

COMPETITIVE INTERACTION BETWEEN TWO *S* ALLELES
IN A SPOROPHYTICALLY-CONTROLLED
INCOMPATIBILITY SYSTEM

K. F. THOMPSON

Plant Breeding Institute, Trumpington, Cambridge

Received 13.iii.71

1. INTRODUCTION

COMPETITIVE interaction between *S* alleles in diploid pollen of artificially-induced or spontaneous autotetraploids has been reported only in species in which self-incompatibility is gametophytically controlled. This interaction, which is known also as mutual weakening of the activities of both alleles of an *S* allele heterozygote, produced self-compatibility in an autotetraploid pear (Lewis and Modlibowska, 1942) and in *Trifolium repens* (Brewbaker, 1954). In *Oenothera organensis* it permitted only the longer growth of pollen tubes (Lewis, 1947). Brewbaker and Natarajan (1960) reported that competitive interaction characterised diploid pollen grains in all *S* allele heterozygotes tested in *Petunia inflata*. Similarly the self-compatibility of some mutants induced by radiation of the pollen of self-incompatible *S* allele heterozygotes in *Petunia* was explained by competitive interaction between different *S* alleles, one of which was carried by a centre fragment produced by the irradiation.

Tetraploid Brassicas, produced by colchicine treatment, were found by Howard (1942) to remain self-incompatible and it has been considered to be characteristic of sporophytically-determined incompatibility systems that competitive interaction between *S* alleles does not occur at either the diploid or tetraploid levels. Crowe (1964) suggested that, in the evolution of sporophytic from gametophytic incompatibility, selection would act against any alleles displaying competitive interaction. In a study of 128 combinations between 28 *S* alleles in *Brassica oleracea* var. *acephala*, in which self-incompatibility is sporophytically determined, Thompson and Taylor (1966*a*) found results suggesting competitive interaction for one combination only. These results, which have been summarised by Thompson (1967), are considered in more detail below because of their uniqueness and importance.

2. MATERIAL AND METHODS

The marrow-stem kale inbreds were obtained by selfing plants selected from a stock produced by Cannell and Sons. The curled kale was selected from plants grown from a packet of Scotch curled kale purchased at Woolworths.

Pollination methods were similar to those described in Thompson and Taylor (1971). Observations on darkening of stigmas were used to detect compatible pollinations in some of the families investigated (Thompson and Howard, 1959; Thompson and Taylor, 1966*a, b*).

3. RESULTS

(a) *Progeny from a cross between curled and marrow-stem kale*

The self-incompatible curled kale selection, plant Cr₁, was found by Thompson and Taylor (1971) to have the two alleles, S₁₅ and S₃₅ (the latter allele had not been identified previously). It was crossed with the self-incompatible marrow-stem kale inbred, plant 220/1, which is homozygous for allele S₂. Three of the five F₁ hybrids were self-compatible and set seed freely in the insect-proofed glasshouse without hand pollination. On crossing the F₁ hybrids as males to inbred 220/1 (the S₂ homozygote), the stigmas darkened only with the two self-incompatible plants. The non-darkening with the self-compatible F₁ plants showed that allele S₂ was active in their pollen.

From one of the self-compatible F₁ hybrids, plant Z209/C, 14 plants of the F₂ generation were selected on the basis of the chemical composition of the pith of their stems. These plants were selfed at the mature flower stage and in the bud and tested for S alleles (table 1). Four plants were self-compatible, one (Z209/20) was partially self-compatible and the other nine were self-incompatible.

TABLE 1
Segregation for self-compatibility and S alleles in selected progeny from selfing the self-compatible plant Z209/C

Progeny No.	Average no. seeds/fruit from selfing		S allele constitution
	at mature flower	in the bud	
1	26.3	23.7	} 2, 15
6	20.8	16.2	
12	21.2	30.0	
13	12.5	13.2	
3	0.2	16.2	} 2, 2
5	0.0	6.5	
15	1.3	13.5	
17	3.5	22.8	
20	10.2	17.6	
22	1.5	19.5	
23	0.0	7.5	
24	1.8	20.7	
4	0.0	27.8	} 15, 15
16	2.7	12.5	

The stigmas of all the F₂ plants darkened when they were crossed by the unrelated inbred, plant A162/2/1, which is homozygous for S₁₆. Testing for alleles present in these plants was therefore possible using stigma darkening as the criterion of compatible pollinations (checks were made, however, by noting fruit development after 5 weeks; seed counts were not made). The stigmas of only two plants, Z209/4 and Z209/16, darkened after pollination with inbred 220/1 (the S₂ homozygote). These two plants, both of which were self-incompatible, are the homozygotes for S₁₅ the recessive (low in the dominance series) allele of plant Cr₁.

The stigmas of eight seedlings darkened after pollination by Z209/4 (homozygous for S_{15} , see above). These eight plants, which were all self-incompatible with the exception of plant 20, which was partially self-compatible, are the homozygotes for allele S_2 .

The remaining four plants, the stigmas of which did not darken after pollination with either 220/1 or Z209/4, are the $S_2 S_{15}$ heterozygotes, both alleles being active in the stigma. They were all self-compatible (table 1), *i.e.* there must be competitive interaction between the two *S* alleles in the pollen or a factor conditioning self-compatibility and independent of the *S* allele system must be present. Although darkening of the stigma did not occur after pollinations with plants 220/1 and Z209/4, some seeds were set. In the self-incompatible plants, on the other hand, appreciable seed setting occurred only after darkening of stigmas.

In the following year a further 18 F_2 plants were selected on vigour and chemical composition from a progeny of the same self-compatible F_1 hybrid, Z209/C. The plants were selfed at the mature flower stage and in the bud and crossed with inbreds, homozygous for alleles S_2 , S_{15} and S_{16} (table 2). The results differ somewhat from those obtained the previous year (table 1). The 12 plants heterozygous for alleles S_2 and S_{15} consisted of eight self-compatibles and four which were only partially self-compatible. The three plants, homozygous for S_2 , and the three homozygous for S_{15} were all highly self-incompatible. One of the partially self-compatible plants, Z209/118, gave 3.2, 20.5 and 26.0 seeds per fruit when crossed with inbreds, homozygous for alleles S_2 , S_{15} and S_{16} respectively; no darkening of the stigma was observed in the S_{15} cross. It would appear, therefore, that in the partially self-compatible plant S_2 was incompletely dominant in the stigma to S_{15} .

Confirmation of partial self-compatibility in plant 106 by a later pollination (table 2) suggests that genetic rather than environmental factors determined the change in dominance relationships and expression of self-compatibility. As five of the 21 *S* allele heterozygotes were partially self-compatible the effect could have been determined by a single recessive gene.

Further unselected F_2 generation plants from selfing Z209/C were studied (table 3). The two plants (133 and 146), which were homozygous for allele S_{15} , showed stigma darkening and produced as high a set of seeds when pollinated with any of three plants (135, 138 and 148), heterozygous for S_2 and S_{15} , as they did when pollinated with an unrelated inbred, homozygous for S_{16} . Both plants were highly self-incompatible and the crossing results suggest that S_2 was dominant to S_{15} in the pollen.

The two plants (151 and 153), which were homozygous for allele S_2 , differed, one plant being self-compatible and the other self-incompatible. Both plants gave high sets of seed per fruit when pollinated by the three plants, heterozygous for S_2 and S_{15} , although there was some reduction in number of seeds for two of the crosses with the self-incompatible plant. Neither of the self-compatible or self-incompatible plants showed darkening of the stigma. These results would suggest either that S_{15} was incompletely dominant to S_2 in the pollen, disagreeing with the conclusion in the previous paragraph or that competitive interaction between alleles S_2 and S_{15} occurred in the pollen. In the latter case, plant 138 showed greater competitive interaction than plants 135 and 148. The difference in seed set between plants 151 and 153 in crosses with plants 135 and 148 may be due to the self-compatibility of plant 151.

Four self-compatible plants (135, 138, 145 and 148), which were $S_2 S_{15}$ heterozygotes, set relatively high numbers of seeds per fruit when pollinated by the two plants (151 and 153), homozygous for S_2 , and the two plants (133 and 146), homozygous for S_{15} . For these pollinations flowers were emasculated before pollen was shed. It should be noted that although plant 151, homozygous for S_2 , was self-compatible, the seed set from pollinations by the four plants, homozygous for S_2 or S_{15} , were consistently lower than seed set on selfing the $S_2 S_{15}$ heterozygotes. The partially self-compatible plant (142) set a high number of seed when pollinated by the S_{15} homozygotes but a relatively low number, similar to those from selfing, when pollinated

TABLE 2
Segregation for self-compatibility and S alleles in further selected progeny from selfing plant Z209/C

Progeny No.	Average no. seeds/fruit from selfing		S allele constitution
	at mature flower	in the bud	
105	26.5	26.7	} 2, 15
107	22.0	25.6	
108	32.2	29.6	
109	28.5	27.7	
124	23.3	29.2	
126	21.8	26.9	
130	19.7	25.3	
131	20.0	29.7	
106	5.5	26.6	} 2, 15
106	7.9*	23.8*	
118	5.5	26.2	
125	6.1	26.6	
127	4.0	14.2	
123	0.0	20.9	} 2, 2
128	1.0	27.5	
129	2.1	25.0	
103	0.0	30.9	} 15, 15
114	0.0	8.7	
136	0.1	11.3	

* Repeat pollinations.

with the two S_2 homozygotes. This result is similar to those obtained earlier with the partially self-compatible S allele heterozygote (plant 118), in which S_2 was incompletely dominant in the stigma to S_{15} .

Taken together all the above results indicate that alleles S_2 and S_{15} must interact in both pollen and stigma so that self-compatibility occurs or that there is a factor independent of the S allele system determining self-compatibility. The curled kale parent plant, Cr_1 , is heterozygous for a dominant self-compatibility gene independent of the S allele system which gives self-compatibility only in the absence of the dominant S gene (Thompson and Taylor, 1971). This gene is obviously not involved in the family from selfing Z209/C because all the S_{15} homozygotes are self-incompatible and it is only the $S_2 S_{15}$ heterozygotes which are self-compatible (tables 1 and 2).

Self-compatibility could also result from reversed dominance of two S alleles in pollen and stigma (Thompson and Howard, 1959). Based on the darkening of stigmas reactions, allele S_2 was completely dominant to S_{15} in the pollen while both alleles were active in the stigma. Although allele S_2 was active in the stigma, self-compatibility could occur if it was incompletely recessive to S_{15} . Pollination of plants, heterozygous for S_2 and S_{15} , with S_2 and S_{15} homozygotes, however, gave equal sets of seed (table 3*b*) showing that the self-compatibility of the heterozygotes was not determined by incomplete reversed dominance. More seeds per fruit were produced by selfing the heterozygotes at the mature flower stage than by pollinating with

TABLE 3

Reciprocal crosses between self-compatible and self-incompatible progeny from selfing Z209/C

(a) Crosses between plants, homozygous for S alleles, as female, with plants, heterozygous for S alleles, as male.

Progeny No.	S alleles	Average no. seeds/fruit				
		by selfing at mature flower	Outcross A162/2/1 16, 16	Crossed by S_2 S_{15} plants as male		
			No.	135	138	148
133	15, 15	0.0	30.7	2, 15	2, 15	2, 15
146		0.7	33.0	33.4	32.4	34.5
151	2, 2	20.1	27.3	30.0	36.2	34.6
153		0.8	25.3	26.7	24.2	23.3
				12.0	27.2	17.7

(b) Crosses between plants, heterozygous for S alleles, as female, with plants, homozygous for S alleles, as male.

Progeny No.	S alleles	Average no. seeds/fruit					
		by selfing at mature flower	Crossed as female by plants				
			A162/2/1 16, 16	151 2, 2	153 2, 2	133 15, 15	146 15, 15
135		26.7	23.1	16.2	17.4	15.8	17.5
135*		—	—	18.1*	21.7*	16.9*	15.9*
145	2, 15	27.7	31.5	22.8	—	13.5	18.0
148		20.9	36.5	12.8	—	14.2	17.4
138		14.4	27.6	7.3	—	7.0	10.5
Mean		22.4	29.7	12.8	19.5	12.6	13.3
142	2, 15	5.5	31.5	5.2	5.3	29.5	29.4

* Simultaneous duplicate pollinations on different branches of same plant.

plants homozygous for S_2 or S_{15} , *i.e.* competitive interaction between alleles S_2 and S_{15} occurred in the pollen as well as in the stigmas.

4. DISCUSSION

In addition to the case of competitive interaction described in this paper for alleles S_2 and S_{15} in kale, the data of Kakizaki (1930) for a self-compatible cabbage plant may afford another example. He found that plant 2, which was self-compatible, gave on selfing a progeny containing 13 self-compatible and 10 self-incompatible plants. Pollination between three of the self-incompatible inbreds showed that there were two reciprocally cross-compatible groups which were either the two homozygotes or a homozygote

and a heterozygote with dominance in pollen and stigma. Self-compatibility could not therefore be due to an S_F allele, nor was it likely to be caused by a dominant self-compatibility gene because 13 self-compatible:10 self-incompatible is a very poor fit to a 3:1 segregation.

Thompson and Taylor (1971) explained the occurrence of self-compatibility in kale using the hypothesis of Sampson (1960) to account for S allele interactions. It was suggested that recessive S alleles are relatively inefficient in the production of the substances responsible for incompatibility so that the threshold for the expression of self-compatibility was only just reached in plants homozygous for recessive S alleles. In a serological study of self-incompatibility antigens from cabbage stigmas Wallace and Nasrallah (1968) found that the quantity of antigen produced by each S allele in an S allele heterozygote was only half that in an inbred homozygous for an S allele. Thus a reduction of the quantity of each specific antigen in plants, heterozygous for two recessive alleles, could make these plants self-compatible even though plants, homozygous for either S allele, would be self-incompatible. If such were so, then competitive interaction to produce partial self-compatibility should occur fairly frequently between S alleles low in the dominance series. A search specifically for competitive interaction between S alleles has not been made in the kales (*B. oleracea* var. *acephala*), but J. G. van Hal (personal communication, 30th December 1969), using ultraviolet fluorescence techniques to observe pollen tube growth, has found recently mutual weakening of activity in several combinations in Brussels sprouts (*B. oleracea* var. *gemmifera*).

5. SUMMARY

1. In the F_2 generation from a cross between marrow-stem and curled kale, plants, heterozygous for the pollen-recessive S alleles, S_2 and S_{15} , were self-compatible. Plants, homozygous for either allele, were self-incompatible.

2. Allele S_2 was completely dominant to S_{15} in the pollen, but both alleles were active in the stigma. Equal numbers of seeds were set when S_2 or S_{15} homozygotes were crossed as males to the heterozygote, but more seed was always set by selfing. Competitive interaction between S_2 and S_{15} was assumed to occur in both pollen and stigmas of plants, heterozygous for these alleles.

3. In five partially self-compatible plants, heterozygous for S alleles, allele S_2 was incompletely dominant to S_{15} in the stigma. This change in self-compatibility and dominance relationships was probably determined by a recessive gene.

6. REFERENCES

- BREWBAKER, J. L. 1954. Incompatibility in autotetraploid *Trifolium repens*. I. Competition and self-compatibility. *Genetics*, 39, 307-316.
- BREWBAKER, J. L., AND NATARAJAN, A. T. 1960. Centric fragments and pollen-part mutation of incompatibility alleles in *Petunia*. *Genetics*, 45, 699-704.
- CROWE, L. K. 1964. The evolution of outbreeding in plants. *Heredity*, 19, 435-457.
- HOWARD, H. W. 1942. Self-incompatibility in polyploid forms of *Brassica* and *Raphanus*. *Nature, Lond.*, 149, 302-303.
- KAKIZAKI, Y. 1930. Studies on the genetics and physiology of self- and cross-incompatibility in cabbage (*B. oleracea* var. *capitata* L.). *Jap. J. Bot.*, 5, 133-208.

- LEWIS, D. 1947. Competition and dominance of incompatibility alleles in diploid pollen. *Heredity*, *1*, 85-108.
- LEWIS, D., AND MODLIBOWSKA, I. 1942. Genetical studies in pears. IV. Pollen-tube growth and incompatibility. *J. Genet.*, *43*, 211-222.
- SAMPSON, D. R. 1960. An hypothesis of gene interaction at the *S* locus in self-incompatibility systems of Angiosperms. *Amer. Nat.*, *94*, 283-292.
- THOMPSON, K. F. 1967. Breeding problems in kale (*Brassica oleracea*) with particular reference to marrow-stem kale. *Rep. Pl. Breed. Inst.*, 1965-66, 7-34.
- THOMPSON, K. F., AND HOWARD, H. W. 1959. Self-incompatibility in marrow-stem kale, *Brassica oleracea* var. *acephala*. II. Recognition of plants homozygous for *S* alleles. *J. Genet.*, *56*, 325-340.
- THOMPSON, K. F., AND TAYLOR, J. P. 1966a. Non-linear dominance relationships between *S* alleles. *Heredity*, *21*, 345-362.
- THOMPSON, K. F., AND TAYLOR, J. P. 1966b. The breakdown of self-incompatibility in cultivars of *Brassica oleracea*. *Heredity*, *21*, 637-648.
- THOMPSON, K. F., AND TAYLOR, J. P. 1971. Self-compatibility in kale. *Heredity*, *27*, 459-471.
- WALLACE, D. H., AND NASRALLAH, M. E. 1968. Pollination and serological procedures for isolating incompatibility genotypes in the Crucifers. *Cornell University Agric. Expt. Station, Memoir 406*. pp. 23.