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Discriminating Styles (DS) and Pollen-mediated Pseudo-self-compatibility (PMPSC) in *Nemesia strumosa* Benth.

Part 1: Characteristics and Inheritance of DS *

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Summary. *Nemesia strumosa* plants were discovered which had styles capable of discriminating among incompatible pollen tubes from different pollinators, allowing growth of some but not others. All but 3 of 26 families tested had at least some members with discriminating styles (DS). Presence and level of DS was independent of *S* genotype. Plants with pseudo-self-compatiblity (PSC) levels greater than 10% had the trait, though many plants with strong DS had PSC levels less than 10%. Self pollination of highly DS plants produced mostly DS offspring, but of differing sensitivities. Some progenies from crosses between a family of highly DS plants and unrelated, probably low DS plants segregated half DS and half non-DS, while others consisted of mostly DS or mostly non-DS. The DS phenomenon is probably caused by PSC genes.

Key words: Self - incompatibility – Pseudo-self-compatibility – Gamete competition – Inheritance

Introduction

Nemesia strumosa Benth., a small, many-flowered diploid annual in the Scrophulariaceae, has been the subject of self-incompatiblity studies in our laboratory since 1969. The initial study was begun with 3 plants from the Harry Saier Seed Co. in Michigan. Though most *Nemesia* plants have a strong gametophytic self-incompatibility system, these 3 plants had a low level of pseudo-self-compatibility (PSC), occasionally setting a few seeds following self pollination. Through heat treatment (Campbell and Ascher 1972) and inbreeding (Henny and Ascher 1976; Robacker and Ascher 1978), the PSC levels were increased such that some plants produced as many seeds from self pollinations as from compatible cross pollinations. Since pollen from such high PSC plants produced no seeds when incompatibly crossed to self-incompatible plants, Henny and Ascher (1976) hypothesized that only the stylar part of the incompatibility reaction was weakened. Further investigations by Henny and Ascher (1977) revealed that some highly PSC plants produced more seeds following self pollination than following incompatible-cross pollination with self-incompatible plants. They termed this phenomenon sporophytic recognition. Flaschenriem and Ascher (1979) discovered an I_7 *Petunia hybrida* plant which was strongly self incompatible, but set varying amounts of seed when incompatibly crossed as female with unrelated plants. The number of seeds produced reflected the self seed setting level of the pollen plants. Reciprocal crosses yielded no seeds, demonstrating that only the style of this I_7 plant functioned abnormally. Since selfs and outcrosses produced progenies with discriminating styles (DS), Flaschenriem and Ascher suggested that this trait was inherited as a simple dominant.

Recently, we discovered that *Nemesia* family 77-12 had discriminating styles similar to those in the *Petunia* plants described above. Following self pollination, these plants produced few or no seeds. As plants in 77-12 were expected to segregate 1 *S*1.2 to 1 *S*1.3, they were crossed as female to *S*2.2 and *S*3.3 plants to determine *S* alleles. Unexpectedly, all crosses produced rather high numbers of seeds. However, crosses of the 77-12 plants as male onto self-incompatible *S*1.3 plants yielded the expected results: half the plants (*S*1.3) were incompatible and half (*S*1.2) were compatible. As in *Petunia*, the style alone behaved abnormally. The following study was undertaken to characterize DS, to determine the prevalence of this phenomenon in our *Nemesia* populations and to discover the mode of inheritance of DS.

Materials and Methods

All *Nemesia* plants in this study were at least distantly related, as they were originally derived from 4 plants. Seedlings were grown

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and pollinated fall through spring in a greenhouse with night temperatures ranging from 10 to 17°C and day temperatures 16 to 30°C, depending upon the time of the year. When flowering was initiated, *S* alleles were determined by crosses with plants of known *S* alleles. Flowers were emasculated one day before anthesis and pollinated the following day. All unpollinated flowers were discarded each day to prevent pollen contamination. Each seedling was assigned an identification code: the first 2 numbers indicated the family to which the seedling belonged, and the last number identified the seedling.

To detect DS, seed sets from self pollinations were compared to those from incompatible-cross pollinations as female. The plants, ranging in age from 4 to 7 months, were self- and incompatibly-cross pollinated, 2 flowers per day for 5 days, giving a total of 10 self and 10 incompatible-cross pollinations per plant. Maximum seed-producing potential of each plant was estimated from 6 compatible crosses performed 2 per day. To determine whether seed sets from self pollinations differed significantly from incompatible-cross pollinations, a t-test was performed when most of the pollinations produced seeds and a Chi-square test was used when many of the self or incompatible-cross pollinations produced no seeds.

A DS value was calculated for each plant to provide a quantitative measure of discriminating sensitivity. The DS value was obtained by subtracting the % self PSC from the % incompatible-cross PSC. Percent self PSC was calculated by dividing the mean self seed set by the mean compatible-cross seed set as female, and multiplying by 100. Incompatible-cross PSC was determined by substituting the mean incompatible-cross seed set for the mean self seed set in the above formula. Incompatible-cross PSC, and therefore the DS value, was obtained for each male-female combination, so that a plant may have different DS values, depending upon the incompatible male used. The higher the absolute DS value, the higher the level of discriminating sensitivity of the style. Positive DS values indicate greater incompatible-cross seed set than self seed set, while negative DS values indicate the reverse.

To obtain a DS value reflecting the maximum discriminating capacity of a plant, special incompatible pollinators were used. Pollinators capable of producing high DS values were selected based on preliminary data from crosses with 77-12 DS plants. Therefore, DS values for self-incompatible or low-PSC plants were obtained from incompatible crosses with pollinators (78-20, 78-21) which exhibited high seed set on DS plants, while the DS values for high self PSC plants were found through crosses with pollinators (77-12) which produced low seed set on DS plants. The highly DS family 77-12 functioned as a low seed-producing pollinator.

Inheritance of DS was investigated by testing for its presence and level in progenies derived from 77-12 plants with high DS values. Families 78-29, 78-30, and 79-7 were obtained from self or sib pollination of 77-12 plants. Four families, 78-10, 78-11, 78-12 and 79-6, came from compatible crosses of 77-12 plants as male to unrelated plants. (Since all plants were derived from only 4 plants, all are actually related; the term unrelated is used when no ancestor was shared for at least 3 generations). Incompatible crosses between 77-12 plants as female and unrelated plants produced progenies 78-31, 78-32 and 79-4. The unrelated plants were not tested for presence of level of DS, but most probably had no or very low levels of DS. An incompatible reciprocal cross between 78-29-19 and 78-45-3 gave families 79-11 and 79-12. The parent 78-29-19 came from a sib cross of 77-12 plants, had a DS value of 51 and low self PSC level. The other parent, 78-45-3, had rather high self PSC, but was not tested for presence of DS.

To determine whether DS arose in 77-12 or occurred earlier, families 78-22 and 78-23, derived from self pollination of the parents of 77-12, 70-311HA-4 and 70-311HA-5, were grown and tested. No seeds of the parents of 77-12 were available. Family 77-12 was only 3 generations removed from the original 3 plants. To discover whether DS appeared in other ancestral *Nemesia* plants, families 79-1, 79-2 and 79-3 were tested.

To discover the prevalence of DS in our *Nemesia* populations, a variety of genotypes, some related and some unrelated to 77-12, were tested for DS. One to 3 plants, randomly selected from each of 26 families, were checked. These plants carried the *S* alleles 1, 2, 3, 5, or 6 and varied in self PSC from 0 to 96%.

Results

Number of discriminating and non-discriminating plants, and progeny DS value ranges for 77-12 and its closely related families are given in Table 1. All 11 plants from family 77-12 had discriminating styles. DS values ranged from 21.9 to 68.8 in a nearly normal distribution (Fig. 1). Progenies 78-22 and 78-23, produced from self pollination of the parents of 77-12, consisted of mostly DS plants, with DS values ranging from low to high. Similar progenies were obtained from selfs and sibs of 77-12 plants. The DS values for family 78-29 appear even more diverse than those of progeny 77-12 (Fig. 1). Again, all plants had DS.

Progenies produced from crosses between 77-12 plants with high DS values and unrelated plants segregated for DS (Table 2). Families 78-10 and 79-6 consisted of mostly discriminating plants, while 78-11, 78-12 and 79-4 were rather evenly divided. Of these progenies, all but 79-4 had 77-12 plants as male parents: 77-12 was the female parent of 79-4. Mostly non-discriminating plants occurred in 78-31, 78-32, 79-11 and 79-12. A 77-12 plant was the female

Table 1. Number of discriminating and non-discriminating plants and the range of DS values in the *Nemesia strumosa* family 77-12 and its closely related families. *S* alleles and PSC levels of parents are given

Parents	Parent S alleles	Parent PSC (%)	Progeny	Progeny self PSC (%)	No. disc.	No. non-disc.	DS range
70-311HA-5 X 70-311HA-4	2.3 X 1.2	6.2 X 5.2	77-12	3.5	11	0	21.9 to 68.8
70-311HA-4 selfed	1.2	5.2	78-22	3.3	5	0	28.9 to 68.0
70-311HA-5 selfed	2.3	6.2	78-23	2.2	5	1	3.5 to 80.5
77-12-7 X 77-12-13	1.3 X 1.2	1.9 X 1.3	78-29	2.7	10	0	10.1 to 75.7
77-12-35 selfed	1.3	2.6	78-30	5.7	4	1	17.8 to 46.6
77-12-32 selfed	1.3	10.3	79-7	8.8	3	0	26.9 to 41.7

parent of progenies 78-31 and 78-32. Only families 78-12, 79-4 and 79-6 had individuals with high DS values.

Self PSC levels in 79-6 ranged from low to high, so 2 sets of pollinators, those capable of producing high, and those which produce low seed sets, were used to detect discriminating styles. Mean seed sets following self and incompatible-cross pollination of 4 typical 79-6 plants appear in Figure 2. The low seed-producing pollinator, 77-12, produced significantly fewer seeds than the high pollinator in every case, and fewer than the self seed set on 79-6-1 and 79-6-8. In addition, the high pollinator 78-20 yielded significantly more seeds than self pollination on every plant but 79-6-1. These 79-6 styles were capable of 3 levels of discrimination.

Progenies 79-11 and 79-12, from reciprocal crosses, were similar to each other in stylar discriminating sensitivity, but different from all other progenies investigated (Table 2). Most of the plants had low self PSC and were non-discriminating when tested with 78-20 and 78-21, the pollinators which produced the seed sets on the low self PSC but highly DS plants in other progenies. The few 79-11 and 79-12 plants which had DS discriminated in the opposite direction from expected, pollination with 78-20 and 78-21 yielding fewer seeds than did self pollination. Progenies 78-12 and 78-32 came from reciprocal crosses between 77-8-14 and 77-12 plants. Because the 77-12 parent was different, these crosses are not true reciprocals. However, both 77-12 plants were *S*1.2, with low self PSC levels and high DS values. The numbers of discriminating

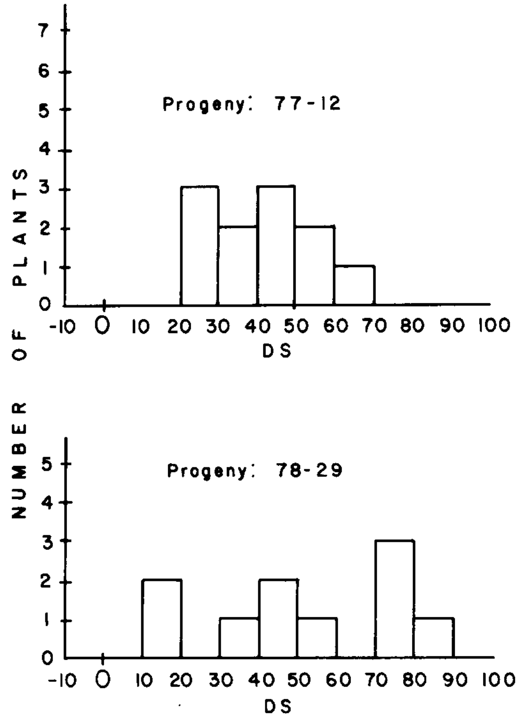

Fig. 1. Frequency histogram of the DS values of *Nemesia strumosa* plants in progenies 77-12 and 78-29

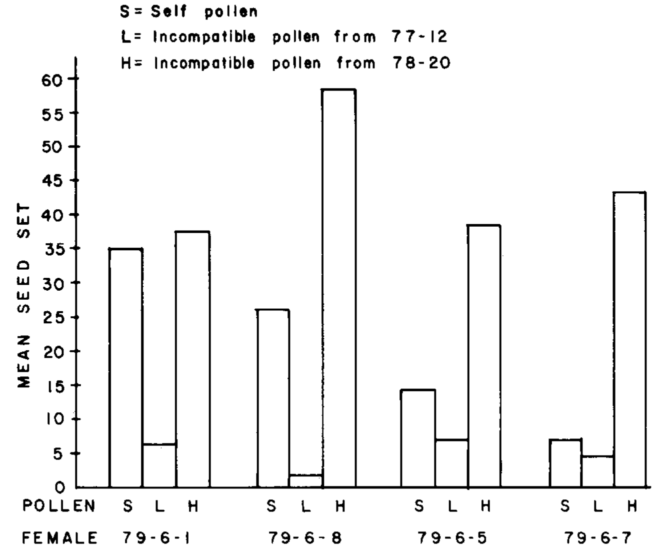

Fig. 2. Self and incompatible cross seed set on 4 *Nemesia strumosa* plants in progeny 79-6

Table 2. Number of discriminating and non-discriminating plants and the range of DS values in the *Nemesia strumosa* progenies from crosses between 77-12 and unrelated plants. The *S* alleles and PSC levels are given

Parents	Parent S alleles	Parent PSC (%)	Progeny	Progeny self PSC (%)	No. disc.	No. non-disc.	DS range
77-27-2 X 77-12-18	3.6 X 1.3	3.2 X 0.0	78-10	1.1	7	1	2.9 to 30.2
77-27-23 X 77-12-16	3.5 X 1.3	0.2 X 0.0	78-11	0.9	4	4	−6.2 to 16.7
77-24-17 X 77-12-24	2.2 X 1.2	90.2 X 1.2	79-6	38.1	8	0	−56.9 to 73.6
77-8-14 X 77-12-17	2.2 X 1.2	0.0 X 1.0	78-12	16.0	11	16	−3.6 to 72.7
77-12-13 X 77-8-14	1.2 X 2.2	1.3 X 0.0	78-32	4.7	2	15	−5.2 to 26.8
77-12-33 X 77-9-12	1.3 X 3.3	0.3 X 0.0	78-31	2.9	3	13	−1.6 to 26.0
77-12-27 X 77-28-8	1.3 X 3.3	6.9 X 48.1	79-4	19.9	3	5	−2.2 to 50.6
78-45-3 X 78-29-19	2.3 X 2.3	68.9 X 1.1	79-11	18.8	5	15	−21.5 to 4.8
78-29-19 X 78-45-3	2.3 X 2.3	1.1 X 68.9	79-12	12.9	4	17	−13.9 to 5.5

Table 3. Number of DS and non-DS plants and the range of the DS values in ancestral *Nemesia strumosa* progenies. The *S* alleles and PSC levels of the parents are given

Parents	Parent S alleles	Parent PSC (%)	Progeny	No. disc.	No. non-disc.	DS range
70-176HA-2 selfed	2.3	4.1	79-1	3	5	0.9 to 61.8
70-176HA-4 selfed	2.3	36.8	79-2	2	2	−12.6 to 47.9
70-284-1 X 76-8-1	1.3 X 3.3	0.0 X 0.0	79-3	1	3	−0.2 to 16.2

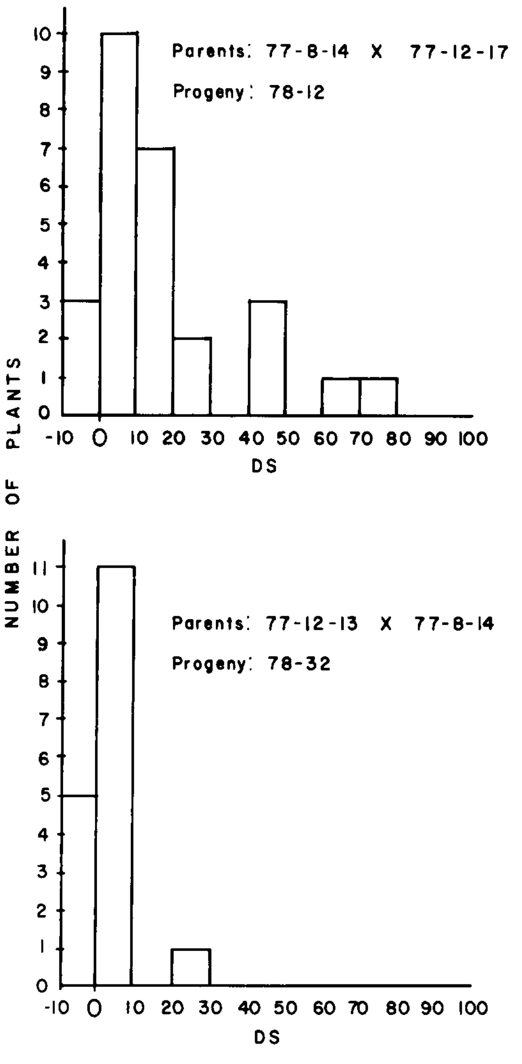

Fig. 3. Frequency histogram of the DS values of *Nemesia strumosa* plants in progenies 78-12 and 78-32, derived from reciprocal crosses between families 77-8 and 77-12

plants in families 78-12 and 78-32 were very different, 78-12 having an equal number of each, while 78-32 had mostly non-discriminating plants. Frequency histograms of the DS values of these 2 families appear in Figure 3.

Highly DS plants were detected in 79-1 and 79-2, progenies derived from the ancestral family of 70-176HA. Only a low level of discrimination was found in 79-3, a family with the ancestral parent 70-284 (Table 3).

Plants with DS were common in *Nemesia*, occurring even in families unrelated to 77-12. Of the 26 families tested for presence of DS, all but 3 had at least some members with DS. Presence and level of DS was independent of the *S* allele genotype, but related to the level of self PSC. Any plant which had a self PSC level greater than 10% had DS, though many plants with self PSC levels less than 10% also had DS.

Discussion

It seems unlikely that DS arose for the first time in family 70-311HA, the parents of 77-12. More likely, the trait was present in one or more of the original 3 *Nemesia* plants or arose sporadically throughout the population, either as a result of mutation or gene recombination. DS cannot be explained through either pollen- or stylar-part mutations, as the highly DS plants and the high pollinator plants 78-20 and 78-21 were self incompatible. Only the combination of a high pollinator crossed onto a discriminating style produced seeds.

Several researchers (Denward 1963; Pandey 1970; Nettancourt et al. 1971; Anderson et al. 1974) suggested that inbreeding produces mutations to new *S* alleles. Despite extensive inbreeding of *Nemesia*, no mutations to new *S* alleles were found. Lundqvist et al. (1973) and Larsen (1977) proposed that some species which appear to have only 1 *S* locus may actually have additional *S* loci which are homozygous and fixed throughout the species (or population investigated) for a particular *S* allele. A mutation to a new *S* allele in plants with only 1 *S* locus or at a hidden second locus would produce plants which were cross compatible as male with all non-mutated plants, but incompatible as female. Plants with DS have the opposite response, being compatible as female, incompatible as male. Nettancourt et al. (1971) reported that mutations to a new *S* allele frequently occurred in the style first. Had this happened in *Nemesia*, the DS plants would have been self compatible, but many were self incompatible.

The high amount of variation in DS ability and the complex inheritance pattern in the progenies from crosses between 77-12 and unrelated plants may indicate that several genes control this trait. Inheritance would be further complicated if the style allowed pollen tubes with particular genes through at a higher rate than expected by chance.

Progenies from incompatible crosses with DS plants as female (78-31, 78-32) tend to have a greater number of non-discriminating plants than when DS plants are male (78-10, 78-11, 79-6; Table 2). However, no differences occurred in progenies 79-11 and 79-12, from a reciprocal cross between the discriminating plant 78-29-19 and 78-45-3. Though 78-45-3 was not tested for DS capacity, one of its siblings had a discriminating style. The similarities of progenies 79-11 and 79-12 can then be explained as a consequence of similar DS abilities in both parents. Additional studies of populations from reciprocal crosses between plants with known DS capacities are needed to clarify these results.

The number of seeds produced following an incompatible pollination is a function of both the pollen and the style. A high DS combined with a high pollinator will give the greatest breakdown of incompatibility, possibly through some failure in recognition. This altered recognition does not appear linked to any particular *S* allele, as high DS plants occur at random among the various *S* classes. However, a non-specificity part of the *S* gene may be altered, though no proposed models could account for the DS behavior. More likely, several genes control the pollen-DS interaction. Mather (1943) and Bateman (1952) proposed that in the evolution of outbreeding systems, many genes evolved which promoted outcrossing. Eventually, the *S* gene and its modifiers predominated. DS may be caused by the modifier genes of the *S* system or a remnant recognition system formed early in the evolution of outcrossing systems. A remnant recognition system would ordinarily be overriden by the *S* system, appearing only when the *S* system was breaking down, otherwise, recognition genes other than *S* could be identified in self-incompatible plants. But a non-*S* recognition system is an unsuitable explanation for DS. First of all, the highly DS family 77-12 was self incompatible, as were its ancestors, suggesting that the self-incompatibility system was quite intact. Secondly, if another recognition system were involved, seed set from compatible as well as incompatible crosses would be altered. But recognition in DS is changed only in incompatible crosses. Finally, when outcrossing became advantageous, it is likely that all the genes which promoted outcrossing eventually worked together as a unit to prevent unfavorable recombinations, cohering to form the *S* system.

Therefore, the DS phenomenon is probably associated with the genes of the *S* system, most likely PSC genes, which by definition, promote a weakening of the self-incompatibility system. Further evidence of the involvement of PSC genes is that all plants with self PSC levels greater than 10% have DS. However, to be highly discriminating, the style may need a special gene combination. For example, most plants in families 79-11 and 79-12 (Table 2), from crosses between unrelated DS plants, lacked DS; the few which had DS responded to pollinators in the opposite way from their parents. This evidence suggests that the genes recombined to either eliminate or change the DS response.

Table 4. Self PSC level of the parents of the high pollinator families 78-20 and 78-21

Seed parent	Self PSC (%)	Pollen parent	Self PSC (%)	Progeny
77-2-9	64.8	76-5-7	6.0	78-20
77-2-22	48.6	76-8-3	1.4	78-21

The factors which cause a plant to be a high pollinator need further investigation. A high level of self PSC appears unimportant, as 78-20 and 78-21 had low self PSC levels but were high pollinators, while 77-12 was both low in self PSC and a low pollinator (Fig. 2). An examination of the self PSC levels of the parents of these pollinators may provide some clues. Families 78-20 and 78-21 each had an intermediate or high self PSC parent, and a low self PSC parent (Table 4). A cross of a high self PSC plant to a low self PSC plant often yields a low PSC progeny (Henny and Ascher 1976; Robacker 1981). The gametes produced by this low PSC progeny will be segregating for PSC genes, possibly producing pollen capable of growing on DS plants. The parents of 77-12 had low self PSC levels (Table 1), and therefore, 77-12 had only low PSC pollen. Another possible explanation for the different pollinating abilities of 78-20 and 78-21 versus 77-12 was relatedness between the pollen and seed plant. Family 77-12 was related to most of the plants in this study, including 79-6 on which it produced few seeds (Fig. 2), while 78-20 and 78-21 were unrelated. Additional investigations into the origin and characteristics of high pollinators and the interaction of these special pollinators with DS will be presented in another paper in this series.

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