THE BEHAVIOR OF LYCOPERSICON INCOMPATIBILITY ALLELES IN AN ALIEN GENETIC MILIEU

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THE properties of a gene can only be adequately described in reference to a particular environment. Although the one gene-one enzyme hypothesis would suggest a certain constant gene product, it is well known that the time and amount of gene action is subject to both internal and external controls. Furthermore, the processes affected by one gene are usually supported or modified by other genes. Thus, there is a danger in cataloguing the properties of a gene, or its relation to a process, without testing such properties in several environments. The most important of the environments to which a gene is exposed is, of course, the environment of the nucleus, as conditioned by the genetic system.

The *S* or incompatibility alleles are the genes giving specificity to incompatibility reactions. When pollen and style express similar phenotypes with respect to these genes, incompatibility occurs by one of the following processes: the pollen fails to germinate; the pollen tubes fail to penetrate the stigma; the growth of the tubes is impeded in the style; or the embryo aborts after fertilization and the ovary abscisses prematurely. Usually the incompatibility system of a species is described in terms of its *S* alleles and their interaction.

The *S* gene has been suggested to be complex in structure. In Primula, several characteristics of style, stamen, and pollen appear to be closely linked in a supergene (ERNST 1936) which can be broken down by rare crossovers. In gametophytic systems of self-incompatibility, independent mutations of pollen and stylar functions have suggested that the *S* gene consists of at least two parts, one of which controls the phenotype of the style, and the other the phenotype of the pollen (LEWIS 1951, 1960). Some controversy exists as to whether the two parts share a common element giving specificity, or are in themselves complete (LUNDQUIST 1965).

The *S* allele has been suggested to produce a dimer molecule (LEWIS 1965) which is identical in pollen and style. After incompatible pollination, similar dimers polymerize to give a tetramer, which, together with a "carrier" molecule, acts to impede pollen tube growth by inhibition of an auxin or stimulation of an inhibitor. A simpler model by ASCHER (1966) suggests that the *S* gene product is a monomer, and that the fusion product, a dimer, acts as a repressor of a high pollen tube growth rate operon. Any theory of the nature of the *S* allele and its function must account not only for incompatible and compatible reactions within

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species, but also for the unilateral incompatibility commonly encountered when a self-fertile species is crossed as male to a related self-incompatible species.

It is interesting to note that theories of *S* allele function have been derived largely from studies of *S* alleles in self-incompatible species. In those few cases in which self-compatible species have been hybridized to their self-incompatible relatives, the *S* allele has normally retained its functions, although these may have been masked by partial or full dominance of the fertility allele (SHERMAN 1939; EAST 1919). In other cases, the *S* allele retained its functions, but was modified by non-allelic genes (EAST 1932; ANDERSON and DEWINTON 1931). Such species crosses have usually not progressed far enough to compare *S* gene behavior in the full genetic environment of the self-compatible species. In Petunia, MATHER (1943) found a weakening and breakdown of incompatibility as the *S* alleles were backcrossed from *P. violacea* to *P. axillaris*. In tomato, *S* alleles backcrossed from self-incompatible *L. peruvianum* var. *dentatum* Dun. to self-fertile *L. esculentum* retain their ability to inhibit growth of pollen with similar alleles or with the allele for self-compatibility (MARTIN 1961). The reaction depends on the presence of a dominant modifier, also from the self-incompatible species. However, the ability of such alleles to function in pollen grains has not been demonstrated.

The tomato material (MARTIN 1961) provides an excellent opportunity to understand the genetic differences between self-compatible and self-incompatible species. The present four studies were made to clarify some unanswered questions concerning the inheritance of self-incompatibility in hybrids of self-compatible with self-incompatible tomato species, to elucidate the properties of the *S* allele in these hybrids, and to attempt to reconstitute a system of self-incompatibility in *L. esculentum.*

MATERIALS AND METHODS

Tiny Tim and Atom, varieties of the garden tomato *Lycopersicon esculentum* Mill. were used as recurrent parents in these experiments. Other *L. esculentum* varieties used in one experiment are listed in Table 1. Genes for self-incompatibility were obtained from the F_1 hybrids of *L. esculentum* × *L. peruvianum* var. *dentatum* Dun. (from Tacna and Moquegua, Peru) and from *L. hirsutum* Humb. & Bonpl. forma *hirsutum*, from Cajamarca, Peru. Self-incompatible derivatives of the *L.esculentum* type were obtained by repeated backcrosses of selected self-incompatible plants to the recurrent parents. Ratios of incompatible to compatible plants were recorded for each generation.

Self-incompatibility of greenhouse-grown plants was judged from fruit-set failure after 5 or more self-pollinations. However, incompatibility was seldom complete, and occasional fruits and seeds were obtained from flowers treated after pollination with 0.1% alpha-naphthalene acetamide in lanolin. In the field, self-incompatible plants set fruit readily (pseudo-compatibility), but their fruits contained very few seeds. Self-compatible plants could be readily identified in field and greenhouse by their seedy fruits.

The *S* alleles present in self-incompatible plants could sometimes be identified by reciprocal incompatibility of the plants with tester stocks consisting of the F_1 species hybrids. In the cross *L. esculentum* × *L. hirsutum*, the F_1's were produced each year from original seed that had been stored.

Repeated tests have shown that fruit setting failure in these materials is always associated

TABLE 1

Segregation for self-compatibility and self-incompatibility in two generations of backcrosses of incompatibility genes from Tiny Tim stock to tomato varieties

		Frequency SC: SI		
Variety	Source	First generation	Second generation	Level of pseudo-compatibility
Pearson	U.S.A.	16:3	25:10	Med.
Marglobe	U.S.A.	12:1	29:7	High
Michigan State Forcing	U.S.A.	26:6	32:11	Low
Red Cherry	U.S.A.	28:6	30:5	Low
Yellow Pear	U.S.A.	20:1	17:5	High
Red top	U.S.A.	5:1	22:8	Med.
Prince Borghese	Europe	6:3	17:6	High
Suttons Best	England	11:3	25:7	Med.
Indigenous variety	Peru	26:8	30:8	Low
Indigenous variety	Mexico	28:5	31:9	High
Indigenous variety	Peru	30:8	27:9	Med.
Indigenous variety	Ecuador	35:6	24:10	High
Cerasiforme	Honduras	17:8	25:7	Low
Cerasiforme	New Caledonia	32:2	28:6	High
Cherry	Galapagos Is.	23:8	24:5	Low
L. pimpinellifolium (Jusl. in L.) Mill.	Peru	21:10	29:9	Low

with pollen tube inhibition (MARTIN 1967). I did not believe that it was necessary to reverify the causes of fruit set failure in these experiments.

Other experimental details are included in the RESULTS section.

RESULTS

Inheritance of self-incompatibility in the backcross hybrids: Previous tests (MARTIN 1963) had revealed that self-incompatibility backcrossed into the *L. esculentum* variety Tiny Tim, was controlled by the dominant *S* allele and a complementary dominant modifier. To test for the presence of additional modifying genes in other tomato varieties, a line of Tiny Tim carrying an *S* allele from *L. peruvianum* variety *dentatum* (6th backcross generation) was crossed to a number of tomato varieties. The ratio of self-compatible to self-incompatible plants was determined in field plantings. Selected self-incompatible plants of each family were then backcrossed as males to plants of the same variety in the greenhouse. The progenies of these crosses were also screened for incompatibility. For both generations, small plantings were made of the parent varieties, to compare with and to verify the hybrid status of the progenies.

In the first generation, most of the progenies showed many more fruitful plants than were expected. Nevertheless, each produced some incompatible plants (Table 1). Progenies of the second generation were observed not only for fruitfulness, but for seediness of the fruit. The latter proved to be a more accurate index of incompatibility. The two generations were, therefore, not rated com-

*parably. As the second generation would have been affected by recessive modi*fiers peculiar to the variety, the data are wholly satisfactory. They suggest that all the varieties are alike in that each segregates 3 to 1 for self-compatibility versus self-incompatibility. In several families there were slight deficiencies for self-incompatible plants. These were probably due to misclassification of partially compatible plants, rather than to the presence of other major modifying genes.

The occurrence of fruitful plants with few seeds suggests that minor genes of each variety weaken the incompatibility. The level of pseudo-compatibility due to such genes was estimated as high, medium, or low, for each generation. The two generations of any particular variety were remarkably similar in this respect.

It appears, therefore, that while these varieties do not differ in major genes, they do differ in polygenes affecting the incompatibility. The principal effect of such polygenes is to weaken or attenuate the incompatibility.

Progressive loss of S *gene properties by backcrossing:* One of the most difficult unanswered problems in the previous study (MARTIN 1963) was the disappearance in backcross hybrids of the ability of self-incompatible lines to cross as males with the self-incompatible parent species, and with other lines known to carry contrasting *S* alleles. This problem was studied in early generations of a cross between the *L. esculentum* cv. Atom, and *L. hirsutum*. The technique used was to develop lines of known *S* allele composition, and to compare the crossability of such lines at different generational levels. Each plant of each generation was tested by at least 10 pollinations to female *L. hirsutum* and to a female sib of contrasting *S* allele. Each test involved at least 250 crosses to at least two testers of each type. The results were measured both as the percentage of plants that crossed to the two female types, and the mean number of seeds per fruit set.

The data show that almost all F_1 plants have a limited ability to backcross as males to *L. hirsutum* (Table 2). They also cross as sibs to produce F_2 generations. These properties are progressively lost in the later generations, as fewer plants are produced that will cross to the two types of females, and as fewer seeds are produced per generation. By the second backcross, self-incompatible hybrids were essentially unilaterally incompatible with female *L. hirsutum*, and reciprocally incompatible with lines of contrasting *S* allele. In each generation most of the

TABLE 2

Crossability of L. esculentum × L. hirsutum *hybrids to female* L. hirsutum *and to female sibs with contrasting* S *alleles, as influenced by generation*

		Success of crosses			
		With female *L. hirsutum*		With contrasting *S* sib	
Variety	Number of hybrids tested	Percent fertile matings	Seeds per fruit	Percent fertile matings	Seeds per fruit
F_1	25	92	3.2	100	12.1
F_2	32	88	1.2	78	5.7
BC	17	24	0.9	29	1.3
F_2 of BC	14	50	0.5	43	2.2
BC_2	28	0	. .	4	0.3

plants that crossed successfully to *L. hirsutum* crossed successfully also to the contrasting allele lines.

The F_2 generations contained plants incompatible with both male tester stocks (F_1's). These segregating hybrids contained two *S* alleles. Such plants did not cross with female *L. hirsutum* any more readily than did the plants carrying a single *S* allele.

These results clearly show that the loss of crossability is not a property of the self-fertility gene, and suggest that a number of genes segregating in the hybrids (sporophytic effects) and in the pollen of individual hybrids (gametophytic effects) control the unilateral crossability.

Behavior of S *alleles in an* L. esculentum *background:* In an attempt to establish a workable system of self-incompatibility in a predominantly *L. esculentum* background, self-incompatible plants of the Tiny Tim type carrying 5 known *S* alleles from *L. peruvianum* var. *dentatum* and *L. hirsutum* were selected. These represented backcross generations 1, 2, 3, 6, and 9. Extensive cross-pollinations were made among plants known to contain different *S* alleles, and fruit-set was encouraged with hormone treatments. In this manner a few fruits and seeds were obtained.

The resulting progeny were screened for self-incompatibility and the fertile, or partially fertile plants were discarded. Using as males two F_1 tester stocks containing S_1 and S_2 alleles, the plants were grouped into 4 classifications $S_1\,S_?$, $S_2\,S_?$, $S_1\,S_2$, $S_?\,S_?$. Plants of contrasting *S* alleles were again cross-pollinated, and again the progeny were grouped and crossed. In subsequent generations crosses were made at random among self-incompatible plants without regard to *S* genes present. The self-incompatible plants of each generation were planted in the field where fruit setting was observed. Small progenies from the open-pollinated plants were then grown in the greenhouse, and tested for self- and cross-incompatibility.

Each generation of open-pollinated progeny segregated for self- and cross-incompatibility. It was not possible to divide the self-incompatible plants into cross-compatible groups, even though the presence of known *S* alleles was demonstrated in some cases (Table 3). None of the crosses among self-incompatible

TABLE 3

Segregation of self-fertility and S *alleles after pseudo-compatible cross-pollinations of self-incompatible tomato lines*

	Segregation of progeny		Fruit set among SI plants			Genotypes found			
Generation	SF	SI	Pollinations	Fruits	Seeds	$S_1S_?$	$S_2S_?$	S_1S_2	$S_?S_?$
Parent	. .	. .	543	13	46	. .	. .	. .	. .
1	28	16	279	16	93	3	1	2	10
2	30	39	617	42	97	5	0	8	26
3	47	20	139	9	38	.	.	.	.
4	19	8	211	15	75	.	.	.	.

plants set sufficient seeds to characterize the reaction as cross-compatible. Instead, the crossing reactions could be said to be pseudo-compatible.

The results clearly demonstrate that S alleles retained their specific activity in the style and continued to impede the growth of pollen containing similar S alleles or S_f. But the specific property that permits pollen containing S alleles to grow in styles containing other S alleles was lost. In this respect the pollen of such lines acts as if it were all S_f. Nevertheless, that contingency was ruled out by the fact that S allele recombinants were detected in 2 generations of progeny from pseudo-compatible crosses. Thus, it appears that genes necessary for the pollen phenotype were lost in the development of BC lines. Such genes were not recovered by recombination in the pseudo-compatible offspring. The prospect of producing a self-incompatible *L. esculentum* population therefore appears to be small. Gradual increases in the ratio of self-fertile to self-incompatible plants through 4 generations of pseudo-compatibility suggests that the stylar behavior is also being weakened, perhaps by selection for modifiers.

Recovery of S *alleles from self-fertile lines:* The relatively high level of pseudo-compatibility of self-incompatible plants in the field permitted an isolation of lines homozygous for the complementary genes responsible for self-incompatibility. A single self-incompatible plant from the cross *L. esculentum* × *L. hirsutum* was planted in an isolated location in the field, and several hundred seeds were obtained by pseudo-compatible self-pollination. One group of progeny consisted of 61 self-incompatible, and 39 self-fertile plants. The seeds of each self-fertile plant were retained, and the plants were crossed in 160 different fruitful combinations. Small lots of 10 to 15 plants of each cross were planted in the field, and screened for self-incompatibility on the basis of seediness of fruits. Seven combinations were selected, which produced only self-incompatible offspring. These involved 9 different parents. The parents, progenies, and F_1 testers were all grown from seed and tested simultaneously.

Of the 7 combinations, only 3 produced all self-incompatible progeny (20–25 plants) in the greenhouse tests. The 3 progenies were produced by 2 males and 3 females. These 5 lines were crossed in all possible combinations, and were self-pollinated.

Compatibility tests were made between the F_1 testers, seedlings from the parents of 100% incompatible progenies, and representative self-incompatible plants. These tests showed that the 5 parents crossed freely as females to both tester stocks, but the reciprocal crosses were almost completely incompatible (unilateral incompatibility). The self-incompatible offspring, however, were reciprocally incompatible with one tester stock. This was taken to mean that each contained one S allele, that of the original self-incompatible parent.

Compatibilities of the progenies of the 5 self-fertile parent stocks are shown in Table 4. When allowances were made for partial fertility, the progenies proved to be either entirely compatible, or entirely self-incompatible. These results could only have been obtained if two parental types were represented among the 5, one homozygous for one dominant complementary gene for self-incompatibility and the other homozygous for the second gene.

TABLE 4

Ratio of self-compatible to self-incompatible plants in progeny of crosses among 5 parent lines

As female	As male M_1	M_2	F_1	F_2	F_3
M_1	16:0	0:23	0:25	0:11	15:0
M_2	0:9	17:0	16:0	14:0	0:19
F_1	0:7	20:0	21:0	8:0	0:17
F_2	0:9	16:0	13:0	20:0	0:14
F_3	14:0	0:21	0:23	0:15	25:0

DISCUSSION

The four experiments reported herein reveal a consistent pattern of behavior that is very useful in understanding the genetic differences between self-incompatible and self-fertile species of Lycopersicon. Two genes of major effect operate, a switch gene that turns the incompatibility on or off (MATHER 1943), and the incompatibility alleles, which give specificity to the incompatibility system. In self-fertile species, the switch gene is represented by its recessive, inactive allele, whereas the *S* gene is represented by a non-specific (S_f) allele devoid of incompatibility properties, and producing pollen incapable of overcoming the effects of normal *S* alleles. Both the normal *S* alleles and their self-fertile counterparts act independently in the style.

The two properties shown by normal *S* alleles in the styles of hybrids of self-fertile × self-incompatible species are the specific property of inhibition of pollen tube growth of pollen containing an identical allele, and the general property of inhibition of pollen tube growth of pollen containing the self-fertility allele. Another property of such alleles, the ability of pollen tubes to grow unimpeded in styles containing different *S* alleles, is weakened or lost due to effects of a polygenic system. The genes of this system seem to have sporophytic effects, in influencing whether a given plant can hybridize, and gametophytic effects, in determining whether a given pollen tube can effect fertilization. Thus, this property of the *S* alleles is shared in part by other genes. The common occurrence of pseudo-compatibility suggests that polygenes may also affect properties of the *S* gene in the style.

Such polygenic modification of incompatibility systems has been previously suggested by MATHER (1943), to account for loss of incompatibility as *S* alleles were transferred from *Petunia violacea* to *P. axillaris*. A self-incompatible species may itself contain such a polygenic system, which on segregation may weaken or destroy the self-incompatibility, as in *Primula sinensis* (MATHER and DE-WINTON 1941) and *Lycopersicon hirsutum* (MARTIN 1963).

It is difficult to see how a self-fertility allele of the *L. esculentum* type could have originated from the typical *S* allele. A one-step mutation would have produced an allele incapable of producing fertile pollen, and thus such an allele could not be fixed. As all *L. esculentum* varieties apparently lack the switch gene also, it is probable that a one-step mutation of that gene caused self-fertility. The loss of *S* gene properties could have proceeded thereafter. It is not necessary to hypothesize a series of mutations of the *S* allele (LEWIS and CROW 1958) to account for the evolution of self-fertility from self-incompatibility.

Self-incompatibility in these studies was always associated with the *S* allele. In addition, unilateral incompatibility between hybrid and *L. esculentum* was only found when hybrids were self-incompatible. Thus, the two properties appear to be common results of the *S* gene–switch gene interaction, and thus *S* alleles are necessary for the existence of unilateral incompatibility. In the cross of the self-fertile *L. esculentum* × self-fertile *L. hirsutum* forma *glabratum* C. H. Mull., some evidence was found of a major gene controlling unilateral incompatibility (MARTIN 1967). This evidence suggests that the gene in question was a relic *S* allele.

Some alteration in current hypotheses of *S* allele structure must be made to accommodate the facts from these studies. There is no *a priori* reason to believe that the tomato self-fertility allele could act as a generalized molecule, fusing with any *S* allele product in the style to produce a tetramer (LEWIS 1965), or a dimer (ASCHER 1966). It would seem more likely that the self-fertility allele of the tomato lacks a function in *L. esculentum.* If this be the case, the initial product of the incompatibility reaction could be inactive, and cross-fertility could be the result of the production of a molecule from two different *S* alleles, that then acts in some fashion as a stimulator of pollen tube growth. I hesitate to offer this as a hypothesis until more is known of the structure of the self-fertility allele.

A final problem remains to be solved. If the genetic action of the *S* allele depends on a highly organized polygenic system, what properties would the self-fertility allele, and the recessive allele of the switch gene, express in the genetic background of a self-incompatible species?

SUMMARY

During the transfer of self-incompatibility from self-incompatible to self-fertile tomato species, two complementary dominant genes from the self-incompatible species segregate, an *S* allele and a switch gene. When both dominant alleles are present in a hybrid, the hybrid is self-incompatible. The genes, and thus self-incompatibility, may be recovered by appropriate crosses among self-fertile hybrids. The growth of pollen tubes containing either the *S* allele or its self-fertility complement is inhibited in the style of self-incompatible hybrids. The specificity of the *S* allele in the style is maintained, as shown by crosses with male F_1 tester plants. However, the specificity of action in the pollen is progressively lost as *S* alleles are transferred into the self-fertile species. This loss appears to be controlled by many genes. In the tomato the evolution of self-incompatibility to self-fertility could have involved either of two processes, a one-step mutation of the switch gene, or a multigenic change in the genetic background, through hybridization.

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