Number, frequency & dominance relationships of S-alleles in diploid *Ipomoea trifida*

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Self-incompatibility genotypes of 224 plants of *Ipomoea trifida* from six populations in Central America have been determined by genetic analysis of segregants in F₁ families derived from cross-pollinations with the most recessive homozygote. A total of 49 different S-alleles was identified in these populations. From analyses of S-allelic interactions in heterozygous plants which were generated from cross-pollinations between plants possessing different S-alleles, a linear dominance hierarchy with six levels has been established among 28 S-alleles in both pollen and stigma. Codominance of alleles occurred more frequently in the stigma (9.2 per cent) than in the pollen (4.9 per cent). Nonlinear dominance relationships were rarely observed. Unequal frequencies of S-alleles have been found in all populations examined, the most common S-allele being, as expected, the most recessive. This suggests that recessive S-alleles are widely distributed throughout Central America. The diversity of the multiple S-alleles observed in the present study also suggests that the southern area of Mexico to Guatemala is a centre of genetic variation in diploid *I. trifida*.

Keywords: allele frequency, allelic interaction, dominance relationship, *Ipomoea trifida*, S-alleles, sporophytic self-incompatibility.

Introduction

Species of the genus *Ipomoea* (Convolvulaceae) possess a sporophytic system of self-incompatibility that is controlled by a single locus with multiple S-alleles (Kowyama *et al.*, 1980). One of the diploid wild species, *I. trifida*, is closely related to the sweet potato, *I. batatas*, and has been used as a genetic resource for sweet potato improvement (Kobayasi & Miyazaki, 1976; Shiotani & Kawase, 1987; Freyre *et al.*, 1991; Orjeda *et al.*, 1991). *I. trifida* is an insect-pollinated perennial which propagates predominantly by seed. It is a weed native to Central America and is distributed from Mexico to Colombia and Venezuela (Austin, 1978; Shiotani *et al.*, 1990). In contrast to the expression of sporophytic self-incompatibility in *Brassica*, *Ipomoea* plants are strongly self-incompatible, there being a complete failure of pollen germination on the stigma surface after an incompatible mating. In addition, no seed is set after bud pollination or physical or biochemical treatments of the flower (Fujise, 1964; Kowyama *et al.*, 1980).

Studies have been made on the number and frequency of S-alleles in wild populations or cultivated varieties of sporophytic self-incompatible species in the Brassicaceae (Bateman, 1954; Sampson, 1967; Ockendon, 1974, 1980, 1982; Stevens & Kay, 1989; Nou *et al.*, 1991, 1993a,b) and of the gametophytic self-incompatible species, *Oenothera organensis* (Emerson, 1939), *Trifolium repens* (Atwood, 1944), *Trifolium pratense* (Williams & Williams, 1947) and *Papaver rhoeas* (Campbell & Lawrence, 1981; Lawrence & O'Donnell, 1981; O'Donnell & Lawrence, 1984). These studies have shown that populations contain nine to 35 different S-alleles. Despite these extensive studies, information about the population genetics of self-incompatibility is still limited to a relatively small number of taxa. Because self-incompatibility is known in at least 71 families and more than 250 genera of flowering plants (Nettancourt, 1977), information from a wider range of taxa would be useful, both in terms of understanding the evolution of self-incompatibility and also its molecular basis.

The chief purpose of the investigation described here is to determine the numbers and frequencies of S-alleles in natural populations of *I. trifida* and the dominance relationships between these alleles.

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Materials and methods

Plant materials

Seed was randomly collected in natural populations from the six localities shown in Fig. 1. The accessions numbered M81 to M84 (Mexico) and G81 (Guatemala) were collected by Shiotani in 1981 and C80 (Colombia) by Dr M. Kobayasi in 1980. Populations M81 and C80 are about 3300 km apart. Between 31 and 41 plants were raised from the seed of each population (Table 2). All of the plants, grown in pots, were treated with short days (10 h) for about 3 weeks to promote flower bud formation before being transferred to a glasshouse.

The correct name of the present species has been the subject of some controversy. Although we referred to it as *I. leucantha* in our previous paper (Kowyama *et al.*, 1980), the species name *I. trifida* is used here following the proposals of Austin (1983) and Shiotani & Kawase (1987).

Determination of *S*-genotypes

All the plants of each accession were classified into several cross-incompatibility groups based on their pollen–stigma compatibilities after diallel test pollination. The test pollinations were classified as compatible or incompatible by examining the germination of pollen on stigmas (see Kowyama *et al.*, 1980 for details). Because the presence of recessive alleles is masked by that of dominant alleles in both stigma and pollen of almost all heterozygotes, all of the original plants were crossed with the most recessive homozygote, S_3S_3 , except for 21 plants which were S_3 -homozygotes. The recessiveness of the S_3 allele had been confirmed by previous work (Kowyama *et al.*, 1980). The resulting F_1 families were then analysed for segregation of the *S*-alleles, eight F_1 plants being raised in each family. To examine the dominance relationships of the *S*-alleles, original plants from different cross-incompatibility groups were cross-pollinated with each other and segregations of *S*-phenotypes were examined in their progenies. Ten to 35 plants, depending on the expected segregation ratio, were raised in each cross. In all, 3067 plants in 174 cross combinations were analysed.

Results

Number of *S*-alleles

As a result of the diallel test pollinations within each population, plants were classified into five (M81) to 16 (M84) incompatibility groups. Figure 2 shows the

Fig. 1. Location of the six populations of *Ipomoea trifida* in Central America. Seed collection sites were Berra de Navidad for M81, Acapulco for M82, Veracruz for M83, Salina Cruz for M84, El Progreso for G81 and Santa Malta for C80.

result of diallel pollination in population G81, in which eight incompatibility groups were detected, so that at least eight *S*-alleles of different *S*-phenotype are present in this population. The plants of the other populations, M82, M83 and C80, were classified into eight, 15 and six incompatibility groups, respectively, giving a total of 58 groups detected in 224 plants from the six populations. Fifty-eight plants, one from each incompatibility group, were reciprocally cross-pollinated; 18 groups were found to occur more than once, i.e. the 58 plants fell into 40 different groups of *S*-phenotype, named S_1 – S_{40} .

F_1 progenies from crosses between the above-mentioned original plants and S_3 homozygotes were tested for their pollen–stigma compatibility, using as testers the 40 plants representing the different *S*-alleles. None of the 203 F_1 families showed a segregation of *S*-phenotype that deviated significantly from the expected ratio of 1:1. Table 1 shows the data obtained from the F_1 families of G81. This analysis revealed the presence of an additional nine *S*-alleles, named S_{41} – S_{49} , which were masked by dominant *S*-alleles in both pollen and stigma of the original heterozygotes. The 49 *S*-alleles detected were confirmed to be different from each other in a full diallel pollination.

As a result of these analyses, each of the *S*-alleles present in the original plants was identified. Table 2 shows the number of *S*-alleles detected in each population and their overall frequencies combined over the six populations. Stevens & Kay (1989) measured the thoroughness of their survey of *S*-alleles by calculating a repeatability statistic, R (Campbell & Lawrence, 1981), modified for a sporophytic system. In brief, $R = 1 - [(n-2)/(m-2)]$, where n is the number of

S- Pheno- Type	<div>♂ ♀</div>	<div>♂ ♀</div>	G81- 1	G81- 2	G81- 3	G81- 5	G81- 7	G81- 8	G81- 9	G81-13	G81-14	G81-16	G81-17	G81-20	G81-27	G81- 6	G81-10	G81-11	G81-15	G81-18	G81-19	G81-26	G81-30	G81-33	G81-38	G81-12	G81-21	G81-24	G81-25	G81-28	G81-32	G81-36	G81-39	G81-29	G81-37	G81-40	G81- 4	G81-23	G81-34	G81-22	G81-31	G81-35	
			G81-1	G81-2	G81-3	G81-5	G81-7	G81-8	G81-9	G81-13	G81-14	G81-16	G81-17	G81-20	G81-27	G81-6	G81-10	G81-11	G81-15	G81-18	G81-19	G81-26	G81-30	G81-33	G81-38	G81-12	G81-21	G81-24	G81-25	G81-28	G81-32	G81-36	G81-39	G81-29	G81-37	G81-40	G81-4	G81-23	G81-34	G81-22	G81-31	G81-35	
S37	G81- 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	G81- 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	G81- 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	G81- 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	G81- 7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	G81- 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	G81- 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	G81-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	G81-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	G81-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	G81-17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G81-20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81- 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81- 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
G81-35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Fig. 2. Diallel test pollinations of 40 plants from population G81. + and - indicate compatible and incompatible pollinations, respectively.

different alleles found in a sample of m alleles examined. The calculated values of R range from 0.76 to 0.96 and indicate a high degree of thoroughness of our study (Table 2). The populations located in a central part of Central America appeared to contain the largest number of alleles, e.g. 19 and 21 S -alleles in populations M83 and G81, respectively. The peripheral populations M81 and C80, on the other hand, appeared to contain only five and six S -alleles, respectively. The S_3 allele was found in all populations examined and the S_{10} allele in four populations. These two S -alleles appear to be widely distributed throughout Central America.

Interaction of S -alleles

An example of the results obtained from reciprocal pollinations between heterozygotes and tester plants is presented in Table 3, from which the dominance

relationships between the six S -alleles can be deduced as follows:

$$S_5 > (S_1 = S_6), (S_1 = S_{16}), (S_1 > S_{19}), (S_{16} = S_6) > S_2$$

in the stigma and

$$S_5 > (S_1 = S_6), (S_1 = S_{16}), (S_1 = S_{19}), (S_{16} > S_6) > S_2$$

in the pollen, where $S_1 = S_{16}$, for example, indicates codominance between these two S -alleles in the heterozygote. Because S_1 , S_6 , S_{16} and S_{19} are codominant in either the stigma or the pollen of heterozygotes, these four S -alleles can be grouped in the same level of dominance.

From test pollinations using other heterozygotes, dominance relationships of 28 S -alleles were classified into six major levels of dominance (Fig. 3). All S -alleles in level B were recessive to alleles in level A and dominant to those in levels C-F. The six S -alleles in level A were the most dominant and S_3 in level F was

Table 1 Segregations and interactions of *S*-alleles in *F*₁ families derived from crosses between G81 plants and *S*₃ homozygotes

<i>F</i> ₁ Family	Segregated		<i>P</i> *	<i>S</i> -allele interaction in pollen**
	Phenotype	Ratio		
G8101	<i>S</i> ₃₇ : <i>S</i> ₄₂	4:4	1.00	<i>S</i> ₃₇ > <i>S</i> ₄₂
G8102	<i>S</i> ₃₇ : <i>S</i> ₉	7:1	0.07	<i>S</i> ₃₇ > <i>S</i> ₉ ***
G8103	<i>S</i> ₃₇ : <i>S</i> ₃	6:2	0.29	<i>S</i> ₃₇ > <i>S</i> ₃
G8104	<i>S</i> ₃₉ : <i>S</i> ₄₁	1:6	0.13	<i>S</i> ₃₉ > <i>S</i> ₄₁
G8105	<i>S</i> ₃₇ : <i>S</i> ₄₁	2:6	0.29	<i>S</i> ₃₇ > <i>S</i> ₄₁
G8106	<i>S</i> ₂₈ : <i>S</i> ₄₃	4:4	1.00	<i>S</i> ₂₈ > <i>S</i> ₄₃
G8107	<i>S</i> ₃₇ : <i>S</i> ₂₅	5:3	0.73	<i>S</i> ₃₇ > <i>S</i> ₂₅
G8108	<i>S</i> ₃₇ : <i>S</i> ₂₅	6:2	0.29	<i>S</i> ₃₇ > <i>S</i> ₂₅
G8109	<i>S</i> ₃₇ : <i>S</i> ₃₉	5:3	0.73	<i>S</i> ₃₇ > <i>S</i> ₃₉
G8110	<i>S</i> ₂₈ : <i>S</i> ₄₃	4:4	1.00	<i>S</i> ₂₈ > <i>S</i> ₄₃
G8111	<i>S</i> ₂₈ : <i>S</i> ₄₄	4:4	1.00	<i>S</i> ₂₈ > <i>S</i> ₄₄
G8112	<i>S</i> ₃₈ : <i>S</i> ₂₇	1:7	0.07	<i>S</i> ₃₈ > <i>S</i> ₂₇
G8113	<i>S</i> ₃₇ : <i>S</i> ₅	5:3	0.73	<i>S</i> ₃₇ > <i>S</i> ₅
G8114	<i>S</i> ₃₇ : <i>S</i> ₅	4:4	1.00	<i>S</i> ₃₇ > <i>S</i> ₅
G8115	<i>S</i> ₂₈ : <i>S</i> ₄₀	6:1	0.13	<i>S</i> ₂₈ > <i>S</i> ₄₀
G8116	<i>S</i> ₃₇ : <i>S</i> ₄₅	4:4	1.00	<i>S</i> ₃₇ > <i>S</i> ₄₅
G8117	<i>S</i> ₃₇ : <i>S</i> ₄₁	5:3	0.73	<i>S</i> ₃₇ > <i>S</i> ₄₁
G8118	<i>S</i> ₂₈ : <i>S</i> ₂₅	2:6	0.29	<i>S</i> ₂₈ > <i>S</i> ₂₅
G8119	<i>S</i> ₂₈ : <i>S</i> ₃	4:4	1.00	<i>S</i> ₂₈ > <i>S</i> ₃
G8120	<i>S</i> ₃₇ : <i>S</i> ₄₆	6:2	0.29	<i>S</i> ₃₇ > <i>S</i> ₄₆
G8121	<i>S</i> ₃₈ : <i>S</i> ₂₇	1:7	0.07	<i>S</i> ₃₈ > <i>S</i> ₂₇
G8122	<i>S</i> ₁₉ : <i>S</i> ₃	4:4	1.00	<i>S</i> ₁₉ > <i>S</i> ₃
G8123	<i>S</i> ₃₉ : <i>S</i> ₂₅	2:6	0.29	<i>S</i> ₃₉ > <i>S</i> ₂₅
G8124	<i>S</i> ₃₈ : <i>S</i> ₄₀	2:6	0.29	<i>S</i> ₃₈ > <i>S</i> ₄₀
G8125	<i>S</i> ₃₈ : <i>S</i> ₃	4:4	1.00	<i>S</i> ₃₈ > <i>S</i> ₃
G8126	<i>S</i> ₂₈ : <i>S</i> ₄₇	4:4	1.00	<i>S</i> ₂₈ > <i>S</i> ₄₇
G8127	<i>S</i> ₃₇ : <i>S</i> ₃₂	6:2	0.29	<i>S</i> ₃₇ > <i>S</i> ₃₂
G8128	<i>S</i> ₃₈ : <i>S</i> ₄₁	6:2	0.29	<i>S</i> ₃₈ > <i>S</i> ₄₁
G8129	<i>S</i> ₅ : <i>S</i> ₃₈	4:4	1.00	<i>S</i> ₅ > <i>S</i> ₃₈ ***
G8130	<i>S</i> ₂₈ : <i>S</i> ₃	6:2	0.29	<i>S</i> ₂₈ > <i>S</i> ₃
G8131	<i>S</i> ₄₀ : <i>S</i> ₃	3:4	1.00	<i>S</i> ₄₀ > <i>S</i> ₃
G8132	<i>S</i> ₃₈ : <i>S</i> ₂₇	2:6	0.29	<i>S</i> ₃₈ > <i>S</i> ₂₇
G8133	<i>S</i> ₂₈ : <i>S</i> ₃₈	5:3	0.73	<i>S</i> ₂₈ > <i>S</i> ₃₈
G8134	<i>S</i> ₃₉ : <i>S</i> ₄₀	3:4	1.00	<i>S</i> ₃₉ > <i>S</i> ₄₀
G8136	<i>S</i> ₃₈ : <i>S</i> ₁₉	6:2	0.29	<i>S</i> ₃₈ > <i>S</i> ₁₉
G8137	<i>S</i> ₅ : <i>S</i> ₃	5:3	0.73	<i>S</i> ₅ > <i>S</i> ₃
G8138	<i>S</i> ₂₈ : <i>S</i> ₃₈	2:5	0.45	<i>S</i> ₂₈ > <i>S</i> ₃₈
G8139	<i>S</i> ₃₈ : <i>S</i> ₃	4:4	1.00	<i>S</i> ₃₈ > <i>S</i> ₃
G8140	<i>S</i> ₅ : <i>S</i> ₂₄	4:2	0.69	<i>S</i> ₅ = <i>S</i> ₂₄

*Binomial probability of obtaining a deviation in either direction from the expected ratio of 1:1 as great as, or greater than, that observed.

***S*_a > *S*_b: *S*_a dominant to *S*_b; *S*_a = *S*_b: codominant.

***These two marked pairs of *S*-alleles showed codominance in the stigma, while the other pairs shown in this table showed identical interactions in both pollen and stigma.

the most recessive allele. Nonlinear dominance relationships among the 28 *S*-alleles were found in only four sets of *S*-alleles, (*S*₅, *S*₉, *S*₃₇), (*S*₅, *S*₁₂, *S*₁₅) and (*S*₁, *S*₆, *S*₁₆) in the stigma and (*S*₁, *S*₆, *S*₁₆) in the pollen. Codominance relationships between two alleles were more frequently observed in the stigma (30/34) than in the pollen (16/34). As expected, the recessive *S*-alleles, *S*₃ and *S*₁₀, occur at higher frequencies, 29.2 per cent and 8.7 per cent, respectively, than the more dominant alleles in natural populations (Table 2), the latter occurring with a considerable variation in frequency ranging from 0.2 per cent to 5.4 per cent.

Discussion

There are four points worth making about these results. Firstly, sporophytic self-incompatibility in *I. trifida* is a stable genetic system because no self-compatible plants were found in any of the six natural populations examined and the strength of the self-incompatibility reaction was not diminished in any combination of *S*-alleles. In *Brassica campestris*, on the other hand, which has the same genetic system of self-incompatibility, self-compatible plants have been found in natural populations with a frequency of 4.5 to 13.8 per cent, their occurrence being attributed to the effects of environment and/or genetic background other than the *S*-locus (Nou *et al.*, 1993b). A wide range of variation in *S*-allele activity and mutual weakening of *S*-alleles in the self-incompatibility reaction have also been reported in other species of *Brassica* (Ockendon & Currah, 1979; Wallace, 1979).

Secondly, a large number of *S*-alleles participates in the insect-mediated pollination of the diploid *I. trifida*, as a total of 49 different *S*-alleles was found in 224 plants from six natural populations (Table 2). A similar number of *S*-alleles appears to occur in other species, 35 having been identified in *Sinapis arvensis* (Stevens & Kay, 1989), 45 in *Papaver rhoeas* (Lawrence *et al.*, 1993) and 49 in *Brassica oleracea* (Ockendon, 1985). There was, however, considerable variation in the number of *S*-alleles in populations of *I. trifida*, more alleles being found in populations located in the central part of Central America than in those from the peripheral regions. Diploid *I. trifida* is widely distributed in the circum-Caribbean region from Mexico to Venezuela and this area is considered to be the centre of origin of this taxon (Austin, 1978). The present study suggests that the southern area of Mexico to Guatemala is the centre of genetic variation of *I. trifida*.

Thirdly, dominance relationships between *S*-alleles fall into a clear hierarchy between the levels of which there is linear dominance in both the pollen and the

Table 2 Number and frequencies of *S*-alleles among 224 plants from six different populations

Population	M81	M82	M83	M84	G81	C80	Total	
No. of plants tested	35	39	38	41	40	31	224	
No. of <i>S</i> -alleles (<i>m</i>)	70	78	76	82	80	62	448	
No. of different <i>S</i> -alleles detected (<i>n</i>)	5	15	19	16	21	6	49	<i>S</i> -allele frequency (%)
Repeatability (<i>R</i>)	0.96	0.83	0.77	0.83	0.76	0.93		
<i>S</i> ₁				5			5	1.1
<i>S</i> ₂				1			1	0.2
<i>S</i> ₃	34	6	28	30	10	23	131	29.2
<i>S</i> ₄			3	3	2		8	1.8
<i>S</i> ₅		13		6	5		24	5.4
<i>S</i> ₆				2			2	0.4
<i>S</i> ₇				1			1	0.2
<i>S</i> ₈	19	1	1				21	4.7
<i>S</i> ₉	6	14			1		21	4.7
<i>S</i> ₁₀	10		2	7		20	39	8.7
<i>S</i> ₁₁	1						1	0.2
<i>S</i> ₁₂		3		7		14	24	5.4
<i>S</i> ₁₃		1				2	3	0.7
<i>S</i> ₁₄						1	1	0.2
<i>S</i> ₁₅						2	2	0.4
<i>S</i> ₁₆				11			11	2.5
<i>S</i> ₁₇				3			3	0.7
<i>S</i> ₁₈				2			2	0.4
<i>S</i> ₁₉				1	2		3	0.7
<i>S</i> ₂₀				1			1	0.2
<i>S</i> ₂₁				1			1	0.2
<i>S</i> ₂₂			11	1			12	2.7
<i>S</i> ₂₃		19	1				20	4.5
<i>S</i> ₂₄		3			1		4	0.9
<i>S</i> ₂₅		3	2		4		9	2.0
<i>S</i> ₂₆		1	2				3	0.7
<i>S</i> ₂₇		1			2		3	0.7
<i>S</i> ₂₈			1		10		11	2.5
<i>S</i> ₂₉			4				4	0.9
<i>S</i> ₃₀			5				5	1.1
<i>S</i> ₃₁		1	4				5	1.1
<i>S</i> ₃₂			4		1		5	1.1
<i>S</i> ₃₃			1				1	0.2
<i>S</i> ₃₄			3				3	0.7
<i>S</i> ₃₅			1				1	0.2
<i>S</i> ₃₆			1				1	0.2
<i>S</i> ₃₇					13		13	2.9
<i>S</i> ₃₈					11		11	2.5
<i>S</i> ₃₉					4		4	0.9
<i>S</i> ₄₀		1			4		5	1.1
<i>S</i> ₄₁		10			4		14	3.1
<i>S</i> ₄₂					1		1	0.2
<i>S</i> ₄₃					1		1	0.2
<i>S</i> ₄₄			1		1		2	0.4
<i>S</i> ₄₅					1		1	0.2
<i>S</i> ₄₆					1		1	0.2
<i>S</i> ₄₇					1		1	0.2
<i>S</i> ₄₈		1					1	0.2
<i>S</i> ₄₉			1				1	0.2

Table 3. *S*-allele interactions in stigma and pollen determined by test pollinations

Female plant	Tester plant used as male						<i>S</i> -allele interaction in stigma
	S_1S_3	S_2S_3	S_3S_5	S_3S_6	S_3S_{16}	S_3S_{19}	
S_1S_2	—	+	+	+	+	+	$S_1 > S_2$
S_1S_5	+	+	—	+	+	+	$S_1 < S_5$
S_1S_6	—	+	+	+	+	+	$S_1 > S_6$
S_1S_{16}	—	+	+	+	—	+	$S_1 = S_{16}$
S_1S_{19}	—	+	+	+	+	+	$S_1 > S_{19}$
S_2S_5	+	+	—	+	+	+	$S_2 < S_5$
S_2S_6	+	+	+	—	+	+	$S_2 < S_6$
S_2S_{16}	+	+	+	+	—	+	$S_2 < S_{16}$
S_2S_{19}	+	+	+	+	+	—	$S_2 < S_{19}$
S_5S_6	+	+	—	+	+	+	$S_5 > S_6$
S_5S_{16}	+	+	—	+	+	+	$S_5 > S_{16}$
S_5S_{19}	+	+	—	+	+	+	$S_5 > S_{19}$
S_6S_{16}	+	+	+	—	—	+	$S_6 = S_{16}$

Male plant	Tester plant used as female						<i>S</i> -allele interaction in pollen
	S_1S_3	S_2S_3	S_3S_5	S_3S_6	S_3S_{16}	S_3S_{19}	
S_1S_2	—	+	+	+	+	+	$S_1 > S_2$
S_1S_5	+	+	—	+	+	+	$S_1 < S_5$
S_1S_6	—	+	+	—	+	+	$S_1 = S_6$
S_1S_{16}	—	+	+	+	—	+	$S_1 = S_{16}$
S_1S_{19}	—	+	+	+	+	—	$S_1 = S_{19}$
S_2S_5	+	+	—	+	+	+	$S_2 < S_5$
S_2S_6	+	+	+	—	+	+	$S_2 < S_6$
S_2S_{16}	+	+	+	+	—	+	$S_2 < S_{16}$
S_2S_{19}	+	+	+	+	+	—	$S_2 < S_{19}$
S_5S_6	+	+	—	+	+	+	$S_5 > S_6$
S_5S_{16}	+	+	—	+	+	+	$S_5 > S_{16}$
S_5S_{19}	+	+	—	+	+	+	$S_5 > S_{19}$
S_6S_{16}	+	+	+	+	—	+	$S_6 < S_{16}$

+, compatible; —, incompatible; $S_a > S_b$, S_a dominant to S_b ; $S_a = S_b$, codominant.

stigma (Fig. 3). In contrast, very complex interactions of *S*-alleles have been reported in *Brassica* (Thompson & Taylor, 1966; Ockendon, 1975; Wallace, 1979). The difference in the pattern of allelic interactions between *Ipomoea* and *Brassica* could be due to the different strengths of *S*-allele activity in these species. The incompatibility reaction between pollen and stigma in *Ipomoea* is a qualitative character because pollen germination occurs on the stigma surface only after compatible pollination. In *Brassica*, on the other hand, the reaction is measured as a quantitative character based on scores of pollen tube penetration into stigma papillae. The degree of dominance between *S*-alleles of *Brassica* is continuous, ranging from weak partial dominance to complete dominance (Ockendon, 1975; Wallace, 1979). It is possible to speculate that the

regular dominance hierarchy observed in *I. trifida* is a reflection of the sequential generation of new *S*-alleles, from recessive to more dominant ones, in the evolution of self-incompatibility in this species, a speculation that could be tested from a molecular biological point of view.

In general, allelic interactions in species with sporophytic systems of self-incompatibility are often different in the pollen and the stigma. The sexual organ $\times$ *S*-allele interactions can be classified into four types (Wallace, 1979), as shown in Table 4. Of 326 allelic pairs that were examined between 28 *S*-alleles (Fig. 3), 94 per cent are of types I and IV and the remainder are of types II and III. Codominance occurs more frequently in the stigma than in the pollen. A similar trend to this has been observed in Brassicaceae

Fig. 3. Allelic interactions and dominance hierarchy among 28 *S*-alleles. $A \rightarrow B$ indicates *A* is dominant to *B*, and $A = B$ that *A* and *B* are codominant.

Table 4 Types of *S*-allele interactions in pollen and stigma

Type	Pollen	Stigma	No. of <i>S</i> -allele pairs (%)
I	$S_a > S_b$	$S_a > S_b$	293 (89.9)
II	$S_a > S_b$	$S_a = S_b$	17 (5.2)
III	$S_a = S_b$	$S_a > S_b$	3 (0.9)
IV	$S_a = S_b$	$S_a = S_b$	13 (4.0)

$S_a > S_b$, S_a dominant to S_b ; $S_a = S_b$, codominant.

(Sampson, 1964; Stevens & Kay, 1989). These features of *S*-allelic interactions provide a model system for investigating the molecular basis of gene expression and dominance relationships between alleles.

Finally, our data have made it clear that frequencies of *S*-alleles are unequal in natural populations, and that, as expected, the most recessive *S*-allele (S_3) occurs with a high frequency even in geographically remote populations (Table 2). Unequal *S*-allele frequencies have also been reported in Brassicaceae (Ockendon, 1974, 1982; Stevens & Kay, 1989). Imrie *et al.* (1972) studied by computer simulation the equilibrium allele frequencies in a sporophytic self-incompatibility system with three or six *S*-alleles and found that, as expected, equilibrium frequencies were dependent on the level of dominance, with alleles increasing in frequency as their degree of dominance decreased. This dominance dependency may be explained in terms of the number of successful matings, i.e. the

more recessive *S*-alleles participate in more matings because of their masking by dominant ones. Theoretical frequencies of *S*-alleles in equilibrium populations containing more alleles could be found from a computer simulation of the polymorphism, taking into account various types of allelic interactions in the dominance hierarchy.

Acknowledgements

We thank Dr M. Kobayasi for providing seeds collected in Colombia. We are grateful to Dr M. J. Lawrence for helpful comments and for improving the manuscript. The present study was supported by a Grant-in-Aid for Special Research on the Cellular and Molecular Basis for Reproductive Processes in Plants (1989–91) from the Ministry of Education, Science and Culture, Japan.

References

- ATWOOD, S. S. 1944. Oppositional alleles in natural populations of *Trifolium repens*. *Genetics*, **29**, 428–435.
- AUSTIN, D. F. 1978. The *Ipomoea batatas* complex. I. Taxonomy. *Bull. Torrey Bot. Club*, **105**, 114–120.
- AUSTIN, D. F. 1983. Variability in sweet potatoes in America. *Proc. Am. Soc. Hort. Sci. Trop. Region*, **27**(B), 15–26.
- BATEMAN, A. J. 1954. Self-incompatibility systems in angiosperms. II. *Iberis amara*. *Heredity*, **8**, 305–332.
- CAMPBELL, J. M. AND LAWRENCE, M. J. 1981. The population genetics of the self-incompatibility polymorphism in *Papaver rhoeas*. I. The number and distribution of *S*-alleles in families from three localities. *Heredity*, **46**, 69–79.
- EMERSON, S. 1939. A preliminary survey of the *Oenothera organensis* population. *Genetics*, **24**, 524–537.
- FREYRE, R., IWANAGA, M. AND ORJEDA, G. 1991. Use of *Ipomoea trifida* (HBK.) G. Don germ plasm for sweet-potato improvement. 2. Fertility of synthetic hexaploids and triploids with $2n$ gametes of *I. trifida*, and their interspecific crossability with sweet potato. *Genome*, **34**, 209–214.
- FUJISE, K. 1964. Studies on flowering, seed setting, and self- and cross-incompatibility in the varieties of sweet potato. *Bull. Kyusyu Agr. Exp. Stn.*, **9**, 123–246 (in Japanese).
- IMRIE, B. C., KIRKMAN, C. J. AND ROSS, D. R. 1972. Computer simulation of a sporophytic self-incompatibility breeding system. *Aust. J. Biol. Sci.*, **25**, 343–349.
- KOBAYASI, M. AND MIYAZAKI, T. 1976. Sweet potato breeding using wild related species. *Proceedings of the 4th International Symposium on Tropical Crops*, pp.53–57.
- KOWYAMA, Y., SHIMANO, N. AND KAWASE, T. 1980. Genetic analysis of incompatibility in the diploid *Ipomoea* species closely related to the sweet potato. *Theor. Appl. Genet.*, **58**, 149–155.
- LAWRENCE, M. J., LANE, M. D., O'DONNELL, S. AND FRANKLIN-TONG, V. E. 1993. The population genetics of the self-incompatibility polymorphism in *Papaver rhoeas*. V. Cross-classification of the *S*-alleles of samples from three natural populations. *Heredity*, **71**, 581–590.
- LAWRENCE, M. J. AND O'DONNELL, S. 1981. The population genetics of the self-incompatibility polymorphism in *Papaver rhoeas*. III. The number and frequency of *S*-alleles in two further natural populations (R102 and R104). *Heredity*, **47**, 53–61.
- NETTANCOURT, D. de. 1977. *Incompatibility in Angiosperms*. Monographs on theoretical and applied genetics, vol 3, Springer, Berlin.
- NOU, I. S., WATANABE, M., ISOGAI, A. AND HINATA, K. 1993a. Composition of *S*-alleles and *S*-glycoproteins between two wild populations of *Brassica campestris* in Turkey and Japan. *Sex. Plant Reprod.*, **6**, 79–86.
- NOU, I. S., WATANABE, M., ISOGAI, A., SHINOZUKA, H., SUZUKI, A. AND HINATA, K. 1991. Variation of *S*-alleles and *S*-glycoproteins in a naturalized population of self-incompatible *Brassica campestris* L. *Jap. J. Genet.*, **66**, 227–239.
- NOU, I. S., WATANABE, M., ISUZUGAWA, K., ISOGAI, A. AND HINATA, K. 1993b. Isolation of *S*-alleles from a wild population of *Brassica campestris* L. at Balcesme, Turkey and their characterization by *S*-glycoproteins. *Sex. Plant Reprod.*, **6**, 71–78.
- OCKENDON, D. J. 1974. Distribution of self-incompatibility alleles and breeding structure of open-pollinated cultivars of Brussels sprouts. *Heredity*, **33**, 159–171.
- OCKENDON, D. J. 1975. Dominance relationships between *S*-alleles in the stigmas of Brussels sprouts (*Brassica oleracea* var. *gemmifera*). *Euphytica*, **24**, 165–172.
- OCKENDON, D. J. 1980. Distribution of *S*-alleles and breeding structure of cape broccoli (*Brassica oleracea* var. 'italica'). *Theor. Appl. Genet.*, **58**, 11–15.
- OCKENDON, D. J. 1982. An *S*-allele survey of cabbage (*Brassica oleracea* var. *capitata*). *Euphytica*, **31**, 325–331.
- OCKENDON, D. J. 1985. Genetics and physiology of self-incompatibility in *Brassica*. In: Sussex, I., Ellingboe, A., Crouch, M. and Malmberg, R. (eds) *Plant Cell/Cell Interactions*, pp.1–6. Cold Spring Harbor Press, Cold Spring Harbor, New York.
- OCKENDON, D. J. AND CURRAH, L. 1979. Breeding structure of a highly selected cultivar of cabbage (*Brassica oleracea* var. *capitata*). *Heredity*, **42**, 373–379.
- O'DONNELL, S. AND LAWRENCE, M. J. 1984. The population genetics of the self-incompatibility polymorphism in *Papaver rhoeas*. IV. The estimation of the number of alleles in a population. *Heredity*, **53**, 495–507.
- ORJEDA, G., FREYRE, R. AND IWANAGA, M. 1991. Use of *Ipomoea trifida* germplasm for sweet potato improvement. 3. Development of $4x$ interspecific hybrids between *Ipomoea batatas* (L.) Lam. ($2n = 6x = 90$) and *I. trifida* (H.B.K.) G.Don. ($2n = 2x = 30$) as storage-root initiators for wild species. *Theor. Appl. Genet.*, **83**, 159–163.
- SAMPSON, D. R. 1964. A one-locus self-incompatibility system in *Raphanus raphanistrum*. *Can. J. Genet. Cytol.*, **6**, 435–445.
- SAMPSON, D. R. 1967. Frequency and distribution of self-incompatibility alleles in *Raphanus raphanistrum*. *Genetics*, **56**, 241–251.

- SHIOTANI, I. AND KAWASE, T. 1987. Synthetic hexaploids derived from wild species related to sweet potato. *Jap. J. Breed.*, **37**, 367–376.
- SHIOTANI, I., YOSHIDA, I. AND KAWASE, K. 1990. Numerical taxonomic analysis and crossability of diploid *Ipomoea* species related to the sweet potato. *Jap. J. Breed.*, **40**, 159–174.
- STEVENS, J. P. AND KAY, Q. O. N. 1989. The number, dominance relationships and frequencies of self-incompatibility alleles in a natural population of *Sinapis arvensis* L. in South Wales. *Heredity*, **62**, 199–205.
- THOMPSON, K. F. AND TAYLOR, J. P. 1966. Non-linear dominance relationships between *S* alleles. *Heredity*, **21**, 345–362.
- WALLACE, D. H. 1979. Interactions of *S* alleles in sporophytically controlled self-incompatibility of *Brassica*. *Theor. Appl. Genet.*, **54**, 193–201.
- WILLIAMS, W. AND WILLIAMS, R. D. 1947. Genetics of red clover (*Trifolium pratense* L.) compatibility. III. The frequency of incompatibility *S* alleles in two non-pedigree populations of red clover. *J. Genet.*, **48**, 69–79.