PHASES IN THE S-GENE EXPRESSION, AND S-ALLELE INTERACTION IN THE CONTROL OF INTERSPECIFIC INCOMPATIBILITY

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SUMMARY

Plants of 19 progeny families, grown from seeds obtained from crosses between five different *S* genotypes of *Nicotiana bonariensis*, were studied for their inter-specific incompatibility behaviour as female parents with pollen of certain S.I. and S.C. species.

The relationship between *S* genotypic constitution and interspecific incompatibility behaviour, first shown by the plants of the parental families studied earlier, was confirmed without exception in all the progenies. All plants of the same *S* genotypic constitution always behaved alike. No dissociation of specific intra- and interspecific incompatibility reactions has been found in a total of 1200 plants so far tested in *N. alata* and *N. bonariensis*.

Study of the 10 genotypes possible from four different *S* alleles, six heterozygous and four homozygous, taken in conjunction with the earlier studies in this species, showed that interspecific incompatibility, including unilateral incompatibility, was the function of *S* alleles. Each *S* allele had its own specific property of accepting or rejecting a class of pollen.

There is a truly sporophytic determination of interspecific incompatibility in the style. In styles heterozygous for *S* alleles there was either independent action, or allelic interaction between the accepting and the rejecting allele showing dominance of one or the other.

The allelic interactions in the style are specific for pollen of particular species, and have no relationship with allelic behaviour in connection with pollen of other species.

Generally, all pollen of a species behaved alike, polymorphism occurring only in the style behaviour. The tester S.I. plant GM8 of otherwise S.C. species *N. glauca* is an exception. The pollen of this plant differed in interspecific incompatibility behaviour from a number of other S.I. plants of this species tested, which were, as expected, all alike in this respect.

The S.I. plant GM8 is *S* heterozygous with two distinct forms of *S* alleles, a usual self-incompatibility allele S_I , and a self-compatibility allele S_C which when present alone in the pollen causes that pollen to be rejected by a S.I. style. The unusual behaviour of the plant GM8 ($S_I S_C$) is due to *S* allele interaction in the pollen. All other S.I. plants, with usual behaviour of their pollen, are *S* homozygous with two different *S* alleles of the same S_I form.

The results suggest a sporophytic action of the S_C gene in the pollen of GM8.

There are two phases in the physiology of the *S*-gene action: (i) a primary *S*-gene action phase which occurs independently in the pollen and style and (ii) a secondary, incompatibility reaction phase which occurs after the pollen and style have come in contact with each other. Allelic interaction may occur in one or both phases.

There are stepwise differences in the extent of rejection of the pollen of S.C. plants having different forms of *S* alleles, starting at the one end with full acceptance of the comparatively young self-compatible " S_0 " class of pollen by S.I. styles to the other end with complete rejection of the comparatively ancient self-compatible " S_{Cn} " class of pollen ($S_{C1}, S_{C2}, \dots$, etc.) by S.I. styles.

It is suggested that the S_0 class of self-compatible pollen, along with the

usual S_I class of pollen, has a gametophytic action of the S gene whereas the S_f , S_C and S_{Cn} classes of pollen containing a relatively "eroded" form of the S gene have a sporophytic or partially sporophytic action.

1. INTRODUCTION

SUPERGENES, closely linked physiologically integrated clusters of loci acting as one gene, are often associated with polymorphism. They have been found in primroses, snails, slugs, *Lepidoptera*, grouse locusts, *Drosophila*, grasshoppers and man, and, no doubt, occur widely in plants and animals alike (Darlington and Mather, 1949; Sheppard, 1953; Darlington, 1958, 1971; Ford, 1964). The supergene S as a gene complex governing morphological and physiological polymorphisms involved in the control of intra-specific incompatibility in heteromorphic and homomorphic flowering plants is well known (East and Mangelsdorf, 1925; Mather and de Winton, 1941; Lewis, 1949, 1954; Mather, 1955). The realisation that the S complex may also be involved in the control of interspecific incompatibility, however, is recent (Lewis, 1954a; Lewis and Crowe, 1958; Pandey, 1961, 1962a, 1964), although certain evidence in this direction was available as early as 1931.

Anderson and de Winton (1931) in *Nicotiana* and Mather (1943) in *Petunia* demonstrated that certain self-incompatibility alleles, when present in the style, had the property of rejecting not only pollen containing the same allele but also pollen of certain related self-compatible species. Recently this finding was confirmed with the rediscovery of such alleles, now called S_{FI} , in *Nicotiana glauca* (Pandey, 1964, 1967). These alleles, of which only two forms, S_{FI10} and S_{FI11} , have been found so far in nature, when present in the style, cause that style to reject pollen of the related self-compatible species *Nicotiana langsdorffii*, whereas the presence of the more numerous S_I alleles allows the style to accept that pollen. The study of the F_1 , F_2 , bud-selfed and backcrossed progeny all suggested that (1) the interspecific incompatibility between *N. glauca* and *N. langsdorffii* was the function of the S_{FI} alleles alone and did not involve any other major factor; and (2) the two specificities controlling intra- and interspecific incompatibility were closely linked so that no dissociation between these two characteristics occurred in the several hundred plants studied.

In a recent study of S -gene polymorphism in the self-incompatible species *N. bonariensis*, a large number of plants comprising four families, 8746, 9503, 9584a and 9584b, grown from seeds obtained from different sources, were tested as females for their cross-compatibility behaviour with two testers, self-compatible species *N. langsdorffii* (N.I.) and a self-incompatible strain (GM8) of an otherwise self-compatible species *N. glauca* (Pandey, 1969a). All four possible kinds of cross-compatibility relationships were observed, thus dividing the plants into four groups, henceforth called "polymorphic classes" (— = Incompatible; + = Compatible):

Polymorphic class	♂ N.I.	♂ GM8
A	—	+
B	+	—
C	+	+
D	—	—

There was a strict relationship between the different polymorphic classes and the *S* genotypic constitutions; all plants of the same *S* constitution were identical in their cross-compatibility behaviour and therefore belonged to the same polymorphic class. However, more than one *S* genotype could belong to the same polymorphic class. Of the six available genotypes (I-VI) two, I and III, were identical with the polymorphic classes A and C respectively; of the remaining four genotypes two, II and VI, fell into the same polymorphic class B and two, IV and V, into class D. These results strongly suggested the involvement of *S* alleles in the control of interspecific incompatibility.

In order to test the above hypothesis further, progeny of a number of crosses, involving five of the six *S* genotypes, were studied for their *S* genotypes and cross-compatibility relationships with the two testers. Also, a comparative study was made of the heterozygous and the *S*-allele homozygous genotypes, obtained through bud-self-pollination, in relation to these two and other testers. The results confirm the major role of the *S* alleles in the control of interspecific incompatibility, and throw significant light on the nature of interspecific incompatibility and *S*-allele polymorphism.

Earlier work on the *S*-allele interactions in the diploid pollen of autotetraploids (Lewis, 1947; Pandey, 1956*a*, 1962*c*) suggested that there are two components of the *S*-gene expression in the control of incompatibility: (1) one which is expressed in the pollen and style independently, before they come in contact with each other, and (2) another which is expressed, as an incompatibility or compatibility reaction, when the two come in contact with each other after pollination. The present study provides a much clearer picture of the two phases in the *S*-gene physiology controlling the incompatibility reaction.

2. MATERIALS AND METHODS

A description of the sources of seed of *Nicotiana bonariensis*, the two regular pollen testers, self-compatible (S.C.) *N. langsdorffii* and a self-incompatible (S.I.) strain GM8 of otherwise S.C. *N. glauca*, and three other testers, *N. alata*, *N. forgetiana* and *N. noctiflora*, has been given in earlier papers (Pandey, 1964, 1967, 1968, 1969*a*). Seed of the sixth tester, *N. longiflora*, varieties *grandiflora* and *breviflora*, was obtained from U.S.D.A., Beltsville, Maryland, U.S.A.

In *N. glauca*, a predominantly self-compatible species, until recently only one S.I. plant, GM8, was available for use in these experiments. A family of bud-selfed progeny of this plant contained three S.I. and two S.C. plants. Tests on these plants showed that plant GM8 was heterozygous, S_1S_c . Fresh seed of this species was obtained from Argentina through the courtesy of the Director, Botany Division, D.S.I.R., Christchurch. All but two of the 30 plants grown from this seed were S.I. (The results of a detailed study of *S*-allele polymorphism in *N. glauca* are to be published later.) In the present study four of the S.I. and the two S.C. plants, arising from the later stock, were included as testers.

The cytological technique involved in the study of pollen-tube growth, the technique of producing selfed-seed in S.I. plants through bud-self-pollination, and the growing conditions of the plants have all been described previously (Pandey, 1962*b*, 1963, 1964).

Two or more crosses were made in each combination studied. All crosses that failed to produce seed were examined for pollen-tube growth in the style.

3. RESULTS AND INTERPRETATIONS

(i) *Crosses between apparently normal parents*

The parental plants for these crosses were drawn from two families, 8746 and 9503, which between them contained five *S* genotypes and all four polymorphic classes: I (A), II (B), III (C), IV and V (D). In all, 19 progeny families were studied. All progenies were selfed, and crossed as females with two interspecific testers, N.I. and GM8, and the six intraspecific testers, the *S* genotypes. After the *S* genotypes in each family were identified the existence of the intra-incompatible inter-compatible groups was confirmed by intercrossing eight plants comprising a few plants from each group. The identities of *S* genotypes were rechecked by crossing between genotypic groups obtained from different families.

The results of test crosses are given in table 1. The same six genotypes found among the original plants were again found in the progeny. No new genotypes were recovered. The *S* genotypes found in each family generally agreed with the expectation based on the 1-locus gametophytic system of incompatibility, and, with a few exceptions, expected genotypes according to the given genotypic constitution of the parents were usually recovered in the progenies. The exceptions included the family 9610, which was expected to yield two genotypes, but in which all 16 plants belonged to one genotype. Similarly, in the family 9577 where four genotypes were expected, only two genotypes were recovered among the 13 plants studied. The lack of certain genotypes in some of the families, where the total number of plants studied was probably high enough to have yielded all the expected genotypes, may be due to *S*-genotype linked zygotic lethality and inviability of seed, and is perhaps associated with the inbreeding depression. The earlier detailed work on this species (Pandey, 1969*a*) had suggested that although seed material was obtained from four different sources they probably had a common origin from a set of two plants differing in both alleles. Same *S* genotypes, totalling six, were recovered from different materials. The conclusion that the main cause of the abnormality lies in linkage and inbreeding depression, and not in any other genetic phenomenon, is supported by the facts (1) that a number of compatible crosses in these experiments yielded poor seed set and gave very poor germination; and (2) that in some families where all expected genotypes were recovered the number of plants in certain genotypes was extremely low—e.g. in families 9611 (17 and 2), 9589 (8 and 1), 9591 (19 and 2) and 9573 (11 and 1)—indicating that in slightly different circumstances these latter genotypes might not have been recovered at all.

The results of crosses of progenies with species testers, N.I. and GM8, were invariably in conformity with that expected on the basis of their *S* genotypes. No exceptional plants dissociating the interspecific and intraspecific specificities were discovered. Where the two parents belonged to the same polymorphic class (D), as in the family 9605 (V $\times$ IV), the progeny, as expected, comprised two polymorphic classes (B and D).

TABLE 1
S genotypes and interspecific incompatibility (polymorphic classes) in parents and progenies of *N. bonariensis*

Cross Genotypic group and polymorphic class (in brackets)	Family No.	Parent and expected progeny <i>S</i> genotypes	No. of plants Observed genotypes and polymorphic classes						
			<i>S</i> ₃ <i>S</i> ₄ (I) A	<i>S</i> ₁ <i>S</i> ₂ (II) B	<i>S</i> ₂ <i>S</i> ₃ (III) C	<i>S</i> ₁ <i>S</i> ₄ (IV) D	<i>S</i> ₂ <i>S</i> ₄ (V) D	<i>S</i> ₁ <i>S</i> ₃ (VI) B	
I(A) × III(C) III(C) × I(A)	9575 9574	<i>S</i> ₃ <i>S</i> ₄ × <i>S</i> ₂ <i>S</i> ₃ → <i>S</i> ₂ <i>S</i> ₃ + <i>S</i> ₂ <i>S</i> ₄ <i>S</i> ₂ <i>S</i> ₃ × <i>S</i> ₂ <i>S</i> ₄ → <i>S</i> ₂ <i>S</i> ₃ + <i>S</i> ₂ <i>S</i> ₄	— 3	— —	8 —	— —	8 13	— —	
I(A) × IV(D) IV(D) × I(A)	9611 9588	<i>S</i> ₂ <i>S</i> ₄ × <i>S</i> ₁ <i>S</i> ₄ → <i>S</i> ₁ <i>S</i> ₄ + <i>S</i> ₁ <i>S</i> ₃ <i>S</i> ₁ <i>S</i> ₄ × <i>S</i> ₂ <i>S</i> ₄ → <i>S</i> ₂ <i>S</i> ₄ + <i>S</i> ₁ <i>S</i> ₃	— 11	— —	— —	17 —	— —	2 17	
I(A) × V(D) V(D) × I(A)	9610 9589	<i>S</i> ₃ <i>S</i> ₄ × <i>S</i> ₂ <i>S</i> ₄ → <i>S</i> ₂ <i>S</i> ₃ + <i>S</i> ₂ <i>S</i> ₄ <i>S</i> ₂ <i>S</i> ₄ × <i>S</i> ₂ <i>S</i> ₄ → <i>S</i> ₂ <i>S</i> ₃ + <i>S</i> ₂ <i>S</i> ₄	— 8	— —	— 1	— —	16 —	— —	
I(A) × II(B)	9577	<i>S</i> ₃ <i>S</i> ₄ × <i>S</i> ₁ <i>S</i> ₂ → <i>S</i> ₂ <i>S</i> ₃ + <i>S</i> ₁ <i>S</i> ₄ + <i>S</i> ₂ <i>S</i> ₄ + <i>S</i> ₁ <i>S</i> ₃	—	—	—	3	—	10	
III(C) × II(B)	9578	<i>S</i> ₂ <i>S</i> ₃ × <i>S</i> ₁ <i>S</i> ₂ → <i>S</i> ₁ <i>S</i> ₃ + <i>S</i> ₁ <i>S</i> ₄	—	15	—	—	—	8	
IV(D) × II(B) IV(D) × III(C) IV(D) × V(D)	9587 9592 9604	<i>S</i> ₁ <i>S</i> ₄ × <i>S</i> ₁ <i>S</i> ₂ → <i>S</i> ₁ <i>S</i> ₃ + <i>S</i> ₂ <i>S</i> ₄ <i>S</i> ₁ <i>S</i> ₄ × <i>S</i> ₂ <i>S</i> ₃ → <i>S</i> ₂ <i>S</i> ₄ + <i>S</i> ₁ <i>S</i> ₃ + <i>S</i> ₂ <i>S</i> ₄ + <i>S</i> ₁ <i>S</i> ₃ <i>S</i> ₁ <i>S</i> ₄ × <i>S</i> ₂ <i>S</i> ₄ → <i>S</i> ₁ <i>S</i> ₃ + <i>S</i> ₂ <i>S</i> ₄	— — —	12 — 8	— — —	— — —	13 1 8	— 3 —	
V(D) × II(B) V(D) × III(C) V(D) × IV(D)	9590 9591 9605	<i>S</i> ₂ <i>S</i> ₄ × <i>S</i> ₁ <i>S</i> ₂ → <i>S</i> ₁ <i>S</i> ₃ + <i>S</i> ₁ <i>S</i> ₄ <i>S</i> ₂ <i>S</i> ₄ × <i>S</i> ₂ <i>S</i> ₃ → <i>S</i> ₂ <i>S</i> ₄ + <i>S</i> ₂ <i>S</i> ₃ <i>S</i> ₂ <i>S</i> ₄ × <i>S</i> ₁ <i>S</i> ₄ → <i>S</i> ₁ <i>S</i> ₃ + <i>S</i> ₁ <i>S</i> ₄	— 19 —	3 — 1	— 2 —	2 — 5	— — —	— — —	
VI(B) × I(A) VI(B) × I(A) VI(B) × III(C) VI(B) × III(C) VI(B) × IV(D)	9552 9576 9551 9573 9608	<i>S</i> ₁ <i>S</i> ₃ × <i>S</i> ₂ <i>S</i> ₄ → <i>S</i> ₁ <i>S</i> ₄ + <i>S</i> ₂ <i>S</i> ₃ <i>S</i> ₁ <i>S</i> ₃ × <i>S</i> ₂ <i>S</i> ₄ → <i>S</i> ₁ <i>S</i> ₄ + <i>S</i> ₂ <i>S</i> ₃ <i>S</i> ₁ <i>S</i> ₃ × <i>S</i> ₂ <i>S</i> ₃ → <i>S</i> ₁ <i>S</i> ₄ + <i>S</i> ₂ <i>S</i> ₃ <i>S</i> ₁ <i>S</i> ₃ × <i>S</i> ₂ <i>S</i> ₄ → <i>S</i> ₁ <i>S</i> ₄ + <i>S</i> ₂ <i>S</i> ₃ <i>S</i> ₁ <i>S</i> ₃ × <i>S</i> ₁ <i>S</i> ₄ → <i>S</i> ₁ <i>S</i> ₃ + <i>S</i> ₂ <i>S</i> ₄	3 2 — — 3	— — 6 1 —	— — 2 11 —	3 10 — — 17	— — — — —		

(ii) *Crosses involving abnormal plants*

In the family 9592 ($S_1S_4 \times S_2S_3$, cf. table 1) which, owing to poor seed set in the cross and poor germination, comprised only four plants there was one unthrifty plant (9592-4) that was female-sterile but pollen-fertile. This plant, as judged from pollen-tube studies, was partly S.C. and had the constitution S_2S_4 (V), and, as expected, belonged to the polymorphic class D. A number of normal and male-sterile plants were crossed as females with plant 9592-4 and the behaviour of their progeny was studied. The results are given in table 2. Of the six families grown, four (8405, 8415, 9655, 8412), in general, produced the expected S genotypic groups; in the remaining two families, however, the groups produced matched the expectation based on one of the alleles of the male parent being S_1 instead of S_2 .

The appearance of the S_1 allele in the progeny where S_2 was expected can, of course, be due to contamination of the pollen in the parental cross, and this cannot be ruled out. However, as already stated, the pollen parent 9592-4 was an exceptional plant in a number of ways. Unfortunately, this plant died before it could be rechecked for its S constitution during the next season. It is possible that this plant was trisomic for the S -bearing chromosome and was triallelic ($S_1S_2S_4$). In some branches the S_1 -bearing chromosome was lost so that only the S_2 or S_4 alleles were present in the pollen, in others the S_2 -bearing chromosome was lost so that only the S_1 or S_4 alleles were present in the pollen. This hypothesis is supported by the fact, shown from the pollen-tube studies, that the plant was self-compatible presumably due to competitive interaction in the pollen (Lewis, 1947; Pandey, 1956a, 1965; Brewbaker and Natarajan, 1960). Apparently, the pollen tubes with two S alleles were inviable, for no triallelic plants were observed in the progeny.

As regards interspecific polymorphism, however, all plants again behaved exactly as expected on the basis of their S genotypic constitution. It did not matter whether the plants were normal or abnormal in other respects, as long as the S genotypic constitution could be determined the interspecific incompatibility relationship with the S alleles was confirmed unequivocally.

(iii) *Bud-selfed progeny*

Bud-selfed progeny were grown from five out of six genotypes. In addition to poor germination of seeds most plants showed varying degrees of male and female sterility. Each plant was selfed and reciprocally crossed with its parental genotype. Plants which as females showed compatibility with the parental genotype were crossed among themselves, and also as male parents with the other five S heterozygous genotypes. The results of these test crosses (table 3) revealed the S genotypic constitution of each plant, including whether it was homozygous or heterozygous for S alleles. In addition to the five parental heterozygous genotypes, four new homozygous genotypes S_1S_1 , S_2S_2 , S_3S_3 and S_4S_4 were recovered in the progeny.

All heterozygous plants behaved exactly like their respective parents in crosses with interspecific testers, N.I. and GM8. The homozygous genotypes, however, varied in this respect. Except for the progeny of the genotypic group III, S_2S_3 , in which both the homozygous genotypes S_2S_2 and S_3S_3 belonged to the same polymorphic class as their parent (C), the progeny of

TABLE 2

S genotypes and interspecific incompatibility (polymorphic classes) in progeny of crosses where one or both parents were sexually abnormal

Parental cross Family and Pl. No.	Progeny family No.	Expected progeny genotypes and groups	Genotypic groups obtained	No. of plants	Interspecific testers		Polymorphic class
					♂ N.I.	♂ GM8	
9503-10 (M.S.*) × 9592-4†	8405	$S_1S_4(IV) \times S_2S_4(V) \rightarrow$ $S_1S_2(II) + S_2S_4(V)$	$\{S_1S_2(II)$ $\{S_2S_4(V)$	18 14	+	—	B D
9503-20 (M.S.*) × 9592-4†	8415	$S_1S_4(IV) \times S_2S_4(V) \rightarrow$ $S_1S_2(II) + S_2S_4(V)$	$\{S_1S_2(II)$ $\{S_2S_4(V)$	15 16	+	—	B D
9503-28 (Normal) × 9592-4†	9655	$S_1S_4(IV) \times S_2S_4(V) \rightarrow$ $S_1S_2(II) + S_2S_4(V)$	$\{S_1S_2(II)$ $\{S_2S_4(V)$	13 9	+	—	B D
9611-12 (Normal) × 9592-4†	8412	$S_1S_2(VI) \times S_2S_4(V) \rightarrow$ $S_2S_4(I) + S_1S_2(II) + S_1S_4(IV) + S_2S_3(III)$	$\{S_2S_4(I)$ $\{S_1S_2(II)$ $\{S_2S_3(III)$	8 5 13	— + +	+ — +	A B C
9503-17 (Normal) × 9592-4 (?)†	9656	$S_2S_4(V) \times S_2S_4(V) \rightarrow$ $S_2S_3 + S_2S_4(V) + S_2S_4$ or $S_2S_4(V) \times S_1S_4(IV) \rightarrow$ $S_1S_2(II) + S_1S_4(IV)$	$\{S_1S_2(II)$ $\{S_2S_4(IV)$	11 19	+	—	B D
9503-21 (Normal) × 9592-4 (?)†	8409	$S_2S_3(III) \times S_2S_4(V) \rightarrow$ $S_2S_4(V) + S_2S_4(I)$ or $S_2S_3(III) \times S_1S_4(IV) \rightarrow$ $S_1S_2(II) + S_1S_3(VI) + S_2S_4(V) + S_2S_4(I)$	$\{S_2S_4(I)$ $\{S_1S_2(II)$ $\{S_2S_4(V)$ $\{S_1S_3(VI)$	9 7 5 4	— + — +	+ — — —	A B D B

+ = Compatible. * Male sterile. † Male fertile, female sterile; pollen shows partial self-compatibility. — = Incompatible.

all other genotypic groups produced two homozygous genotypes which belonged to two different polymorphic classes, one being the same as that of its parent. Each homozygous genotype had its own characteristic polymorphic class irrespective of the parental genotypic group from which individual plants were derived.

TABLE 3

S genotypes and interspecific incompatibility (polymorphic classes) in bud-selfed progeny

Bud-selfed parent			Family No.	Total No. of plants	Progeny—No. of plants in each genotype (polymorphic class)	
Genotype	Group	Polymorphic class			Heterozygous	Homozygous
S_3S_4	I	A	9546	8	7— S_3S_4 (A)	1— S_3S_3 (C)
S_1S_2	II	B	9521	10	6— S_1S_2 (B)	3— S_1S_1 (B); 1— S_2S_2 (C)
S_2S_3	III	C	9519+9518	7	5— S_2S_3 (C)	1— S_2S_2 (C); 1— S_3S_3 (C)
S_2S_4	V	D	9681+9682	27	16— S_2S_4 (D)	3— S_2S_2 (C); 8— S_4S_4 (D)
S_1S_3	VI	B	8488	9	3— S_1S_3 (B)	4— S_1S_1 (B); 2— S_3S_3 (C)

(a) *Dominance interaction in the style*

The comparative behaviour of the four homozygous and six heterozygous *S* genotypes in respect to their interspecific incompatibility with two testers, N.I. and GM8, are highlighted in table 4. It can be seen from the table that styles with genotypes S_1S_2 and S_1S_3 reject GM8 pollen by virtue of the presence of the allele S_1 , for homozygous styles having S_2 or S_3 allele alone accept GM8 pollen. Styles having the genotype S_2S_3 , as expected, accept both N.I.

TABLE 4

Comparative interspecific incompatibility (polymorphic classes) in the 10 S-homozygous and -heterozygous genotypes possible from four different S alleles

<i>N. bonariensis</i> <i>S</i> genotypes	Interspecific testers		Polymorphic class
	♂ N.I.	♂ GM8	
Homozygous			
<i>S</i> ₁ <i>S</i> ₁	+	—	B
<i>S</i> ₂ <i>S</i> ₂	+	+	C
<i>S</i> ₃ <i>S</i> ₃	+	+	C
<i>S</i> ₄ <i>S</i> ₄	—	—	D
Heterozygous			
<i>S</i> ₁ <i>S</i> ₂ (II)	+	—	B
<i>S</i> ₁ <i>S</i> ₃ (VI)	+	—	B
<i>S</i> ₂ <i>S</i> ₃ (III)	+	+	C
<i>S</i> ₁ <i>S</i> ₄ (IV)	—	—	D
<i>S</i> ₂ <i>S</i> ₄ (V)	—	—	D
<i>S</i> ₃ <i>S</i> ₄ (I)	—	+	A

+ = Compatible. — = Incompatible.

and GM8 pollen. Styles with genotypes S_1S_4 and S_2S_4 reject the pollen of both testers by virtue of the presence of the allele S_4 , for homozygous styles having the allele S_4 reject both types of pollen. These reactions of *S* alleles in the diploid style have been termed *independent*, and are characteristic of

stylar *S* gene action in the gametophytic system of incompatibility (Lewis, 1947, 1954*b*; Pandey, 1956*a*).

When the genotype S_3S_4 is considered, however, a different picture emerges. Styles of this constitution accept GM8 pollen although they contain the allele S_4 which normally causes rejection of both N.I. and GM8 pollen. It is apparent that in this case the sister allele S_3 , which causes acceptance of GM8 pollen, is dominant over the rejecting allele S_4 . Another interesting feature highlighted by this interaction is the independence of the two faculties of the S_3 allele, one in relation to the N.I. pollen and the other to the GM8 pollen. The S_3 allele when present alone causes acceptance of both types of pollen while S_4 pollen when present alone causes rejection of both types of pollen. When in association, as in S_3S_4 styles, S_3 is dominant over S_4 only in relation to GM8 pollen but not in relation to N.I. pollen. In the latter case S_4 allele is able to assert its independent action (or dominance over S_3).

(iv) *Other pollen testers*

(a) *N. noctiflora*, *N. forgetiana*, *N. alata* and *N. longiflora*

Having obtained, in *N. bonariensis*, plants homozygous for *S* alleles, and thus having available all the 10 possible *S* genotypes arising from four

TABLE 5

Interspecific incompatibility in the 10 S-homozygous and -heterozygous genotypes in N. bonariensis, possible from four different S. alleles, when tested with the pollen of four other Nicotiana species

<i>N. bonariensis</i> ♀ Parent		♂ Testers						
		<i>N. noctiflora</i> (S.I.)	<i>N. forgetiana</i> †		<i>N. alata</i>		<i>N. longiflora</i> (S.C.)	
Genotype	Plant No.		S.I.	S.C.	S.I.	S.C.	var. <i>grandiflora</i>	var. <i>breviflora</i>
Homozygous								
S_1S_1	8488-10/1	—	+	+	+	+	—	—
S_2S_2	9548-1	—	+	+	+	+	—	—
S_3S_3	8488-5/1	+	+	+	+	+	—	—
S_4S_4	9681-5	+	+	+	+	+	—	—
Heterozygous								
S_1S_2	9587-19	—	+	+	+	+	—	—
S_3S_4	9588-20	+	+	+	+	+	—	—
S_1S_3	9611-15	—	+	+	+	+	—	—
S_2S_4	9503-15	+	+	+	+	+	—	—
S_2S_3	9589-8	+*	+	+	+	+	—	—
S_1S_4	9608-15	+*	+	+	+	+	—	—

+ = Compatible. — = Incompatible. * Partial compatibility. S.I. = Self-incompatible. S.C. = Self-compatible.

† Tests with the pollen of this species give inconclusive results, producing seed-set in most crosses.

S alleles (six heterozygous and four homozygous), it was thought desirable to extend the testing of these plants to include pollen of certain other species and strains of *Nicotiana*. They were crossed as females with normally S.I. species *N. noctiflora*, *N. forgetiana* and *N. alata*, and two strains, var. *grandiflora* and var. *breviflora*, of the S.C. species *N. longiflora*. Two plants, one S.I. and the other S.C., were used in each of the species *N. forgetiana* and *N. alata*. The results are given in table 5. The pollen of *N. forgetiana* and

N. alata, whether from S.I. or S.C. plant, was accepted by all *N. bonariensis* styles although with *N. forgetiana* an occasional inconsistent incompatibility was observed. (The cause for this is unknown and may be related to the extreme disparity between the lengths of the styles of the two species involved. A similar situation has been noted before between *N. bonariensis* and *N. alata* (Pandey, 1969a). It may be recalled that in an earlier test where *N. forgetiana* plants of hybrid origin were used as testers *N. bonariensis* styles showed polymorphism (Pandey, 1969b).) Pollen of the two varieties of S.C. *N. longiflora* was, on the other hand, rejected by all *N. bonariensis* styles. *N. bonariensis* did not show polymorphism with regard to the different forms of any of the three species, *N. forgetiana*, *N. alata* and *N. longiflora*.

Dominance interaction in the style

N. bonariensis styles show polymorphism with regard to the pollen of S.I. *N. noctiflora*, as already shown previously (Pandey, 1969b). In the present experiments, styles homozygous for the S_1 or S_2 allele reject pollen of *N. noctiflora*, whereas those homozygous for the S_3 or S_4 allele accept them. As expected, the heterozygous S_1S_2 styles reject the pollen of *N. noctiflora* while the heterozygous S_3S_4 styles accept them. Again, as expected on the basis of the gametophytic system of incompatibility, the heterozygous S_1S_3 styles, owing to the presence of the allele S_1 , reject the pollen of *N. noctiflora*. The situation in the heterozygous S_2S_4 styles, however, is contrary to expectation. These styles, despite the presence of the rejecting allele S_2 , accept the pollen of *N. noctiflora*. This would suggest that the accepting allele S_4 is dominant over the rejecting allele S_2 . A similar interaction also occurs in heterozygous styles of the genotypes S_2S_3 and S_1S_4 , although in these the dominance of S_3 over S_2 and of S_4 over S_1 is incomplete.

(b) *N. glauca*

The results of crosses of *N. bonariensis* with seven testers from *N. glauca* are given in table 6. The testers include, in addition to the previously used S.I. plant GM8, two S.C. and four S.I. plants derived from the new family 8468. *N. bonariensis* shows polymorphism with regard to the pollen of *N. glauca*. As in previous studies (Pandey, 1968, 1969a, b), pollen of the two S.C. plants is generally rejected by S.I. plants of *N. bonariensis*. In the present study, however, there is an incomplete rejection of this pollen by one genotype, the homozygous S_3S_3 . All other homozygous and heterozygous genotypes show complete rejection in the stigma. Thus, in the same species *N. glauca*, the S.C. plants 8468-4 and 8468-9 have the S_C type of allele whereas the S.I. plants generally have the usual S_I type of alleles.

Unique reaction of the GM8 pollen

As regards pollen of S.I. plants the results show a difference between the pollens of S.I. testers, a case not recorded previously. The homozygous S_4S_4 styles reject GM8 pollen but accept pollen of the four other S.I. testers. The remaining three homozygous styles, S_1S_1 , S_2S_2 and S_3S_3 , individually, behave alike with all five S.I. testers—the S_1S_1 styles reject all pollen and the S_2S_2 and S_3S_3 styles accept all pollen.

A similar difference in the pollen reaction among the S.I. testers is evident in tests with heterozygous plants, the pollen of GM8 again being different from the pollen of other S.I. testers. Considering the four new

testers first, the heterozygous genotypes S_3S_4 , S_2S_3 and S_2S_4 accept their pollen since the homozygous genotypes, S_2S_2 , S_3S_3 and S_4S_4 , the three alleles concerned, also accept them. On the other hand, the heterozygous S_1S_2 , S_1S_4 and S_1S_3 genotypes reject their pollen since they all contain the allele S_1

TABLE 6*

Interspecific incompatibility in the 10 S-homozygous and -heterozygous genotypes in N. bonariensis with the pollen of six self-incompatible (GM8— S_1S_C , five others— S_1S_I) and two self-compatible (S_CS_C) plants of N. glauca

N. bonariensis ♀ Parent		♂ Testers N. glauca						
		Plant No.						
		S.I. GM8	S.I. 8468-8	S.I. 8468-11	S.I. 8468-14	S.I. 8468-17	S.C. 8468-4	S.C. 8468-9
Homozygous								
S_1S_1	8488-10/3	—	—	—	—	—	—	—
S_2S_2	9548-1	+	+	+	+	+	—	—
S_3S_3	8488-5/1	+	+	+	+	+	+	+
S_4S_4	9681-5	—	+	+	+	+	—	—
Heterozygous								
S_3S_4	9588-20	+	+	+	+	+	—	—
S_1S_2	9587-19	—	—	—	—	—	—	—
S_2S_3	9589-8	+	+	+	+	+	—	—
S_1S_4	9608-15	—	—	—	—	—	—	—
S_2S_4	9503-15	—	+	+	+	+	—	—
S_1S_3	9611-15	—	—	—	—	—	—	—

* Symbols same as in table 5.

† Partial compatibility

which in the homozygous condition is known to cause rejection of this class of pollen.

Considering the GM8 pollen, five of the six heterozygous genotypes behaved with this pollen the same way as they did with the other testers. However, one, S_2S_4 , behaved differently. The latter rejected the pollen of GM8 while it accepted the pollen of other testers. The difference in the two groups of testers, GM8 on the one hand and the four new testers on the other, lies in their reaction with the allele S_4 . This allele accepts the pollen of the new testers but rejects that of GM8. The heterozygous genotype S_2S_4 , therefore, rejects the pollen of GM8.

4. DISCUSSION

(i) S alleles and interspecific incompatibility

The association between S alleles and interspecific crossability behaviour discovered in the previous study of four families of *N. bonariensis* (Pandey, 1969a) is fully substantiated by the present study of a large number of their progeny. Hybridisation between plants made no difference in the expression of this characteristic, the segregant progeny again showing the crossability behaviour peculiar to each S genotypic constitution. In a total of approximately 600 plants studied (96—original families, 502—progenies) not a single plant was found which varied from the crossability behaviour characteristic of its S genotype. A similar result has been reported from a previous

study of approximately 600 plants in *N. alata* (Pandey, 1964). The relationship, therefore, within the limits tested (1200 plants), is unbroken and complete.

The production and testing of *S* homozygous plants further confirmed the central role of the *S* alleles in the control of interspecific incompatibility. Each allele had its own characteristic behaviour in relation to pollen of one species, and this behaviour varied in relation to pollen of different species. In table 7 the four *S* alleles of *N. bonariensis* studied have been classified into two groups, "accepting" and "rejecting", in relation to the pollen of three species, *N. langsdorffii*, *N. noctiflora* and *N. glauca*. It is obvious from the table that each allele is independent in its reaction to pollen of different species.

The incompatibility involved in these studies including others reported previously (Pandey, 1967, 1969*a*) is of the unilateral type, the reciprocals being compatible, and owes this reaction to the presence of specific *S* alleles

TABLE 7

S-allele specificity in the styles of *N. bonariensis* causing rejection or acceptance of pollen from different species

Classes	♂ N.I.	♂ <i>N. noctiflora</i>	♂ <i>N. glauca</i> *
"Accepting" alleles	S_1, S_2, S_3	S_3, S_4	S_2, S_3, S_4
"Rejecting" alleles	S_4	S_1, S_2	S_1

* The pollen of GM8, which is heterozygous $S_I S_C$, is excluded from this compilation, which is concerned only with usual $S_I S_I$ plants of *N. glauca*.

in the style and pollen. It supports the earlier conclusion, that different forms of unilateral interspecific incompatibility observed among S.I. and S.C. species (*i.e.* S.I. $\times$ S.I., S.I. $\times$ S.C., S.C. $\times$ S.I. and S.C. $\times$ S.C.) are all primarily based on the specificity of *S* alleles (Pandey, 1968).

(ii) *Determination of interspecific incompatibility: gametophytic or sporophytic?*

(a) *Allelic interactions in the style*

The comparative study of all the possible 10 genotypes, four homozygous and six heterozygous, from four different *S* alleles, revealed certain interesting features of the *S* gene in relation to interspecific incompatibility. One of the basic characteristics of the normal gametophytic system of intraspecific incompatibility is an independent action and full expression of both alleles in the diploid style. In a pollination a haploid pollen grain containing a S_I allele which is also present in the style is invariably rejected irrespective of the presence of other kinds of stylar sister allele(s). The presence in the style of an associated compatible allele has no effect on the rejection caused by the incompatible allele. No interaction of *S* alleles occurs.

In the present study, as indeed in similar other studies involving pollen-style incompatibility, in an incompatible pollination of usual S.I. styles containing both a rejecting and an accepting allele it is impossible to distinguish between (1) independent action of the rejecting allele and (2) allelic interaction resulting in dominance of the rejecting allele over the accepting allele. Both alternatives would show identical responses—rejection of the pollen. The occurrence of only one allele out of four showing this kind of

rejection makes three (50 per cent.) of the six possible *S*-heterozygous styles incompatible with one type of pollen. Such is the case with the allele S_4 in relation to the pollen of *N. langsdorffii* and with the allele S_1 in relation to the pollen of the usual S.I. plants of *N. glauca* (cf. tables 4, 6 and 7). Thus, the function of the rejecting allele is exaggerated in expression in plant populations.

In investigations of interspecific incompatibility where usually all pollen of a species behaves alike and the determining variation occurs only in the stylar alleles, the action of the two categories of *S* alleles, one accepting and the other rejecting the pollen, can be studied with a greater degree of discrimination than is possible in the study of intraspecific incompatibility. Here it is possible to test, unequivocally, the third possibility, (3) dominance

FIGS. 1-4.—Diagrammatic presentation of *S* allele interaction in the style of *Nicotiana bonariensis*. Of the three possible interactions, (i) dominance of the incompatible allele over the compatible allele, (ii) independent action of the incompatible allele, and (iii) dominance of the compatible allele over the incompatible allele, the first two cannot be distinguished and this has been shown by two parallel lines; the third has been shown by an arrow pointing from the dominant to the recessive allele. Arrow with broken lines shows incomplete dominance. The encircled alleles are "rejecting" alleles, the others are "accepting" alleles.

Fig. 1.—Pollen of *N. langsdorffii*. Fig. 2.—Pollen of *N. noctiflora*. Fig. 3.—Pollen of *N. glauca*, strain GM8. Fig. 4.—Pollen of *N. glauca*, usual S.I. plants.

of the accepting allele over the rejecting allele. If in a heterozygous style the accepting allele expresses itself despite the presence of an associated rejecting allele, the dominance of the former over the latter is indisputable. Such is the case with the heterozygous genotypes S_2S_4 and S_3S_4 . Styles of the constitution S_2S_4 accept the pollen of *N. noctiflora*, the accepting allele S_4 being dominant over the rejecting allele S_2 . Similarly, styles of the constitution S_3S_4 accept the pollen of GM8, the accepting allele S_3 being dominant over the rejecting allele S_4 (cf. tables 5 and 6). A diagrammatical representation of *S* allele interactions in relation to four different pollen testers has been given in figs. 1-4. These results undoubtedly show that the *S* gene action in the style with regard to interspecific incompatibility is truly sporophytic. The allelic interactions are specific for the pollen of particular species and have no relationship with the behaviour of the individual alleles towards the pollen of other species (cf. table 7).

(b) *Inherent ability of genes for allelic interaction*

In the gametophytic system of intraspecific incompatibility, although the expression of incompatibility on the female side occurs in the diploid tissue of the sporophyte, the gametophytic type of reaction is assured by the independence of *S*-gene action. Thus, in the evolution of gametophytic

system of incompatibility there has been a selection for independent action of *S* alleles in the style. This selection apparently did not affect the inter-specific incompatibility-controlling-elements of the *S* complex, the alleles of which may show ability to interact. A similar ability to interact is displayed in the diploid pollen of autotetraploids, where again no selection has operated to exclude interaction.

Since the 1-locus multiallelic gametophytic system is believed to be the primary form of self-incompatibility (Whitehouse, 1950; Lewis and Crowe, 1958; Pandey, 1960*b*), the expression of dominance or independent action of *S* alleles whenever two alleles occur in the same cell which has not been subjected to selection favouring any particular kind of allelic interaction, shows that each allele, in a specific genetic environment, may have its own peculiar ability to interact with another allele. A particular allelic interaction may, however, be selected under certain circumstances either through modification of the genetic environment, or exclusion of alleles with unwanted interactions, or both (Pandey, 1957*a*, 1960*a*). Allelic interactions in the pollen and style, showing considerable variation between different pairs of alleles, is a common feature of the homomorphic sporophytic system of incompatibility, which is believed to be secondarily derived from the originally gametophytic system (Pandey, 1960*b*) and has no requirement for selection against allelic interactions.

(c) *Absence of competitive interaction in the style*

By necessity, competitive interaction which brings about a breakdown of self-incompatibility (Lewis, 1947; Pandey, 1956*a*) can not occur in the styles of fully functional gametophytic and sporophytic systems of intra-specific incompatibility. In the present study of *S* gene interaction, in cases where two different incompatibility (rejecting) alleles occur together in the same style, there is an absence of competitive interaction, although no selection could have operated to eliminate it. The heterozygous styles S_1S_4 , having both S_1 and S_4 alleles incompatible with GM8 pollen, reject this pollen. Similarly, heterozygous styles S_1S_2 , having both S_1 and S_2 alleles incompatible with *N. noctiflora* pollen, reject this pollen. In the earlier studies in *N. alata* (Pandey, 1964), heterozygous styles $S_{F10}S_{F11}$, having both S_{F10} and S_{F11} alleles incompatible with *N. langsdorffii* pollen, rejected this pollen.

All evidence suggests that competitive interaction is a peculiar property of the pollen and is limited to polyploid plants (or plants with pollen having more than one *S* allele) of the gametophytic system of incompatibility. In all other situations only a dominant or independent type of interaction occurs. It has been proposed that competitive interaction is a secondary phenomenon of the *S* gene action in the male gametophyte. It occurs late during germination, or even later in the pollen tube, when the specific incompatibility precursors are converted into specific incompatibility substances, and probably involves competition for the specific enzyme(s) presumably responsible for the conversion (Pandey, 1962*c*).

(d) *Allelic interactions in the pollen*

In the extensive study in *Nicotiana* illustrating the *S*-gene control of inter-specific incompatibility, polymorphism has been discovered on the female side only. No polymorphism was found on the male side in a number of

S.I. species studied, all pollen of S.I. plants of the same species behaving identically (Pandey, 1969*a, b*).

Why, then, should the pollen of S.I. GM8 be different from that of the other new S.I. plants of *N. glauca*? The answer appears to lie in the fact, stated earlier in Materials and Methods, that GM8 is heterozygous, containing two different forms of *S* alleles, a normal self-incompatibility allele S_I , and a mutant self-compatibility allele S_C which, when present in pollen, causes that pollen to be rejected by a S.I. style. The reaction of pollen of this heterozygous plant $S_I S_C$ is apparently different from that of plants which have both alleles of the same form ($S_I S_I$). It is an example of *S* allele interaction in the pollen. And this leads to a second question.

The second question is: Why is it that only styles with the allele S_4 react differently with the pollen of GM8 while styles with the other alleles, S_1 , S_2 and S_3 , behave alike with both classes of S.I. testers, GM8 as well as the four new testers? The answer seemingly lies simply in differences in allelic characteristics. If the four new testers are assumed to belong to the common class of S.I. plants having two different alleles of the same S_I form of the gene—which they have proved to be in tests so far (unpub.)—then the absence of a difference in the reactions of the alleles S_1 , S_2 and S_3 as regards the two classes of pollen testers may mean that GM8 pollen is equivalent to that of the normal S.I. group of plants when reacting with styles having S_1 , S_2 or S_3 , but it is different when it reacts with styles having S_4 . This would suggest that in the pollen of GM8 ($S_I S_C$), in the case of the former reactions the S_I allele is either dominant over the S_C allele or has an independent action, while in the case of the latter reaction the S_C allele is dominant over the S_I allele. *These results imply that there is a sporophytic reaction in the pollen of GM8.*

(e) *Phases in the S-gene expression and the nature and evolution of dominance*

The present results in the pollen of *N. glauca* plant GM8 ($S_I S_C$), where two different forms of alleles occur in the same plant and show allelic interaction, strongly suggest that in this plant there is a precocious, sporophytic (premeiotic, or precytokinetic) action of the specificity producing elements either of the S_C allele alone, or of both the S_C and S_I alleles. In the incompatibility reaction in the pollen of GM8 the dominance relationship—whether the S_I is dominant over the S_C or the S_C is dominant over the S_I —is not fixed but can be either way, being determined late, after pollination, by the specific S_I allele present in the style (cf. tables 4 and 6). This suggests that in GM8 pollen, in spite of eventual dominance interaction both alleles are initially expressed, producing their respective, specific, primary incompatibility precursors. One way this could be possible is if the S_C action occurs sporophytically but the S_I action is still gametophytic. Thus in 50 per cent. of the pollen, having the allele S_I , both alleles will be expressed independently without interaction during this pre-pollination phase. The other 50 per cent. of the pollen, having the expression of the S_C allele alone, would normally be rejected by a style having a S_I allele. In the pollen having the S_I allele, the expression of dominance interaction occurs at the pollen germination and tube growth phase, after the pollen has come in contact with the stigma. As discussed above, this presumably relates to the process of conversion of the incompatibility precursors into incompatibility substances. The situation may be described as “partly sporophytic”.

Ordinarily, in interspecific incompatibility between S.I. species, where the different alleles of a pollen parent belong to the same usual S_I form, e.g. S_1S_2 , all pollen of the species behave alike irrespective of the possible differences in the allelic primary specificity. This indicates that there is no interaction and that the action of the primary specificity elements, under these conditions, may be gametophytic. (Normally, the interspecific specificity, however, does not interfere with the intraspecific specificity for as discussed above, the former is physiologically expressed only when the intraspecific, secondary specificity is unable to express itself owing to the disparity in the polygenic backgrounds of the pollen and the style. Thus the expression of the two specificities alternates between the two.) For, if the action of the primary specificity elements of the S_I alleles is assumed to be sporophytic one might expect variation in the pollen reaction owing to interaction between the two allelic primary specificities, as suggested from the observations in the pollen of GM8.

The S -gene studies thus provide a special insight into the still incompletely understood problem of the nature and evolution of allelic dominance. In the present context they underline the significance of the phase difference between the "primary gene action" occurring before pollination and the "incompatibility reaction" occurring after pollination, and elucidate how interaction could occur at the incompatibility reaction rather than the primary gene action phase. Natural selection modifying genetic interactions may, possibly, occur at one or the other, or both, phases. Further light on this subject, with particular reference to the expression of the primary specificity, may be shed when detailed studies of the new S.I. *N. glauca* plants are completed.

(f) S_I and S_C alleles and the time of the S -gene action

N. glauca is the only species in the genus *Nicotiana* which has two distinct forms of pollen in the same species: (1) pollen of S.I. plants which are accepted by S.I. styles, and (2) pollen of S.C. plants which are rejected by S.I. styles. The species therefore offers a rare opportunity to study interaction between distinctive forms of alleles which normally occur in different species. Investigation of allelic interactions in the latter situation through studies of interspecific hybrids is generally complicated by pollen sterility and polygenic effects (Pandey, 1968).

The present study has shown that *there is sporophytic action of S alleles in the style for the determination of interspecific incompatibility. It has also shown that when two distinct forms of S alleles, for example S_I and S_C , occur in the same plant, the pollen reaction, at least in relation to the S_C allele, may also be sporophytic.* There are cases reported in the literature in which a precocious action of a particular unit, or of a major part, of the S gene complex leading to varying degrees of sporophytic reaction is assumed to occur on account of the presence of a certain additional mutant allele, or an alien cytoplasm from a S.C. species. In a cross between S.I. *Solanum sparsipilum* (*S. aracc-papa*) and S.I. *S. chacoense* (*S. subtilius*) (Pushkarnath, 1953) the determination of the incompatibility alleles in the F_1 hybrids is sporophytic, presumably due to the presence of the mutant allele "R" at another locus (Pandey, 1957b). In *Oenothera*, interspecific hybrids between S.I. *O. pallida* (σ) and S.C. *O. trichocalyx* (ϕ), having S_I and S_f forms of alleles respectively, showed partially sporophytic control of incompatibility (Crowe, 1955; Pandey, 1968). It

was suggested that in *Oenothera* hybrids having the cytoplasm of the S.C. species, the S -gene elements controlling the production of the presumed specific pollen growth substance acted precociously before cytokinesis (sporophytic) while the elements controlling the presumed specific incompatibility precursors acted, as is normal for gametophytic action, after cytokinesis. Similarly, a partly sporophytic action of S alleles, owing to the presence of a duplicate S locus with two forms of mutant alleles, has been proposed to explain intraspecific incompatibility in *Solanum pinnatisectum* (Pandey, 1962*d*). Sporophytic control of incompatibility has been suggested to occur in interspecific *Lycopersicon* hybrids between S.I. and S.C. species having S_I and S_C forms of alleles respectively (McGuire and Rick, 1954).

In hybrids between S.I. *Nicotiana alata* (δ) and S.C. *N. langsdorffii* (φ), which have been tested on a very limited scale (Anderson and de Winton, 1931; Pandey, 1964), the situation is ambiguous. As stated earlier, *N. alata* has two kinds of self-incompatibility alleles, the more numerous S_I alleles ($S_1, S_2, S_3, \dots$, etc.) which when present in the style allow that style to accept the S_I pollen of *N. langsdorffii* and the S_{FI} alleles, of which only two S_{F10} and S_{F11} have been found, which when present in the style allow that style to reject the S_I pollen. The interspecific hybrids having the constitution $S_I S_I$, ($S_1 S_1, S_2 S_2$, etc.) are usually self-compatible. This may suggest that the S gene action is gametophytic, but the alternative that the S gene action in the pollen is sporophytic with the S_I allele being dominant over the S_I alleles tested, cannot be ruled out. Similarly in the $S_{F10} S_I$ and $S_{F11} S_I$ hybrids which are both self-incompatible the reaction can be interpreted on the basis of either the gametophytic or the sporophytic action. There is a possibility that further work on hybrids might reveal cases of dominance of a S_I allele over the S_I or of the S_I over a S_{FI} allele, thus providing a clear evidence for sporophytic action.

It has been proposed earlier (Pandey, 1968, 1969*b*) that, intraspecifically, in the pollen the secondary specificity which governs the intraspecific incompatibility is dominant (epistatic?) over the primary specificity which governs the interspecific incompatibility. Interspecifically, however, when the polygenic backgrounds of the two species are sufficiently distinct, the dominance of the secondary specificity over the primary specificity is lost and the primary specificity is expressed. With this hypothesis, in interspecific pollinations between two S.I. species where both have usual S_I alleles, the action of the primary as well as the secondary specificity elements could be gametophytic, but whether or not the actual expression of the interspecific incompatibility occurs—conversion of specific precursors into specific incompatibility substances—is governed by the relative polygenic backgrounds of the pollen and style.

(iii) *Different forms of self-compatibility alleles and the time of their action in the pollen*

In *N. bonariensis*, homozygous S.I. styles $S_1 S_1$, $S_2 S_2$ and $S_4 S_4$, as expected, reject the pollen of S.C. plants of *N. glauca* ($S_C S_C$). However, homozygous styles of the constitution $S_3 S_3$ partially accept them. Unpublished results in *N. glauca* show that there are within this species rare S_I alleles which when present in the homozygous condition in the style allow that style to accept S_C pollen, and the resultant hybrid plants from such pollinations

($S_I S_C$) are self-compatible. There are other similar examples of rare clones in S.I. *Solanum* species (11 clones in *S. chacoense* and 1 clone each in *S. soukupii* and *S. vernei*), styles of which accept the pollen of S.C. *Solanum verrucosum* ($S_C S_C$), while the majority of other clones of these species reject this pollen (Grun and Radlow, 1961; Pandey, 1962b). As in *N. glauca*, S.I. plants are also found infrequently in S.C. *S. verrucosum* (Magoon *et al.*, 1958; Cipar *et al.*, 1964).

The S.C. plants in *N. alata* and *N. forgetiana* used as pollen testers in the present crosses behaved identically with the S.I. plants. Their pollen was acceptable on all S.I. styles (cf. table 5). Similar behaviour of pollen (having the allele named here " S_0 ") occurs in S.C. plants obtained through spontaneous and induced mutations in the regulatory (not specificity) elements of the *S* gene (Lewis, 1951; Pandey, 1956b).

S_I pollen of *N. langsdorffii* is accepted by most S.I. styles and rejected by some (Anderson and de Winton, 1931; Pandey, 1964, 1967, 1969a). In contrast, S_C pollen of *N. glauca* (and *S. verrucosum*) is rejected by most S.I. styles and accepted by some. As opposed to both S_I and S_C pollen, which are accepted by at least some S.I. styles, there is a third class of pollen, having the allele called here " S_{Cn} ", which are invariably rejected by all S.I. styles. The last class of pollen occurs in a large number of wholly S.C. species, which are further divisible into groups having distinct forms of self-compatibility alleles, designated here as S_{C1} , S_{C2} , S_{C3} , etc. This class is discernible on the basis of unilateral cross-incompatibility behaviour among these S.C. species (Pandey, 1968, 1969b).

Thus, there is a stepwise gradation in the cross-incompatibility behaviour of the pollen of S.C. plants, which is revealed by the increasing degree of rejection of these pollens by S.I. styles. The increase in rejection of the pollen is associated, in a general way, with the relative age of self-compatibility in the parent species, the older the self-compatibility the greater the rejection, the rejection becoming finally complete in species which are wholly S.C. and in which the self-compatibility is relatively ancient. The suggested allelic progression in the pollen rejection by S.I. species is as follows:

There appears to be a progressive relationship between the two phenomena, the extent of incompatibility of the pollen, and the time of the *S* gene action. The normal S_I and, usually, the recently mutated S_0 alleles, give the least cross-incompatible pollen and show fully gametophytic action; S_I and S_C alleles give progressively more cross-incompatible pollen and show relatively precocious, and hence partially sporophytic, *S*-gene action (as discussed earlier in (ii) (f)); and finally, S_{Cn} alleles (S_{C1} , S_{C2} , S_{C3} , ...) give entirely cross-incompatible pollen and show fully sporophytic action.

The stepwise gradation in the extent of rejection of the pollen, having different forms of self-incompatibility and self-compatibility alleles, strongly supports the erosion hypothesis of the evolution of *S*-gene polymorphism (Pandey, 1968, 1969b). The massive evidence obtained from the study of

incompatibility in the genus *Nicotiana* is in full agreement with the previously proposed theory of the evolution of the S-gene complex.

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