

# Genetic control of self-incompatibility in *Anagallis monelli* (Primulaceae: Myrsinaceae)

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The genetic control of self-incompatibility (SI) was studied in the Mediterranean short-lived perennial species *Anagallis monelli* (Primulaceae: Myrsinaceae). Arrays of siblings, including families derived from reciprocal crosses, were cross-pollinated in full diallels, and compatibility groups were assessed from a census of fruit-set. Two, three and four intercompatible and intraincompatible groups were found. These crossing relationships fit the model for gametophytic SI controlled by a single polymorphic gene locus in families derived from parents with one or no S alleles in common (two vs. four compatibility groups), whilst one genotype was presumed to be missing in the small families that showed only three compatibility groups.

**Keywords:** *Anagallis monelli*, homomorphic SI, Myrsinaceae, Primulaceae, self-incompatibility.

## Introduction

Three major systems of self-incompatibility (SI) are known in flowering plants: homomorphic, gametophytic SI (GSI), homomorphic, sporophytic SI (SSI), and heteromorphic SI (HetSI) (see recent review by Hiscock & Kùes, 1999). Both homomorphic systems have a genetic control usually of one gene with multiple alleles; however, while in GSI the S-genotype of the haploid (usually two-celled) pollen phenotype effectively determines the SI reaction, in SSI the incompatibility reaction of the (usually three-celled) pollen is determined by both S-alleles present in the pollen parent. Moreover, whereas the S-alleles show codominance in GSI, in all SSI cases analysed thus far the S-alleles show a hierarchy of dominance–recessive interactions. HetSI is controlled by one gene with two alleles — one dominant and one recessive in the more common distylic condition — and two epistatic genes each with two dominant–recessive alleles in the rarer tristylic condition (Ganders, 1979).

Present knowledge of the distribution of these SI systems in angiosperm families is very variable. Data for HetSI, comprising some 165 genera and 25 families (Ganders, 1979), are mostly complete, mainly because the presence of this SI mechanism is usually signalled by evident floral di- or trimorphy (although it should be noted that the number of HetSI taxa for which the genetic control of SI has been rigorously characterized

are few). In contrast, SSI seems to be restricted to only five (eight according to some authors; see discussion below) families: Asteraceae (Gerstel, 1950; Hughes & Babcock, 1950), Brassicaceae (Bateman, 1954), Betulaceae (Thompson, 1979), Caryophyllaceae (Lundquist, 1990) and Convolvulaceae (Martin, 1968).

The occurrence of GSI is more problematical. Generally this is regarded as the most widespread SI system; estimates range from it occurring in around half of the flowering plant species (Brewbaker, 1959), in 71 families (Richards, 1986), or 60–90 families Weller *et al.* (1995). However, detailed studies on the genetic control that clearly establishes GSI (i.e. involving diallel crosses within progeny arrays) have been undertaken for very few species from some 15 to 17 families (Mau *et al.*, 1991). Most cases of alleged GSI encountered in the SI literature derive from reports for taxa in which ‘self-incompatibility’ has been determined, and for which a syndrome of characters (absence of floral heteromorphy, two-celled pollen, absence of pollen inhibition at the stigma) favour a GSI interpretation over other SI mechanisms. However, exceptions are known for each of these syndrome characters, e.g. two-celled pollen with SSI in *Betula*, three-celled pollen with GSI in Cactaceae and Poaceae; moreover, it is likely that reports of assumed gametophytic SI for some species, particularly in studies that do not include observations of postpollination events, may actually refer to cases of ‘late-acting SI’ or early inbreeding depression (Seavey & Bawa, 1986; Sage *et al.*, 1994; Gibbs & Bianchi, 1999; Lipow & Wyatt, 2000).

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Mechanisms of SI seem to be mostly mutually exclusive at the family level because, with the recent exception of the Polemoniaceae, with GSI in *Phlox* (Levin, 1993) and SSI in *Linanthus* (Goodwillie, 1997), GSI and SSI are not found in the same family, and likewise there are no examples of SSI and HetSI reported in the same family (cf. Ganders, 1979). However, both HetSI and GSI are found in a number of families (de Nettancourt, 1977). This is partially a reflection of the fact that only a few families have widespread HetSI (e.g. Primulaceae, Plumbaginaceae, Rubiaceae). A more common situation is for occasional HetSI genera or species to be found in families that otherwise lack SI or in which other taxa have GSI (e.g. *Jepsonia* is the only genus with HetSI in the Saxifragaceae, and only four of some 400 *Hypericum* species have HetSI). It is likely that HetSI has evolved independently more frequently than homo-morphic systems.

Two opposing theories have been proposed for the evolution of HetSI: the more traditional view holds that the diallelic incompatibility mechanism evolved first, and was followed by the accumulation of floral morph features that promote disassortative pollination between the two compatible genotypes in the population and thus diminish pollen wastage. A more recent hypothesis (Lloyd & Webb, 1992) proposes that floral heteromorphy, via approach herkogamy, precedes the acquisition of SI. The former theory would imply that there may be some species with diallelic SI that lack the associated floral morph features. The only such case in the literature to our knowledge is the SI reported in *Capsella grandiflora* (Riley, 1936), for which control by two genes each with two alleles (as in tristylous species) was proposed. Lewis (1949) noted that this example supported the view that incompatibility came first and heterostyly (when it occurs) followed as a reinforcement.

The case of *Capsella grandiflora* needs further investigation because it seems unlikely that a genus of the SSI family Brassicaceae shows diallelic SI. However, SI species that lack obvious floral dimorphy in such predominantly HetSI families as the Primulaceae and Rubiaceae are an obvious focus of interest, and such taxa would seem to be *a priori* potential candidates for 'cryptic' diallelic SI. Given that a number of species of the Primulaceae tribe Anagallideae have been reported to have nonheterostylic but uncharacterized SI, e.g. *Lysimachia nummularia* (Dahlgren, 1922), *Trientalis arctica* (Pojar, 1974) and *T. borealis* (Anderson & Beare, 1983; Barrett & Helenurm, 1987), and *Anagallis monelli* (Gibbs & Talavera, 2001), the objective in the present study was to clarify the genetic basis for SI in this latter species.

## Materials and methods

### The species

*Anagallis monelli* is a profusely flowering, short-lived perennial species distributed in the western Mediterranean. Its reproductive biology was studied in the field by Gibbs & Talavera (2001), who reported it to be self-incompatible. Cytological studies by Talavera *et al.* (1997) established that *A. monelli* is diploid ( $2n=20$ ). Chromosome pairing at microsporogenesis is regular, with 10 bivalents in all Metaphase I plates analysed ( $n=100$ ), and the pollen has simultaneous formation, and is two-celled at time of release from the anther (S. Talavera, unpubl. data).

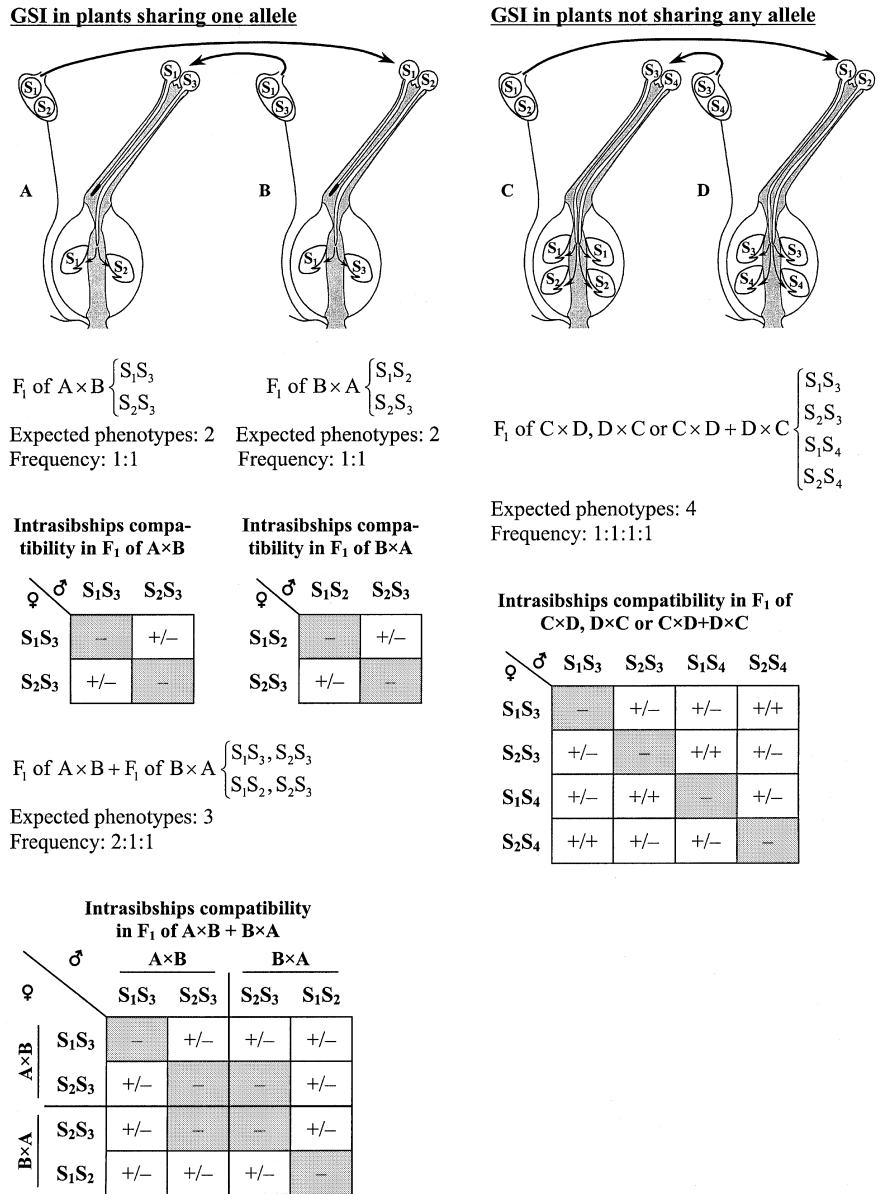
### Parents and progeny

In July 1996, 200 fruit were collected from 10 plants (20 from each) in a population of *A. monelli* growing in open *Quercus suber*–*Pinus pinea* woodland near Hinojos, Huelva province, SW Spain. The fruit were stored in the dark at room temperature until sowing. In September, seeds were bulked and put to germinate on moist filter

		♂ PARENT PLANTS								
		2	42	52	47	24	34	36	40	63
♀ PARENT PLANTS	2	0/2 –	0/5 –	3/3 <b>D1</b>	2/2 +	2/2 +	2/2 +	2/2 +	2/2 +	2/2 +
	42	0/5 –	0/2 –	?	?	2/2 +	2/2 +	2/2 <b>D2</b>	?	2/2 +
	52	0/2 +?	?	0/2 –	2/2 +	?	?	?	1/2 +	?
	47	2/2 +	?	1/2 +	0/2 –	2/2 +	1/2 +	?	2/2 +	2/2 +
	24	2/2 +	2/2 +	?	2/2 +	0/2 –	2/2 +	?	2/2 <b>D3</b>	2/2 <b>D4</b>
	34	2/2 +	2/2 +	?	2/2 +	2/2 +	0/2 –	?	1/2 +	2/2 <b>D5R</b>
	36	1/1 +	3/3 <b>D2R</b>	?	?	?	?	0/2 –	?	?
	40	2/2 +	?	0/2 +?	2/2 +	2/2 <b>D3R</b>	1/1 +	?	0/2 –	2/2 +
	63	2/2 +	2/2 +	?	2/2 <b>D6</b>	2/2 <b>D4R</b>	2/2 <b>D5</b>	?	2/2 +	0/2 –

**Fig. 1** Results of diallel cross in a sample of nine plants of *Anagallis monelli* used to generate seed for progeny arrays. For each cell, top left = number of fruits/number of pollinations. Each cross scored as compatible (+) or incompatible (–) or not attempted (?). D1, etc. are family numbers, D3R indicates reciprocal family. Note: all plants were self-incompatible, and that of the nine parental plants, only 2 and 42 were fully interincompatible.

**Fig. 2** Model for GSI: expected compatibility interactions and phenotypes generated in a full sib diallel between two plants with (left-hand column) one S-allele in common, and (right-hand column) no S-allele in common. (+/+ denotes fully compatible cross, +/- semicompatible cross and -/- incompatible cross). Reciprocal differences in incompatibility between types are indicated by shaded cells. A and B: When two plants with one S allele in common are crossed, the plants are fully semicompatible. If the F1 are crossed, then two phenotypic groups are obtained, independently of the direction of the cross (A x B or B x A). If all members of the F1 generation are crossed reciprocally (A x B + B x A), they form four phenotypes, two in each direction of the cross. Two of these four phenotypes are different from each other (one in A x B and one in B x A) and two are similar. Consequently, we would expect with the results of such reciprocal crosses to find three phenotypes with a frequency of 2:1:1. C and D: When plants that do not have an allele in common are crossed, the plants are fully or semi compatible. Crosses of the F1 of these plants, independent of direction of the cross (C x D or D x C) form four groups of phenotypes with a frequency of 1:1:1:1. This same result is obtained with the progeny of the two reciprocals of such crosses.



paper in sterile Petri-dishes (50 seeds per dish) and watered as necessary. Seedlings were subsequently transferred to a peat:vermiculite (3:1) compost in 15 x 15 cm pots and the plants grown on in a glasshouse at Seville. When flowering commenced (November–December), 43 plants were transferred to an acrylic net (0.4 mm mesh) enclosure to avoid contaminant pollination, and were maintained at 16 h light, with temperatures 18–30°C and watered every six hours.

A diallel cross with these 43 plants indicated that they were all SI, and that 25 phenotypic cross-intercompatible groups were present. In the present study progeny from 10 of these crosses were used (Fig. 1), each assigned the prefix D, and with the suffix R to indicate reciprocal crosses. In order to simplify the results of the

original diallel with 43 plants, Fig. 1 includes only the nine plants that produced the 10 progeny families (sibships). Compatibility relationships between these nine plants and fruit-set in all crosses are also included in the same figure.

Seeds of each family were sown in Petri dishes as before except that the first watering consisted of a 0.2% solution of KNO<sub>3</sub>. Germination exceeded 80% in all cases. Plants of the sibships D1 and D6 were cultivated in St Andrews, and the remaining families were cultivated and studied in Seville. From the onset of flowering all plants at Seville were maintained under insect-proof mesh cages as before but with temperature control between 18 and 24°C, whilst those at St Andrews were grown in a glasshouse with 16 h light regime at 23°C but

♀	D5																													D5R							
	1	8	9	10	11	12	14	16	17	18	20	22	23	24	26	2	3	4	5	6	7	13	15	19	21	25	27	28	29	31	34	36	39	30	35	38	
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	3	1	2	2	2	2	4	2	2	1	4	1	2	3	2	1	1	5	2	
3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6	3	3	1	2	2	2	4	2	2	1	4	1	2	3	2	1	1	6	2	
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	6	1	1	1	3	2	2	1	2	1	2	1	2	2	2	2	2	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	1	1	1	1	2	2	4	3	2	1	4	4	1	3	3	3	1	2	1	
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6	2	2	2	2	2	6	2	2	1	4	1	2	3	3	2	1	2	1	3	
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	1	1	4	1	1	1	5	1	1	1	2	2	1	3	3	2	2	3	
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2	2	1	1	1	2	1	1	2	1	2	2	1	1	2	2	5	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	1	1	3	3	2	2	3	3	4	1	2	4	2	1	2	3	2	2	3	
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	5	5	1	1	3	2	4	3	2	2	3	2	2	3	2	2	1	3	1	1	
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	2	2	1	2	2	2	2	2	2	0	1	1	1	1	1	1	
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	4	1	1	5	2	2	4	1	2	2	2	1	1	2	2	2	4	
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	2	1	3	2	2	2	1	2	1	1	2	3	1	1	2	2	2	2	2	
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	3	2	4	5	2	4	2	2	2	2	2	2	2	
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2	2	5	2	1	2	2	4	4	1	1	4	1	1	2	1	1	
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	3	2	2	1	2	1	1	4	4	1	3	2	4	1	4	4	1	3	
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6	1	1	2	2	2	2	2	2	2	2	1	3	1	2	2	2	2	2	2	
2	4	1	3	2	2	1	1	1	1	1	1	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	
3	6	1	2	2	2	1	1	1	1	1	1	2	2	2	2	3	3	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	
4	1	2	2	4	2	2	1	1	1	1	1	2	2	2	2	7	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
5	4	1	5	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
6	1	2	1	2	1	1	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
7	2	1	2	4	2	1	1	1	1	1	1	4	3	2	4	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	
13	2	1	2	2	1	2	2	2	4	1	3	2	2	2	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
15	1	2	1	1	1	1	1	1	1	2	2	2	2	2	2	0	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	
19	3	1	3	3	2	4	2	4	4	6	1	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
21	2	2	4	4	2	2	3	3	2	2	2	1	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
25	2	3	2	2	2	2	2	2	2	2	2	3	2	2	2	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
27	1	2	2	2	2	2	1	1	1	1	2	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
28	4	2	2	2	3	5	2	3	5	2	2	2	2	2	2	3	1	1	1	1	1	1	1	2	2	2	2	1	2	4	1	2	2	2	2	2	
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	
31	2	2	2	1	1	1	1	1	6	2	0	1	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
34	2	1	1	3	2	1	2	1	1	4	2	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
36	1	1	2	1	3	1	1	1	1	1	1	1	1	1	1	0	5	6	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	1	3	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
0	2	1	2	1	4	3	3	3	1	1	2	2	3	1	2	3	1	2	1	4	4	3	4	4	5	5	3	5	3	5	0	1	0	1	0	1	
1	2	1	5	2	4	5	4	3	1	2	2	2	1	2	4	2	2	2	1	4	4	5	7	6	5	5	5	5	5	2	1	1	1	1	1	1	
2	4	2	1	1	5	2	2	2	5	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
35	2	1	1	1	3	1	4	1	1	1	1	2	3	4	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38	2	1	1	1	5	1	5	1	1	1	1	2	3	5	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Fig. 3 *Anagallis monelli*: results of full sib diallel in families D5 (1–29) and reciprocal D5R (31–38) and D5 + D5R (0/2, etc. = number of fruits/number of pollinations). Reciprocal differences in incompatibility between types are indicated by shaded cells. Numbered progeny have been rearranged into incompatibility types, based upon crossing relationships.

uncovered since there was initially negligible insect activity. Diallel crosses were carried out in each of the families D1–D6 and also between all members of reciprocal families for which these were available [(D2 × D2R) – (D5 × D5R)]. Initially, two flowers per cross were pollinated, but in cases where reciprocal crosses were incongruent, additional pollinations were undertaken (two to seven times) in each direction. In

total, some 6000 pollinations on 99 plants were carried out.

*Theoretical model for GSI*

In this model it is hypothesized that incompatibility is controlled by a single multiallelic gene with allelic codominance. As a consequence, crosses between two plants with both S-alleles in common (also self-pollina-

♀	♂	D4				D4R							
		2	5	3	4	6	7	11	8	9	10	12	
D4	2	0/1	0/2	2/2	2/2	2/2	2/2	2/2	—	2/2	2/2	2/2	
	5	0/2	0/2	2/2	2/2	2/2	2/2	2/2	—	2/2	2/2	2/2	
	3	2/2	2/2	0/2	0/2	0/2	0/5	0/5	1/1	2/2	2/2	2/2	
	4	2/2	2/2	0/2	0/2	0/4	0/2	0/2	1/1	2/2	2/2	2/2	
D4R	6	2/2	2/2	0/2	0/4	0/2	0/2	0/2	1/1	2/2	2/2	2/2	
	7	2/2	2/2	0/5	0/2	0/2	0/2	0/5	1/1	2/2	2/2	2/2	
	11	2/2	2/2	0/5	0/2	0/2	0/5	0/2	1/1	2/2	2/2	2/2	
	8	1/1	1/1	1/1	1/1	3/3	2/2	1/1	0/2	0/1	0/1	0/1	
	9	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/1	0/2	0/2	0/2	
	10	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/1	0/2	0/2	0/2	
	12	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/1	0/2	0/2	0/2	

incompatible  
  compatible  
 — no data

**Fig. 4** *Anagallis monelli*: results of full sib diallel in families D4 (2–5) and reciprocal D4R (6–12) and D4 + D4R (0/2, etc. = number of fruits/number of pollinations). Reciprocal differences in incompatibility between types are indicated by shaded cells. Numbered progeny have been rearranged into incompatibility types, based upon crossing relationships.

tions) are fully incompatible. Crosses between plants with one S-allele in common are ‘half-compatible’ (owing to functioning of pollen bearing the dissimilar allele), and plants with no S-alleles in common are fully intercompatible. The latter two situations, with the respective number of expected phenotypes and their frequencies in the progeny, are expressed in Fig. 2. In a diallelic SI model, e.g. as for SI in distylous *Primula*, crosses between compatible phenotypes (long-style × short-style) ss × Ss will always produce progeny with the same two phenotypes in a 1:1 ratio. The same would of course apply for cryptic diallelic SI with no floral dimorphy.

Chi-squared tests were used to determine the goodness of fit between the observed results and expected segregation ratios.

**Results**

In the 253 self-pollinations carried out on the 99 plants from 10 families, only one fruit was obtained: from two self-pollinations on plant number 31 of the D5R family. As a further 14 self-pollinations with flowers of this

♀	♂	D1											
		1	4	5	7	12	2	3	6	8	11	9	10
D1	1	0/2	0/4	0/6	0/3	0/5	2/2	5/5	3/3	3/3	2/2	2/2	3/3
	4	0/2	0/2	0/2	1/10	0/7	3/3	2/2	2/2	2/2	7/9	2/2	2/2
	5	2/9	3/11	0/2	0/2	0/2	1/1	2/2	2/2	4/4	2/2	2/2	2/2
	7	0/3	0/2	0/2	0/2	0/6	3/3	2/2	4/4	2/2	2/2	2/2	5/5
	12	0/5	0/5	0/2	0/2	0/2	5/15	7/16	2/7	4/4	5/6	3/5	3/3
	2	2/2	2/2	3/4	2/2	0/4	0/2	0/4	1/4	0/3	1/6	2/2	2/2
	3	2/2	4/4	2/4	4/4	7/10	2/2	0/2	1/8	0/10	0/3	3/3	2/2
	6	3/3	2/3	2/2	4/5	4/4	0/2	0/2	0/2	0/2	1/6	4/4	2/2
	8	4/4	3/3	3/3	4/4	4/4	0/2	0/2	0/4	0/2	0/2	4/4	4/4
	11	5/5	4/4	3/3	2/2	2/2	0/2	0/2	0/3	0/3	0/3	3/3	2/2
D1	9	2/2	2/2	2/2	3/3	3/3	2/2	4/4	3/3	2/2	0/2	5/5	
	10	3/3	3/3	3/3	3/3	3/3	2/2	2/2	2/3	3/3	5/5	0/2	

incompatible  
  compatible  
  incongruity

**Fig. 5** *Anagallis monelli*: results of full sib diallel in families D1 (0/2, etc. = number of fruits/number of pollinations). Reciprocal differences in incompatibility between types are indicated by shaded cells. Numbered progeny have been

plant produced no fruit, it can be concluded that all plants studied were self-incompatible.

The diallel crosses within the 10 families of *A. monelli* produced three kinds of intra-incompatible and reciprocally intercompatible groups: families D1 and D6 each showed four compatibility phenotypes; families D4, D4R, D5 and D5R showed two compatibility phenotypes, whilst families D2, D2R, D3 and D3R showed three such phenotypes (Figs 3, 4, 5, 6, 7, 8). The four compatibility phenotypes, as in D1 and D6 (Figs 5 and 6), are what would be expected with single locus multiallelic GSI in crosses between progeny derived from parents which did not share an S allele in common (cf. Fig. 2). For both of these families, the frequencies observed for the four phenotypes conform with the expected mendelian ratios (Table 1). Diallel crosses that produce only two compatibility groups, as in families D4 and D5 and their reciprocals (Figs 3 and 4), would be expected with diallelic SI, but also under the GSI system in families derived from parents that shared one S allele in common (cf. Fig. 2). That the latter case is indeed the explanation in these families is confirmed when the results of the crosses between members of the

		♂																
		D6																
♀	D6	1	7	10	13	18	2	5	8	9	12	3	4	6	11	15	16	17
		1	0/2	0/3	0/2	1/3	0/3	2/2	2/4	4/4	1/1	2/2	2/2	3/3	2/2	1/1	2/2	2/2
7	0/3	0/2	0/3	0/3	0/5	2/2	4/4	2/2	2/2	3/3	2/2	2/2	2/2	2/2	2/2	2/2	2/1	
10	1/3	0/2	0/2	0/8	0/5	2/2	2/2	2/2	2/2	3/3	2/2	2/2	2/2	2/2	2/2	3/3	2/2	
13	0/6	0/2	0/10	0/2	0/5	4/4	3/3	5/7	3/4	2/2	2/2	2/2	2/2	3/3	2/2	2/2	2/3	
18	1/6	0/4	0/5	0/5	0/2	4/4	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/4	4/4	2/2	
2	2/2	2/2	2/2	2/2	2/3	0/2	1/7	0/5	0/6	0/3	2/2	5/5	2/2	2/2	2/2	2/2	2/2	
5	1/1	3/3	2/2	3/3	3/3	0/4	0/2	1/7	0/7	2/11	5/5	2/2	3/3	2/2	1/2	2/2	2/2	
8	4/4	4/4	2/2	2/2	2/2	0/0	0/3	0/2	0/3	0/3	3/3	2/2	2/2	2/2	1/2	2/2	2/2	
9	1/1	3/3	2/2	2/4	2/2	0/2	2/7	0/3	0/2	0/2	2/2	2/2	4/4	2/2	2/2	2/2	2/2	
12	1/1	2/2	2/2	4/4	4/4	0/6	0/6	0/2	1/7	0/2	2/2	2/2	1/1	2/2	5/5	3/3	4/4	
3	2/2	3/3	5/5	2/2	3/3	2/2	5/5	2/2	2/2	2/2	0/2	0/4	0/5	0/3	0/4	4/4	2/2	
4	2/4	2/2	3/3	2/2	2/2	3/5	2/2	3/4	3/3	2/2	0/3	0/2	0/4	0/2	0/3	2/2	2/2	
6	2/2	2/2	2/2	2/2	3/3	2/2	3/3	3/3	2/2	3/3	0/3	0/4	0/2	0/3	0/7	2/2	1/1	
11	2/2	3/3	2/2	2/2	3/3	2/2	2/2	2/2	2/2	2/2	0/3	0/3	0/3	0/2	0/4	4/4	2/2	
15	1/1	2/2	2/2	2/2	2/3	3/4	2/2	2/2	2/4	4/4	0/4	0/4	1/8	0/2	0/2	2/2	2/2	
16	1/1	3/3	3/3	3/3	3/3	2/2	2/2	3/3	2/2	5/5	3/3	2/4	4/3	3/3	0/2	1/10	0/2	
17	1/1	2/2	2/2	2/2	4/4	2/2	2/2	3/3	2/2	3/3	3/3	2/2	4/4	2/2	2/2	0/7	0/2	

Fig. 6 *Anagallis monelli*: results of full sib diallel in families D6 (0/2, etc. = number of fruits/number of pollinations). Reciprocal differences in incompatibility between types are indicated by shaded cells. Numbered progeny have been rearranged into incompatibility types, based upon crossing relationships.

two reciprocal families are considered. Thus, crosses between plants of D5 with their reciprocals in D5R showed that each family has one compatibility phenotype which is also present in the reciprocal, and each has another compatibility phenotype which is restricted to that family. Crosses between plants in family D4 with their reciprocals in D4R show a similar pattern, as expected under the GSI model. Again, the frequencies observed for the three phenotypes conform with the expected mendelian ratios (Table 1).

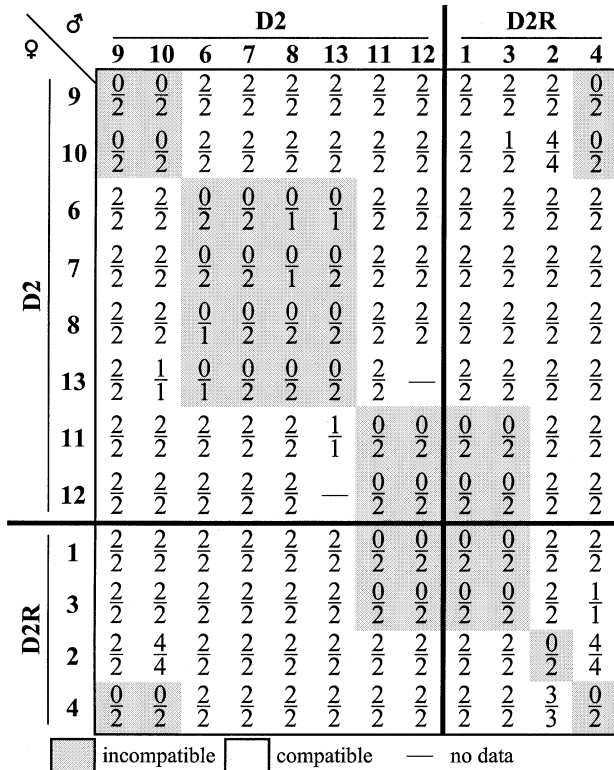
The families D2 and D3 and their reciprocals (Figs 7 and 8) showed three compatibility phenotypes, and given the small size of these families, it is likely that in each case one S phenotype was missing. For all four families the proportions of the phenotypes present in each diallel do not contradict this assumption of a mendelian segregation of 1:1:1:1, e.g. 4:2:2:0 in D2 and D2R (see Table 1). Again, the results of the reciprocal crosses between members of these families confirm this

interpretation, because the diallel between members of D2 and D2R (Fig. 7) shows that two of the phenotypes present in D2 are also present in D2R, and similarly with the diallel between plants of D3 and D3R (Fig. 8).

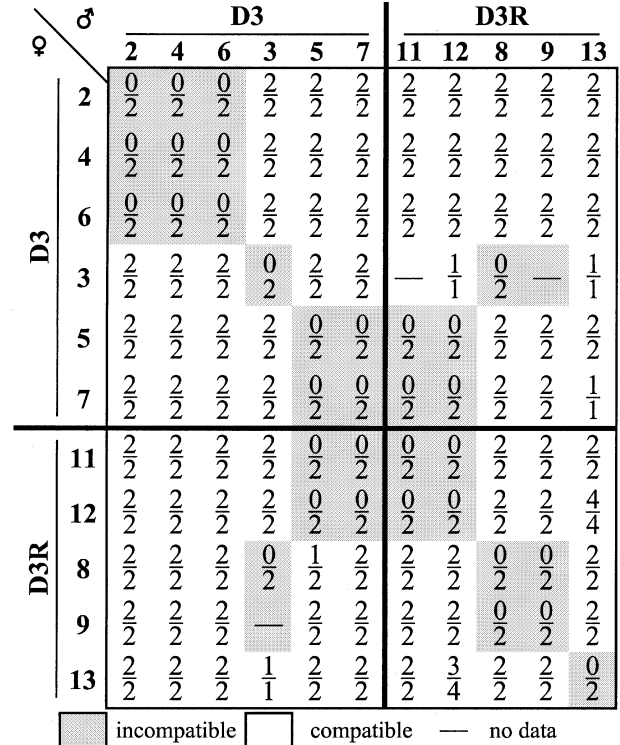
### Discussion

The diallel crosses carried out with 10 families of *A. monelli* provide convincing evidence for the presence of single locus, multiallelic homomorphic gametophytic self-incompatibility in this species. It is notable that in such diallels, the results of crosses between reciprocal families can provide valuable confirmatory evidence of fit with the GSI model.

Although the results clearly indicate a GSI type control of SI in *A. monelli*, some incongruent results were obtained. These were of two types: the first, which were relatively trivial, resulted from several inconsistent results from reciprocal crosses. Usually, these took the



**Fig. 7** *Anagallis monelli*: results of full sib diallel in families D2 (6–13) and reciprocal D2R (1–4) and D2 + D2R (0/2, etc. = number of fruits/number of pollinations). Reciprocal differences in incompatibility between types are indicated by shaded cells. Numbered progeny have been rearranged into incompatibility types, based upon crossing relationships.



**Fig. 8** *Anagallis monelli*: results of full sib diallel in families D3 (2–7), D3R (8,9,11–13) and D3 + D3R (0/2, etc. = number of fruits/number of pollinations). Reciprocal differences in incompatibility between types are indicated by shaded cells. Numbered progeny have been rearranged into incompatibility types, based upon crossing relationships.

**Table 1** Diallel crosses in *Anagallis monelli*. Comparison of observed phenotype frequencies with those expected with single locus control homomorphic gametophytic SI. (Families D1 and D6 are unilateral crosses; for D2–D5 results are for unilateral, reciprocal and combined crosses)

Sibship	No. of progeny	Phenotype frequencies			$\chi^2$	d.f.	P
		Observed	Expected				
D1	12	5:5:1:1	1:1:1:1	5.33	3	0.149 NS	
D2	8	4:2:2:0	1:1:1:1	4.00	3	0.261 NS	
D2R	4	2:1:1:0	1:1:1:1	2.00	3	0.572 NS	
D2 + D2R	12	4:4:3:1	1:1:1:1	2.00	3	0.572 NS	
D3	6	3:2:1:0	1:1:1:1	3.33	3	0.343 NS	
D3R	5	2:2:1:0	1:1:1:1	2.20	3	0.532 NS	
D3 + D3R	11	4:3:3:1	1:1:1:1	1.73	3	0.631 NS	
D4	4	2:2	1:1	0	1	1.000 NS	
D4R	7	3:4	1:1	0.14	1	0.705 NS	
D4 + D4R <sup>1</sup>	11	5:3:3	2:1:1	0.10	2	0.956 NS	
D5	29	14:15	1:1	0.03	1	0.853 NS	
D5R	7	4:3	1:1	0.14	1	0.705 NS	
D5 + D5R <sup>1</sup>	36	19:9:8	2:1:1	0.17	2	0.920 NS	
D6	17	5:5:5:2	1:1:1:1	1.59	3	0.662 NS	

<sup>1</sup>Because the expected number of phenotypes is 3, each family will have a different number of individuals, the frequency given is the weighted average of the frequency in the population.

form of occasional fruit formation where the 'expected' result was for incompatibility. In most cases, such fruits were found to contain only 1–2 seeds and were presumably the rare consequence of a few pollen tubes crossing the SI 'barrier' in the style. However, several of these anomalous fruits were well filled with seeds but most of these occurred in the St Andrews open glasshouse and were presumed to be the result of contaminant cross-pollinations. Although, overall, less than 0.1% of untreated flowers formed fruits in this glasshouse, the occasional presence of syrphid flies caused some fruit formation in nontreated flowers and, most likely, in some of the hand-pollinated flowers. In some cases, pollinations which were 'expected' to set fruits (as established in the reciprocal cross) did not do so, presumably because the pollen used had poor viability or the stigmas were passed their prime. It is worth noting that in virtually all published cases of genetic control of SI, some incongruent results have been obtained (see Hiscock, 2000, for discussion of such cases with SSI taxa).

The occurrence of GSI in *Anagallis monelli* indicates that the uncharacterized but apparently non-heteromorphic SI reported in other genera of the tribe Anagallideae, e.g. *Lysimachia nummularia* L. (Dahlgren, 1922), *Trientalis arctica* (Pojar, 1974) and *T. borealis* (Anderson & Beare, 1983; Barrett & Helenurm, 1987) is also most likely to be of the GSI type. This raises an interesting situation for the evolution of HetSI which predominates in the Primulaceae, because a phylogenetic classification for this family proposed by Anderberg & Ståhl (1995), based on a cladistic analysis of habit, morphological and anatomical characters, indicated that the Anagallideae (sub Lysimachieae) was a paraphyletic grade located near the base of the family. This view would imply that the largely heteromorphic tribe Primuleae, e.g. *Primula*, *Dodecatheon*, *Dionysia*, *Vitaliana* is a more derived clade that evolved from a group which had GSI, or perhaps more likely an offshoot which had lost such ancestral GSI. However, a more recent phylogenetic treatment of the order Primulales by Källersjö *et al.* (2000), based on three chloroplast DNA sequences and morphology, has proposed that monophyly in this group is best maintained by transferring the Anagallideae (sub Lysimachieae), together with some other genera (e.g. *Coris* and *Cyclamen*) to the family Myrsinaceae, and *Samolus* to the Theophrastaceae. In this interpretation, the Primulaceae *Sensu stricto* becomes an almost exclusively HetSI family (with some SC taxa), whilst the inclusion of *Anagallis*, *Trientalis* and *Lysimachia* in the Myrsinaceae introduces taxa with GSI to this family. To our knowledge, SI has not been reported previously in any traditional Myrsinaceae, e.g. *Myrsine*, *Ardisia*.

Molecular studies on SI have indicated that despite an apparently uniform genetic control, homomorphic gametophytic SI consists of two very different mechanisms: that found so far exclusively in the Papaveraceae, in which SI is characterized by the involvement of a  $Ca^{2+}$  dependent signal transduction pathway (Franklin-Tong *et al.*, 1994), and that found in the families Rosaceae, Scrophulariaceae and Solanaceae (see Hiscock & Kües, 1999 for a recent review) in which S-RNases are implicated. It is unknown which of these systems (if either) the remaining 15 or so families with proven GSI belong to; however, it is tempting to speculate that the S-RNase system is more likely to occur in taxa which show stylar rejection of incompatible pollen, e.g. as in *A. monelli* (Gibbs & Talavera, 2001) and the majority of GSI families, whilst the *Papaver* system, if it occurs in other families, is more likely to be found in taxa with a stigmatic level of inhibition (e.g. as in the Onagraceae, Commelinaceae).

Of more immediate concern is the paucity of species for which rigorous analyses of the kind of genetic control have been made, and therefore convincing proof of the occurrence of GSI, SSI or HetSI obtained. Charlesworth (1985) commented that: 'The data on homomorphic self-incompatibility are so fragmentary that it seems premature to draw any conclusions from them...'. This situation has scarcely changed in the intervening 15 years. In addition, unfortunately, this problem is unlikely to be resolved by the kind of diallel studies report herein, for various reasons. First, although *A. monelli* is in many ways an ideal species for this experimental approach, because many small potted plants that flowered prolifically for many months could be cultivated in the glasshouse, nevertheless, 6000 pollinations were made in this study. Most herbaceous or small shrubby plant species which would permit experimental progeny arrays to be grown from seed would produce only limited numbers of flowers in a limited flowering season, and this can be a serious constraint for a diallel pollination programme. However, a much more serious problem is that the majority of angiosperm species are woody taxa for which the possibilities of such experimental studies are very limited. The main hope for advances in our knowledge of the occurrence of homomorphic SI mechanisms must lie with the development of molecular probes for known SI genes, e.g. as in the attempt to probe SSI *Corylus avellana* genome with *Brassica* SLG and SRK genes by Hampson *et al.* (1996).

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