Theor. Appl. Genet. 61, 289–296 (1982)

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

Part 2: Origin of PMPSC and Nature of the DS-PMPSC Interaction *

C. D. Robacker and P. D. Ascher
Department of Horticultural Science and Landscape Architecture University of Minnesota, St. Paul, Minn. (USA)

Summary. *Nemesia strumosa* pollinators which produced significantly more seeds than other pollinators following incompatible pollinations onto discriminating styles (DS) had pollen-mediated pseudo-self-compatibility (PMPSC). Level of PMPSC was not affected by relatedness between the pollen and style. When a plant expressed PMPSC, either it or its ancestors had intermediate or high pseudo-self-compatibility (PSC) levels, though these conditions did not guarantee PMPSC. Except within families, self PSC level was not related to PMPSC level. Generally, members of the same family showed similar patterns of DS and PMPSC response. When differences occurred, they were related to the level of discriminating sensitivity and self PSC respectively. Incompatible diallel crosses among related and unrelated plants revealed similar patterns of seed set, indicating that the interaction between DS and PMPSC pollinators was of a general nature. Both PMPSC genes and DS genes are PSC genes, though not the same genes. The products of PMPSC genes and DS genes interact to determine self or incompatible-cross seed set level.

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

Introduction

Self-incompatible or pseudo-self-compatible (PSC) plants with discriminating styles (DS) are capable of distinguishing among incompatible pollen tubes from different sources, permitting some to escape the incompatibility reaction, but not others. This form of gamete competition has been reported in several species. Hodgkin (1977) found that the number of incompatible pollen tubes which penetrated the stigma in *Brassica oleracea* was a function of both pollen and stigma genotypes. Furthermore, the ability of pollen to overcome the incompatibility reaction when crossed onto another stigma was independent of its ability to penetrate its own stigma. In contrast, Flaschenriem and Ascher (1979) discovered an inbred line in *Petunia hybrida* in which the level of seed set following incompatible crosses was related to the level of self PSC of the pollen plant used. The term "pollen-mediated PSC" (PMPSC) was applied to pollinators which produced seeds following such incompatible crosses to plants with DS.

Nemesia strumosa Benth., an ornamental annual in the Scrophulariaceae, has the gametophytic self-incompatibility system. Henny and Ascher, through selection for self seed from self-incompatible plants, obtained high PSC plants (1976). Self pollination of many of these high PSC plants produced a greater seed set than did incompatible-cross pollinations with low PSC plants (1977). This phenomenon, called sporophytic recognition, was found in 95 out of 274 plants studied. Robacker and Ascher (1981) discovered high DS in the *Nemesia* family 77–12. However, the response was opposite that observed by Henny and Ascher (1977): the 77–12 plants set fewer seeds following self pollination than incompatible-cross pollination with certain pollinators. The level of discriminating capacity was highly variable from plant to plant, and the inheritance of the ability to discriminate was complex, suggesting that the trait may be controlled by several genes, perhaps PSC genes. Plants with DS were widespread in *Nemesia.* All but 3 of 26 families tested had at least some members with DS.

To determine whether sporophytic recognition and DS are part of the same phenomenon, both high and low self PSC genotypes were tested in various male-female combinations. Additional studies, described below, were conducted to provide information on the origin and characteristics of PMPSC plants and the nature of their interaction with DS.

Materials and Methods

Nemesia seeds were sown from late summer through early winter in milled sphagnum moss and germinated under in-

* Scientific Journal Series Paper No. 11, 719 of the Minnesota Agricultural Experiment Station

0040-5752/82/0061/0289/$ 1.60

termittent mist. Following germination, the seedlings were placed in a 1:1:1 peat, soil, and perlite medium. Daylight greenhouse temperatures ranged from 16 to 30 °C, while night temperatures varied from 10 to 17 °C, depending upon the time of year. When flowering began, the plants were self and compatibly-cross pollinated as female to determine self PSC levels. Percent self PSC was calculated by multiplying the ratio of mean self seed set to mean compatible cross set as female by 100. Self PSC, therefore, measures the level of breakdown of the incompatibility reaction between the pollen and style of the same plant, as opposed to the level of breakdown which occurs following incompatible pollinations with pollen from other plants. Mean self and compatible-cross seed sets were usually based on 10 and 6 pollinations respectively, though occasionally fewer, as some developing seed capsules were lost. To average environmental effects, only 2 self and/or cross pollinations were performed on each day. Flowers were pollinated on the day of anthesis. Those to be cross pollinated were emasculated one day before anthesis.

Since only discriminating styles detect PMPSC, detection of DS and PMPSC was accomplished in the same experiments. Therefore, any plant which set significantly different numbers of seeds following different incompatible pollinations was classified as having DS. Conversely, those plants which, after incompatible-cross pollination, produced significantly more seeds than other pollen donors on these discriminating styles were labeled as having PMPSC. To test for DS and PMPSC, plants were self and incompatibly-cross pollinated with a variety of pollinators. In addition, maximum seed set for each plant was determined through compatible pollinations. Ten pollinations of each type were performed, 2 per day for 5 days. All pollinations to be compared were done at the same time on the same days. Significant differences in seed set from incompatible pollinators were detected using either a Chi-square test, a t-test, Duncan's New Multiple Range Test, or a combination of these (Steel and Torrie 1960). When all the crosses yielded seeds, Duncan's Test was performed. The Chi-square test was used to detect significant differences among pollinators when either all the pollinators frequently produced no seeds or when some pollinators produced seeds and others did not. The categories compared were number of pollinations yielding seeds and number failing to yield seeds. Those pollinators which, according to the Chi-square test, yielded seeds significantly more often, were further tested: when only 2 pollinators produced seeds frequently, they were compared with a t-test and when 3 or more pollinators consistently produced seeds, Duncan's Test was performed.

All pollen plants were tested for male fertility through compatible crosses to insure that any differences in seed set from the incompatible pollinations reflected PMPSC differences rather than fertility differences. To do this, each group of pollen plants being compared for PMPSC were compatibly crossed onto the same seed plant on the same days. Each pollen plant was crossed 4 times, 2 crosses per day. Duncan's New Multiple Range Test was used to determine whether differences in male fertility occurred.

A 3-number identification code was assigned to each seedling. The first number indicates the year in which the seed was sown, the second identifies the family, and the third labels the individual seedling. Siblings, therefore, have the same first 2 numbers.

All *Nemesia* plants tested came originally from 4 plants. Three were from the Harry Saier Seed Co. in Michigan and one plant was from Bodger's Seed Co. in California. The plants studied in this paper were derived through selfs and intercrosses and are at least 3, though usually more, generations removed from the original plants. Therefore, though all plants are at least distantly related, the term related will be applied to plants which have a common ancestor within 3 generations. Otherwise, they will be designated unrelated. Pollen parents examined for PMPSC came from 38 families, ranged in self PSC from 0 to 96%, and carried the *S* alleles 1, 2, 3, 5, or 6. From 1 to 4 plants were tested in each family.

Robacker (1981) observed that plants in 79–6, a family related to 77–12, produced few seeds when incompatibly crossed as female with 77–12, but many seeds when crossed with the unrelated family 78–20. Progeny 78–20 had low self PSC, but came from a cross of high by low self PSC parents, while 77–12, its parents and ancestors, all had low self PSC. Experiments were done to determine whether relatedness between the seed plant and pollinator or the PSC ancestry of the pollinator were factors in PMPSC expression. In one experiment, self seed set of 77–12 was compared with that produced following incompatible crosses as female to unrelated self-incompatible plants with no PSC ancestry (78–37). A second experiment contrasted 77–12's self seed set to seed set from incompatible pollinations as female with unrelated low PSC plants of high PSC ancestry (78–20 and 78–21) and with related plants of various PSC levels and high PSC ancestry (79–4 and 79–6). The importance of relatedness in determining PMPSC was also investigated in genotypes unrelated to 77–12. Again, crosses of pollinators related and unrelated to the seed plant were performed and the resulting seed sets compared.

The effect of PSC ancestry on PMPSC was further studied by assigning a rating of 1 to 4, denoting the level of self PSC of parents and ancestors, to each of the 68 pollinators tested for PMPSC. A rating of 1 indicated that both parents, in the case of a cross, or the one parent, in the case of a self, had high self PSC. PSC levels of 70% or greater were considered high, 20% or lower were low, and those between 20 and 70% were considered intermediate. When only 1 of 2 parents was high in self PSC, or one or both parents were intermediate in self PSC, the progeny was rated a 2. A rating of 3 was assigned when the parents were low in self PSC, but one or more grandparents or ancestors had intermediate or high self PSC levels. Finally, a 4 rating indicated that the parents and ancestors all had low or 0 self PSC levels. The relationship between PMPSC and self PSC ancestry was examined using this classification.

To test for differences in level of PMPSC expression, pollen plants were incompatibly crossed onto several plants with DS. When possible, the same group of seed plants was used. However, because of different *S* alleles in the pollen plants and the limited number of flowers available on each seed plant, different plants were sometimes used. In any case, the females were selected from a variety of families to make the comparison among pollen plants unbiased.

As a test of general versus specific response (the concept here is similar to general and specific combining ability, but concerns interaction between pollen and style genes which regulate pollen tube growth), the pattern of DS-PMPSC expression was determined by incompatible diallel crosses of groups of males to groups of females. Eleven sets of diallel crosses were performed, involving a total of 38 different pollinators and 33 seed plants. If the pattern of response was the same, that is, PMPSC plants produced seeds on the same females, then the response was general. A specific response would occur when only particular male-female combinations yielded seeds. As a sub-experiment, siblings were included among the males and females crossed in diallels to determine whether the pattern of response among siblings was more similar than among non-siblings. Furthermore, seed sets from self pollination were compared to seed sets produced from incompatible sibling pollinations. The siblings frequently differed from each other in self PSC level.

The inheritance of PMPSC was investigated by evaluating the PMPSC levels of plants from families 78-47 and 78-49, produced from self or sib pollinations of the high PMPSC family 76-2. Seed sets from incompatible crosses of 78-47-7 and 78-49-13 were compared to those from the high PMPSC plant 76-2-40, and the unrelated high PMPSC plant 78-21-7, when incompatibly crossed onto 5 seed plants.

Results

Relatedness (other than sibling relationships) between the pollen and style plants had no detectable effect on the amount of seed produced (Table 1). All pollen plants compared in Table 1 had ancestral PSC ratings of 2. On plant 78-19-1, the unrelated pollen plant 76-2-40 yielded significantly more seeds than either the related pollen plants or self pollination. The opposite occurred with 78-34-1 and 78-41-2, on which the related pollen plant, 78-45-12, produced significantly more seeds than the unrelated plants. Plant 78-38-9 set significantly more seeds when pollinated with either a related or an unrelated plant, than when self pollinated.

Incompatible crosses onto 77-12 with related and unrelated plants of high or low PSC ancestry revealed that high PSC ancestry was important in determining PMPSC. Self versus incompatible-cross pollination with 78-37, a low PSC family with PSC ancestors and unrelated to 77-12, produced no significant differences in seed set on four 77-12 plants with DS (Table 2). However, significant differences were obtained among incompatible pollinations of eight 77-12 plants with self, 79-4, 79-6, 78-20, and 78-21 pollen (Table 3). Half of these 77-12 styles were capable of discriminating among 3 levels of PMPSC. Self pollen expressed little or no PMPSC and yielded the fewest seeds. On 5 out of the 8 77-12 plants tested, 78-20 or 78-21 produced more seeds than did 79-4 or 79-6, with no significant differences occurring on the other 3 plants. Families 78-20, 78-21, 79-4, and 79-6 had intermediate or high PSC parents, but 79-4 and 79-6 were related to 77-12. Interestingly, however, 78-20 and 78-21 had much lower self PSC levels than did 79-6, evidence that level of self PSC is not a good predictor of level of PMPSC.

The importance of PSC ancestry in predicting whether a plant can express PMPSC was further demonstrated by the 68 pollinators tested for PMPSC. Of the 15 plants with an ancestral PSC rating of 4, only 1 expressed PMPSC, and that plant did so in only one cross. The mean ancestral PSC rating for plants capable of demonstrating PMPSC was 2.1, while the mean rating for plants which expressed no PMPSC was 2.8. Plants with ancestral PSC ratings of 1, 2 and 3 fell into both categories.

PMPSC plants vary in their level of expression, as measured by the number of DS plants on which a pollen plant can exhibit PMPSC (Table 4). The plants listed in the top part of Table 4 were highly PMPSC, expressing PMPSC on nearly all DS plants. Plants with intermediate PMPSC levels are given in the middle of the table: these plants produced seeds on some plants with DS, but not on others. At the bottom of the table are plants which failed to demonstrate PMPSC on any DS. All plants appearing in this no-PMPSC group had an ancestral PSC rating of 4. Tests of male fertility showed that most of the pollinators produced equal amounts of seeds following compatible-cross pollinations. The few exceptions were either discarded or noted. A comparison of the seed sets obtained when the high PMPSC plant 76-2-40, the intermediate PMPSC plant 78-39-2, and the no-PMPSC plant 78-30-5 were incompatibly crossed onto 6 female plants is given in Table 5. Both the individual cross results and means over the 6 females show that 76-2-40 consistently produced the most seeds and 78-30-5 the least. Similar

Table 1. Mean self and incompatible-cross seed set of *Nemesia strumosa* plants with discriminating styles pollinated with related and unrelated plants to determine whether relatedness affected seed set levels. Different letters following seed sets indicate significant differences at the 5% level on the female

Female	Mean self seed set	Related male	Mean incompatible cross seed set	Unrelated male	Mean incompatible cross seed set
78-19-1	1.1 a	78-20-1	1.9 a	76- 2-40	6.0 b
		78-21-7	1.7 a	78-25-7	1.4 a
78-34-1	0.6 a	78-45-12	1.8 b	78-20-1	0.6 a
		78-41-1	0.3 a		
78-38-9	3.8 a	78-45-12	21.0 b	78-20-1	24.3 b
				78-41-1	10.3 a
78-41-2	7.7 b	78-45-12	9.5 b	78-20-7	2.3 a
		78-35-9	3.4 a		

Table 2. Seed set from self pollination and incompatible-cross pollination of the *Nemesia strumosa* family 77–12. Males used in the incompatible cross were from family 78–37, a low PSC family with no PSC ancestors and unrelated to 77–12

Plant	Mean self seed set	Mean incompatible cross seed set	Difference	Significance
77–12–39	1.6	14.2	12.6	ns
77–12–40	2.0	1.0	1.0	ns
77–12–41	1.3	0.9	0.4	ns
77–12–42	1.2	0.1	1.1	ns

linator. Seed set from the intermediate- and no-PMPSC plants 78–16–9 and 78–23–4, respectively, were quite similar. The data in Table 4 provides evidence that level of self PSC is not a good predictor of PMPSC. Both the high and intermediate PMPSC plants have similar self PSC levels, the high PMPSC plants ranging from 0.0 to 36.3% and the intermediate from 0.0 to 28.4%. Even among the low PMPSC plants, one plant had a self PSC level of nearly 17%.

Generally, members of the same family show similar patterns of DS and PMPSC response, such that siblings with DS permit growth of pollen tubes from the same

Table 3. Seed set from self and incompatible-cross pollination of the *Nemesia strumosa* family 77–12. Self PSC levels of the males used in the incompatible crosses are given. Different letters following seed sets on the same plant indicate significant differences at the 5% level

DS plant	Mean self seed set	Related male	Self PSC (%)	Incompatible cross seed set	Unrelated male	Self PSC (%)	Incompatible cross seed set
77–12–53	0.1 a	79–6–3	60.7	17.5 b	78–20–1	0.0	26.0 b
77–12–54	0.6 a	79–6–4	69.9	14.6 b	78–20–1	0.0	11.2 b
77–12–58	0.0 a	79–6–5	24.9	2.2 b	78–20–1	0.0	28.8 c
77–12–59	2.1 a	79–6–1	75.2	17.7 b	78–20–1	0.0	29.2 c
77–12–55	7.9 a	79–4–2	5.4	18.1 b	78–21–8	1.7	19.9 c
77–12–56	1.3 b	79–4–3	6.3	0.0 a	78–21–8	1.7	11.7 c
77–12–57	3.8 a	79–4–5	70.1	14.5 b	78–21–8	1.7	11.5 b
77–12–60	1.9 a	79–4–1	18.6	1.1 a	78–21–8	1.7	21.6 b

Table 4. PMPSC expression of pollinators with varying self PSC levels and PSC ancestry, on plants with discriminating styles

Pollinator	S alleles	Ancestry rating	Mean self PSC (%)	PMPSC expressed	PMPSC not expressed
76– 2–40	3.3	2	10.5	12	0
78–16–8	2.3	2	7.8	4	0
78–20–1	2.2	2	0.0	7	1
78–21–8	3.3	2	1.7	7	0
78–45–12	2.2	2	1.5	6	0
78–49–8	6.6	2	36.3	5	0
78–16–9	2.3	2	0.0	1	3
78–21–7	3.3	2	26.2	3	2
78–39–2	3.3	2	28.4	5	3
78–47–7	3.3	3	3.0	3	2
78–49–13	3.3	2	18.5	2	2
78–22–11	1.1	4	16.8	0	4
78–23–4	2.3	4	2.9	0	4
78–30–5	3.3	4	2.0	0	9
78–39–1	3.3	2	2.4	0	4

types of comparisons can be made with the data in Table 6. The high PMPSC plant 76–2–40 again either equaled or exceeded the seed set from crosses with the intermediate PMPSC plants 78–47–7, 78–49–13, and 78–21–7. Again, in Table 7, seed set was highest when the high PMPSC plant 78–16–8 was used as the pol-

pollinators and that sibling pollinators can grow on the same DS plants. However, some differences do occur. These appear to the associated with the level of self PSC. Table 8 lists the number of similar and different DS responses of several sibling pairs. The few cases in which siblings differed in response can be attributed to

Table 5. Self, incompatible-cross and compatible-cross seed sets of *Nemesia strumosa* plants with differing levels of DS and PMPSC. Different letters following seed sets for a female indicate significant seed set differences at the 5% level. Self PSC of male is in parenthesis beneath plant number

Female	Self	Male					Compatible
		76–2–40 (10.5%)	78–30–5 (2.0%)	78–36–18 (14.5%)	78–39–1 (2.4%)	78–39–2 (28.2%)	
77–12–39	4.1 a	33.7 c	3.7 a	–	–	21.0 b	49.2
78–23–8	0.1 a	21.1 c	0.0 a	–	0.9 a	5.8 b	53.1
78–29–6	0.2 a	13.8 c	0.0 a	0.3 a	0.2 a	2.0 b	42.2
78–31–1	0.3 a	0.1 a	0.0 a	0.5 a	0.0 a	0.0 a	46.3
78–31–13	17.1 b	14.7 b	2.1 a	2.2 a	2.0 a	2.6 a	39.9
78–31–25	1.6 a	21.8 b	0.5 a	0.2 a	0.3 a	1.4 a	50.2
Mean	3.9	17.5	1.0	0.8	0.7	5.5	

Table 6. Self, incompatible-cross and compatible-cross seed sets of *Nemesia strumosa* plants with differing levels of DS. Pollinators 76–2–40, 78–47–7 and 78–49–13 are closely related. Different letters following seed sets for a female indicate significant seed set differences at the 5% level. Self PSC of male is in parenthesis beneath plant number

Female	Self	Male				Compatible
		76–2–40 (10.5%)	78–21–7 (26.2%)	78–47–7 (3.0%)	78–49–13 (18.5%)	
78–21–8	0.7 a	2.8 a	0.6 a	0.9 a	5.2 a	41.4
78–21–11	4.5 a b	13.8 c	3.3 a	10.0 b c	11.8 c	34.2
78–31–14	0.2 a	6.7 b	0.6 a	4.6 b	2.8 b	53.3
78–39–1	1.1 a	7.6 b	8.0 b	1.5 a	0.8 a	45.0
78–39–2	11.5 a	36.6 b	32.6 b	17.5 a	11.3 a	40.8
Mean	3.6	13.5	9.0	6.9	6.4	

Table 7. Mean self, incompatible-cross and compatible-cross seed sets of *Nemesia strumosa* plants with differing levels of DS. Different letters following seed sets on a given female indicate significant differences at the 5% level. Self PSC of male is in parenthesis beneath plant number

Female	Self	Male					Compatible
		78–16–8 (7.8%)	78–16–9 (0.0%)	78–23–4 (2.9%)	78–38–6 (15.4%)	78–45–3[a] (68.9%)	
78–16–15	2.4 a	1.8 a	2.9 a	0.8 a	2.5 a	2.3 a	74.9
78–38–18	14.9 a	25.8 b	16.4 a	14.1 a	16.0 a	25.6 b	26.7
78–39–17	0.6 a	9.9 b	2.4 a	0.5 a	4.4 a	4.4 a	35.7
78–45–2	39.6 b	40.2 b	34.3 a b	24.2 a	32.2 a b	37.9 b	43.9
78–45–6	5.8 a b	13.6 c	4.6 a b	1.8 a	2.8 a b	9.1 b c	50.5
Mean	12.7	18.3	12.1	8.3	11.6	15.9	

[a] 78–45–3 had a lower level of male fertility than the other pollinators with which it is compared

differences in level, not type, of discriminating capacity. The low self PSC plants 78–31–1 and 78–21–8 had non-discriminating styles. A comparison of the self and incompatible cross seed sets of the 78–31 siblings is presented in Table 5, the 78–21 and 78–39 siblings in Table 6, the 78–45 siblings in Table 7 and the 78–35 siblings in Table 9.

Results from comparisons of PMPSC responses in siblings are summarized in Table 10. Sibling pairs from families 78–16, 78–39, and 78–49 demonstrated similar PMPSC responses on some DS plants and different responses on others. In all 3 families, the sibling with the higher self PSC level had the higher PMPSC level when differences occurred. The seed set data for siblings

Table 8. Number of similar and dissimilar seed set responses of *Nemesia strumosa* sibling pairs when incompatibly crossed as seed plants. When siblings differed in DS response, the plant with the higher level of discrimination is marked with an asterisk

Seed plants				No. of similar DS responses	No. of differ. DS responses
Sibling	Self PSC (%)	Sibling	Self PSC (%)		
78-10-6	0.7	78-10-9	4.7	3	0
78-11-2	0.0	78-11-5	0.8	3	0
78-21-8	1.7	78-21-11*	13.3	1	3
78-31-1	0.6	78-31-13*	42.8	4	1
78-31-1	0.6	78-31-25*	3.2	4	1
78-31-25	3.2	78-31-13	42.8	5	0
78-35-38	39.7	78-35-9	59.4	3	0
78-39-1	2.5	78-39-2	29.2	5	0
78-45-6	11.6	78-45-2	90.2	5	0

Table 9. Mean seed set from a diallel of *S* 2.2 *Nemesia strumosa* plants. Different letters following seed sets within a female indicate significant differences at the 5% level. Self PSC of male is in parenthesis beneath plant number

Female	Male				Compatible
	78-20-7 (0.3%)	78-35-9 (19.5%)	78-35-38 (58.8%)	78-45-12 (1.5%)	
78-20-7	1.2 a	0.6 a	0.5 a	3.8 a	39.5
78-35-9	19.4 b c	14.4 a b	8.2 a	21.4 c	24.2
78-35-38	29.1 b	11.2 a	12.8 a	26.1 b	32.2
78-45-12	0.1 a	0.2 a	0.1 a	0.8 a	52.8

Table 10. Number of similar and dissimilar PMPSC responses of sibling pollen plants crossed onto plants with DS. When plants differed in PMPSC, the plant with the higher PMPSC is indicated with an asterisk

Pollen plants				No. of similar PMPSC responses	No. of differ. PMPSC responses
Sibling	Self PSC (%)	Sibling	Self PSC (%)		
76-2-25	0.0	76-2-38	24.0	1	0
78-2-6	0.0	78-2-9	0.0	1	0
78-16-9	0.0	78-16-8*	7.8	2	3
78-35-9	19.5	78-35-38	58.8	3	0
78-38-4	0.4	78-38-3	1.4	1	0
78-39-1	2.4	78-39-2*	28.2	3	2
78-39-6	0.2	78-39-2	28.2	1	0
78-49-3	6.1	78-49-8*	36.3	2	3

78-39-1 and 78-39-2 are given in Table 5, 78-16-8 and 78-16-9 in Table 7 and 78-35-9 and 78-35-38 in Table 9.

Seed set from self pollination is usually equal to that from incompatible sib pollination. The mean self seed set on 78-21-11 was 4.5, not significantly different from the mean of 3.3 seeds which resulted from sib pollination with 78-21-7 (Table 6). Similarly, no significant differences in seed set occurred from self or sib pollination of 78-16-15 (Table 7), 78-45-2 and 78-45-6 (Table 7), and 78-35-9 and 78-35-38 (Table 9).

Four diallels, representative of the 11 performed, are presented in Tables 5, 6, 7, and 9. Patterns of seed set from diallel crosses among related and unrelated plants were remarkably similar. Though differences among families occurred, the differences could be attributed to the level of discriminating ability and PMPSC, not the type, indicating that the interaction between DS and PMPSC pollinators is of a general rather than a specific nature. In other words, a group of pollinators crossed onto a group of discriminating styles can be ranked in order of capacity to grow on the DS, and this order of ranking will remain the same for all DS. For example, in Table 7, 78–16–8 ranks first, 78–45–3 ranks second, 78–16–9 and 78–38–6 ranks third and 78–23–4 ranks fourth. These rankings reflect increasing levels of PMPSC. Again, the level of self PSC has little relationship to the PMPSC level. The female plants can also be ranked according to sensitivity of discrimination. In Table 7, plant 78–45–6 has the greatest discriminating ability, followed by 78–45–2, 78–38–18, and 78–39–17, with 78–16–15 being non-discriminating. Only one case has been found in which the order of ranking of pollen plants was not the same on all DS, that is, the pollen-style interaction was of a specific rather than a general nature. The pollen plant 78–21–7 in Table 6 ranked higher than 78–47–7 and 78–49–13 on the female 78–39 plants, but lower on 78–21–11. Since 78–21–7 and 78–21–11 are siblings, and sibling intercrosses tend to match the self seed set, this "sibling effect" appears to override the effect of level of PMPSC.

Results in Table 6 show that the high PMPSC of family 76–2 was weakened in the 2 descendents of family 76–2 tested. Both 78–47–7 and 78–49–13 failed to demonstrate PMPSC on the DS of 78–39, but did express PMPSC equal to that of 76–2–40 on the other 3 families tested.

Discussion

The DS-PMPSC interaction is most likely the same phenomenon as the sporophytic recognition behavior reported by Henny and Ascher (1977). Sporophytic recognition, which results in lower incompatible cross seed set than self seed set, may be explained by the low PMPSC of the plants used in the incompatible cross pollinations. These low PMPSC pollinators were low in self PSC and probably had low PSC ancestors.

DS and PMPSC vary in level of expression and DS does not appear to be simply inherited (Robacker and Ascher 1981), suggesting that both traits may be under multigenic control. PSC genes have been generally viewed as a multitude of genes with small effects which weaken the self-incompatibility reaction. Since the effect of the DS-PMPSC interaction is to weaken the incompatibility reaction, PMPSC genes and DS genes are, by definition, PSC genes. This idea is supported by the relationship of self PSC expression to both PMPSC and DS.

1) Relative level of PMPSC among siblings is correlated to level of self PSC (Table 10).

2) Plants with low self PSC and no PSC evident in ancestors fail to express PMPSC (Table 4; Henny and Ascher 1977).

3) All plants with self PSC levels greater than 10% have DS (Robacker and Ascher 1981).

Level of PMPSC, however, is independent of DS sensitivity and self PSC. Plants with high DS may be self incompatible and lack PMPSC when incompatibly crossed to other plants with DS (see 78–39–1 in Tables 4 and 6). Similarly, highly PMPSC plants may be self incompatible and lack DS when crossed with other high PMPSC plants (see 78–21–8 in Tables 4 and 6). Therefore, the genes which produce PMPSC are different than those which govern DS and both sets may be different than those maximizing expression of self PSC.

Additional argument for considering the DS-PMPSC interaction and self PSC separate but related phenomena comes from the following two observations. First, if PMPSC is a measure of pollen tube inability to perceive or react to incompatibility and DS measures stylar inability to mediate the incompatibility reaction, then a plant which has both PMPSC and DS should have high self PSC and usually does. However, such a plant may be self incompatible as was plant 78–21–11 (Tables 4, 6) which had DS and probably high PMPSC (since its siblings all expressed high PMPSC). Second, because DS implies gamete selection, progenies from incompatible crosses between highly DS and highly PMPSC plants should combine the genes for maximum breakdown of both pistil and pollen tube portions of the self-incompatibility reaction and, therefore, express elevated levels of self PSC. However such progenies reflected the self PSC levels of the parents and were frequently fully self incompatible (Robacker and Ascher 1981).

Genes conferring DS and PMPSC as well as those directly responsible for self PSC may be remnants from the development of the self-incompatibility system. Theoretically, as self incompatibility evolved, genes ever more efficient in enhancing this system were favored (Mather 1943; Bateman 1952). But less efficient genes, namely PSC genes, were not entirely eliminated. On the other hand, however, these genes may simply represent mutation-induced variation in the multigenic complex supporting the self-incompatibility reaction, which, when selection favors inbreeding, recombine to produce PSC. In any case, the literature is replete with reports of PSC in self-incompatible species. For example, Henny and Ascher (1976) demonstrated the presence of PSC in a self-incompatible population of *Nemesia strumosa* by obtaining, in only 3 generations of inbreeding and selection, plants capable of setting 100% of compatible-cross seed set after self pollination. Interestingly, both DS and PMPSC were present in these plants (Henny and Ascher 1977), although PMPSC was low.

The most highly DS plants observed in both *N. strumosa* (Robacker and Ascher 1981) and *Petunia hybrida* (Flaschenriem and Ascher 1979) appeared in self-incompatible, inbred lines. Yet high PMPSC in these species is limited to plants of intermediate or high PSC or those with intermediate or high PSC ancestry. These data suggest that development of DS preceeds development of PMPSC in the evolution of high self PSC. DS and PMPSC probably occur in other species expressing PSC and may account for some of the unusual observations reported following inbreeding of these plants (Denward 1963; Pandey 1970; Nettancourt et al. 1971).

Additional study of the DS-PMPSC phenomenon should provide useful information concerning the genetic basis for conversion of a self-incompatible to a self-compatible species through PSC. Further, valuable information about the mechanism of self incompatibility can be obtained from investigating the gradual destruction of the system.

Literature

Bateman, A.J. (1952): Self-incompatibility systems in angiosperms. I. Theory. Heredity **6**, 285–310

Denward, T. (1963): The function of the incompatibility alleles in red clover (*Trifolium pratense* L.). III. Changes in the *S* specificity. Hereditas **49**, 285–329

Flaschenriem, D.R.; Ascher, P.D. (1979): Pollen tube expression of pseudo-self-compatibility (PSC) in *Petunia hybrida.* Theor. Appl. Genet. **54**, 97–101

Henny, R.J.; Ascher, P.D. (1976): The inheritance of pseudo-self-compatibility (PSC) in *Nemesia strumosa* Benth. Theor. Appl. Genet. **48**, 185–195

Henny, R.J.; Ascher, P.D. (1977): Sporophytic recognition of pollen *S* alleles in the gametophytic self-incompatibility system of *Nemesia strumosa* Benth. Theor. Appl. Genet. **49**, 15–19

Hodgkin, T. (1977): The role of pollen and stigma in determining the level of partial self-compatibility in Brussels sprouts. Euphytica **26**, 401–408

Mather, K. (1943): Specific differences in *Petunia.* I: Incompatibility. J. Genet. **45**, 215–235

Nettancourt, D.de.; Ecochard, R.; Perquin, M.D.G.; Drift, T. van der.; Westerhof, M. (1971): The generation of new *S* alleles at the incompatibility locus of *Lycopersicum peruvianum* Mill. Theor. Appl. Genet. **41**, 120–129

Pandey, K.K. (1970): New self-incompatibility alleles produced through inbreeding. Nature **227**, 689–690

Robacker, C.D.; Ascher, P.D. (1981): Discriminating styles (DS) and pollen-mediated pseudo-self-compatibility (PMPSC) in *Nemesia strumosa* Benth. I: Characteristics and inheritance of DS. Theor. Appl. Genet. **60**, 297–302

Steel, G.D.; Torrie, J.G. (1960): Principles and procedures of statistics. New York, Toronto, London: McGraw Hill.

Received May 22, 1981
Communicated by H. F. Linskens

Dr. C. D. Robacker
Department of Plant and Soil Science
Texas Tech. University
Lubbock. Tex. 79409 (USA)

Prof. Dr. P. D. Ascher
Department of Horticultural and Landscape Architecture
305 Alderman Hall
1970 Folwell Avenue
St. Paul, Minn. 55108 (USA)