Gametophytic–sporophytic incompatibility in the Cruciferae—*Raphanus sativus*

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The established sporophytic incompatibility system in the Cruciferae is really a gametophytic-sporophytic system. This is demonstrated by the finding of a gene *G* which is gametophytic in its action in the pollen and complements the *S* gene. Six *S* alleles and two *G* alleles have been identified. Concomitant *G* and *S* allelic matching is essential to give the incompatibility reaction in some matched combinations of *S* alleles. In other *S* allelic combinations the difference between the two *G* alleles is not expressed. It is concluded that *G* is an essential part of the incompatibility recognition process but not used for the multiple allelic variation of the system. It is suggested that *G* is a relic of a primitive system into which the *S* gene has been incorporated. *G* and *S* are linked.

The inbreeding used in the tests revealed an instability of one *S* allele which gave rise to three distinct fully operative alleles. One of the derived alleles was unstable and could revert back to the original.

INTRODUCTION

Self-incompatibility in *Raphanus sativus* is thought to be based on a single *S* gene with sporophytic control of the pollen, dominance and codominance of the alleles in both pollen-mother-cells and in styles, and is typical of the well established self-incompatibility system found throughout the Cruciferae and the Compositae. The results in all the published work on this system, in as many as seven different species, contain a significant proportion of unexpected and unexplained anomalies, frequently in the nature of a positive result where a negative is expected (Hughes and Babcock, 1950; Bateman, 1954; Sampson, 1956, 1964; Putrament, 1960; Kroh 1956; Verma *et al.*, 1977; Hinata and Nishio, 1978). In another major incompatibility system, the gametophytic system, such unexpected results are rare and usually totally absent (cf. Lewis, 1962). Since the methods used, and the conditions under which the mating tests were made, were almost identical in the two systems, the cause of the unpredicted anomalies in the sporophytic system cannot be attributed to trivial noise in the system, and may be highly significant.

Raphanus sativus was chosen for the study of these anomalies because Sampson (1957) and

Putrament (1960) had obtained many exceptional results which could not be explained on a single *S* gene hypothesis. We used, therefore, material similar to that of Putrament (1960).

MATERIAL AND METHODS

Seeds of the open-field variety Rzodkiewka Gruntowa Würzburgska of *R. sativus* were kindly supplied by Dr Putrament. Plants were raised and brought to maturity in an insect-proof greenhouse in the spring and summer of 1977. To obtain seed for a further generation fresh open flowers were pollinated for compatible crosses, and for self-pollinations buds one day from flower-opening were used, fresh open flowers were used to test cross-pollinations and selfings for incompatibility. The methods for fixation, staining and mounting the pollinated stigmas followed Zuberi *et al.* (1981). The mounted stigmas were assessed for compatible pollen grains (faintly stained, shrunken and with pollen tubes penetrating the stigma) and incompatible pollen grains (darkly stained, fully turgid and either ungerminated or with a short swollen tube adhering to, but not penetrating stigmatic papilla). Most of the assessments were made

by a quick visual inspection which provided an obvious difference, all incompatible (–) or all compatible (+). There were some cases which appeared to have both + and – pollen grains, if there were more than 20+ grains they were recorded as +–. These +– pollinations were repeated several times and the majority could be accepted as a definite +– category. The intrafamily incompatibility tests were done as quickly as possible without repeats of clear-cut cases so that major incompatibility groups could be defined before the plants had finished flowering. The results which were then found to be exceptions to the main grouping were repeated until a firm decision +, – or +– could be made. All assessments of mounted stigmas were done by a coded blind method.

RESULTS

Second generation

Six families derived from intercrossing and one from budselfing plants were grown and analysed. Five of the crossed families were partially analysed without showing any anomalies from the expected results on a one *S* sporophytic gene basis. These were discarded leaving one crossed and one budselfed family for complete analysis. Crossed family 3/78 consisted of twenty-nine plants which gave a matrix of $29 \times 29 = 841$ matings. These were all tested by the pollen-stigma method with adequate repeats as described in the methods. A summary of the matrix is given in fig. 1; from which it is evident that there are four incompatibility groups which give a pattern typical of the one gene

No. plants		10	8	5	6
	Pollen				
	Stigma	$S_{1,2}$	$S_{1,4}$	$S_{2,3}$	$S_{3,4}$
10	$S_{1,2}$	–	58 – 22+, +–	–	+
8	$S_{1,4}$	–	–	+	+
5	$S_{2,3}$	–	+	+	–
6	$S_{3,4}$	+	+	+	–

Figure 1 Mating matrix of twenty-nine plants of fam. 3/78 from the cross $B_6 S_{1,3} \times B_{11} S_{2,4}$. The dominance relationship of $S_{2,4}$ is not known

sporophytic system. Four *S* alleles are allocated to the groups. The dominance and co-dominance are all normal; active alleles are indicated by a dot over the number for the pollen and an underlining for the stigma. Note that all matings within squares marked – or + are consistent. One block $S_{1,2} \times S_{1,4}$, which is mainly –, as expected, contains some + and +– reactions which are exceptional to the one *S* gene system. This anomalous block is shown in full in fig. 2. These results were unsuccessfully tested against a hypothesis based upon a second *S* sporophytic gene, particularly difficult to explain was the group allocated to $G_{1,2}$ which rejects the pollen of the other two groups. Further work (see below) suggested a gametophytic gene which perfectly explains the observations in fig. 2. Compared with later work the assessment of + and +– was partly subjective (see methods) and the difference between them less rigorous. Note that the segregation of *G* phenotypes only occurs in one of the cross-incompatible combinations; the *G* alleles are segregating in other combinations but, in these, the difference between G_1 and G_2 is not expressed.

The bud-selfed family was from plant B6, one parent (with allocated genotype $S_{1,3}$) of crossed family 3/78 described above. The family consisted of 23 plants and the matrix of 23×23 pollinations was completed (see fig. 3). Three major groups corresponding to the expected three genotypes $S_{1,3}$, $S_{1,1}$ and $S_{3,3}$ were obtained in the proportion of 12, 4, 4. Two of the $S_{1,3}$ plants were incompatible as males on all three major groups, indicating pollen co-dominance, $S_{1,3}$. S_1 was dominant over S_3 in the ten other plants. In addition there were three anomalous plants, two of which were com-

Pollen		Stigma		
		$S_{1,4}$		
		$G_{1,2}$	$G_{1,1}$	$G_{2,2}$
$S_{1,2}$	$G_{1,2}$	– – –	– – – –	–
		– – –	– – – –	–
		– – –	– – – –	–
		– – –	– – – –	–
	$G_{1,1}$	– – –	– – – –	–
		– – –	– – – –	–
		– – –	– – – –	–
		– – –	– – – –	–
	$G_{2,2}$	– – –	– – – –	–
		– – –	– – – –	–
		– – –	– – – –	–
		– – –	– – – –	–

Figure 2 Detailed enlargement of cross-incompatible block $S_{1,2} \times S_{1,4}$ of the matrix of family 3/78 given in Fig. 1. *G* is a proposed gametophytically operating gene with typically oppositional action.

Group	No. of plants	Pollen Stigma	$S_{1,3}$	$S_{1,3}$	$S_{3,3}$	$S_{1,1}$	$S_{1,6}$	$S_{3,5}$ $7^9/8$	$S_{3,6}$ $7^{14}/8$
1	10	$S_{1,3}$	—	—	—	—	—	+	+
1 ^a	2	$S_{1,3}$	—	—	—	—	—	+	+
2	4	$S_{3,3}$	+	—	—	+	+	+	+
3	4	$S_{1,1}$	—	—	+	—	—	+	+
4	1	$S_{1,6}$	—	—	+	—	—	+	—
5	1	$S_{3,5}$	+	—	—	+	+	—	+
6	1	$S_{3,6}$	+	—	—	+	—	+	—

Figure 3 Mating matrix of family 7/78 of B6 $S_{1,3}$ bud-selfed. Groups one, two and three are expected. The sub-group 1a is due to a difference in allelic dominance in the pollen. Groups four, five and six are unexpected; the explanation of the alleles S_5 and S_6 is deferred until after the analysis of the later generations.

patible as pollen parents on all the three groups and as female parents were compatible with $S_{1,3}$ and $S_{1,1}$ but not with $S_{3,3}$. The third plant was compatible both as male or female parent only with $S_{3,3}$. To explain these anomalous plants we have to invoke two further alleles S_5 and S_6 , which either were from stray contaminating pollen from other plants in the greenhouse or were cryptically present in the parental plant B6 and were revealed by inbreeding. Further evidence on this is deferred until after the consideration of later generations. There were no anomalies like those in the crossed family described previously. The parent plant B6 must be heterozygous for $G_{1,2}$ from the results in fig. 2; the G alleles must be segregating but not expressed.

Third generation

Many bud-selfed and crossed pollinations were made within family 7/78 to supply third generation families, but seed yield from bud-selfing was so low that no families of sufficient size were obtained. Six crossed families were raised and five were analysed in detail (see table 1 for a summary). The four mating groups that segregated in family 1/79 confirm the proposed S -locus genotypes of the 7/78 parents, $S_{3,5} \times S_{1,3}$ but provide no information on the origin of the unexpected S_5 allele. The + and — blocks were homogeneous except for the block $S_{1,5} \times S_{1,3}$ which contained + and — matings characteristic of G allele segregation. The detailed results for this block are given in fig. 4. Again as in family 3/78 cf. fig. 2 there were three sub-groups related to the gametophytically-acting gene G . Apart from one mating between $G_{1,1}$ and $G_{2,2}$ the

fit is perfect. The absence of $S_{1,3} G_{1,1}$ class is due to linkage of S and G (see below). This class would require a crossover gamete from both the male and female plants.

Table 1 Summary of third generation families

1/79	Parental Genotypes $7^9/78 S_{3,5} G_{1,2} \times 7^7/78 S_{1,3} G_{1,2}$. Thirty plants, complete matrix analysed. Genotypes of 4 Groups found, as expected, 9 $S_{1,3}$, 6 $S_{3,3}$, 7 $S_{1,5}$, 8 $S_{3,5}$. Mating reactions normal without anomalies except in one block. Segregation of + anomalies in $S_{1,5} \times S_{1,3}$ incompatible block. The + anomalies grouped into three genotypes $G_{1,2}$, $G_{1,1}$, and $G_{2,2}$.
4/79	Parental Genotypes $7^{14}/78 S_{3,6} G_{1,2} \times 7^9/78 S_{3,5} G_{1,2}$. Nineteen plants, complete matrix analysed. Genotypes of 3 groups found but not as expected, 7 $S_{1,3}$, 9 $S_{1,6}$, 3 $S_{3,6}$. Note the absence of S_5 and the unexpected appearance of S_1 . S_1 is assumed to have arisen from S_5 as a manifestation of $S_1 \leftrightarrow S_5$ instability. The absence of $S_{3,3}$ is not significant since the number expected is only one. Segregation of + — anomalies corresponding to the three genotypes $G_{1,2}$, $G_{1,1}$ and $G_{2,2}$ occurred only in the $S_{1,6} \times S_{1,3}$ incompatible group. Note the change of dominance of the derived S_1 e.g., $S_{1,6}$ instead of $S_{1,5}$ with the original S_1 in family 7/78.
2/79	Parental Genotypes $7^9/78 S_{3,5} G_{1,2} \times 7^{22}/78 S_{1,1} G^?$. Thirteen plants, complete matrix analysed. Genotype of 3 groups found, but not as expected, 2 $S_{3,5}$, 2 $S_{1,3}$, 9 $S_{3,5}$. Mating reactions normal, without exception. No + — segregation found.
5/79	Parental Genotypes $S_{1,1} \times S_{3,3}$. Eleven plants, complete matrix analysed. $S_{3,5}$, $S_{3,6}$ genotypes of 2 groups found but not expected. Mating reactions normal without exception. No + — segregation found.
6/79	Parental Genotypes $S_{1,1} \times S_{3,3}$. Twelve plants, complete matrix analysis. Genotypes of 2 groups found but not expected. Mating reactions and absence of + — anomalies as in 5/79.

Intercrosses between third generation families

The unexpected segregations of S_5 and S_6 in family 7/78 and the appearance of a hitherto undetected allele S_x in the third generation were checked by making a test of all the alleles in the families in a complete matrix of interfamilial crosses. Sixteen plants, representing all four genotypes, were used from family 1/79 and one plant from each genotype in the other four families were intercrossed in all combinations. The total matrix was twenty-five used as male by twenty-six used as female. The results with one exception were completely consistent and as expected from our assumptions. This cross-checking also included a $+$ – result of G allelic segregation in crosses between $S_{1,5}$ from family 1/79 with $S_{1,3}$ from family 4/79 which is a striking confirmation of $+$ – segregations within family 1/79. The one exception was that $S_{1,6}$ stigmas from family 4/79 accepted the pollen of $S_{1,3}$ from family 1/79 indicating that the S_6 allele in the family 4/79 is dominant in both pollen and stigma or more probably the S_1 is recessive in pollen and stigma.

We consider that this comprehensive cross-checking confirms the allocation of S and G alleles and, particularly, confirms G allele manifestation in particular S allele combinations. It also emphasizes the importance of the interpretation of the S_5 and S_6 alleles in the original family 7/78 by the unexpected presence of S_5 in families 5/79 and 6/79. The unexpected new allele S_x , which has originated from S_1 on three separate occasions (families 2/79, 5/79 and 6/79, table 1) is proved to be the same allele in all three families. We favour an explanation based upon an instability of S_1 but defer a full discussion until the final section.

Fourth generation

The object of the fourth generation tests was to confirm the S genotypes and the segregation of G alleles in the $+$ – incompatibility block of 1/79 (see table 1 and fig. 4). This block, $S_{1,5} \times S_{1,3}$ is S incompatible and any matings that are compatible within the block are due to compatibility of the G alleles. The G compatible crosses are of two types, (1) homozygote $G_{1,1}$ or $G_{2,2}$ stigmas crossed with pollen of a heterozygote $G_{1,2}$. This produces all heterozygotes, $G_{1,2}$, because only the unmatched allele in the pollen will function. (2) crossing different homozygotes $G_{1,1} \times G_{2,2}$ which also produces all heterozygotes. Neither of these crosses would give segregation of G genotypes in the next generation. We, therefore, concentrated on pro-

Pollen \ Stigma		$S_{1,3}$					
		$G_{1,2}$		$G_{2,2}$		$G_{2,2}$	
$S_{1,5}$	$G_{1,2}$	–	–	–	–	–	–
		–	–	–	–	–	–
	$G_{2,2}$	+	+	+	–	–	–
		+	+	+	–	–	–
	$G_{1,1}$	+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	–

Figure 4 Mating matrix of nine $S_{1,3}$ plants with seven $S_{1,5}$ plants of fam. 1/79. The mating reactions conform with the G allelic allocation with the addition of a secondary factor which modifies the $G_{2,2}$ and $G_{1,1}$ into two subgroups.

ducing seeds from the incompatible matings by bud-pollination. These would be of the type $G_{1,2} \times G_{1,2}$, $G_{1,2} \times G_{2,2}$ or $G_{1,1}$, all of which will give segregation of G genotypes in the next generation. Seven families from $S_{1,5} \times S_{1,3}$ were raised and the expected four S genotypes $S_{3,5}$, $S_{1,1}$, $S_{1,5}$, $S_{1,3}$. An interfamilial mating matrix of plants from the seven families without exception confirmed the S genotypes.

Because of the reliance on the partial breakdown of incompatibility reaction in bud-pollination all the families contained only a few plants but family 7/80 was large enough to detect $G_{1,2}$ segregation. Details of the origin and progeny of family 7/80 are given in table 2 which fully agrees with expectation.

Return to original material with new technique

Most of the S and G analysis of the three generations just described was done before we adopted the novel idea of an S - G system and before the technique of restricted pollination first used by us in *Brassica campestris* (Zuberi and Lewis, 1988) to detect segregating $+$ and $-$ pollen grains from the same anther - a *sine-qua-non* for gametophytic

Table 2 Mating reactions of plants in family 7/80 which demonstrate the expected segregation of $G_{1,2}$ and $G_{2,2}$

		$S_{1,3}$					
		$G_{1,2}$		$G_{2,2}$			
$S_{1,5}$	$G_{1,2}$	–	–	–	–	–	–
	$G_{2,2}$	+	+	–	–	–	–

control. To test the hypothesis in *Raphanus*. We chose two crosses from family 7/78; (see fig. 3) $7^{14} S_{3,6} \times 7^4 S_{1,1}$ and $7^{16} S_{1,1} \times 7^{14} S_{3,6}$.

We had evidence that plant 14 was heterozygous for $G_{1,2}$. Because the compatibility of the S genotypes overrules any G incompatibility the G alleles would be transferred through the pollen without restriction and therefore we should obtain more than one G genotype in the progeny.

Furthermore, the progeny of the cross should be $S_{1,3}$ and $S_{1,6}$. These are both cross-incompatible so the family would be one group with all mating combinations S incompatible. Fifty per cent of the matings in this block will be $S_{1,3} \times S_{1,6}$ and the reciprocal, a combination of which we know from family 4/79 allows the expression of the G gene. These families were analysed by Verma in Chandigarh, India, by using the restricted pollination method and counting the numbers of + and - pollen grains on the stigmas. This method, which is highly successful in *B. campestris*, Zuberi and Lewis, 1988, is not quite so decisive in our material of *Raphanus* because more of the incompatible pollen does not germinate and only a minority produce the short tube which adheres to the stigmatic papillae. The compatible pollinations which we designate with a + have from 20 to 73 compatible pollen on a stigma, the - pollinations have no compatible pollen. The mating matrices of the two families are given in figs 5 and 6. The results in fig. 5 are particularly clear, and they confirm the segregation of alleles of the gametophytic gene

G and also the oppositional action found in previous families and characteristic of the gametophytic system. The second family, fig. 6, also conforms to the G scheme with one unexpected plant which can be explained on S_1 instability which has previously been described. The best, but not perfect, fit is to assume that the exceptional plant is $S_{3,5}$ by mutational change of $S_1 \rightarrow S_x$.

Intercrosses of plants from the two families were analysed as a further check. Sixty-three crosses were found to be - and all but five of these were expected on the G scheme. Six crosses were found to be +, of which three were expected. It should be emphasized that although the S genotypes of the parents were comparable in the two families the actual individuals $S_{1,1}$ parents were different. We are not surprised that some discrepancies arise as we cannot rule out the segregation of undetected genes that modify the system. The most important aspect of the study of these Verma families is the demonstration that pollen grains from a single plant or even a single anther segregate compatible and incompatible reactions on a single stigma or even a single papilla, as shown in fig. 7.

Linkage between S and G

None of the families analysed were raised specifically to test for linkage of S and G ; however, two families 3/78 and 1/79 with a total of thirty-four relevant plants are available for testing linkage

Pollen Stigma		$S_{1,3}$		$S_{1,6}$	
		$G_{1,2}$	$G_{2,2}$	$G_{1,1}$	$G_{2,2}$
$S_{1,3}$	$S_{1,2}$	- - - -	- - - -	- - - -	-
		- - - -	- - - -	- - - -	-
		- - - -	- - - -	- - - -	-
		- - - -	- - - -	- - - -	-
$S_{1,6}$	$G_{1,1}$	+ + + +	+ + +	- - -	-
		+ + + +	+ + +	- - -	-
		+ + + +	+ + +	- - -	-
		+ + + +	+ + +	- - -	-
$S_{1,3}$	$G_{2,2}$	+ + + +	- - -	- - -	-
		+ + + +	- - -	- - -	-
		+ + + +	- - -	- - -	-
		+ + + +	- - -	- - -	-

Figure 5 Mating matrix of eleven plants of family Verma 1, 1984, from a cross between $7^{14}/78 S_{3,6} \times 7^4/78 S_{1,1}$ especially chosen for study because the two S genotypes produced, $S_{1,3}$ and $S_{1,6}$, are cross incompatible and are known to allow G allele expression. Compatible crosses are expected only in the $S_{1,3} \times S_{1,6}$ and reciprocal combinations.

Pollen Stigma		$S_{1,3}$			$S_{1,6}$		$?S_{3,x}$
		$G_{2,2}$	$G_{1,2}$		$G_{1,1}$	$G_{2,2}$	?
$S_{1,3}$	$G_{2,2}$	- - - -	- - - -		- - - -		- -
	$G_{1,2}$	- - - - - -	- - - - - -	sterile	- - - - - -	sterile	+ + +
$S_{1,6}$	$G_{1,1}$	+ + + +	+ + + +	male	- - - -	male	- -
	$G_{2,2}$	- -	+ +		- -		-
$?S_{3,x}$	?	- -	+ +		+ +		-

Figure 6 Mating matrix of family Verma 2, 1984, from a cross $7^{16}/78 S_{1,1} \times 7^{14}/78 S_{3,6}$

of *S* and *G*. Both parents of both families are heterozygous for *S* and *G* so all parental gametes are testable for recombination. The total number of recombinant gametes found is seventeen (9 out of 36 in fam 3/78 and 8 out of 32 in family 1/79. This gives a good fit to 25 per cent recombination. The results from family 3/78 are given in detail in table 3. The family alone is not sufficient evidence that linkage is involved but a similar analysis of family 1/29 of 32 gametes gives a $\chi^2[5]$ for free recombination of 10.594, $P=0.06$ and for 25 per cent recombination of $\chi^2[4]=4.4379$, $P=0.7$. Perhaps the most convincing evidence for linkage is that only one plant that required recombinant gametes from both parents was found. The expec-

tancy on free recombination is 4.25 and with 25 per cent recombination 1.06. The assumed linkage explains the unequal distribution of *G* genotypes found in families V1 and V2. We consider this demonstration of linkage to be of some importance because of the similarity with the linkage we have found in *B. campestris* (Zuberi and Lewis, 1988).

DISCUSSION

Evidence of gametophytic action of the *G* gene

Incompatibility in the Cruciferae has been repeatedly demonstrated to be controlled by a single gene which imprints its product on the pol-

Table 3 Genotypes of progeny obtained from part of family 3/78 (see fig. 2) with expected frequencies based upon 25 per cent recombination which was calculated from the 9 recombinant gametes found out of a total of 36 gametes used in the generation of eighteen plants

Parents $\frac{S_1 G_1}{S_3 G_2} \times \frac{S_2 G_2}{S_4 G_1}$							
	N obs	Exp free	Exp 25 per cent recom		N obs	Exp free	Exp 25 per cent recom
$\left. \begin{matrix} \frac{S_1 G_1}{S_2 G_2} \\ \frac{S_1 G_2}{S_2 G_1} \end{matrix} \right\}$	6	4.5	5.627	$\left. \begin{matrix} \frac{S_1 G_2}{S_4 G_1} \\ \frac{S_1 G_1}{S_4 G_2} \end{matrix} \right\}$	3	4.5	3.372
$\frac{S_1 G_1}{G_2 G_1}$	2	2.25	1.686	$\frac{S_1 G_1}{S_4 G_1}$	4	2.25	5.065
$\frac{S_1 G_2}{S_2 G_2}$	2	2.25	1.686	$\frac{S_1 G_2}{S_4 G_2}$	1	2.25	0.499

$\chi^2[5]$ on free recombination 3.35, $P=0.50$.
 $\chi^2[4]$ on 25 per cent recombination 0.97, $P=0.92$

Figure 7 (a) Compatible and incompatible pollen from a single anther in the cross $V_1^{11} \times V_1^8$ ($G_{1,1} \times G_{1,2}$) fig. 5. (b) Incompatible and compatible pollen on the same stigmatic papilla in $V_1^9 \times V_1^2$ ($G_{1,1} \times G_{1,2}$). (c) Stigma of diploid selfed with four incompatible pollen. (d) Stigma of tetraploid selfed with seven incompatible pollen grains and with thirteen faintly stained and with tubes entering the style.

len indirectly through the sporophyte. The genetics is extremely complex with multiple alleles and dominance and codominance and with individual alleles frequently expressing different dominance relationships in pollen and stigma. The initial discovery by Hughes and Babcock (1950), Gerstel (1950) and Bateman (1954) of this system and the subsequent work of others has been a major achievement which has resulted in consistent and highly predictable rules of segregation and mating patterns between pollen and stigma. Equally precise but less complex are the corresponding rules in the gametophytic system where the pollen is imprinted directly from the gene within it.

We have presented results in two species, *R. sativus* and *B. campestris* (Zuberi and Lewis, 1988), which confirm the rules of the *S* sporophytic gene, but also reveal a second gene, complementary to *S*, which obeys the rules of the gametophytic system. This is in contrast to the published reports of work which have contained odd exceptional results which have either not been explained or have

elicited a second gene either unspecified or sporophytic in action (Putrament, 1960, Kakizaki, 1930 and Kroh, 1956). Some of these results have been repeated in the same species but with different stocks and have not been confirmed. The reason for this is that the families tested were both too small and not numerous enough and often not completely analysed so that the few exceptional results which are rare features of the published work have been too scattered to be of any analytical value. We have used comparatively large families and have analysed most of them completely and the unexpected results repeated to consistency. But despite this only by analysing several large families was it possible to observe the gametophytic gene. For example, in the results presented here we should emphasise that out of six crossed and one bud-selfed families in the first generation only one crossed family 3/78 showed the segregation of the *G* gene. It was the complete analysis of this family that convinced us that a second incompatibility gene was segregating and the pattern of matings

produced, fig. 2, forced us to adopt a gametophytically active gene. No combination of sporophytic *S* alleles can generate three groups two of which are reciprocally cross compatible and a third group that is compatible as male onto these two and as female incompatible with all groups. The pattern is, however, characteristic of the two homozygotes and the one heterozygote derived from a plant or plants heterozygous for a gametophytic gene. Although we believe this gene is probably very similar to the gametophytic gene, traditionally designated *S* in the gametophytic system, we have designated it *G* where it could be confused with the *S* gene with which it is associated. It is worth noting that because *G* is manifest only in one *S* cross-incompatible group out of the nine present in family 3/78 and cannot by its action be expressed in *S* compatible combinations, a large family and complete analysis are essential to reveal the convincing pattern of mating shown in fig. 2. Another large family of 30 plants, 1/79, yielded a nine by seven matrix of cross-incompatible plants in which *G* segregation was expressed, but the pattern of matings did not contain the $G_{1,1}$ genotypes in the $S_{1,3}$ male partners of the cross; this is due partly to chance but mainly to linkage of *S* and *G* which is another factor which confirms the reality of the *G* gene but does contribute to the difficulty of the first analysis. The family 4/79 shows a similar incomplete pattern of *G* segrega-

tion. Other third generation families contribute in a negative way in that *G* segregation is not manifest either because of *G* homogeneity or the absence of a *G*-revealing combination of *S* alleles. The fourth generation families mainly contribute in showing that the *G* gene can be silent in one generation, for the reasons stated, and be manifest in the next generation.

The final confirmation came from fam. V_1 , grown from stored seed of parents of known *S* and *G* genotypes, which would give two *S* heterozygotes that would reveal *G* manifestation and thus give us the maximum *G* information. The results were as predicted with $G_{1,1}$, $G_{2,2}$ and $G_{1,2}$ showing their characteristic oppositional mating reactions.

Is the G gene common to the sporophytic system?

Although the published results on other species with the sporophytic system are excellent demonstrations of the *S* gene, they are inadequate for a complete demonstration of the presence and action of *G*. However, the number and nature of anomalous results can be usefully compared with our results. In table 4 we present our assessment of the number of anomalous results in all published work where the presentation provides an adequate basis for a count. These exceptions are all of the type + or + - in an otherwise incom-

Table 4 Exceptional compatible matings in expected incompatible mating combinations

	Incompatible* cross matings	Exceptional + and + - matings	per cent	Reference
Cruciferae				
<i>Brassica campestris</i>	334	22	6.6	Zuberi <i>et al.</i> 1981
<i>Brassica campestris</i>	390	72	18.4	Hinata, K. and Nishio, T. 1978
<i>Brassica oleracea</i>	585	28	4.8	Hinata, K. and Nishio, T. 1978
<i>Brassica oleracea</i>	128	10	7.8	Sampson, D. R. 1956
<i>Eruca sativa</i> ¹				Verma <i>et al.</i> 1977 Verma, S. C. 1986
<i>Iberis amara</i>		2		Bateman, A. J. 1954
<i>Raphanus raphanistrum</i>	76	7	9.2	Kroh 1956
<i>Raphanus raphanistrum</i>	—	5		Sampson, D. R. 1964
<i>Raphanus sativus</i>	16	1	6.2	Sampson, D. R. 1957
<i>Raphanus sativus</i>				Putrament, A. 1960
<i>Raphanus sativus</i> 3/78	486	22	4.5	Lewis, Verma, Zuberi 1988
<i>Raphanus sativus</i> 1/79	414	33	7.9	Lewis, Verma, Zuberi 1988
<i>Sinapis arvensis</i>		2		Ford, M. A. and Kay, Q. O. N. 1985
Compositae				
<i>Cosmos bipinnatus</i>		1		Crowe, L. K. 1954
<i>Crepis foetida</i>	383	27	7.0	Hughes, M. B. and Babcock, E. B. 1950
<i>Parthenium argentatum</i>				
Tab. 1	173	0	0	Gerstel, D. U. 1950
Tab. 4	33	2	6.0	

*Includes the exceptions. ¹ impossible to make a precise grouping. Blank spaces: data presentation not suitable for counting.

patible (–) *S* combination. Only three incompatible – anomalies in a compatible (+) combination can be found among all the reports. It is clear that the percentage of anomalies ranges from 4.5–9.2 per cent with one report on *B. campestris* as high as 18.4 per cent that could be due to an abnormally high proportion of *S* combinations that allow *G* gene expression. These figures are comparable with the 4.5 and 7.9 per cent obtained in two families of *Raphanus* and 6.6 per cent obtained in *B. campestris*. The most problematical species is *Eruca sativa* in which the number of mating groups was so large that a precise fit to any system of genes was not possible and in which three complementary sporophytic genes were considered to be a minimum requirement (Verma *et al.*, 1977; Lewis, 1977). These data were derived from seed-set as a criterion of compatibility which is not reliable and speedy enough to provide a fine analysis. Verma (unpublished) has subsequently obtained strong evidence for + and – pollen grains on the stigma from the same anther in some cross-pollinations in *Eruca* but a thorough re-examination is now required. The table includes three species with the sporophytic *S* gene but in the Compositae. Twenty-seven anomalies were reported in *Crepis*. Two anomalies were reported in *Parthenium* and one in *Cosmos*. This indication that the *G* gene is present in the Compositae is an important factor in assessing the importance and basic nature of the *G* gene in the sporophytic system.

G expression

One of the most obscure aspects of the *G* gene is its dependence on particular *S* allele combinations for its expression. Complete expression in *Raphanus* was found in only four *S* combinations; these are:

$S_{1,2} \times S_{1,4}$ in fam. 3/78, $S_{1,5} \times S_{1,3}$ in fam. 1/79

$S_{1,6} \times S_{1,3}$ in fams 4/79, V_1 and

V_2 , $S_{1,3} \times S_{1,6}$ fams V_1 & V_2

We have tried to relate the expression of *G* to the dominance relations of *S* alleles and to the particular *S* allele. There appears to be no correlation with dominance but there is some evidence for correlation with particular *S* alleles.

Modification of *S* dominance

Both in *Raphanus* and *Brassica* we have found differences in the dominance relations between the same pair of alleles. In *Raphanus* fam. 7/78 (fig.

3) there were ten plants that were $S_{1,3}$ and two $S_{1,3}$, i.e., S_3 in the same family was either recessive or co-dominant to S_1 in the pollen. In *Brassica* fam. 1/80 there were two plants of $S_{3,4}$ and five of $S_{3,4}$ which shows a difference both in pollen and stigma. In a Japanese cultivar of *R. sativus* Verma *et al.* (1986) have identified $S_{1,2}$, $S_{1,2}$, $S_{1,2}$, $S_{1,2}$ and $S_{1,2}$. Similar dominance differences have been found by Hinata and Nishio (1978). Sampson (1957, 1964), with *Brassica campestris* and *R. raphanistrum* found several differences in dominance and concluded that modifiers were acting in both pollen and stigma to alter the dominance of *S* alleles. Dominance modification, therefore, appears to be a common feature of the *S* system, and the clean-cut segregation of different types of dominance in a family supports a genetic origin. We do not think that the change of *S* dominance has a direct bearing on the expression of *G* but it is probable that the modifiers could be having their effect both on *S* and *G*. That dominance changes due to modifiers are common with the *S* gene is not surprising since the whole system of multiple alleles depends on a complex balance of linear and cyclical dominance and co-dominance in both pollen and style.

Molecular implications of the *G* gene

The oppositional action of *G*, and its complementary combination with the oppositional action of *S* must imply that both *G* and *S* are producing molecules capable of high specificity and recognition, properties that are usually associated with large proteins or glycoproteins. Attempts to identify the incompatibility protein in the pollen of the sporophytic system have failed although *S* proteins from the stigma were demonstrated (Nasrallah, 1974; Sedgley, 1974). In contrast *S* proteins have been readily demonstrated in the pollen of gametophytic systems in *Oenothera* (Lewis, 1952) and in *Petunia* (Linskens, 1960). The *S* protein in the Cruciferae has been assumed to be located in the pollen extine and synthesised in the tapetum (Heslop-Harrison 1968, Dickinson and Lewis, 1973). The tapetal origin has been seriously questioned and is not consistent with some observations (Wallace, 1979). The demonstration of the *G* gene and its action has convinced us that an important incompatibility protein is synthesised within the pollen grain and is for a brief period uncombined within the intine and, at pollen maturity, combines with the extine-held *S* protein. It might therefore be possible to extract three different proteins from each *S* allele, a *G* protein, an *S* protein, and a *GS*

protein. In the stigma the *G* and *S* products must similarly combine in order to give the *I* reaction which, as we have said, now depends upon allelic matching in both *G* and *S*.

It is probable that both products are synthesised in the stigma in the same cell, the papilla, but this is not certain. It is not impossible that the sequential timing of *S* translation followed by *G* translation in the pollen tissue is also followed in the stigma, either in the same cell or in different cells: the *S* gene might be translated in the basal cells of the stigmatic surface and the *G* gene in the papilla. With the *S* product diffusing into the papillae at the critical change from the bud stage to a mature stigma when the recognition system is finally established, it would appear that the *GS* product is the active recognition molecule which acts as a trigger to stop or start all the processes leading to full pollen germination penetration and tube growth. This is evident from the fact that we could not see, at least under the light microscope, any difference between the start of compatible growth given by *G* mismatching or *S* mismatching of alleles, although detailed E.M. and biochemical studies might reveal some difference.

The effect of polyploidy (see later) which causes a breakdown in the system in *G* hetero-allelic diploid pollen of *Raphanus*, is similar to its effect in the *S* gametophytic system in other plant families. This persuades us to think that the *G* gene is a relic of an old system which has been built upon and partly superseded by the *S* gene in the present sporophytic system, and has developed into the *S* gametophytic system in other plant families. The suggestion that the *G* gene is a forerunner of the gametophytic system may be confirmed or refuted by a comparison of the gene product and the DNA of the genes in the two systems.

Generation of new *S* alleles

The origin of the many *S* alleles in both the gametophytic and sporophytic system has long been a problem. Mutagenic treatment and spontaneous mutations using sensitive selection techniques on the pollen have not yielded any new fully active *S* alleles in the gametophytic system (Lewis, 1951; de Nettancourt, 1977). But new *S* alleles have been reported in the gametophytic system after inbreeding in *Trifolium pratense* (Denwood, 1963), *Lycopersicum peruvianum* (de Nettancourt *et al.* 1971) and *Nicotiana* (Pandey, 1970). No mutations in the sporophytic system have been reported where they would not be expected because the stylar sieving system for mutations does not work because of sporophytic control of the pollen. On the other hand none have been reported after inbreeding.

In our work on *B. campestris* (Zuberi and Lewis, 1988) we have no evidence for *S* gene changes except in dominance which is transitory and dependent upon the presence of a modifier gene. In *Raphanus* we have unexpectedly found a wave of *S* gene instability giving us three different alleles from the unstable *S*₁. The data are scattered throughout the families and are here brought together in table 5. The instability and generation of the new alleles *S*₅, *S*₆, and *S*_x all come from *S*₁ in the bud-selfed family 7/78. The original family contained three new alleles, one *S*₅ and two *S*₆. Three different *S*_{1,1} homozygotes were used as parents in five different cross families. Four of the families produced the new alleles *S*₅ and *S*_x, one family, the only one from plant (4), produced only *S*₁ and no new alleles.

Although the families do not show the same *S*_{1,1} plant used as a male and female, the results already show that the mutational change can occur

Table 5 Mutational changes arising from *S*₁ in the bud-selfed family 7/78, and in other families derived from plants of fam. 7/78. The numbers in brackets after the *S*_{1,1} parents are the individual numbers given to the plants

			<i>S</i> ₁	<i>S</i> ₅	<i>S</i> ₆	<i>S</i> _x	
7/78	<i>S</i> _{1,3} bud-selfed	<i>S</i> ₁ →	21	1	2	0	
2/79	<i>S</i> _{3,5} × <i>S</i> _{1,1(22)}	<i>S</i> ₁ →	2	2	0	9	<i>S</i> _{1,1} as ♂
5/79	<i>S</i> _{1,1(16)} × <i>S</i> _{3,3}	<i>S</i> ₁ →	0	5	0	6	<i>S</i> _{1,1} as ♀
6/79	<i>S</i> _{1,1(16)} × <i>S</i> _{3,3}	<i>S</i> ₁ →	0	6	0	6	<i>S</i> _{1,1} as ♀
V ₁	<i>S</i> _{3,6} × <i>S</i> _{1,1(4)}	<i>S</i> ₁ →	11	0	0	0	<i>S</i> _{1,1} as ♂
V ₂	<i>S</i> _{1,1(16)} × <i>S</i> _{3,6}	<i>S</i> ₁ →	9	0	0	1	<i>S</i> _{1,1} as ♀
4/79	<i>S</i> _{3,6} × <i>S</i> _{3,5}	<i>S</i> ₅ →	16	0	0	0	<i>S</i> ₅ as ♂

through the egg cell or through the pollen. This is different from the results of de Nettancourt with *Lycopersicum* where the mutational change was first manifest in the stigma in the form of self-compatibility and transferred to the next generation as a fully operative new *S* allele.

The Radish results show that the change occurs in one generational step, occurs in both male and female tissue, and different *S*₁ alleles differ in their mutability. Finally the mutational change in some cases is reversible as shown by fam. 4/79 where an *S*₅ which originated from *S*₁ in the previous generation mutates back to *S*₁ in all the tested plants.

We will not speculate on the mechanism of the changes but only note the fact that *S*₁ homozygotes are the richest source of mass change rules out classical crossing-over or gene conversion as a possible mechanism.

Effect of polyploidy

Tetraploidy causes a partial or complete loss of self-incompatibility in the one-gene gametophytic system in the dicotyledons. It has been generally accepted that this weakening effect of polyploidy is confined to the interaction of different *S* alleles in heteroallelic diploid pollen grains (Lewis, 1954). Since Howard's (1942) report it has been generally accepted that the sporophytic system in the Cruciferae and Compositae is not affected by polyploidy. This has been logically explained on the basis that the incompatibility gene in the sporophytic system is not translated in the pollen which is usually haploid, but in diploid tissue of the germ line or even in the normally polyploid cells of the tapetum. This conclusion about the negative effect of polyploidy was based upon the absence of seed after selfing and on the logical reason for its absence.

The effect of tetraploidy is also absent in the one- and two-gene gametophytic systems in the monocotyledons. Fearon *et al.* (1984) summarised the situation thus: "The disruptive effect of polyploidy on self-incompatibility appears to be confined to dicotyledons with one-locus gametophytic systems of incompatibility".

The photograph (fig. 7) is an induced tetraploid of *R. sativus* var. Scarlet-Globe taken by D. Lewis in 1943. It was not published for the lack of an explanation and was forgotten until rediscovered by accident when writing this paper. The photograph shows thirteen faintly stained shrunken pollen grains with tubes and seven fully stained turgid pollen grains which are incompatible. This is a

good fit to the expected 2:1 output of heterozygotes to homozygotes from a tetraploid duplex heterozygote. The only note made at the time is in the Annual Report of the John Innes Horticultural Institution (Crane, 1943). "Dr Lewis has found that in the diploid (*Raphanus*) self-pollen germinates very poorly and no tubes penetrate the stigma. In the tetraploids, although self-incompatible, self-pollen germinates and the tubes penetrate some distance, about 3 mm, down the style." This partial disruption without giving full self-compatibility is typical of several species with the one-gene gametophytic system in dicotyledons. We venture to suggest that this disruption is due to the interaction of *G*₁ and *G*₂ in diploid pollen grains. If this is so we would expect about half of the plants tested not to show this effect because half should be homozygotes *G*_{1,1} and *G*_{2,2} if our suggestion that the *G* gene exists in only two alleles is correct. Even if this connection with the effect of tetraploidy turns out to be spurious, it does not invalidate our basic hypothesis, but if it is supported then it is important in the speculation about *G* as an ancestral incompatibility gene. It would confine its ancestry to the dicotyledons.

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