Theor. Appl. Genet. 53, 135-141 (1978)

Restoration of Pseudo-self-compatibility (PSC) in Derivatives of a High-PSC x No-PSC Cross in *Nemesia strumosa* Benth*

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

Summary. Mean PSC increased following each generation of recurrent selection in F_1, F_2 and F_3 *Nemesia strumosa* families derived from a cross of a 100% PSC plant to an unrelated 0% PSC plant. The first 100% PSC individuals occurred in the F_4. Populations derived through sib pollination tended to have higher PSC means than lines derived through self pollination. One F_3 family showed a three-fold higher PSC level when pollinated in the greenhouse than when pollinated in the growth chamber, while another F_3 family similarly pollinated showed no change in PSC.

Key Words: Self incompatibility – Pseudo-self-compatibility – Inbreeding – Selection

Introduction

Self incompatibility acts as a physiological barrier to inbreeding by preventing self fertilization even though the plant has functional gametes. In the gametophytic system, incompatibility is controlled by a series of *S* alleles. When the *S* allele in the pollen matches the *S* alleles in the style, the pollen tube fails to reach the ovary. Various disruptions of the self-incompatibility reaction may occur, however, resulting in seed set from incompatible pollinations; these disruptions have been termed pseudo-self-compatibility (PSC). A common type of PSC, caused by multigenic modifiers and influenced by environment, produces a wide range in ability to produce self seed, from very few seeds in some plants to many seeds in others.

Several researchers have studied the genetics of PSC. Atwood (1942) and Leffel (1963) found no relationship between particular *S* alleles and level of PSC in *Trifolium*. Inbreeding with selection to obtain plants consistently high in PSC has often been unsuccessful (Ockendon 1973; Duncan et al. 1973). Townsend (1965), in studies of the inheritance of PSC in tetraploid *Trifolium hybridum*, found that the mean PSC level of a progeny was somewhat similar to that of the parent plant, although a wide range in PSC occurred. After selfing for three generations, the overall level of PSC was high. Similarly, Takahashi (1973) selfed *Petunia hybrida* plants of various PSC levels and although the progeny obtained showed a wide variability in PSC, many were similar to the parent. Crossing a highly compatible with a highly incompatible plant yielded an F_1 progeny with a PSC level slightly lower than that of progeny obtained by selfing the highly compatible plant. Nasrallah and Wallace (1968) inbred lines of cabbage for 10 years and found plants which bred true for high and low PSC. A cross of a high PSC plant as female with a low PSC plant gave an F_1 progeny with high PSC, while the reciprocal cross gave an F_1 with low PSC. This suggests a maternal effect. However, the F_1 from the cross with the high PSC plant as female set more seed when backcrossed with pollen from the high PSC parent than with pollen from the low PSC parent, suggesting a pollen effect.

Nemesia strumosa Benth., a member of the Scrophulariaceae, has the gametophytic incompatibility system (Riley 1933). Henny and Ascher (1976) found low levels of PSC in selected populations of *Nemesia* and, through inbreeding and selection, increased mean PSC from 2.0% in an F_1 to 70.1% in the F_4. Since the PSC increased from very low to very high in so few generations, Henny and Ascher suggested that a relatively small number of modifying genes were involved. Intercrossing *Nemesia* plants high in PSC produced a progeny segregating both high and intermediate level PSC plants. This broad progeny distribution is similar to that observed by Townsend (1965) and Takahashi (1973). In contrast, a high crossed with a

* Scientific Journal Series Paper Number 10,356 of the Minnesota Agricultural Experiment Station

0040/5752/78/0053/0135/$ 1.40

low gave a progeny of mostly low PSC plants, indicating that PSC in *Nemesia* is phenotypically recessive. Takahashi's *Petunia* data show somewhat the same recessive nature, although not so great as in *Nemesia*. The style determines the level of PSC: pollen from *Nemesia* plants of any PSC level produce a strong self-incompatibility reaction on a self-incompatible style.

All of the plants used by Henny and Ascher were originally derived from three plants. Because of the close relationship of the descendants of these plants, all might be expected to carry recessive modifiers for the self-incompatibility reaction and crosses among them might give especially high PSC progeny. To determine whether these genes could be transferred to an unrelated incompatible line, a 100% PSC individual was crossed to a 0% PSC plant from a population which lacked PSC. This 0% PSC population was obtained from a commercial source. The resulting F_1 had a mean PSC level of 0.9%, which was lower than any of the F_1 progenies of similar crosses among the related plants. This raised the question of whether high PSC plants could be obtained through inbreeding this F_1 progeny. Our purpose, therefore, was to select and inbreed PSC plants in an attempt to increase PSC to levels comparable to those of the original material.

Materials and Methods

Nemesia strumosa seeds were placed in petri dishes lined with filter paper saturated with 100 ppm gibberellic acid. After soaking for 24 to 48 hours, the seeds were planted in sphagnum moss and placed under mist until germination. When the second or third set of true leaves appeared, the seedlings were transplanted to a 1:1:1 peat:perlite:soil medium. Each seedling received a three-number code: the first two numbers indicating the family and the last number the seedling.

Pollinations were performed the day of anthesis. In order to average environmental effects, no more than two pollinations of each self or cross were done on the same day. Flowers to be cross pollinated were emasculated the day before anthesis to prevent contamination by self pollen. All unused flowers were removed each day to avoid contamination from insect pollination and to insure uniform floral age at pollination. Routine checks for pollen fertility were made. Maximum seed set for each plant was determined by using the plant as female in a compatible cross. Mean self and cross seed sets were based on at least nine and four pollinations, respectively. To calculate the % PSC for each plant, the mean self seed set was divided by the mean compatible cross seed set and these values were plotted in a frequency histogram.

Progeny B-1, produced by crossing the 100% PSC plant 73-20-34 with the 0% PSC plant B74-1-2, was the source of our plants (Fig. 1). Self or sib pollinations of individuals from B-1 gave rise to three F_2 families, 75-3, 76-1 and 75-2. The highest PSC plant in each of these F_2 families was selfed to yield the three F_3 families 76-2, 76-4 and 76-3. To produce the F_4, high PSC plants of families 76-2 and 76-3 were selfed and sibbed. This gave rise to the self progenies 77-5 and 77-3 and the sib progenies 77-6 and 77-4.

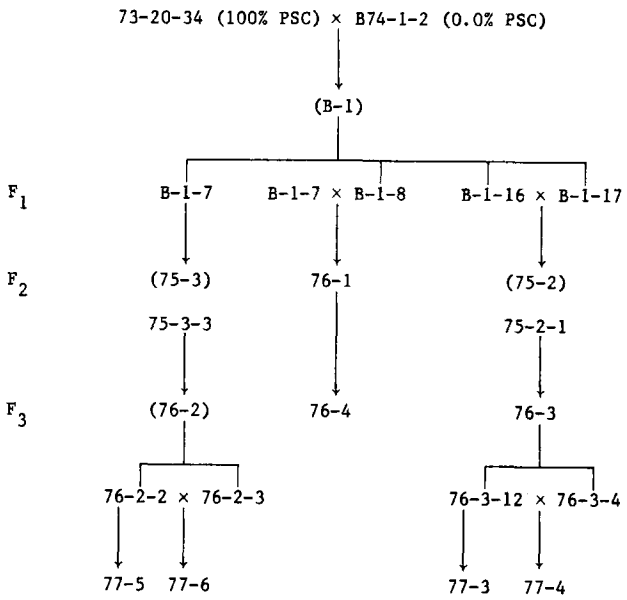

Fig. 1. A pedigree diagram of the plants used to study the inheritance of PSC in *Nemesia strumosa*

In a greenhouse maintained at about 13°C nights and 19°C days, the F_2 families 75-2 and 75-3 were grown and pollinated in the winter while 76-1 was pollinated in the same greenhouse in the spring. The F_3 progenies 76-2 and 76-3 were raised in the summer. Because high temperatures may affect self seed set (Campbell and Ascher 1972) and cause poor growth of *Nemesia*, the F_3 plants were placed under fluorescent lights in an air-conditioned laboratory. When near flowering, the plants were transferred to and pollinated in a growth chamber held at 16°C nights and 19°C days. To make the environmental conditions in which 76-2 and 76-3 were pollinated more similar to the environment of the F_2 pollinations, the plants were relocated into the greenhouse in the fall and the pollinations were repeated. The following winter the other F_3 family, 76-4, was pollinated and the F_4 families 77-3, 77-4, 77-5 and 77-6 were grown in the greenhouse at temperatures approximating 13°C nights and 20°C days. Populations 77-3, 77-4, 77-5 and 77-6 were pollinated in late spring when day and night temperatures ranged from 21 to 31°C and 12 to 20°C, respectively.

Results

Mean PSC increased gradually from the F_1 generation B-1 to the F_3 generation 76-2. Progeny B-1 had an overall mean PSC of 0.9% (Henny and Ascher 1976). Self pollination of B-1-7 produced the F_2 progeny 75-3 with a mean PSC level of 6.8% and a range extending from 0% to 23.9% (Fig. 2). In the F_3 family 76-2, the mean PSC of the plants when pollinated in the growth chamber was 9.3%, only a small increase over the F_2. The range extended from 0.0% to only 20.6% (Fig. 2). Seed set from compatible pollinations remained high although many of the plants were partially male sterile. However, when family 76-2 was pollinated in the greenhouse, the mean PSC

increased to 23.1% while the range broadened to 0.5% to 77.4% (Fig. 2). All plants except 76-2-7 increased their % PSC about three-fold in the greenhouse and therefore maintained the same relative level of PSC (Table 1). Mean compatible seed set was higher in the growth chamber than in the greenhouse (Table 2) while incompatible was lower. These two factors combined to cause the large difference in PSC observed. Many of these greenhouse pollinations were done on cuttings taken from the plants when seedlings. The cuttings were more vigorous than the stock plants, many of which became fasciated in the greenhouse. Male sterility was not a problem in the greenhouse.

Self pollination of 76-2-2 gave the F_4 progeny 77-5. Mean PSC in the F_4 increased to only 30.5%, with a range of 1.2 to 87.0% (Fig. 3). Again, the anthers released pollen only sporadically. Progeny 77-6, the F_4 from sib pollination, had a mean PSC of 37.7%, similar to the mean PSC of 77-5. The PSC range narrowed, however, to 24% to 53.3% (Fig. 3). This narrow range may be due to small sample size. More pollen was available in this population than in 77-5.

Through sib pollination within B-1, two F_2 families were produced. One of these, 75-2, had a mean PSC level of 2.3%. Four of the six seedlings were 0% PSC; the highest had a PSC level of 13.5% (Fig. 4). This high PSC plant was self pollinated to obtain the F_3 family 76-3. In the growth chamber the mean PSC for 76-3 was 11.3%, varying from 0.0% to 40.8% (Fig. 4). About half the plants were 0.0% PSC. Inbreeding depression was evident in progeny 76-3. Many of the seed pods from compatible pollinations swelled normally but on ripening contained small, aborted ovules, suggesting female sterility. However, 76-3 produced abundant pollen. When family 76-3 was pollinated in the greenhouse, the mean PSC was 13.8% and the

Table 1. The % PSC of the F_3 *Nemesia strumosa* progenies 76-2 and 76-3 as determined from pollinations in the growth chamber and in the greenhouse

Seedling number	% PSC in growth chamber	% PSC in greenhouse
76-2-1	4.1	13.4
76-2-2	20.6	77.4
76-2-3	12.7	37.3
76-2-4	0.7	4.7
76-2-5	9.2	20.9
76-2-7	17.8	7.5
76-2-8	0.0	0.5
76-3-2	0.0	0.0
76-3-3	2.4	3.2
76-3-4	37.7	33.3
76-3-5	9.2	5.6
76-3-7	0.0	0.0
76-3-8	0.0	1.9
76-3-9	0.0	0.0
76-3-10	11.9	24.7
76-3-12	40.8	55.4

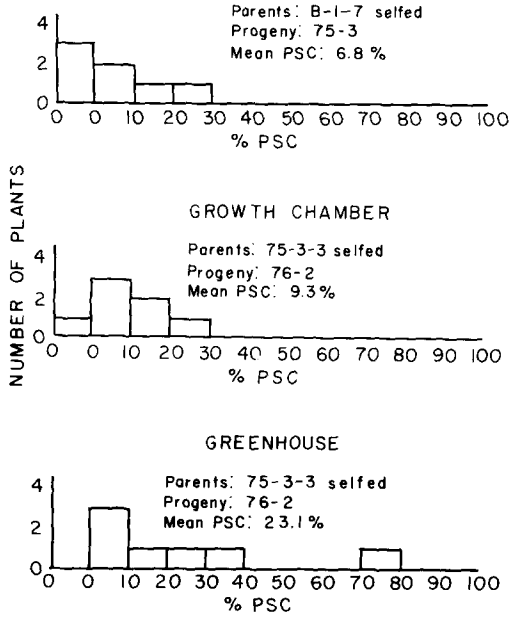

Fig. 2. Distribution of % PSC in *Nemesia strumosa* in the greenhouse for the F_2 progeny 75-3 and the F_3 progeny 76-2, and in the growth chamber for the F_3 progeny 76-2

Table 2. Mean self and compatible cross seed sets and % PSC of *Nemesia strumosa* progenies 76-2 and 76-3 in the growth chamber and in the greenhouse

Progeny code	Location	Mean self seed set	Mean compatible cross seed set	% PSC[a]
76-2	growth chamber	4.9	55.9	9.3
76-2	greenhouse	9.6	47.6	23.1
76-3	growth chamber	4.2	33.3	11.1
76-3	greenhouse	4.8	35.2	13.8

[a] % PSC was calculated by averaging the % PSC of all individuals in a progeny rather than by dividing the figures in the mean self seed set column by those in the mean compatible cross seed set column

range was 0.0% to 55.4% (Fig. 4) which is similar to the PSC observed in the growth chamber. A comparison of the % PSC in the growth chamber and in the greenhouse is shown in Table 1. Some plants slightly increased or decreased their PSC levels in the greenhouse as compared to the growth chamber but all maintained the same relative degree of PSC. The F_4 generation 77-3, produced through self pollination of the 55.4% PSC plant, increased to 78.4% PSC with a range from 22.4 to 100.0% (Fig. 5). More than 1/3 of the plants had PSC levels over 90%. Sib pollination of the two highest F_3 plants yielded the F_4 family 77-4. With a mean PSC of 92.8% and a range of

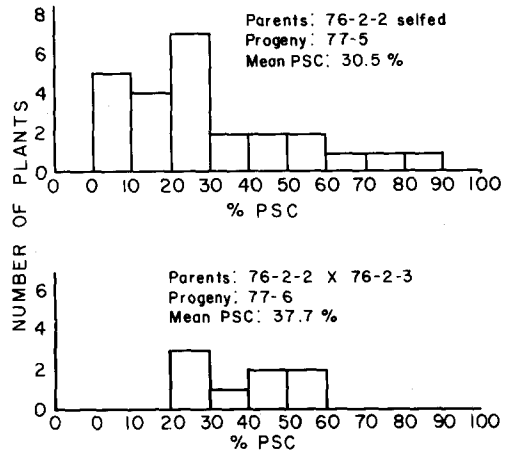

Fig. 3. The frequency distribution of % PSC in the F_4 *Nemesia strumosa* progenies 77-5 and 77-6

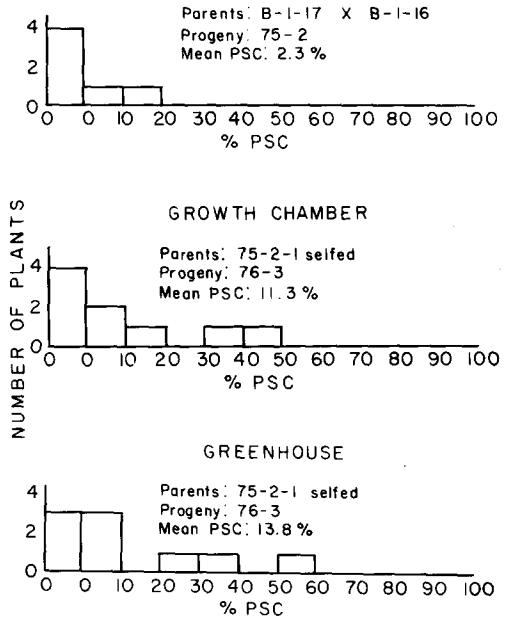

Fig. 4. Distribution of % PSC in *Nemesia strumosa* in the greenhouse for the F_2 progeny 75-2 and the F_3 progeny 76-3, and in the growth chamber for the F_3 progeny 76-3

73.4% to 100.0% PSC (Fig. 5), this population was the highest PSC population obtained. Twenty-two out of 30 plants had PSC levels greater than 90%.

The other F_2 produced through sib pollination of B-1 was 76-1. This F_2 had a mean PSC of 1.1% (Fig. 6). Only

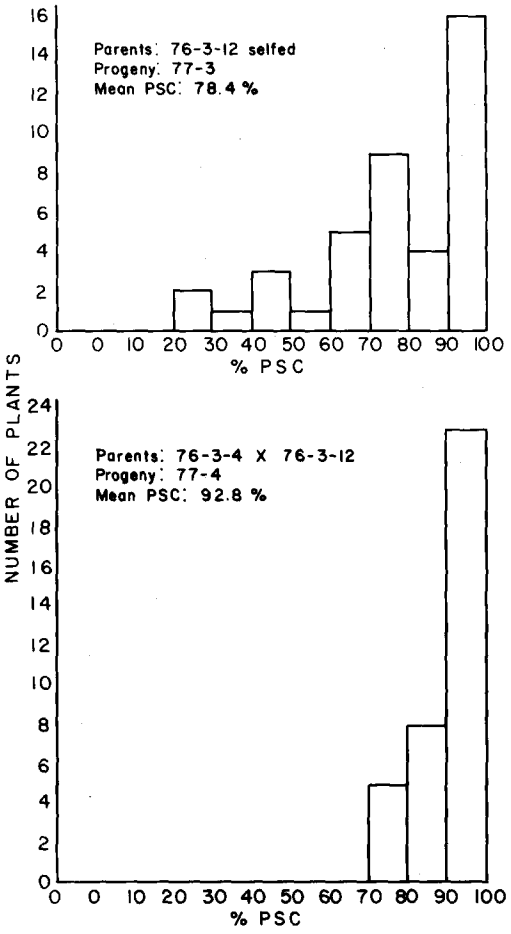

Fig. 5. The frequency distribution of % PSC in the F_4 *Nemesia strumosa* progenies 77-3 and 77-4

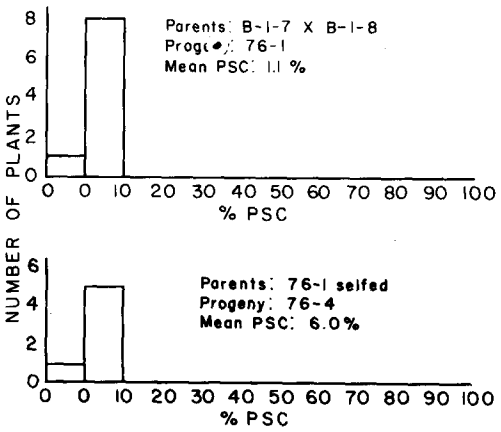

Fig. 6. Distribution of % PSC in the F_2 *Nemesia strumosa* progeny 76-1 and the F_3 progeny 76-4

Table 3. Mean self and compatible cross seed sets and % PSC of the *Nemesia strumosa* parent plants and their F_1, F_2, F_3, and F_4 progenies

Generation	Progeny code	Mean self seed set	Mean compatible cross seed set	% PSC[a]
Parent	73-20-34	41.5	39.9	100.0
Parent	B74-1-2	0.0	61.3	0.0
F_1	B-1	0.8	78.1	0.9
F_2	75-3	3.9	52.6	6.8
F_3	76-2	9.6	47.6	23.1
F_4	77-5	7.1	25.7	30.5
F_4	77-6	13.2	34.5	37.7
F_2	75-2	1.5	56.7	2.3
F_3	76-3	5.3	34.1	13.8
F_4	77-3	23.6	27.6	78.4
F_4	77-4	37.3	36.9	92.8
F_2	76-1	0.9	80.9	1.1
F_3	76-4	2.8	43.2	6.0

[a] % PSC was calculated by averaging the % PSC of all individuals in a progeny rather than by dividing the figures in the mean self seed set column by those in the mean compatible cross seed set column

one plant out of 10 was 0% PSC but the highest PSC plant was only 3.7%. When selfed, this plant yielded the F_3 generation 76-4. Mean PSC increased to 6.0%, again with only one plant out of six 0.0% PSC. The highest PSC plant was 9.3% (Fig. 6).

Table 3 compares self seed set, compatible cross seed set, and % PSC for all the generations discussed above. Note particularly the drop in compatible cross seed set from the F_1 to the F_4 generations because of inbreeding depression. A further indication that this decrease in compatible seed set is due to inbreeding depression is shown in that the F_4 families produced through sib pollination had higher compatible seed sets than the F_4 families produced through self pollination. The increase in % PSC from the F_1 to the F_4 results from a progressive increase in self seed set and a decrease in cross seed set.

Discussion

Transfer of PSC from a 100% PSC line to an unrelated 0.0% PSC line was sucessful. In only four generations of inbreeding and selection a 100% PSC progeny was produced. Basically, the progress made from the F_1 to the F_4 generations of the high PSC family 77-3 is comparable to that observed by Henny and Ascher (1976) in the three generations of inbreeding and selection that produced the high PSC family 73-19. However, unlike the plants used in this study, the parents producing the first generation leading to 73-19 were related. This was a low PSC × low PSC cross and actually resembles the sib cross used to generate the F_2 which led to 77-3.

Comparison of the PSC means and ranges of each of the F_2, F_3 and F_4 families from the high times low cross shows variation among families. Variation among F_2 families is expected if, as is likely, the parent plants of the F_1 were not homozygous. Although the highest PSC plant in each F_2 was selected to produce the F_3, the plants undoubtedly differed in PSC genotype, therefore increasing the variation among lines. The selected F_3 plants demonstrated that dominance or epistasis give phenotypes which mask the potential for producing high PSC offspring as the lower PSC F_3 progeny produced the higher PSC F_4 progeny (Figs. 2, 3, 4 and 5).

The importance of environment on % PSC is demonstrated by the F_3 generations which were pollinated in both the growth chamber and the greenhouse. For population 76-2 in particular, very different PSC levels occurred in the two environments although the highest PSC plants were highest in both environments and the lowest PSC plants were lowest in both environments. Therefore, it may be better to think of each PSC value as a range rather than a fixed number. However, assigning PSC values to plants remains a useful technique for classifying plants so long as the plants compared are pollinated in similar environments.

Determination of the inheritance of PSC, both in terms of the number of genes and the type of gene action, is complicated by the effect environment has on expression of PSC. Environment blurs the effects of individual genes,

producing the same continuous distribution as occurs with a large number of genes. Evidence such as response to selection suggests, however, that PSC is controlled by a small number of genes with rather high additive genetic variance.

Dominance or epistasis or both also contribute to the inheritance of PSC. A model which explains the behavior of PSC as due to recessive alleles at five or six loci fits some, but not all, of the data. Crossing a 100% PSC plant to an unrelated 0% PSC plant produced an F_1 in which most plants were 0% PSC. This suggests that PSC genes are recessive at most loci. If PSC in *Nemesia* is controlled by a small number of recessive genes (i.e. five or six), some high PSC plants would be expected in the F_2 from this high times low cross. Our highest PSC F_2 segregate had a PSC level of 23.9%, although we tested only 22 F_2 plants. Richards and Thurling (1973) observed PSC levels in F_1, F_2 and backcross progenies derived from a high by low cross of *Brassica campestris*. The *Brassica* F_1 and F_2 progenies had PSC levels similar to those of our F_3 *Nemesia* progenies. They found that no 100% PSC plants occurred in the F_1, F_2 or backcross progenies, although these progenies were not large. Examination of a larger F_2 population or of a larger backcross progeny of an F_1 to the 100% PSC parent may give better information about gene number and gene action. Support of the hypothesis of a small number of recessive genes will be provided if high PSC segregates are found in a large F_2 or backcross progeny. Henny and Ascher (1976) crossed the 100% PSC plant 73-20-34 to the 0% PSC sib, 73-20-33, and obtained several high PSC plants. If 73-20-33 carried recessive PSC genes at many loci, then these high PSC plants are expected. A cross of 73-20-34 to 74-2-4, a low PSC plant with no high PSC relatives, produced no high PSC segregates, suggesting that 74-2-4 carried few recessive PSC genes. These data also fit the recessive gene model.

Support for epistasis may be seen in the rather gradual increase in PSC from the F_1 to the F_2 to the F_3, followed by a large jump to the F_4 in families 77-3 and 77-4. The gradual increase may be due to additive and dominant gene action while the sharp jump to the attainment of a combination of genes producing total disruption of the incompatibility reaction. Furthermore, a 100% PSC plant, according to the recessive gene hypothesis, should carry only recessive PSC genes and therefore produce only high PSC progeny. Although Henny's data (1976) show no very low segregates from self pollination of high PSC plants, intermediate level plants occur. This implies that not all loci in a 100% PSC plant must carry recessive PSC alleles. Perhaps a particular number or combination of loci is required for complete self incompatibility breakdown.

The tendency for higher PSC progenies to occur in lines produced through sib pollination rather than through self pollination also suggests epistatic gene action. In the F_3, some PSC genes were probably fixed in the homozygous state, different genes being fixed in different individuals. Both self and sib pollination will uncover some recessive genes and therefore give rise to increased PSC in later generations but sib pollination will also produce new gene combinations which may lead to even higher PSC progenies. If a large F_2 of a high times low plant yields no high plants, a backcross of the F_1 to the high parent gives no high progeny and a true breeding 100% PSC plant cannot be obtained, an epistatic gene action model would be even more compelling.

PSC, like self incompatibility, is not understood biochemically. Malfunctions at any of many steps of the self-incompatibility reaction may cause PSC. Many genes must exist which code for enzymes and other substances necessary for the functioning of the self-incompatibility system. Minor coding mistakes which reduce the efficiency of a reaction or the quantity of a necessary substance, may weaken the incompatibility reaction and produce the PSC phenotype. Some mistakes may cause more drastic effects on the incompatibility reaction and be more subject to environmental modification than others. Therefore, a particular combination of PSC genes may dramatically affect the PSC phenotype. Genetically, this may appear as epistasis. Genes producing the normal self-incompatibility reaction are expected to be dominant over these PSC genes.

Acknowledgement

We thank Dr. R.J. Henny for providing necessary information and plant material for this research.

Literature

Atwood, S.S.: Genetics of self-compatibility in *Trifolium repens*. J. Amer. Soc. Agr. **34**, 353-364 (1942)

Campbell, R.J.; Ascher, P.D.: High temperature removal of self-incompatibility in *Nemesia strumosa*. Incompatibility Newsletter **1**, 3-5 (1972)

Duncan, J.F.; Anderson, M.K.; Taylor, N.L.: Effect of inbreeding on pseudo-self-compatibility in red clover (*Trifolium pratense* L.). Euphytica **22**, 535-542 (1973)

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

Leffel, R.C.: Pseudo-self-compatibility and the segregation of gametophytic self-incompatibility alleles in red clover, *Trifolium pratense* L. Crop Sci. **3**, 377-380 (1963)

Nasrallah, M.E.; Wallace, D.H.: The influence of modifier genes on the intensity and stability of self-incompatibility in cabbage. Euphytica **17**, 495-503 (1968)

Ockendon, D.J.: Selection for self-incompatibility in inbred lines of brussel sprouts. Euphytica **22**, 503-509 (1973)

Richards, R.A.; Thurling, N.: The genetics of self-incompatibility in *Brassica campestris* L. ssp. *oleifera* Metzg. I. Characteristics of *S*-locus. Control of self-incompatibility. Genetica **44**, 428-438 (1973)

Riley, H.P.: Self-sterility in *Nemesia*. Science 77, 326-327 (1933)

Takahashi, H.: Genetical and physiological analysis of pseudo-self-compatibility in *Petunia hybrida*. Jap. J. Genet. **48** (1), 27-33 (1973)

Townsend, C.E.: Self-compatibility studies with tetraploid alsike clover, *Trifolium hybridum* L. Crop Sci. **5**, 295-29 (1965)

Received June 21, 1978
Communicated by H.F. Linskens

Dr. Carol D. Robacker
Prof. Dr. Peter D. Ascher
Department of Horticultural Science
and Landscape Architecture,
University of Minnesota
Saint Paul (USA)