S_2 styles, self-pollination of s.i. hybrids does not in any case lead to full compatibility. Ten such F_2 plants, five from progeny of each of s.i. plants 1 and 2, were tested by self-pollinating

Table 2

Parent and plant no.	Tested with pollen from				
	Self	s.i.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
s.i. 1 selfed					
1	—	f, f	s, s	f, f	s, s
2	f, f	p, f	s, s	f	s, s
3	s, s	s, f	s, p	f, f	s, f
4	s, s	f, f	s, s	f, f	s, s
5	—	f, f	s, s	f, f	s, s
s.i. 2 selfed					
1	p, f	p	s, s	f, f	s, s
2	p, p	f, f	s, s	f, f	s, s
3	—	p, p	s, p	f, f	s, s
4	f, f	f, f	s, s	f, f	s, s
5	—	p, f	s, s	f, f	s, s

In Tables 2-11 each letter represents one pollinated flower. s indicates a successful pollination, p a partially successful pollination, and f a pollination which failed to give seed.

two flowers, crossing two flowers with pollen from s.i. F_1 hybrids, two with pollen from s.c. F_1 hybrids, two with pollen from *P. axillaris* and two with pollen from S_1S_2 *P. violacea*. The results are given in Table 2. Two pollinations is a somewhat small number on which to base a conclusion concerning the incompatibility of a given cross, because faulty management could lead to serious misjudgment; but, as will be seen later, the total pollination programme was large and it was thought more desirable to try all crosses with a few flowers each, than a few crosses on an extensive scale. Reliance for the detection of false results must be placed in the comparative behaviour of single plants with various kinds of pollen and of different plants with the same pollen. In actual practice very little difficulty was experienced in picking out the trustworthy results.

In the case of the F_2 plants from self-pollinated s.i. hybrids, *P. axillaris* pollen completely failed to cause seed setting. In other words, we must suppose that no S_2S_2 individuals were present, at least among the ten plants tested. This is confirmed by the failure, complete or nearly complete, of s.i. pollen on all ten plants. s.c. pollen, on the other hand, was, as might be expected, successful in all cases, only two tests out of twenty giving partial takes with the rest fully compatible. Heterozygous *P. violacea*

pollen is also compatible as would be expected. Not all the F_2 plants were tested by self-pollination, because three were male sterile and one nearly so. The remaining six consisted of two which failed completely with their own pollen, one which showed very slight signs of self-compatibility, one which was definitely partially self-compatible and two fully self-compatible. If, as test crosses with *P. axillaris* pollen and s.c. pollen lead us to believe, no $S_a S_a$ plants were present, we must have either fully self-compatible $S_1 S_a$ plants or fully self-compatible $S_1 S_1$ plants. The former possibility is the more likely as the s.c. F_1 's are partially self-compatible; but, in any case, it is clear that at least one non-allelomorphic gene, whose effect is to weaken the action of S_1 on $S_1 S_a$ plants, must be postulated. If only one were present, it must be nearly recessive, since the parental s.c. hybrids were less self-compatible than these F_2 individuals. It would, however, appear that this gene could not be the same as the one responsible for weakened action of S_1 in the F_1 hybrid, as compared with the parental *P. violacea*. The possibility of several modifying genes will be discussed later.

Table 3

Parent and plant no.	Tested with pollen from				
	Self	s.c.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
s.c. 3 selfed:					
1	—	p, p	p, p	f	s, s
2	s, s	s, s	s, p	s, p	s, s
3	s, s	s	p, p	p, f	s, s
4	—	s, p	s, s	p, p	s, s
5	s, s	s, s	p, p	f, f	s, p
s.c. 4 selfed					
1	—	s, s, f	s, f, f	f, f	s, s
2	s, s	s, s	s, f	s, s	s, s
3	—	s, p	s, s	p, p	s, s
s.c. 5 selfed					
1	—	s, s	s, p	p, f	s, s
2	s, s	s, p	p, p	p, f	s, f

The F_2 from the self-pollinated s.c. hybrids is less informative (Table 3). It is known that S_a pollen is not quite wholly inhibited in $S_2 S_a$ styles, so we may expect $S_2 S_2$, $S_2 S_a$ and $S_a S_a$ plants, though the last may be rare. All the plants tested proved to be self-compatible and all but two were reasonably compatible with s.c. pollen. All were, as might be anticipated, compatible with s.c. and *P. violacea* pollen. When, however, we turn to the tests with *P. axillaris* pollen we see that two plants were clearly compatible and a number of others partially so, whereas s.c. F_1 hybrids were, at best, only slightly compatible with this type of pollen. It might be supposed that the two especially compatible plants were of the constitution $S_a S_a$, but it so happens that one of them was descended from s.c. plant 4, which, as we saw earlier, was unique among the s.c. hybrids in completely refusing S_a pollen from *P. axillaris*. Furthermore, this same plant set only one capsule out of two flowers tested with s.c. pollen. This may be due to accidental mismanagement; but, coupled with the ancestry of the plant, it strongly suggests that the operation of the incompatibility system has changed. The production of a plant which accepts *P. axillaris* pollen but partially refuses s.c. pollen in the F_2 progeny of an F_1 whose behaviour leads us to expect no $S_a S_a$ individuals is very suggestive of modifying genes.

The remaining F_2 's from s.i. \times s.c. and s.c. \times s.i. are expected to contain a type absent from both of the F_2 's considered so far, viz. S_1S_2 . The first of these crosses, s.i. \times s.c., is $S_1S_a \times S_2S_a$ and, since S_a pollen has never been observed to be effective in s.i. styles, should give S_1S_2 and S_2S_a plants in equal numbers. The former should fail to set seed after self-pollination and also after pollination by the s.i. parent. The latter type should set seed both after selfing and after crossing with the s.i. parent. Table 4 shows that five plants set with s.i. pollen and five failed or nearly failed. Where selfing was possible, some plants being male sterile, its results agreed with those from the s.i. tests. This agreement is especially striking in two cases where both self-pollination and pollination by s.i. nearly, but not quite, proved incompatible.

Table 4

Parents and plant no.	Tested with pollen from				
	Self	s.i.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
s.i. 3 \times s.c. 3					
1	p, f	p, f	f, f	f, f	s, p
2	f	f, f	f, f	f, f	s, p
3	s, s	s, s	s, s	f, f	s, s
4	p, p	p, f	f, f	f, f	s, s
5	—	s, s	s, s	p, p	s, s
s.i. 1 \times s.c. 4					
1	p, p	s, s	s, s	s, s	s, s
2	—	s, s	s, s	f, f	s, s
3	—	f, f	f, f	f, f	p, f
4	s, s	s, s	s, p	s, p	s, s
5	—	f, f	f, f	f, f	f, f

These five plants, which fail or nearly fail, are thus S_1S_2 and the other five are S_2S_a . The latter set seed with pollen from s.i. hybrids, s.c. hybrids and heterozygous *P. violacea* as would be expected. Two of them failed completely with pollen from *P. axillaris* and one set poorly; but the remaining two set good capsules—behaviour which was not shown by the s.c. hybrids of the previous generation, though these were of the same constitution in regard to the S gene. Evidently a recessive modifier or a number of modifiers have segregated.

The S_1S_2 plants of this F_2 show two remarkable properties. In the first place they refuse to set with pollen from the s.c. hybrids, though their S_2S_a sisters set with the same pollen. Just as was observed in the case of the cross of s.c. hybrid on to S_1S_2 and S_2S_2 *P. violacea*, described in the previous section, S_1 is strengthening the action of the S_2 allelomorph. There can thus be no doubt that S_1 and S_2 display this peculiar relation, which shows that they must govern some common reaction, as well as the reactions by which they differ.

The other remarkable property of the S_1S_2 F_2 plants is that they set seed more or less readily after pollination with S_1S_2 *P. violacea*. Thus the S_1S_2 system in the F_2 is internally efficient, though it shows more pseudo-compatibility than does *P. violacea*, but it is, so to speak, out of phase with the S_1S_2 system of *P. violacea*. The same incompatibility allelomorphs are at work but they operate in a somewhat different way. We must assume that this is due to modifying genes.

The reciprocal F_2 from s.c. \times s.i. is also expected to contain S_1S_2 plants together with S_1S_a and perhaps also a few S_2S_a and S_aS_a in the first of the two progenies tested. The ten plants tested from F_2 's of this kind (Table 5) are less helpful than those from the

reciprocal cross. Only two of them fail with s.i. pollen, one of which also nearly fails with s.c. pollen and with its own pollen. This must be an S_1S_2 individual, and it agrees with what we should expect from a plant of this constitution by refusing *P. axillaris* pollen but partially setting with *P. violacea* pollen. The remaining plants are difficult to classify as all set more or less well with all the pollen tried except, in some cases, with that from *P. axillaris*. Only the last plant in the table seems exceptional in refusing to set with its own pollen; but an exceptional failure always raises the suspicion of mis-managed pollination. The second plant which fails with s.i. pollen was not tested with its own pollen but is compatible with s.c., and so is most likely of the constitution S_1S_2 .

Table 5

Parents and plant no.	Tested with pollen from				
	Self	s.i.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
s.c. 3 × s.i. 3					
1	s, s	s, s	s	p, p	s, s
2	s, s	s, s	s, s	p, f	s, s
3	—	s, s	s, p	s, p	s, s
4	s, s	s, s	s, p	p, p	s, s
5	s, s	p, p	s, s	f, f	s, s
s.c. 4 × s.i. 1					
1	—	f, f	s	f, f	s, s
2	p, f	f	p, f	f, f	s, f
3	s, s	s, s	s, s	p, p	s, s
4	s, s	s, p	s, s	s, s	s, s
5	f, f	s, s	s, p	s, s	s, s

Table 6

Parents and plant no.	Tested with pollen from				
	Self	s.i.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
<i>P. axillaris</i> × s.i. 3					
1	s, s	s, p	s, s	s, s	f
2	s, s	p, p	s, p	s, s	f, f
3	s, s	s, p	s, s	p, f	f, f
4	s, s	p, p	s, s	s, s	p, f
5	s, s	p, p	s, s	s, s	f
<i>P. axillaris</i> × s.i. 1					
1	s, s	s, s	s, s	s, s	s, p
2	s, s	p, p	p, p	s, s	p, f
3	s, s	p, p	p	s, s	f, f
4	s, s	p, p	s, s	s, s	f, f
5	s, s	p, p	s, s	s, s	p, f

No certain conclusion can be drawn from the rest, except that they do not fully conform to any simple scheme based on the interrelations of S_1 , S_2 and S_3 . Modifiers are presumably at work.

Progenies were also tested from the crosses *P. axillaris* × s.i. and *P. axillaris* × s.c. (Tables 6, 7). These require little comment. All but one plant set well with *P. axillaris* pollen, and even this exceptional individual gave a partial set. All set with their own pollen and gave a poor set or no set at all with *P. violacea* pollen. Reasonably good sets were obtained from all with s.i. and s.c. pollen. In fact they behave very nearly like *P. axillaris* itself; for, it will be remembered, though S_2S_3 styles permit the growth of S_1 and S_2 pollen, crosses of *P. axillaris* by *P. violacea* do not always set seed. The back-cross to *P. axillaris* would appear to have introduced so many modifying genes that the incompatibility allelomorphs if present have largely ceased to be operative.

The backcrosses with heterozygous *P. violacea* were all made with the parent species as male. The cross S_1S_a (hybrid) $\times S_1S_2$ (*P. violacea*) is expected to give S_1S_2 and S_2S_a plants in equal numbers, just as was the cross s.l. \times s.c. Thus the results of this backcross form a test of the interpretation of the F_2 . Applying the same criteria as with the F_2 , viz. the failure to set after self-pollination and after pollination by s.l., we find that four plants fall into the category S_1S_2 , the other six being S_2S_a (Table 8). Furthermore, we find, exactly as before, that the S_1S_2 plants fail with pollen from s.c. hybrids, though the S_2S_a plants set with the same pollinator. In fact the only difference, a very slight one, is that none of the S_2S_a 's set freely with *P. axillaris*, whereas plants of this con-

Table 7

Parents and plant no.	Tested with pollen from				
	Self	s.l.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
<i>P. axillaris</i> \times s.c. 3					
1	s, s	s, p	s, s	s, s	f, f
2	s, s	p, p	p, p	s, s	f, f
3	s, s	s, p	p, p	s, s	f, f
4	s, s	p, p	s, s	s, s	f, f
5	s, s	p, p	s, p	s, s	f, f
<i>P. axillaris</i> \times s.c. 4					
1	s, s	s, s	s, s	s, s	f, f
2	s, s	p, p	s, s	s, s	f, f
3	s, s	p	s, s	s, s	f, f
4	s, s	p, p	s, p	s, s	f, f
5	s, s	p, p	p, p	s, s	f, f

Table 8

Parents and plant no.	Tested with pollen from				
	Self	s.l.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
s.l. 3 \times <i>P. violacea</i> (S_1S_2)					
1	—	f, f	f, f	f, f	f, f
2	p, p	s, s	p, p	f, f	s, s
3	f, f	f, f	f, f	f, f	f, f
4	s, s	s, s	s, s	p, p, f	s, s
5	—	s, s	s	f, f	s, p
s.l. 1 \times <i>P. violacea</i> (S_1S_2)					
1	—	s, s	s, s	f, f	p, p
2	f, f	f, f	f, f	f, f	p
3	p, p	p, p	s, p	p, f	s, s
4	s	s, p	s, s	p, p	s, s
5	f, f	f, f	f, f	f, f	s, s, p, p

stitution in the F_2 did set seed. This is quite in keeping with the supposition, made on the basis of the F_2 results, that *P. axillaris* carries modifiers reducing the efficiency of S_2 in inhibiting S_a pollen; for the more genes from *P. violacea* that an S_2S_a plant carries the less chance there is of *P. axillaris* pollen being successful in its styles. We may observe also that, as before, pollen from heterozygous *P. violacea* causes seed setting on the reconstituted S_1S_2 plants; but, again as would be expected on the modifier view, these sets are less frequent and less complete than those observed with the F_2 plants.

A backcross of s.l. hybrids with pollen from the homozygous (S_2S_2) *P. violacea* should give exactly the same results as the backcross with pollen from heterozygous *P. violacea*. The five plants tested bear out this view as far as the tests go. Only one S_1S_2 plant seems to be present (Table 9).

Specific differences in *Petunia*

The backcross of s.c. by heterozygous *P. violacea* is $S_2S_2 \times S_1S_2$ and so should give equal numbers of S_1S_2 and S_1S_a plants. These are more difficult to discriminate than are S_1S_2 and S_2S_2 . The only reliable test is that S_1S_a should accept pollen from s.c. hybrids which S_1S_2 plants will, as we have seen, reject. One S_1S_a and nine S_1S_2 may be recognized by this means (Table 10). All ten should fail with their own and with s.i. pollen, as S_1S_a plants reject or nearly reject S_1 pollen, unlike S_2S_2 which accepts

Table 9

Parents and plant no.	Tested with pollen from				
	Self	s.i.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
s.i. 1 × <i>P. violacea</i> (S_2S_2)					
1	s, s	s, s	s, s	f, f	s, s
2	—	f, f	p	f, f	s, s
3	—	s	s, s	f, f	s, s
4	—	s, s	s, s	f, f	s, s
5	p, p	s, s	s, s	f, f	p, p

Table 10

Parents and plant no.	Tested with pollen from				
	Self	s.i.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
s.c. 3 × <i>P. violacea</i> (S_1S_2)					
1	f, f	f, f	f, f	f	f, f
2	f, f	f, f	s, p	f, f	s, s
3	f, f	p, f	f, f	f, f	s, s
4	—	f, f	f, f	f, f	s, s
5	p	f, f	p, f	f, f	s, s
s.c. 4 × <i>P. violacea</i> (S_1S_2)					
1	—	p, f	f, f	f, f	p, p
2	f	f, f	f, f	f, f	p, f
3	—	p, f	f, f	f, f	s, s
4	f, f	f, f	f, f	f, f	p, f
5	—	f	f, f	f, f	p, f

Table 11

Parents and plant no.	Tested with pollen from				
	Self	s.i.	s.c.	<i>P. axillaris</i>	<i>P. violacea</i>
s.c. 3 × <i>P. violacea</i> (S_2S_2)					
1	f, f	s, s	s, s	f, f	s, s
2	—	p, p	s, p	f, f	s, s
3	f, f	s, s	s, p	f, f	s, s
4	—	s, s	p, p	f, f	s, s
5	f, f	s, s	s, s, p, f	f	s, f
6	—	s, s	s, s	f, f	s, s
7	f, f	s, p	p, p	f, f	s, s
8	—	s	s, s	f, f	—
9	—	s, s	s, f	f, f	s, s
10	—	s, s	s, p	f, f	s, s

S_2 pollen from hybrids. This expectation is borne out. No plants accept *P. axillaris* pollen, but the S_2S_2 plants accept pollen from heterozygous *P. violacea* rather better than do the S_1S_2 plants in the s.i. backcross. Whether the difference is significant is difficult to say, and perhaps it should not be stressed.

The backcross of s.c. by homozygous *P. violacea* was only partially successful, but ten plants obtained in this way were tested (Table 11). The cross $S_2S_a \times S_2S_2$ should give equal numbers of S_2S_a and S_2S_2 plants. No S_1S_2 are expected, and so no plant should fail with s.i. pollen. None, in fact, do fail. The four plants tested with their own

pollen fail, but this may have little significance as male sterility, complete and partial, was rife in this family. We may note that male sterility is more common in those families which have more *P. violacea* genes. It is not found in the backcross with *P. axillaris*. Evidently small upsets of the incompatibility mechanism are apt to lead to male sterility. The ten plants all fail to set seed with *P. axillaris* pollen, but all set well with heterozygous *P. violacea*, apart from one odd failure. This, of course, accords with expectation.

One of the crucial tests of the hypothesis is that, while s.i. should give, exactly the same classes of progeny when backcrossed by *P. violacea* pollen from both homozygous and heterozygous plants, this is not expected of s.c. \times *P. violacea*. The results described above show clearly that this is the case, the differences in the latter crosses being of the kinds expected.

Thus we see that the four assumptions necessary to account for the behaviour of the F_1 plants, crossed *inter se* and with their parents, are regularly borne out by the behaviour of the plants obtained in F_2 and in the various backcrosses. The interpretation is confirmed.

6. THE EVOLUTION OF THE INCOMPATIBILITY SYSTEM

The operation of gametic incompatibility requires the existence of a minimum of three allelomorphs at the controlling S locus; for all individuals must be heterozygous, and heterozygotes of the same constitution are inter-incompatible. Once a system of at least three allelomorphs is established, the number should tend to grow by mutation, because each new allelomorph has an advantage over the pre-existing more widespread members of the series. When, however, there are many allelomorphs relative to the population size, the frequencies of some of them become so low that they will be lost by random extinction. Thus the number maintained in a population is dependent on the mutation rate, including immigration, and on the population size (Wright, 1939).

The critical stage in the development of a gametic incompatibility system is clearly the establishment of the first three S allelomorphs. East (1929) has supposed that incompatibility allelomorphs accumulate in a self-compatible species, under cover of the compatibility allelomorph, until a sufficient number exist, when the incompatibility system is brought into being, by segregation, in a fully developed state. Though this could happen in *Nicotiana Langsdorffii*, where pollen carrying the compatibility allelomorph, S_1 , can grow down a style carrying any of the incompatibility allelomorphs, it is clearly impossible in *Petunia*. S_a pollen functions poorly, if at all, on any but $S_a S_a$ styles. We must suppose that the incompatible species arose from a compatible ancestor, since a mutation to S_a in *P. violacea* would be eliminated immediately; but we cannot postulate that it arose in the way East suggests because a mutation to S_1 or S_2 in a population otherwise homogeneous for S_a would have the effect of making the mutant individual virtually female sterile. Even in the case of *Nicotiana*, where mutant incompatibility allelomorphs could persist under cover of the compatibility allelomorph, it is doubtful whether they would so persist; for the pollen carrying S_1 , etc., would be at a disadvantage as compared with that carrying S_1 . It could not function in any style carrying the same incompatibility gene. This disadvantage would increase as the incompatibility allelomorph spread in the population.

It might be supposed that S_1 , say, would sometimes persist and even spread in such a population by virtue of the fact that plants carrying S_1 would be somewhat more crossbred than those of the constitution $S_1 S_1$, crossbred plants being supposed to have

on the average a fitter genotype than inbreds. Since, however, it cannot be supposed that the advantage of outbreeding arises suddenly, at the same time as the S_1 mutation occurs, this view demands that the self-compatible species has persisted for some time while waiting, so to speak, for mutations suitable for converting it into the fitter incompatible type. This seems to be highly improbable, as, indeed, is the sudden rise of a marked advantage in outbreeding. It certainly must be presumed that outcrossing is advantageous in incompatible species, for otherwise any suitable mutant compatibility gene would soon oust its incompatibility allelomorphs; but it is much more reasonable to suppose that the change in external conditions, acting directly or through change in population size, to which the increased advantage of outbreeding is attributable, arose gradually.

Hence an incompatibility system cannot be supposed to come into being, by segregation or by any other means, in perfect form. It must develop slowly, each allelomorph gradually showing a stronger incompatibility reaction as the changing conditions gradually favoured increased outbreeding. The system of breeding is an adaptive character and must be supposed to show the same kinds of selective change as any other adaptive character (Mather & de Winton, 1941; Mather, 1943).

Such a gradual transition from self-compatibility to self-incompatibility could be brought about in two ways. First, it may be achieved by the replacement of the compatibility gene by weak incompatibility allelomorphs, which in their turn give way to somewhat stronger incompatibility allelomorphs, and so on. The existence of incompatibility allelomorphs having different strengths is well known. For example, S_{15} in *Nicotiana* is so weak that a homozygous plant will set seed quite readily with its own pollen, while other allelomorphs in the same species seldom, if ever, permit pseudo-compatibility. In *Petunia* S_1 and S_2 differ in this very way, too. There is no evidence that development by replacement of this kind has occurred, but it is clearly a possibility, even though, perhaps in some ways, not a highly likely one. Allelomorphic replacement cannot in any case be regarded as proceeding to the extreme, for otherwise S_{15} would have long ago disappeared from *Nicotiana*.

The second way in which an incompatibility system might develop is by the selection of non-allelomorphic genes which modify the strength of the reaction controlled by the S allelomorphs. The evidence for the existence of such modifiers is extensive. In crosses between the cultivated *Antirrhinum majus* and related wild species Tseng (1938) found a non-allelomorphic gene for self-compatibility. Brieger (1930) has described such genes in *Nicotiana Sanderae*, which is a garden form of hybrid origin. He was able to isolate one major modifier and was of the opinion that a number of minor modifiers also existed. Wergin (1936) and Harland & Atteck (1933) found evidence of such modification in *Petunia* as we have already seen.

The present results allow us to go somewhat further in the analysis of the incompatibility system. Three allelomorphs, S_1 , S_3 and S_a , were segregating in the material, and their general properties were established. But it was necessary to postulate non-allelomorphic genes affecting the following reactions at least:

- (1) The growth of S_2 pollen, from s.c. hybrids, in S_3S_2 and S_3S_a styles.
- (2) The growth of S_a pollen in S_2S_a styles.
- (3) The growth of S_1 pollen on F_2 plants in their own styles, probably of the constitution S_1S_a .

- (4) The growth of S_a pollen in F_2 styles of constitution S_2S_a and S_2S_2 .
 (5) The growth of S_a pollen in S_2S_2 plants from the cross s.i. \times *P. violacea*.
 (6) The growth of S_1 and S_2 pollen in the styles of S_1S_2 having various origins.

Some of these various results could perhaps be ascribed to the action of a single gene, but the complexity and number of the various modifications strongly indicates a number of such non-allelomorphic modifiers. To take two specific examples, S_1 pollen from s.i. hybrids grows better in S_1S_a styles than does S_1 pollen down the S_1S_2 styles of *P. violacea*, while S_1 pollen from F_2 plants can grow better still; and S_1 and/or S_2 pollen from *P. violacea* grows poorly in S_1S_2 styles within *P. violacea*, but grows better in S_1S_2 styles of plants from the backcross s.i. \times *P. violacea* and best of all in S_1S_2 styles of some F_2 plants. Such results could be explained in terms of single modifying genes, but such genes must have very special properties. They are, however, just the type of result to be expected if a number of genes, each having a small effect, were at work. In other words, the evidence strongly suggests that the self-compatible *P. axillaris* carries a number of modifying genes which individually and collectively weaken the action of the incompatibility system of *P. violacea* to various degrees.

When S_1 and S_2 are transferred to *P. axillaris*, by appropriate crosses, it would appear likely that they cease to control any incompatibility reaction at all. The modifiers of the self-compatible species have removed the reaction which the S allelomorphs control. The existence of an incompatibility reaction is thus polygenically determined, and its strength is under polygenic control. The S allelomorphs only control the direct working of the reaction once it is established. They are merely switch allelomorphs. Thus the rise of outbreeding, depending on the operation of incompatibility allelomorphs, would be due to selection of the polygenic combinations controlling the strength of the reaction. There might also, of course, be some selection of allelomorphs at the S locus according to their efficiency of action with the existing polygenic background.

Though it is perhaps too much to say that the *Petunia* crosses have proved that the strength of the incompatibility reaction is under polygenic control, it is clear that they are well in keeping, and, indeed, strongly suggest such an interpretation. And this view enables us to see exactly how incompatibility systems develop. In so far as the rigour of the incompatibility reaction is polygenically controlled, it may show any strength between complete compatibility and complete incompatibility. The system can show a gradually increasing efficiency as external conditions gradually favour more rigorous outbreeding. The change from compatibility to incompatibility will be smooth and will occur when circumstances favour such a change, and to the extent that circumstances favour it. The same S allelomorphs can operate the mechanism at every stage, but the incompatibility that they control will change smoothly in the way that only such characters as are under polygenic control can change. Furthermore, as soon as conditions favour the rise of an incompatibility system, it can begin to develop, for one of the most striking properties of polygenic variability is that it can exist as potential variability in the genotype, having but little phenotypical effect. Thus self-compatible species can carry all the polygenes necessary for the development of an incompatibility system, if conditions should favour one (see Mather, 1941, 1943). Such a development would be slow, as the release of polygenic variability from the potential to the free state is slow, though it would presumably be speeded up by the development of incompatibility (Mather, 1943); but then external conditions would be changing only slowly, and polygenic change,

though slow, can proceed to great lengths, because large amounts of variability can exist in the potential state.

On this polygenic view we must anticipate that related species will frequently have well-developed incompatibility systems depending on the same **S** allelomorphs, but differing in the polygenic combinations on which the efficient action of the switch allelomorphs depends; for selection to maintain polygenic balance will proceed independently in the species, and these will in consequence digress in their polygenic combinations (Mather, 1943). On intercrossing two such related species we should expect that recombination between the highly selected polygenic combinations, now brought together for the first time, would destroy their balance and lead to a more or less complete breakdown in the efficiency of the incompatibility mechanism (Mather, 1941). Pseudo-compatibility would occur in the F_2 and later generations. This is exactly what appears to happen in *Nicotiana Sanderae*. The incompatible species *N. alata* and *N. Forgetiana* rarely show pseudo-compatibility and then only under special circumstances; but *N. Sanderae*, which is a horticultural 'species' derived by hybridization of *N. alata* and *N. Forgetiana*, frequently shows pronounced pseudo-compatibility (Brieger, 1930).

Different polygenic combinations will thus be capable of giving a variety of incompatibility systems with the same **S** allelomorphs. We have seen how, in *Petunia*, two or more incompatibility systems of different strengths can be built up all having S_1 and S_2 from *P. violacea*, but with different mixtures of polygenes from the two parents. It is then found that S_1 and S_2 operate less efficiently between systems than they do within systems. The polygenes are determining the system, the **S** allelomorphs merely control its direct working. Thus within a system S_1 and S_2 can share some property not shared by either allelomorph when it is operating with a different system of polygenes. The behaviour of S_1 , as compared with S_a , in strengthening the action of S_2 in *Petunia* is further evidence of this phenomenon. So we can see that allelomorphs having little if any effect on the breeding system in a compatible species can be built up into an efficient incompatibility system by selection of polygenes which affect all the **S** allelomorphs alike. It is not necessary to suppose that each allelomorph has its own specific polygenic modifiers.

On this view, it is clear that any new allelomorphs at the **S** locus, arising by mutation from pre-existing allelomorphs, would immediately be effective in operating the incompatibility system. There would be no necessity for selection of modifiers to enable them to work efficiently, for this selection would already have taken place in the development of the system depending on the original allelomorphs. If one adopted some alternative interpretation of the development of an incompatibility system, such as the allelomorphic replacement view, or the view that each allelomorph had its own specific modifiers, this conclusion would not follow. We should then expect that at least some mutant allelomorphs would not operate efficiently until sufficient time had elapsed for them to be rendered efficient. Or, on the replacement view, they might never be efficient. Inefficient allelomorphs, whether they were inefficient because of their own nature, or because of their failure to work efficiently in the existing polygenic background, would doubtless ultimately be eliminated by selection, only the efficient ones remaining, and then it would be difficult to distinguish such a system from that in which all new allelomorphs were immediately efficient. But clearly the latter would be the more advantageous as it would involve less loss of efficiency immediately after mutation at the **S** locus.

It is not to be supposed that, once an incompatibility system has developed, the species

will always find it advantageous to maintain such a mechanism indefinitely. Indeed, it has been shown (Mather, 1943) that inbreeding and inbreeding mechanisms not infrequently supervene in outbreeding species. It is likely that this has happened in some cases of outbreeding by gametic incompatibility. *Antirrhinum majus* is self-compatible, but most if not all of its wild relatives are incompatible. It is then not without significance that self-compatibility in *Antirrhinum majus* is dependent on a single non-allelomorphic major modifier, which suppresses the action of the S allelomorphs. Although the rise of the outbreeding system depends on polygenic modification, once established its action may be vitiated by a much simpler genetical change. This also has been the case in the heteromorphic incompatibility system of *Primula* (Mather & de Winton, 1941), where, once the heterostylic mechanism has been built up, a homostylic inbreeding mechanism can supervene by a change in the switch gene. It is the mechanism, not the direction, of mating control which must arise by gradual polygenic selection. Once such control is established by polygenic change, it can be put to various uses by relatively simple adjustment of either switch gene or some major non-allelomorphic modifier, to give outbreeding or inbreeding or a mixture of the two.

7. SUMMARY

Incompatibility is classified according to whether it depends directly for its action on morphological variation (*heteromorphic incompatibility*) or not (*homomorphic incompatibility*). The latter type is again divisible into *gametic* and *zygotic* according to whether it is the genotype of the male gamete, or that of the zygote producing the male gamete, whose relation to the constitution of the zygote producing the female gamete determines the reaction.

Self-compatibility in an otherwise gametically incompatible species may depend on compatibility allelomorphs of the incompatibility genes, or on non-allelomorphic modification. The latter kind, leading to pseudo-compatibility, is associated with a hybrid origin of the individuals behaving in this way or of their ancestors.

Incompatibility in garden *Petunias* depends on a series of S allelomorphs of the kind described in *Nicotiana* and *Veronica*, but is often complicated by non-allelomorphic modifying genes. The cause of this complicated inheritance is to be sought in the origin of the horticultural strains from hybrids between the self-compatible *Petunia axillaris* and the self-incompatible *P. violacea*.

P. axillaris was crossed with a single plant of *P. violacea*, which was shown to carry two S allelomorphs, S_1 and S_2 , controlling a characteristic and uncomplicated gametic incompatibility reaction. The species cross only succeeds with *P. axillaris* as female. It gives two types of offspring in equal numbers, one freely self-compatible (s.c.) and the other nearly self-incompatible (s.i.). These classes are respectively S_2S_a and S_1S_a , where S_a is the allelomorph of S_1 and S_2 , that *P. axillaris* must be presumed to carry. A homozygous, S_2S_2 plant of *P. violacea*, when crossed on to *P. axillaris* gave only s.c. progeny.

Crosses between s.c. and s.i. are compatible both ways. Both types set seed with pollen from S_1S_2 *P. violacea*, but give no seed in the reciprocal cross. Both are compatible as pollen parents on to *P. axillaris*, but no seed is set on s.i. plants, and only very little on s.c. plants, when they are pollinated by *P. axillaris*. Pollen from S_2S_2 *P. violacea* gives a full set on s.i. plants, but nearly fails on s.c. plants. Pollen from both types of

hybrid gives seed when used in the reciprocal cross, though s.c. pollen is slightly less compatible than s.i. pollen.

The results are explicable in terms of the four assumptions:

- (1) S_1 and S_2 act as oppositional factors of the *Nicotiana* type.
- (2) S_a never gives efficient pollen growth in any style containing S_1 or S_2 , but never inhibits the growth of S_1 or S_2 pollen.
- (3) *Petunia axillaris* contains a gene or genes, non-allelomorphic with S , which weaken the incompatibility reaction of S_1 and, especially, S_2 .
- (4) When present in the same style, S_1 strengthens the action of S_2 in inhibiting the growth of S_a pollen from s.c. hybrids.

The behaviour of plants in the various kinds of F_2 and in backcrosses with both parental species verifies these postulates and also shows that S_1S_2 individuals of different origin may be markedly self-incompatible and yet set seed fairly freely when intercrossed.

Incompatibility systems cannot come into being, either by segregation or any other means, in perfect form. They must arise gradually, as changing external circumstances favour increased outbreeding. This gradual change is brought about by the selection of polygenes controlling the strength of the reaction, so that the move is from full self-compatibility through the various degrees of partial incompatibility to full incompatibility.

P. axillaris and *P. violacea* differ in the polygenes which they carry, those of the former species changing and weakening the action of the S allelomorphs on which the operation of incompatibility in the latter species depends. The S allelomorphs act only as the switch on which the immediate working of the system depends. The efficiency of the incompatibility system of controlled outbreeding depends on the polygenes which determine the behaviour of all the S allelomorphs. The occurrence of more pseudo-compatibility in the hybrid *Nicotiana Sanderae* than is found in either of its parent species is predictable from this polygenic view.

Self-compatible protospecies may be supposed to contain all the polygenic variability necessary for the development of an incompatibility system at such time as external circumstances favour such a development. Once a controlled outbreeding system, depending on gametic incompatibility or some other means, has been developed by polygenic selection, it may be changed to give controlled inbreeding by simple genetical adjustment of the switch gene or of a major non-allelomorphic modifier. This appears to have happened in *Antirrhinum majus*, which is a self-compatible species in a group otherwise self-incompatible.

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