Theor. Appl. Genet. 49 (1977) 15-19

THEORETICAL AND APPLIED GENETICS

Sporophytic Recognition of Pollen *S* Alleles in the Gametophytic Self-Incompatibility System of *Nemesia strumosa* Benth.[1]

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Summary. Several seedlings of *Nemesia strumosa* with various levels of pseudo-self-compatibility (PSC) often produced more seed after self pollination than when pollinated using pollen from incompatible plants bearing the same S alleles. Sporophytic recognition of self pollen apparently increases PSC levels above those attributable to modifying genes which interfere with normal stylar activity.

Introduction

Self incompatibility promotes genetic diversity in plants by preventing self fertilization even though an individual contains functional ovules and pollen. Gametophytic and sporophytic self incompatibility are two types of incompatibility systems existing in homomorphic flowering angiosperms. Both systems depend on a series of alleles at the S locus to control the incompatibility reaction. In the gametophytic self-incompatibility system (East and Mangelsdorf 1925), the particular S allele in the pollen grain determines specificity. Matching specificity between the S allele in the haploid pollen tube with either of the two in the diploid style inhibits normal pollen tube growth. Slow growing pollen tubes rarely traverse over half the total stylar length in the time needed to insure fertilization. In the sporophytic system (Hughes and Babcock 1950; Gerstel 1950), pollen specificity is determined by the S genotype of the diploid pollen producing parent, regardless of the individual S allele carried in the pollen grain. Matching identity between either or both of the two pollen specificities with either or both of the two stylar specificities results in failure of the pollen tubes to penetrate the stigmatic surface.

Pseudo-self-compatibility (PSC) occurs when plants with a functional self-incompatibility system yield seeds following self pollination. PSC levels are usually low but can reach levels approaching true self compatibility. This inefficiency of the incompatibility mechanism has been attributed to polygenic modifying genes inhibiting S gene activity (Atwood 1942; Mather 1943; Denward 1963; Takahashi 1973). In *Nemesia strumosa*, where the gametophytic system of self incompatibility operates (Riley 1933), we found progenies containing individuals expressing levels of PSC ranging from none or slight to those resembling self compatibility. This phenomenon was a stylar-conditioned effect as plants with high levels of PSC set no seed when crossed as male with tester plants of the same S genotype (Henny and Ascher 1976).

Although pseudo-self-compatible nemesias bore pollen with a functional incompatibility reaction, oftentimes the self seed yield of an individual was higher than the seed set from crosses involving incompatible male plants bearing the same S alleles. If PSC in *Nemesia* was indeed simply stylar conditioned, then self and cross pollination with males of the same S genotype should produce nearly identical seed sets. For example, an $S_{2.3}$ plant seemed less able to prevent fertilization by its own pollen than by pollen from other incompatible $S_{2.2}$, $S_{3.3}$ or $S_{2.3}$ plants. It is as though identical S alleles from separate plants have sporophytic differences which are somehow recognized in the style and serve as the basis for enhancement or reduction of the gametophytic incompatibility reaction. We first noticed the differences between self and tester cross sets while studying PSC in *Nemesia*. The unique nature of the results caused us to investigate them further.

Materials and Methods

Nemesia seedlings were grown under continuous lighting until near the flowering stage in plastic pots containing equal parts peat moss, perlite and soil. A numerical code was given each plant with the first

[1] Scientific Journal Series Paper Number 9546 of the Minnesota Agricultural Experiment Station.

two segments showing the progeny code and the last segment the seedling number; seedling 73-20-28 refers to the 28th plant in progeny 73-20. Some plants (populations 73-1, 73-13 and 73-15) were tested in growth chambers held at 13°C nights and 19°C days with a 16-hr photoperiod while all others were tested during the fall, winter and spring seasons in greenhouses held at 13°C nights. Flowers were emasculated and pollinated on the day of anthesis. Ripe seed pods were harvested and seed made. Most plants were self pollinated 5-7 times or more with tester crosses being made at least twice and often 5-7 times also. Throughout this paper the term tester cross will refer to tester plants, of known S genotype, used as male on individuals which contained the same S alleles. Tester plants, both homozygous and heterozygous for S alleles, were selected for their strong incompatibility reaction. The mean seed set was computed for each type of pollination. Chi-square tests were conducted to test the hypothesis that mean self and tester cross seed sets, from each type of tester used, were equal. Differences at the 95.0% level were considered significant.

Results

A summary of Chi-square test results for each of 14 progenies (Table 1) and a sample of seedlings showing typical differences (Tables 2-4) are given. Chi-square tests showed a significant difference between self and tester cross seed sets in 95 of the 274 plants tested. Among those 95 plants there were 142 comparisons between self and tester cross seed sets which were significantly different. Tester cross seed set was significantly less than self in 137 of those cases.

Of the 95 plants with significant differences, 64 were $S_{2.3}$ seedlings spaced throughout 6 populations (Table 2). Each of the plants had been tested with $S_{2.2}$, $S_{3.3}$ and $S_{2.3}$ testers. Paired comparisons of the $S_{2.2}$ tester with the self seed set (Table 2) showed that in 56 of 64 cases there was a significant difference. Tester crosses with $S_{3.3}$ and $S_{2.3}$ testers were significantly different than selfs 22 and 34 of 64 times respectively. In only 5 instances, once with $S_{2.2}$ and $S_{3.3}$ testers and 3 times with $S_{2.3}$ testers, did the tester cross seed set significantly exceed selfs (Table 2).

In population 73-1, 6 of 11 seedlings produced significantly less seeds when an $S_{3.3}$ tester was used as male, compared to selfs (Table 3). Two seedlings (73-1-1 and 73-1-4) were crossed with one $S_{2.2}$ plant (72-4-3) to give progenies 73-13 and 73-15 respectively. Progeny 73-13 yielded 4 of 9 and progeny 73-15 produced 5 of 11 $S_{2.3}$ seedlings with significantly less seeds from $S_{2.2}$ and $S_{3.3}$ testers than selfs (Table 4). In 2 of 18 comparisons among plants where significant differences were found, one of the two homozygous tester crosses did exceed self seed set (Table 4) but each time the larger tester cross seed sets were not significantly different from the self while the lower seed sets were.

Table 1. A summary of Chi-square test results, in 14 separate progenies, showing the number of $S_{2.2}$, $S_{2.3}$ and $S_{3.3}$ *Nemesia* seedlings with significant differences between their mean self and tester cross seed sets

Population	S Genotype and Number of Plants Tested			Number of Plants with a Significant Difference[a]
	$S_{2.2}$	$S_{2.3}$	$S_{3.3}$	
73-1	-	-	11	6
73-13	-	9	-	4
73-15	-	11	-	5
74-4	6	-	-	2
74-4	-	-	12	1
74-4	-	18	-	7
74-5	-	42	-	23
74-6	-	35	-	5
74-7	-	16	-	0
74-8	-	-	14	2
74-9	-	17	-	14
74-12	-	6	-	0
74-15	-	19	-	8
74-16	-	12	-	2
74-17	-	10	-	6
74-17	19	-	-	2
74-18	-	-	8	1
74-18	-	9	-	7
Totals	25	204	45	95

[a] Significantly different at the 95% level.

One $S_{2.3}$ seedling (73-20-34) averaged 42.6 seeds per selfing. However, seed set with $S_{2.2}$ testers was a significantly less 20.7 seeds while $S_{3.3}$ testers averaged 28.8 seeds which was not significantly different from self set. An $S_{3.3}$ seedling (74-1-5) had a mean of 40.2 seeds per self pollination but a significantly less 12.0 seeds with $S_{3.3}$ testers. One or the other, or both plants were a parent in several of the progenies in this study (progenies 74-4, 74-5, 74-6, 74-8, 74-15, 74-17 and 74-18).

Discussion

The PSC we observed in *Nemesia strumosa* is not solely caused by modifying genes reducing the level of stylar activity in the self-incompatibility reaction.

Table 2. The mean seed sets of *Nemesia* seedlings, from six progenies, which showed significant differences between self and tester cross seed sets (determined by Chi-square tests). All seedlings were $S_{2.3}$ and were tested with $S_{2.2}$, $S_{3.3}$ and $S_{2.3}$ testers

Seedling	Type of Tester (Male)			
	Self	$S_{2.2}$	$S_{3.3}$	$S_{2.3}$
74-4-5	35.0	7.3[a]	21.7	44.3
74-4-11	34.4	24.0	34.0	57.5[b]
74-4-12	37.4	10.5[a]	23.2	18.3[a]
74-4-19	60.7	11.3[a]	48.8	46.7
74-4-33	41.5	19.3[a]	30.7[a]	21.5[a]
74-4-34	41.6	21.8[a]	47.3	54.5
74-4-41	40.0	15.5[a]	49.5	47.0
74-5-6	45.6	17.5[a]	36.8	29.2
74-5-7	33.3	15.5[a]	41.8	17.0[a]
74-5-9	41.1	11.3[a]	32.5	19.0[a]
74-5-10	55.1	34.2[a]	24.0[a]	52.0
74-5-13	60.9	28.5[a]	49.5	46.0
74-5-14	42.6	19.7[a]	31.0	30.8
74-5-18	38.4	33.0	26.0	17.3[a]
74-5-19	19.8	9.0[a]	40.0[b]	24.8
74-5-20	51.0	34.0	26.8[a]	24.0[a]
74-5-25	58.9	17.0[a]	43.8	24.0[a]
74-5-26	58.2	19.0[a]	22.2[a]	33.2[a]
74-5-28	46.8	7.5[a]	40.7	44.0
74-5-29	57.4	29.2[a]	28.2[a]	49.2
74-5-31	51.5	27.5[a]	33.5	39.0
74-5-32	59.1	12.4[a]	23.8[a]	19.8[a]
74-5-37	61.1	36.0[a]	37.7[a]	37.2[a]
74-5-38	62.0	28.2[a]	46.2	48.0
74-5-42	31.5	10.0[a]	11.5[a]	21.8
74-5-43	43.1	20.0[a]	35.0	29.3
74-5-44	55.6	25.5[a]	53.5	37.2
74-5-45	45.7	25.0[a]	46.5	45.8
74-5-47	30.0	8.5[a]	28.8	23.3[a]
74-5-48	60.8	27.5[a]	53.8	46.6
74-9-7	18.2	0.3[a]	20.5	3.0[a]
74-9-11	14.9	0.5[a]	1.7[a]	1.2[a]
74-9-13	27.1	4.0[a]	24.8	29.5
74-9-14	10.3	0.0[a]	5.0	4.8
74-9-16	15.6	0.2[a]	9.5	13.2
74-9-18	37.3	11.0[a]	32.7	13.2[a]
74-9-19	25.1	6.5[a]	8.3[a]	12.2[a]
74-9-21	10.7	0.0[a]	1.2[a]	3.2[a]
74-9-22	16.0	0.4[a]	20.2	19.2
74-9-23	5.4	4.0	15.2[b]	13.0
74-9-26	18.2	2.8[a]	20.0	11.2
74-9-27	20.6	3.7[a]	25.4	8.4
74-9-28	13.6	0.8[a]	4.5[a]	3.8[a]
74-9-30	4.1	0.0[a]	7.0	6.6
74-15-1	0.0	0.0	5.5[b]	0.0
74-15-2	6.6	0.2[a]	5.2	0.0[a]
74-15-3	11.3	6.8[a]	0.0[a]	2.8[a]
74-15-4	17.3	1.0[a]	15.5	12.0
74-15-8	21.8	4.5[a]	11.2	1.7[a]
74-15-12	10.5	2.5[a]	12.0	1.0[a]
74-15-16	35.9	15.3[a]	39.2	17.8
74-15-22	4.4	23.5[b]	11.5	0.0[a]
74-17-1	9.3	1.3[a]	0.6[a]	1.1[a]
74-17-13	17.4	5.0[a]	19.7	7.4[a]
74-17-17	14.0	2.9[a]	12.4	1.0[a]
74-17-19	16.6	2.1[a]	9.2[a]	5.9[a]
74-17-22	20.0	2.4[a]	11.3	2.5[a]
74-17-25	22.6	6.6[a]	4.0[a]	6.2[a]
74-18-7	20.0	3.3[a]	8.9[a]	14.0
74-18-8	37.1	10.1[a]	39.3	13.7[a]
74-18-12	40.9	7.6[a]	15.8[a]	16.8[a]
74-18-14	58.5	6.2[a]	14.0[a]	12.0[a]
74-18-15	6.9	1.4	11.0	1.0[a]
74-18-17	39.1	8.7[a]	27.0	12.4[a]

[a] Significantly less than self at the 95% level.
[b] Significantly greater than self at the 95% level.

While reduction in stylar activity does account for various levels of PSC, especially when measured by crosses with males of identical S genotype, estimates of self seed yield are inflated by increased seed production on certain individuals able to sporophytically recognize self pollination. Styles of these individuals appear to be more capable of carrying out the self-incompatibility reaction with matching S alleles from other plants than with matching S alleles from self pollination. High-temperature-induced PSC (Campbell and Ascher 1972) should not have been a factor in the cool greenhouses or growth chamber. Similarly, tester cross results rule out the presence of pollen-part or stylar-part mutants or S_f alleles (Henny and Ascher 1976).

According to different gene action models (Ascher 1966; Lewis 1965), the incompatibility substance may be formed by the combination of half-repressor molecules (either monomer or dimer), one each from the style and pollen. The key to the reaction is the successful joining of the half-repressors into the functional incompatibility substance inside the pollen tube. Stylar conditioned PSC could be caused by genetic factors limiting substrates for or synthesis of the stylar incompatibility substance, its secretion into the style, or its transport into the pollen tube. Because of deficiency of stylar molecules, less functional repressor would be formed leading to PSC.

Both of the gene action models for self incompatibility offer an explanation for the apparent sporophytic recognition of self pollination we observed in *Nemesia*. These models call for a polymerization of similar monomers to explain recognition, but this polymer must function as a repressor. Small errors

Table 3. The Chi-square test results comparing the equality of mean seed sets from self and tester cross pollinations on 11 $S_{3.3}$ *Nemesia* seedlings in progeny 73-1

Seedling No.	Self	$S_{3.3}$ Tester as Male	P
1	21.7	8.0	0.025-0.01
2	14.4	7.3	0.25 -0.10
3	1.4	1.0	0.90 -0.75
4	16.0	3.7	0.01 -0.005
5	13.1	1.8	0.005
6	14.2	7.0	0.25 -0.10
7	13.0	1.7	0.005
8	15.1	5.0	0.025-0.01
9	11.7	10.0	0.75 -0.50
11	16.1	3.6	0.005
12	4.1	2.2	0.50 -0.25

Table 4. The Chi-square test results comparing the equality of mean seed sets from self and tester cross pollinations on 9 $S_{2.3}$ *Nemesia* seedlings in population 73-13 and 11 $S_{2.3}$ seedlings in population 73-15

Seedling	Self	$S_{2.2}$ Tester as Male	$S_{3.3}$ Tester as Male	P
73-13-1	1.3	0.0	0.0	0.25 -0.10
73-13-2	0.5	1.0	0.3	0.90 -0.75
73-13-3	18.4	0.0	3.0	0.005
73-13-4	10.1	0.0	6.3	0.25 -0.10
73-13-5	0.0	0.0	0.0	0.995
73-13-6	14.2	0.5	20.5	0.005
73-13-7	14.0	0.7	2.0	0.005
73-13-8	1.0	0.5	0.0	0.50 -0.25
73-13-9	14.0	0.0	5.6	0.005
73-15-1	14.8	0.0	0.0	0.005
73-15-2	13.7	0.0	12.8	0.005
73-15-3	0.9	0.0	0.0	0.50 -0.25
73-15-4	10.8	7.5	5.8	0.50 -0.25
73-15-5	7.8	10.4	0.0	0.01 -0.005
73-15-6	5.0	0.0	1.0	0.05 -0.025
73-15-7	2.0	0.0	1.8	0.50 -0.25
73-15-8	2.8	0.0	0.7	0.25 -0.10
73-15-9	1.2	0.0	0.0	0.50 -0.25
73-15-10	0.0	0.0	0.0	0.995
73-15-11	2.5	0.0	0.0	0.025-0.01

in the synthesis of these monomers might hinder their ability to join, or if joined may affect the regulatory function of the polymer. If genetically determined, these small errors should occur in both the pollen- and the stylar-portions of the recognition molecules for by both theories, pollen and stylar specificity are coded by the same gene. Upon self pollination, these small errors may act synergistically, resulting in less functional repressor than an incompatible outcross. Then incompatible tester crosses (tester plants as male) would measure the amount of stylar conditioned PSC in a plant determined by modifying genes other than the S gene and self seed set above this level would be due to the synergistic effects of combining small structural errors occurring in the transcription and translation of the half-repressor molecules coded by the specificity (S) gene.

In essence we are talking about a form of genetic complementation. Small errors in the half-repressor molecules of a particular allele may be complemented by subtle structural differences in the half-repressor molecules of the same allele from a plant with a different genetic background. Such complementation would lead to a more efficient incompatibility reaction and less PSC.

We cannot state conclusively that this phenomenon is linked to a particular S allele, although a higher frequency was associated with the $S_{2.2}$ and $S_{2.3}$ genotypes compared with $S_{3.3}$. The differences between self and tester cross seed set were usually realized in plants expressing a fairly high level of PSC. As tester cross seed sets only rarely exceeded self, such highly PSC plants are necessary for they allow a range within which differences can be detected. We are now able to generate progenies of *Nemesia* with high levels of PSC. Future research will be conducted to clarify the inheritance of these differences. Our explanation of the current results may not be the only one possible, but it does allow for differential inhibition of identical S alleles, from separate plants, in a single style.

Literature

Ascher, P.D.: A gene action model to explain gametophytic self incompatibility. Euphytica 15, 179-183 (1966)

Atwood, S.S.: Genetics of pseudo-self-compatibility and its relation to cross-incompatibility in *Trifolium repens*. J. Agric. Res. 64 (12), 699-709 (1942)

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

Denward, T.: The function of the incompatibility alleles in red clover (*Trifolium pratense* L.). Hereditas 49, 189-334 (1963)

East, E.M.; Mangelsdorf, A.J.: A new interpretation of the hereditary behavior of self-sterile plants. Proc. Nat. Acad. Sci. (Wash.) 11, 166-171 (1925)

Gerstel, D.U.: Self-incompatibility studies in guayule. II. Inheritance. Genetics 35, 482-506 (1950)

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

Hughes, M.B.; Babcock, E.B.: Self incompatibility in *Crepis foetida* L. subspecies *Roeadifolia* (Biel) Schinz et Keller. Genetics 35, 570-588 (1950)
Lewis, D.: A protein dimer hypothesis in incompatibility. Proc. XI Int. Cong. Genet., The Hague, In: Genetics Today, Ed. Geerts, S.J. 3, 656-663 (1965)
Mather, K.: Specific differences in Petunia. I. Incompatibility. J. Gen. 45, 215-235 (1943)
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*. Japan. J. Gen. 48, 27-33 (1973)

Received May 30, 1976
Communicated by H.F. Linskens

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