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Sexually localized expression of pseudo-self compatibility (PSC) in *Petunia × hybrida* Hort.

2. Stylar inactivation *

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Summary. A previously identified *S*-linked stylar-inactivation PSC factor (Flaschenriem and Ascher 1979b) was studied for its location relative to *S*. Plants exhibiting complete stylar-inactivation PSC were those with higher multigenic PSC background level than plants with only *S*-linked partial stylar-inactivation PSC. A pollen-mediated pseudo-self compatibility (PMPSC) adjustment factor was offered as a device to focus on stylar-inactivation PSC by removing some male origin, multigenic PSC. The stylar inactivation factor was not tightly linked to *S* but affected expression of only the allele to which it was linked. A three part interacting association of genetic material governing self incompatibility (SI) is proposed. The parts of *S* are the SI identity gene, *S*-specific PSC genes and, finally, PSC genes which are not *S*-specific in action. The complete association is termed the SI-complex.

Key words: Self-incompatibility – Pseudo-self compatibility – Stylar part mutation – *Petunia × hybrida*

Introduction

Self-incompatibility (SI) is a physiological mechanism which impedes inbreeding by minimizing self fertilization in spite of functional male and female gametes. Disruption of an SI system resulting in self seed set is termed pseudo-self compatibility (PSC). Self-compatible species are those in which an SI system has not been detected.

The primary factor in the function of the single locus gametophytic SI system is a single gene, *S*, with multiple alleles (East and Mangelsdorf 1926). *S* confers an identity to the haploid pollen and two independent identities to the diploid style. A match between pollen and either stylar *S* identity prevents fertilization. Reports of mutations of *S* occur in the literature (Lewis 1948, 1949, 1960; Lewis and Crowe 1953, 1954; Pandey 1956, 1959, 1970; Denward 1963; Nettancourt et al. 1971, 1975), although there is ambiguity as to the strict meaning of *S* in some of the reports. The physiological complexity of the SI reaction, comprising as many as eight distinct stages of recognition and growth of pollen tubes (Ferrari and Wallace 1977), suggests that many genes are involved. Numerous researchers observed single genes, non-allelic to *S*, which modify the SI reaction resulting in PSC (Brieger 1927; Wergin 1936; Townsend 1969). Others reported multigenic combinations of modifiers allowing for various levels of seed set (Atwood 1942; Pandey 1956; Denward 1963; Henny and Ascher 1976).

Petunia × hybrida Hort., is a horticulturally derived diploid species exhibiting a single-locus-gametophytic SI system (Linskens 1975). The species originated from a cross of *P. violacea* and *P. axillaris* (Mather 1943; Natarella and Sink 1974). Although Mather (1943) considered the former self incompatible and the latter self compatible, Kojan (1950) and Bali (1971) reported that *P. axillaris* was self incompatible, bearing the one-locus gametophytic system. Apparently the *P. axillaris* used by Mather was highly PSC. The interspecific hybrid origin from two species expressing PSC and the history of horticultural breeding and selection for seed set in isolated lines has apparently resulted in considerable genetic erosion of self incompatibility in *P. hybrida*.

In an F_1 *Petunia* progeny (77–93) resulting from a 0% PSC ($S_{3.3}$) × 100% PSC ($S_{1.1}$) compatible cross, Flaschenriem and Ascher (1979b) reported a PSC factor linked to *S* which resulted in loss of stylar activity of the linked *S* allele. Tests of the F_1 with unrelated $S_{1.1}$ and $S_{3.3}$ male testers revealed that S_1 pollen tubes grew through the $S_{1.3}$ style while S_3 pollen tubes did not. Two F_2 populations segregated 1 : 1 for $S_{1.1}$ and $S_{1.3}$ genotypes confirming the inactivation of the style for S_1

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only. The parents of the F_1 had been inbred seven generations from commercial F_1-hybrid *Petunia* cultivars. The observed stylar-inactivation PSC phenotype fits the definition of a mutation to the stylar activity part of *S* (Lewis 1949).

A linkage test placed the S_1 allele stylar-inactivation PSC factor at 28 map units from *S*, a location similar to a linked stylar inactivation PSC gene found in *Nicotiana* (Brieger 1927). A second estimate by Flaschenriem and Ascher using the F_2 progenies also arrived at 28 map units. However, in the initial test, problems arose in *S* allele determination: 14 plants of the 102 plant population had to be disregarded. This occurred because all plants were tested as females for *S* alleles and seed set differentials for 14 plants were too small to assign *S* allele genotypes with confidence. In addition, the F_1 individual used in the linkage test cross was only partially stylar inactive (53.9% PSC), while several of its full sibs were 100% PSC.

Our objective was to define the difference between partial and complete stylar-inactivation PSC through analysis of linkage populations arising out of both types of parents. Further, a numerical factor for pollen-mediated pseudo-self compatibility (PMPSC) (Flaschenriem and Ascher 1979a; Robacker and Ascher 1981, 1982) was computed to direct attention specifically to the stylar inactivation portion of the total PSC phenotype. Finally, the previously reported linkage distance between *S* and the stylar-inactivation PSC factor was re-evaluated.

Materials and methods

Remnant seed from Flaschenriem and Ascher (1979b) was used for all three populations studied. The populations designated 79-1, 80-1, and 80-2 resulted from the crosses shown in Fig. 1. For population 77-612 (Flaschenriem and Ascher 1979b) only the extant seed count data were again analyzed in this investigation. The population itself was not studied. The 77-612 seed counts were converted to PSC as described here.

A preliminary study with population 79-1 was performed during 1979 at the same time of year as the work with 80-1 and 80-2. The methods employed in 1979 were as previously described (Flaschenriem and Ascher 1979b) while 80-1 and 80-2 were studied using the methods detailed below.

Seeds were treated for 24 h with 100 ppm gibberellic acid (GA_3). They were allowed to germinate on milled sphagnum moss under intermittent mist in the greenhouse. At the second true leaf stage, seedlings were potted and placed in a greenhouse set at 21/15 °C (day/night). Four-hour-duration night interruption incandescent lighting was supplied to assure flowering. Regular and ordinary fertilization and pesticide applications were made throughout the experiments. Due to space limitations, plants were pruned to one flowering shoot and staked. Pollinations were done successively up the shoot, but no more than one flower opened on a plant on any given day. All pollinations were performed on the day of anthesis. Flowers were emasculated one day prior to anthesis for cross pollinations and protected with glassine bags. Self pollinations were not emasculated, but were bagged immediately following manipulation. Seed capusules were havested prior to dehiscence and seeds counted using an electronic seed counter with an error of ±2–4%.

For *S* allele determination, paired pollinations were repeated three times using plants as males on an $S_{1.2}$ (4.1% PSC) clone and an $S_{2.3}$ (2.8% PSC) clone. Except for one plant in 80-2, differential seed set indicated the *S* allele constitution of the males tested. These pollinations were conducted between February and April, 1980.

To measure PSC, five self pollinations and three compatible outcross pollinations were performed on each test plant. Selfs and outcrosses were alternated twice, then three selfs were made followed by a final outcross. Pollinations were performed between April and June, 1980. For each plant, the self seed set values were divided by the mean seed set of compatible pollinations and those quantities were multiplied by 100 to give percent PSC.

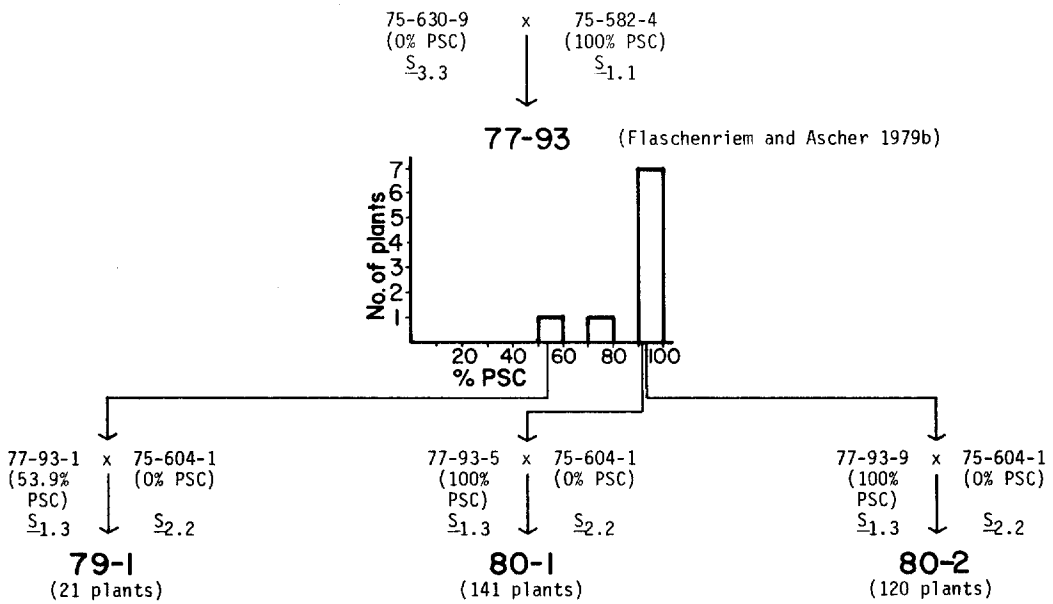

Fig. 1. Origin of populations studied for behavior of *S*-linked stylar-inactivation PSC factor

Since Flaschenriem and Ascher (1979b) concluded that stylar-inactivation PSC due to the linked factor was purely a female phenomenon, we attempted to focus on the female component of PSC by adjusting the data to remove male-gametophyte PSC, PMPSC (Flaschenriem and Ascher 1979a; Robacker and Ascher 1981, 1982). The same two females were used for *S* allele determination with every member of the test populations and those females exhibited stylar discrimination (DS). Therefore, the behavior of each male on the female with the same *S* genotype was a measure of PMPSC, or PSC attributable to the male gametophyte. Percent PMPSC for each male on the matching *S* genotype female was calculated as [(mean incompatible-cross set/mean compatible seed set for the female) × 100] – percent PSC of the female. The figure was then subtracted from the previously computed percent PSC to arrive at the adjusted value.

Results

Population 77-612 (Flaschenriem and Ascher 1979b)

Using the previously reported seed counts (Flaschenriem and Ascher 1979b), the data were computed as percent PSC, means were calculated and distributions displayed according to *S* genotype (Fig. 2a). Shading indicates the extended tails of the two subpopulation distributions. The total population exhibits positive skewness ($P < 0.05$) as does the $S_{2.3}$ subpopulation. As reported previously, the *S* genotype segregation was 1 : 1 with 43 $S_{1.2}$ and 45 $S_{2.3}$.

Population 79-1

This 21 plant population arose from remnant seed from the lot which produced population 77-612 (Flaschenriem and Ascher 1979b). The distributions and means in Fig. 2b illustrate the expected 1 : 1 (11/10) segregation of *S* allele genotypes. Only incomplete PMPSC values were available for this population so no adjusted data are presented. Although the *S* subpopulations appear skewed they were not significantly so, probably due to the small number of plants involved.

Population 80-12

PSC distribution and means of all 141 plants of this population appear in Fig. 3a. The 77 $S_{1.2}$ and 64 $S_{2.3}$ genotypes approximate a 1 : 1 ratio (χ^2 0.25 < P < 0.50) and the entire population exhibits a negative skewness ($P < 0.01$). Each subpopulation has skewness ($S_{1.2}$ $P < 0.01$) or very nearly does ($S_{2.3}$ 0.05 < P < 0.10).

Figure 3b illustrates this population following adjustment for PMPSC. The entire population shifted toward lower PSC with the mean decreased by 19.9%. Now both subpopulations are clearly skewed ($P < 0.01$) in opposite directions and the extended tails of the distributions become obvious.

Population 80-2

This population, like 80-1, resulted from a test cross in which one parent was a 100% PSC plant, that is, one with complete stylar-inactivation PSC. There is apparent variability, however, between individuals with complete stylar-inactivation PSC since population 80-2

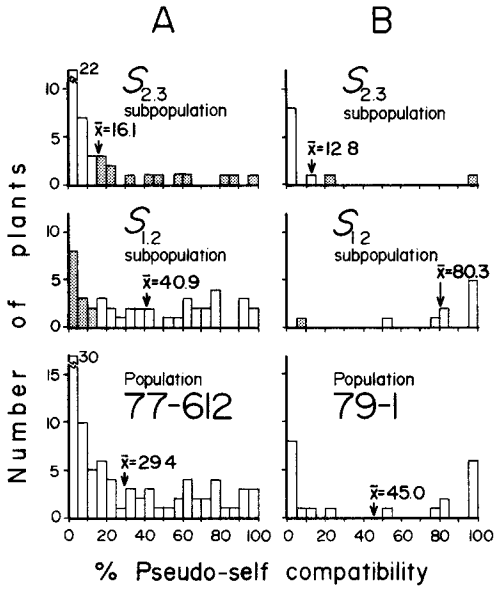

Fig. 2. PSC distributions and means of (**A**) populations 77-612 and (**B**) population 79-1 and their *S*-allele subpopulations. Shading indicates the extended tails of the subpopulation distributions

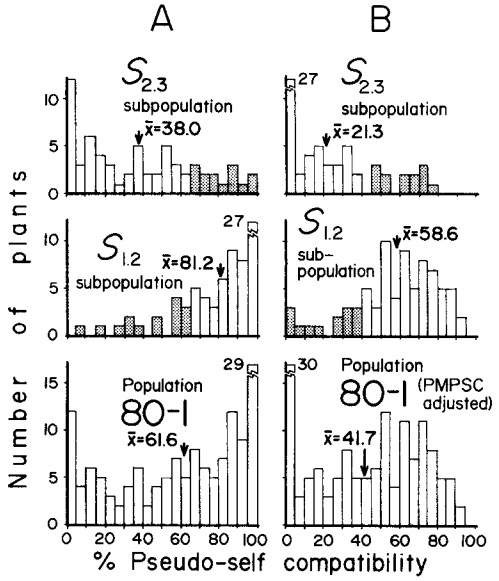

Fig. 3. PSC distributions and means of population 80-1 (**A**) unadjusted or (**B**) PMPSC adjusted with their *S*-allele subpopulations. Shading indicates the extended tails of the subpopulation distributions

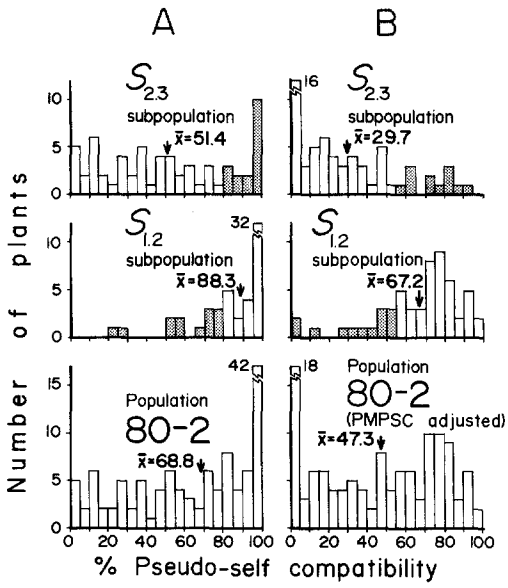

Fig. 4. PSC distributions and means of population 80-2 (**A**) unadjusted or (**B**) PMPSC adjusted with their *S*-allele subpopulations. Shading indicates the extended tails of the subpopulation distributions

has a higher mean PSC and is more negatively skewed than population 80-1 (Fig. 4 a). This is confirmed by the test for skewness ($P \ll 0.01$). Segregation of *S* genotypes is 56 $S_{1.2}$ and 63 $S_{2.3}$, a 1 : 1 ratio by χ^2 test ($0.25 < P < 0.50$). Again, there is skewness in the distribution of the $S_{1.2}$ class ($P \ll 0.01$), but the $S_{2.3}$ class is not significant in that respect.

Adjustment for PMPSC results in altered distributions and means (Fig. 4 b). Both subpopulations are significantly skewed ($P \ll 0.01$) and the population mean is shifted 21.5% PSC downward.

Discussion

This stylar-inactivation PSC factor is evidently not a null mutation. It does not entirely void stylar function for the linked *S* allele. Rather, based on the observed PSC distribution in the F_1 (77-93) of Flaschenriem and Ascher (1979 b), it is likely that less than 50% of the PSC found in the original $S_{1.1}$ 100% PSC parent (75-582-4) is attributable to the factor.

The *cis* position *S* allele specificity and partial stylar-inactivation PSC exhibited by this factor make it phenotypically identical to a mutation in the stylar activity part of the *S* locus in the Lewis model for SI (Lewis 1949, 1960). The Lewis stylar-activity-part concept is contained in numerous subsequent models (Linskens 1965; Pandey 1967). Generally, it is described as a tightly linked, complete null. However, close examination of the reports (Lewis and Crowe 1954; Pandey 1956, 1970; Nettancourt et al. 1971) indicates that in every case in which stylar part mutations have been reported, partial or incomplete types have been found. Lewis and Crowe (1954) speculated about partial stylar part mutants in *Prunus avium* saying that "these types are presumably due to mutations similar to those giving complete self-fertility but in which the activity is reduced but not lost" (p. 362). In *Nicotiana,* Pandey (1970) found that a reduction in seed set was "a general feature of the spontaneous [stylar part] mutants," with few "producing more than 50% of the normal seed set" (p. 501). Linkage data are lacking for these stylar part mutations. The Lewis concept of tight linkage within the parts of *S* may have resulted from his observations of pollen part mutations (Lewis 1949) or related work in fungi (Lewis 1954).

Our data indicate that the PSC differences among linkage test populations not attributable to the *S*-linked PSC factor are differences of non-*S* specific, multigenic PSC. The differences occur between the two populations with 100% PSC parents as well as between those and the population derived from a 53.9% PSC parent. Some of the non-linked PSC in the test populations may be due to PSC genes introduced by the 0% PSC $S_{2.2}$ parent (75-604-1). In spite of its typically non-PSC behavior, it was minimally responsive to heat treatment indicating the presence of PSC genes (Flaschenriem 1978). Also, the original $S_{3.3}$ parent (75-630-9), which was 0% PSC, produced a progeny from bud selfing which exhibited a low level (0.9%) of PSC (Flaschenriem and Ascher 1980). Most of the multigenic PSC gene complement, however, is likely derived from the original 100% PSC $S_{1.1}$ parent (75-582-4).

Adjustment of PSC data by subtraction of the PMPSC value for male gametophyte PSC was an attempt to remove some of the multigenic PSC. The relationship between PSC and PMPSC is not clear. Robacker and Ascher (1982) found that PSC and PMPSC levels were independent in *Nemesia strumosa,* but argued that both are involved in the evolution of high PSC. An interrelationship was observed in certain genotypes or within certain families in *Nemesia.* Work in *Petunia* (Flaschenriem and Ascher 1979 a; Dana 1982) revealed that, in some genotypic combinations, PMPSC is highly correlated with PSC while in others it is not. The inconsistency of the observed relationship between PSC and PMPSC notwithstanding, it seems reasonable that a value derived solely from male gamete performance should be a measure of genotype largely independent of genes for a purely female type of PSC. The adjustment procedure is offered as a contribution to the effort of sorting the obviously complex components of PSC. It is improbable, however, that the relationship is a simple additive one.

A relationship appears to exist between the PMPSC adjustment values and the specific *S* alleles involved. When each *S* genotype subpopulation is examined separately, there is a curious disparity between the location of peaks in the $S_{1.2}$ adjustment value distributions and those peaks in the distributions of the $S_{2.3}$ values (Fig. 5). The possibility that these peaks are a function of the female tester cannot be ruled out. Perhaps the entire DS/PMPSC interaction is implicated. At present we simply note the association between certain pollen *S* genotypes and PMPSC behavior.

We return to consideration of the extended tails of the *S* genotype subpopulation distributions, both with and without adjustment of PMPSC. Since the distributions are not normal as would be the expectation for many freely segregating PSC genes, each with small effect, the extended tails are interpreted to mean that a recombinational event has occurred between *S* and the *S*-linked factor discovered by Flaschenriem and Ascher (1979 b). The fact that $S_{1.2}$ genotypes occur with very low or no PSC and $S_{2.3}$ genotypes appear with high PSC means that the factor originally linked to S_1 has crossed-over to the chromosome bearing S_3. The shaded areas of the

Table 1. PSC phenotypic classes within *S* genotypes of population tested for linkage of stylar-inactivation PSC factor

Population	No. of plants	Phenotypic classes						Total of non-parentals	Non-parentals as % of population
		$S_{1.2}$			$S_{2.3}$				
		High PSC parental (not shaded)	Low PSC non-parental (shaded)	Total	High PSC parental (not shaded)	Low PSC non-parental (shaded)	Total		
77-612	88	30	13	43	32	13	45	26	29.5
79-1	21	9	1	10	9	2	11	3	14.3
80-1	141	62	15	77	50	14	64	29	20.6
80-2	119	43	13	56	46	17	63	30	25.2
Total	369	144	42	186	137	46	183	88	23.8
80-1 (PMPSC adj.)	141	63	14	77	51	13	64	27	19.1
80-2 (PMPSC adj.)	119	43	13	56	51	12	63	25	21.0
Total (PMPSC adj.)	260	106	27	133	102	25	127	52	20.0

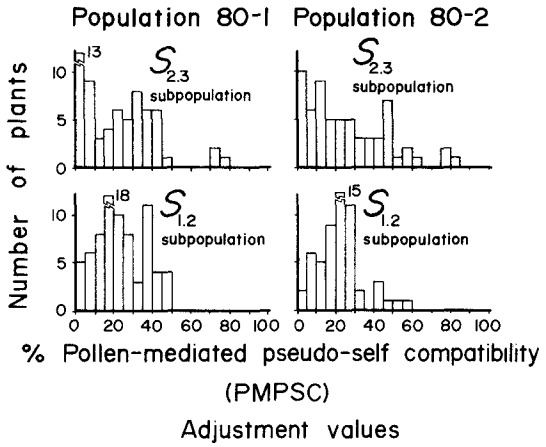

Fig. 5. PMPSC adjustment values for populations 80-1 and 80-2 associated with specific *S*-allele genotypes

skewed tails are approximately equal within each population based on the expectation that in a recombinational event, number of cross-over products of each type will be approximately equal. Therefore, the previously reported linkage map distance of 28 units (Flaschenriem and Ascher 1979 b) must be reconsidered. Both our unadjusted PSC data and those distributions adjusted for PMPSC indicate a linkage distance less than 28 units (Table 1). Without further, extensive progeny testing of every individual in each population, a conclusive statement on map distance is not justified. However, these data suggest that a location closer to 20 units from *S* may be more accurate.

Thus, we have a phenotype which is fully in keeping with that of stylar-activity-part mutation. However, the skewed distributions suggesting some 20 map units between the linked stylar-inactivation PSC factor and *S* does not fit the Lewis definition of absolute linkage. The factor is involved in stylar expression of PSC in the SI system, affects only the linked *S* allele, but may not be tightly linked to *S*.

Gametic basis for single-locus SI redefined

We now propose that the single-locus-gametophytic SI system be viewed as a three part association of genetic material and relationships. The first part is *S*, the specificity or identity gene which is basic to the system. It is a discrete gene.

The second part involves genes which interact only with a specific *S* allele. The stylar-inactivation PSC factor discovered by Flaschenriem and Ascher (1979 b) would be such a locus. So, presumably, are the genes responsible for other reported stylar part mutations. The gene identified by Brieger (1927) in *Nicotiana* and a temperature sensitivity gene in *Tradescantia* (Qaraeen 1980) fit in this category. It is likely that, in some reported cases of unexpected *S* allele ratios (Harland and Atteck 1933; Robacker and Ascher 1981), the genes responsible are *S* specific. The PMPSC behavior of 80-1 and 80-2 in the present work suggests that *S*-allele specific genes might be operative in PMPSC also (Fig. 5). Such loci may be linked to *S*, the specificity gene, but need not be. Pollen-inactivation PSC mutations which result in loss of pollen activity for one *S*-allele only and are usually asumed to be tightly linked to *S* are of this type. Our work shows that a pollen-inactivation PSC, *S*-specific factor may not be closely linked to *S* (Dana 1982; Dana and Ascher 1986). Also, complementation between a pollen part mutation and a normal allele has been observed (Lewis 1958)

suggesting that pollen activity genes may be of a specific or non-specific nature. All of the breakdown of SI due to these loci should correctly be termed PSC since they are not true mutations to *S* identity.

The third part of the association is composed of the many genes contributing to SI which are not *S* allele specific. These genes, either singly or in polygenic combinations, lead to highly variable self seed setting in an SI species. Their expression may be complex, given the possibility of interaction with all parts of the SI system. They may be of minor or relatively major phenotypic importance. Such loci are frequently relied upon to explain otherwise inexplicable results (Wergin 1936; Tseng 1938; Townsend 1979). Those genes involved in expression of DS and PMPSC (Flaschenriem and Ascher 1979a; Robacker and Ascher 1981, 1982) may be of this nature.

This conceptual redefinition demands some revision in terminology. The *S* designation should be reserved exclusively for the locus conferring identity, or the Lewisian *S* specificity part. All other loci involved as well as *S* should be considered part of the SI-complex. This is different from the *S* gene complex (Pandey 1962) which assumed tight linkage and multipartite *S*. Only true mutations or alterations of specificity would be *S* gene mutations. This would include mutations to S_f (Takahashi 1973). All others, including changes in pollen or stylar activity, would be a form of PSC, since the matching *S* allele identities in any self pollination would not be violated.

This re-definition of concept and terminiology, incorporating, as it does, our observations that linkage between *S* and stylar-activity factors and *S* and pollen-activity factors (Dana and Ascher 1986) may not be close, is offered here as a departure point for further study. With a less rigid concept of *S* and related genes, research may proceed without the constraint that some existing models and long standing assumptions have caused.

The concept of a single identity and numerous specific or non-specific related genes for pollen tube-style interaction is in agreement with the gradual evolutionary development theory of SI (Mather 1943). All genes in the SI-complex, excluding *S*, are of the type which influence the expression of *S*. The maintenance of these genes in some portion of the population of an SI species would be highly advantageous and enable the species to respond to changes in environment favoring inbreeding or outcrossing. It has been suggested that all SI systems exist in an equilibrium between PSC and non-PSC depending on environment (Robacker and Ascher 1981). A recombinable package of coordinated units comprising the SI complex would enable a species to make a more rapid response to such environmental fluctuations.

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