

Single Gene Control of Postzygotic Self-Incompatibility in Poke Milkweed, *Asclepias exaltata* L.

Sara R. Lipow* and Robert Wyatt†

*Department of Botany and †Institute of Ecology, University of Georgia, Athens, Georgia 30602

Manuscript received April 28, 1998

Accepted for publication November 4, 1999

ABSTRACT

Most individuals of *Asclepias exaltata* are self-sterile, but all plants lack prezygotic barriers to self-fertilization. To determine whether postzygotic rejection of self-fertilized ovules is due to late-acting self-incompatibility or to extreme, early acting inbreeding depression, we performed three diallel crosses among self-sterile plants related as full-sibs. The full-sibs segregated into four compatibility classes, suggesting that late acting self-incompatibility is controlled by a single gene (S-locus). Crosses between plants sharing one or both alleles at the S-locus are incompatible. An additional diallel cross was done among full-sib progeny from a cross of a self-sterile and a self-fertile plant. These progeny grouped into two compatibility classes, and plants within classes displayed varying levels of self-fertility. This suggests that the occasional self-fertility documented in natural pollinations is caused by pseudo-self-fertility alleles that alter the functioning of the S-locus.

BOTH self-incompatibility (SI) and early acting inbreeding depression can lead to failure of self-pollinations to set fruit. Differentiating between these causes of self-sterility is usually straightforward. Early acting inbreeding depression results from the expression of detrimental embryonic genetic load (Charlesworth and Charlesworth 1987) and is manifested as abortion of selfed ovules. In contrast, most types of SI, including the well-described gametophytic and sporophytic systems, are prezygotic barriers that involve active recognition and rejection of self-pollen (de Nettancourt 1977, 1997). In gametophytic SI, incompatibility occurs when the haploid genotype of the pollen tube matches the diploid genotype of the female sporophytic tissue. Incompatible tubes typically are rejected in the style, although stigmatic rejection also occurs (see Frankl in *et al.* 1995). In sporophytic SI, incompatibility is determined by the diploid genotype of the male parent, and incompatible pollen is usually rejected on the stigma. The locus responsible for self-incompatibility differs in different systems and is typically referred to as the S-locus (de Nettancourt 1997).

Distinguishing between SI and severe, early acting inbreeding depression is more difficult in taxa with putative late-acting SI systems. In some cases of late acting SI, self-pollen tubes enter ovules but selfed ovules never mature into seeds (reviewed by Charlesworth 1985; Seavey and Bawa 1986; Sage and Williams 1994). In these species, growth of pollen tubes following self- and cross-pollination is similar (Waser and Price 1991; Gibbs and Bianchi 1993). Importantly, extreme in-

breeding depression could also cause selfed ovules to abort (Wiens *et al.* 1987; Krebs and Hancock 1990; Seavey and Carter 1994).

Three criteria have been proposed to differentiate late-acting SI from early acting inbreeding depression (reviewed by Charlesworth 1985; Seavey and Bawa 1986; Sage and Williams 1994). First, the hypothesis of late-acting SI predicts that rejection of selfed ovules should occur at a uniform stage across individuals, since the specific action of one or a few genes may control the rejection. In contrast, developmental stages at which ovules abort due to inbreeding depression should vary among individuals; different genes are responsible for abortion in different individuals, and the developmental stages at which these genes are expressed varies (Seavey and Bawa 1986; Wiens *et al.* 1987; Sage and Williams 1994). Second, there are no known cases in which all individuals of a population are self-sterile due to inbreeding depression, although complete self-sterility has been found in individual plants (Wiens *et al.* 1987; Krebs and Hancock 1990; Waser and Price 1991; Seavey and Carter 1994). Thus, if nearly all individuals in a population are self-sterile, then late-acting SI is implicated. The converse is not true; given the frequent occurrence of pseudo-self-fertility alleles that mitigate the effects of SI (reviewed by Levin 1996), variable expression of self-sterility among individuals could indicate either late-acting SI or extreme inbreeding depression (see Lipow *et al.* 1999). Third, if several closely related species do not self and all lack prezygotic barriers to prevent double fertilization, then late-acting SI, but not inbreeding depression, is implicated (Lipow and Wyatt 1999). This is because the genetic load required for complete self-sterility based on inbreeding depression is so unusually high that it is rarely, if ever, reached

Corresponding author: Sara Lipow, Department of Forest Science, University of Oregon, Corvallis, OR 97331-5752.
E-mail: sara.lipow@orst.edu

by any species (Waser and Price 1991). Thus, it is extremely improbable that each of several related species would have independently accumulated a sufficiently high genetic load. On the other hand, true SI is normally conserved within plant families.

Although the criteria outlined above can provide results that support a hypothesis of late-acting SI, only genetic analysis can unequivocally establish the underlying genetic basis of self-sterility (Charlesworth 1985; Seavey and Bawa 1986). Because only one or a few genes typically control SI, self-incompatible species usually show segregation within families for alleles at the gene(s). Thus, related plants segregate into a limited number of intraincompatible classes. Some or all of these classes are compatible with each other, and the pattern of intercompatibility depends on the particular genetic system involved. In contrast, since inbreeding depression is caused by many loci, related plants do not segregate into discrete classes.

Importantly, SI is rarely absolute and, regardless of its type, genetic studies almost always reveal anomalous self-fertile plants or specific crosses that do not behave as predicted. For instance, Ascher (1984) found that 4 of 10 individuals of *Petunia violacea*, a species with gametophytic SI, were self-fertile to various degrees. Similarly, *Lolium perenne* expresses two-locus gametophytic SI, but Cornish *et al.* (1979) identified two plants that were homozygous at the S-locus because they had arisen from self-fertilizations. Examples from sporophytic systems include the high rate (25.4%) of observed self-fertility among 12 families of *Iberis amara* (Bateman 1954) and the 17.5% of crosses among individuals of *Crepis foetida* found to deviate from their expected behavior (Hughes and Babcock 1950). These latter deviations are composed of crosses that were expected to be compatible but proved to be incompatible and crosses that were expected to be incompatible but proved to be compatible. Such deviations are problematic. According to Ascher (1984), occasional self-fertility "appears ubiquitous among SI angiosperms: it has been observed in all species subjected to serious SI studies, all too often confounding experiments designed to elucidate genetic control of pollen-pistil specificity."

Unfortunately, genetic analysis of late-acting SI is even more difficult than genetic analysis of prezygotic SI. With prezygotic SI, the compatibility of a cross can be assessed quickly by examining pollen germination and/or pollen tube growth in hand-pollinated pistils. Such examinations also permit crosses involving 100% compatible pollen to be distinguished from crosses involving a smaller percentage of compatible pollen. With late-acting SI, however, one often has to wait until fruits mature to assess compatibility. Additionally, crosses involving a mixture of compatible and incompatible pollen will produce ovaries containing some compatibly fertilized ovules and some incompatibly fertilized ovules, and there may be no easy way to determine

whether such ovaries are capable of maturation. Finally, early acting inbreeding depression may cause some crosses among related plants to fail, even when late-acting SI is the primary cause of cross-sterility. Indeed, in highly self-sterile populations, inbreeding depression regularly leads to lower fruit-set from inbred crosses than from crosses among unrelated plants (Klekowski 1988; Levin 1989; Seavey and Carter 1994). Perhaps because of these problems, the few attempted analyses of segregation patterns for taxa suspected of possessing late-acting SI have been equivocal. For example, studies of *Theobroma cacao* suggest that incompatibility interferes with gametic fusion, but through some poorly understood gametophytic-sporophytic system (Knight and Rogers 1955; Cope 1962). Additionally, three groups have reported results from small, full-sib diallels among individuals of *Gasteria* spp. and variously argued for one (Brewbaker and Gorez 1967), two (Naaborgh and Willemse 1992), or three (Brandham and Owens 1978) genetic loci controlling the presumed postzygotic SI system.

Despite the difficulties inherent in genetic characterization of late-acting SI, earlier evidence suggesting that milkweeds possess late-acting SI prompted us to conduct such an analysis. At least seven species of *Asclepias* rarely, if ever, produce fruit following self-pollination (Sparrow and Pearson 1948; Wyatt 1976; Kephart 1981; Kahn and Morse 1991; Sage and Williams 1991; Wyatt *et al.* 1996). In four of these species, growth of self- and outcross-pollen tubes has been reported to be indistinguishable from germination to ovule penetration, and in *Asclepias syriaca* self-pollen was slightly more successful than cross-pollen (Kahn and Morse 1991). Moreover, detailed studies of self-pollinated flowers of *A. syriaca* and *A. exaltata* show that male gametes are released into the female gametophyte and that initial development of endosperm occurs. Subsequently, the selfed ovules consistently fail; selfed zygotes do not undergo mitosis, and the endosperm stops growing (Sparrow and Pearson 1948; Sage and Williams 1991). Moreover, other members of the Asclepiadaceae, including *Gonolobus suberosus* (Lipow and Wyatt 1998) and *Periploca aphylla* (Lipow 1998), as well as *Apocynum cannabinum* of the closely related Apocynaceae (Lipow and Wyatt 1999), are also entirely self-sterile, but appear to lack prezygotic barriers to double fertilization. Below we characterize the genetic basis of this self-sterility in *A. exaltata*.

MATERIALS AND METHODS

Species description: *A. exaltata* L. is a perennial herb native to woodland habitats from northern Georgia to Maine and westward to Minnesota and Iowa (Woodson 1954). It occupies forest clearings and roadsides sheltered by forests. Mature plants usually produce one to three stems, each of which typically bears one to six

		MALE PLANT							
		1	2	3	4	5	6	7	8
FEMALE PLANT	1	0 2	0 2		1 2	1 2	0.50 2		
	2	0 2	0 2	1 2 D4	0.67 3	0.75 4	1 2 D2		
	3		1 2	0.80 5	1 3	0.67 3	1 4	0.67 3	0.83 6
	4	1 3 D1	1 1	1 2	0 4	0.67 3	0.67 3		0.5 2
	5	1 2	1 1	0.80 5	0.50 2	0 4	0.20 5 ^a	1 2	0.75 ^a 4
	6	1 3	0.50 2	0.83 6	1 2 D3	0 10	0 6	1 4	0.83 6
	7		1 1	0.60 5	1 4	1 3	0.75 4	0 4	1 3
	8		1 1	0.88 7	0.67 3	0.25 ^a 4	0.75 4	1 3	0 4

^aIndicates crosses discussed in text.

Figure 1.—Eight field-collected plants of *A. exaltata* were crossed in a near-complete diallel including self-pollinations. The top number shows the proportion of pollinated umbels that matured fruits and the bottom number represents the number of umbels pollinated. In all cases, five flowers per umbel were pollinated with pollen from a single donor. The four crosses from which families were generated for subsequent diallels D1–D4 are indicated (see materials and methods).

umbels of 10–25 flowers (Shannon and Wyatt 1986). As in all milkweeds, pollen is produced in discrete sacs termed “pollinia.” Pollinia contain ~180 pollen grains, which is more than the number necessary to fertilize all of the 60–80 ovules in a single ovary (Wyatt 1976; Bookman 1983a). Pollinia are transported between plants by strong-flying insects, such as bees and butterflies (Broyles and Wyatt 1990). The gynoecium consists of two ovaries, of which only one usually matures into a follicle. In most milkweeds, including *A. exaltata*, fruit-set is low, typically <5% in natural populations (Wilbur 1976) and ranging from 15 to 25% after hand-pollination (Queller 1985). Like all species of *Asclepias*, *A. exaltata* is diploid with $n = 11$ (Woodson 1954).

Diallel crosses: Rootstocks were collected from eight adult plants, separated by at least 2 m, in a natural population of *A. exaltata* (Brasstown Bald, Union County, GA). The plants were grown in a pollinator-free greenhouse at the University of Georgia, where they were crossed in a near-complete diallel including self-pollinations (referred to as the “parental diallel”). Seven of the eight plants proved to be entirely self-sterile, whereas one plant (3P) was self-fertile.

Four families of plants (denoted D1–D4) were grown from seeds produced in the parental diallel. Plants within families were crossed in the greenhouse in diallels including self-pollinations. Reciprocal backcrosses

between these plants and their parents were also performed. The parents of D1, D2, and D3 were all entirely self-sterile (female \times male: 4P \times 1P, 2P \times 6P, 6P \times 4P, respectively), but D4 (2P \times 3P) was generated from a self-sterile plant (2P) crossed to a self-fertile plant (3P). All plants within a family were related as full siblings and were grown from seeds from a single fruit.

For all diallels, we pollinated five flowers per umbel, and, at most, two umbels per flowering stem. During the hand-pollinations, a pair of anther wings of a recipient flower was splayed open using a large-diameter sewing needle. A single pollinium from a flower of a pollen donor was then inserted into the exposed stigmatic chamber, convex margin first, and the anther wings were gently pressed back together. This relatively complicated pollination method was necessary because milkweeds have pollinia and a complex floral morphology (Wyatt and Broyles 1994). These features increase the difficulty of performing large numbers of pollinations but have the advantage of decreasing the likelihood of pollen contamination. A single pollen donor was used per flowering stem to minimize the potential for pollen competition. To lower resource investment in fruit maturation (Chaplin and Walker 1982; Bookman 1983b, 1984), all but one fruit per umbel was removed after determining fruit-set for the umbel. Fruits were not removed until they had reached a minimum size of

TABLE 1
Fruit- and seed-set following cross-pollinations of field-collected plants of *A. exaltata*

Diallel	Fruit traits		Seed traits		
	Fruit-set (<i>N</i>)	Proportion of umbels maturing fruit	<i>N</i>	Mean (SE) filled seeds	Mean (SE) proportion of filled seeds
Parental	0.343 (760)	0.730 (152)	52	79.73 (2.85)	0.923 (0.015)
D1	0.508 (325)	0.800 (65)	8	58.16 (5.71)	0.895 (0.037)
D2	0.184 (125)	0.520 (25)	4	69.00 (7.26)	0.964 (0.016)
D3	0.307 (75) ^a	0.600 (15) ^a	9	59.33 (7.83)	0.930 (0.029)
D4	0.422 (90)	0.722 (18)	7	46.25 (13.67)	0.942 (0.033)

^a Excludes female-sterile plant 10 (see text).

2 cm and had matured for at least 2 wk. We assumed that these fruits would have completed development, because fruits of this size and age rarely abort spontaneously (Queller 1985; Shannon and Wyatt 1986). Hand-pollinations were performed over a 3-year period from 1995 to 1997, and numbers of filled (and presumably viable) and unfilled (and presumably inviable) seeds were counted for most fruits produced during the first 2 years.

RESULTS

Parental diallel: Seven of the eight field-collected plants did not set fruit following self-pollination (Figure 1). Fruit set of the eighth plant (3P), however, was equivalent after self- (44.0%, $N = 25$) and cross-pollination (46.7%, $N = 105$). The selfed fruits contained fewer filled seeds (27.0 ± 9.41 ; mean \pm SD) than fruits from cross-pollinations (Table 1), but plants were successfully grown to maturity from these seeds. Cross-pollinations among the parental plants were performed for 47 of the 56 possible combinations (crosses). For these pollinations, per-flower fruit-set averaged across all crosses was 36.0%, and 75.6% of umbels produced one or more fruits (Table 1). In all but five crosses, one or more fruits were produced on at least half of the pollinated umbels. Four of the exceptional crosses represented reciprocal pairs ($2P \times 1P$ and $1P \times 2P$ and $5P \times 6P$ and $6P \times 5P$). Three were entirely incompatible, maturing no fruits, and the fourth ($5P \times 6P$) matured only a single fruit from 25 pollinated flowers. This fruit, however, contained only 11 seeds, of which 4 were shrunken and inviable. This seed number is much lower than the mean of 79.7 (Table 1), and the next lowest seed number from outcrossing was 35. We therefore consider the cross $5P \times 6P$ to be incompatible. Finally, the fifth exceptional cross, $8P \times 5P$, produced only two fruits from 20 pollinated flowers, but in this case we suspect that the apparent incompatibility is due to chance. Although the probability of obtaining only two fruits from 20 pollinations is 0.01 (calculated by assuming that fruit-set is binomially distributed around the mean value of

0.360), one aberrant cross out of 47 crosses is not unexpected. Moreover, the fruits from $8P \times 5P$ had seed numbers within the normal range (64 and 42), and the reciprocal cross ($5P \times 8P$) had average fruit-set (35.0%, $N = 20$).

General results from D1, D2, and D3: Outcross-, full-sib-, backcross-, and self-pollinations were performed for D1, D2, and D3. In all diallels, fruits were produced by more than half of the outcrossed umbels (Table 1). Fruit-set following full-sib crosses, which involved an average of 17.1 pollinated flowers on 3.4 umbels, displayed a clear bimodal distribution when umbels were treated as replicates (Figure 2). All pollinated umbels matured fruit from 178 of 811 crosses (22%). No umbels matured fruit for 511 of 811 crosses (63%). For the remaining 15% of the crosses, however, fruits matured on only some pollinated umbels. This between-umbel variation probably had two causes: (1) low overall fruit-set that is characteristic of milkweeds and results in failure of some compatible pollinations, and/or (2) "leakiness" in the presumed late-acting SI system, which allowed occasional fruit production from what should

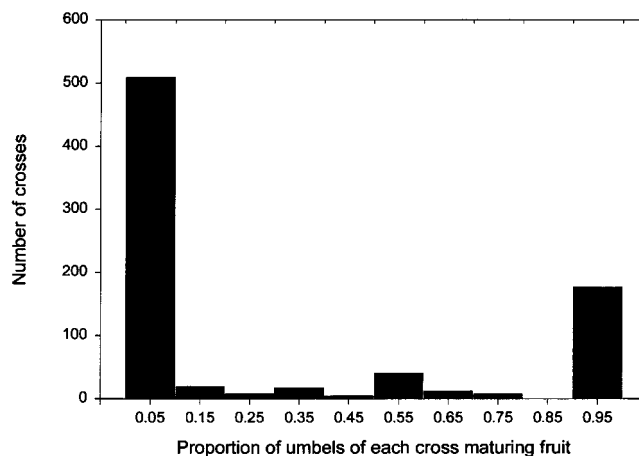


Figure 2.—Frequency histogram showing the proportion of umbels that matured fruit for crosses among plants of *A. exaltata* related as full siblings. The histogram includes data from D1, D2, and D3 (see materials and methods).

TABLE 2

Categorization of full-sib crosses according to the proportion of pollinated umbels setting at least one fruit and taking into account the total number of umbels pollinated

Category	Criteria for crosses with small sample sizes		Criteria for crosses with large sample sizes	
	Proportion of umbels maturing fruit	No. of umbels pollinated	Proportion of umbels maturing fruit (x)	No. of umbels pollinated
Incompatible	0	3	$x \leq 0.20$	>3
Probably incompatible	0	2	$0.20 < x < 0.33$	>3
Compatible	1	2	$x > 0.50$	≥ 3
Probably compatible	1	1	$x = 0.50$	>3
Ambiguous	0.50	2	$0.33 \leq x < 0.50$	≥ 3
Insufficient data	—	0	$x = 0$	1

See materials and methods.

have been incompatible pollinations. An example of a “leak” is the low fruit- and seed-set described above for the cross 5P \times 6P.

Crosses were repeated until we were reasonably certain that they were either compatible or incompatible. Constraints imposed by flower availability and mortality of a few plants, however, prevented us from repeating crosses indefinitely. Thus, to minimize misclassification of crosses attributable to low overall fruit-set or to leaks in incompatibility, we assigned crosses to five categories based on proportions of umbels setting fruit and on sample sizes (Table 2).

We compared fruit-set per umbel and seed-set for fruits produced from compatible pollinations and from

presumably leaky, incompatible pollinations. The analyses included all umbels setting fruit from full-sib crosses, excluding those assigned to the ambiguous category. Analysis of variance was used to examine variation in the number of filled seeds and the proportion of filled seeds (Table 3). Fixed effect models that included leak and female plant as main effects were analyzed using the GLM procedure of SAS (SAS Institute 1985), after an angular transformation had been applied to the proportion of filled seeds. The analyses showed that, for all three diallels, seed number was lower in fruits from leaky, incompatible pollinations than from compatible pollinations, and this variation was highly significant. The proportion of filled seeds was also lower in fruits

TABLE 3

Analysis of variance and mean values for the number of filled seeds and the proportion of filled seeds in fruits produced from leaky, incompatible crosses and from compatible crosses

Diallel	Fixed effect	d.f.	No. of filled seeds			Proportion of filled seeds		
			Type III SS	F	P	Type III SS	F	P
D1	Leak	1	6139.1	17.85	<0.0001	1.196	20.73	<0.0001
	Female plant	14	40495.0	8.41	<0.0001	0.159	2.76	0.001
	Model r^2		0.568			0.323		
D2	Leak	1	6016.2	10.48	0.002	0.151	4.01	0.050
	Female plant	13	16347.9	2.19	0.220	1.050	2.15	0.025
	Model r^2		0.394			0.360		
D3	Leak	1	7035.3	17.59	<0.0001	0.007	0.17	0.667
	Female plant	13	7815.7	1.50	0.137	0.102	2.46	0.008
	Model r^2		0.387			0.303		
	Type of cross	N	Mean	SD		Mean	SD	
D1	Compatible	115	65.85	23.84		0.902	0.116	
	Incompatible leaks	53	37.96	23.18		0.756	0.259	
D2	Compatible	58	66.52	24.57		0.826	0.156	
	Incompatible leaks	13	45.77	34.29		0.565	0.116	
D3	Compatible	58	53.69	22.19		0.796	0.153	
	Incompatible leaks	31	30.48	17.65		0.781	0.169	

The analysis includes data from D1–D3.

TABLE 4
Analysis of variance and mean values for the number of fruits produced per umbel from leaky, incompatible crosses and from compatible crosses

Diallel	Fixed effect	n.d.f.	d.d.f.	Type III <i>F</i>	<i>P</i>	Deviance	Dispersion
D1	Leak	1	213	32.53	<0.0001	257.18	1.06
	Female	14	213	1.76	0.046		
	Model						
D2	Leak	1	84	7.07	0.009	90.01	1.01
	Female	15	84	1.51	0.117		
	Model						
D3	Leak	1	91	9.85	0.002	111.01	1.12
	Female	11	91	1.33	0.222		
	Model						
	Type of cross		<i>N</i>	Mean	SD		
D1	Compatible		176	2.597	1.243		
	Incompatible leaks		53	1.528	0.799		
D2	Compatible		82	2.098	1.193		
	Incompatible leaks		19	1.421	0.090		
D3	Compatible		77	2.021	1.207		
	Incompatible leaks		27	1.481	1.087		

The analysis includes results from D1–D3 and uses a generalized linear model that takes into account the binomial distribution of the data (see materials and methods). n.d.f., numerator degrees of freedom; d.d.f., denominator degrees of freedom.

from leaky, incompatible pollinations, and this effect was significant for D1 and D2. Finally, significant variation attributable to female plant was detected for seed number only in D1; for the proportion of filled seeds, however, significant variation due to the female plant was detected in all three diallels.

To compare variation in fruit-set per umbel from leaky pollinations and compatible pollinations, we employed GLIMMIX, a recently developed SAS macro that is an extension of generalized linear mixed-model theory. GLIMMIX can account for the binomial distribution of data such as fruit-set (Littell *et al.* 1996). The models we analyzed included “leak” and “female plant” as fixed effects and used restricted maximum likelihood to estimate variance components. The results showed that, on umbels that set fruit, the number of fruits was significantly lower from leaky, incompatible pollinations than from compatible pollinations, for all three diallels (Table 4). The effect of female plant on fruit-set per umbel, however, was significant only for D1. Finally, the dispersion factor, which measures whether the conditional error of variance associated with flowers within umbels fits the assumed binomial distribution, was very close to one for all three diallels (see Littell *et al.* 1996). This suggests that the probability of each pollinated flower maturing fruit was not influenced by other flowers in the umbel.

Specific results from D1: Figure 3 shows the proportion of umbels maturing fruit from self-pollinations, from crosses between full-sibs, and from reciprocal backcrosses between progeny and their female parent. Unfortunately, the male parent (1P) died before back-

crosses could be done. Figure 4 reports the same results except that the data from each pair of reciprocal crosses (*e.g.*, 11 × 6 and 6 × 11) have been pooled. The net effect of pooling is to move crosses from the “probably compatible” category to the “compatible” category, or from the “probably incompatible” category to the “incompatible” category, or to resolve ambiguities apparently caused by small sample sizes for certain crosses.

There are eight cases, however, for which the coding of crosses changes more drastically or becomes more ambiguous when the data from reciprocal crosses are pooled. Three of these (3 × 1, 3 × 24, and 3 × 4P) are probably due to sampling error (small sample sizes). Four other cases (6 × 23, 8 × 15, 5 × 15, and 9 × 15) were categorized as compatible or probably compatible when plants 15 and 6 served as the female parent but variously categorized as incompatible, probably incompatible, or ambiguous when plants 15 and 6 served as male parent. We believe that “leaks” may have been especially common for plants 15 and 6. These were the only two plants in D1 that matured fruits from self-pollinations, and in all cases the fruits contained only small numbers of seeds, all of which were unfilled. More importantly, on these plants we regularly observed swelling of both ovaries of flowers that were either unpollinated or pollinated with only a single pollinium. These ovaries appeared to begin maturation and often persisted on the plants 1–3 wk longer than ovaries of other plants. We believe that this unusual behavior, although not directly responsible for either SI or inbreeding depression, was related to the unusually high fruit production from crosses that we would have otherwise expected

		MALE PLANT																
		7	10	16	15	5	8	24	23	11	9	3	6	1	32	17	4P	
FEMALE PLANT	7	0	0	0	0	0	.06	.06	.17	.08	0	0	.75	1	1	1	.05	
		6	5	10	7	10	16	17	18	13	6	8	4	2	2	6	19	
	10	0	0	0	0	0	.06	0	0	0	0	0	1	1	.5	.6	0	
		6	6	5	6	7	16	6	5	6	3	8	2	1	8	5	8	
	16	0	0	0	0	0	.1	.06	0	.09	0	.1	1	1	.56	.5	0	
		9	7	11	10	13	10	16	11	11	5	10	3	2	9	6	9	
	15	0	0	.17	.2	.71	.67	1	.4	0	.5	.75	1	1	1	1	.2	
		6	4	6	5	7	3	2	5	5	4	4	3	0	2	2	15	
	5	0	0	.1	0	0	0	.13	.06	1	.6	.5	.75	1	1	.6	0	
		5	8	10	11	10	7	8	17	5	5	2	4	1	4	10	7	
	8	.13	0	.13	.25	0	0	0	.25	1	1	.67	1	1	1	1	0	
		16	11	8	8	14	17	20	12	3	1	3	4	1	4	2	12	
	24	.09	0	.33	0	0	.11	0	0	.2	.67	.2	.09	0	.2	.11	.02	
		11	5	9	9	5	9	8	9	5	3	5	11	1	5	9	6	
	23	0	0	0	0	0	.06	0	0	.63	.5	1	0	0	0	0	0	
		7	6	11	10	6	18	6	5	8	2	3	8	2	6	6	13	
	11	0	0	0	0	1	.67	1	.67	0	0	0	0	0	0	0	0	
	8	7	7	6	3	3	3	6	6	5	7	10	1	11	7	7		
9	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0		
	3	3	4	2	1	1	1	1	3	3	3	3	1	3	2	3		
3	0	.5	.25	1	1	1	1	1	0	0	0	1	0	.17	0	.5		
	3	2	8	1	2	1	1	2	8	5	5	1	2	6	5	2		
6	1	.5	.6	1	1	1	.13	.56	0	.5	.4	.1	0	.14	0	.2		
	1	4	5	1	2	1	8	9	6	2	5	10	0	7	3	5		
1	0	1	1	0	1	1	0	0	0	0	1	0	0	0	0	0		
	1	1	1	0	1	1	3	1	1	2	1	2	2	2	3	2		
32	.75	1	.67	1	1	1	0	0	0	0	.08	0	0	0	0	0		
	4	2	6	3	4	3	7	11	10	4	13	10	1	12	10	12		
17	.56	1	1	.5	.75	.75	0	.13	0	0	0	0	0	0	0	0		
	9	2	3	4	4	4	10	23	9	3	6	10	1	10	8	9		
4P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	a		
	4	2	2	2	3	4	2	2	2	3	2	2	2	2	2	2		

■ compatible,
 ■ probably compatible,
 ■ ambiguous,
 ■ probably incompatible,
 ■ incompatible,
 ■ insufficient data

^a See Figure 1

Figure 3.—Plants of *A. exaltata* related as full-sibs were crossed in a diallel (D1), including self-pollinations and backcross-pollinations to the female parent (4P). The top number shows the proportion of umbels that matured fruit, and the bottom number shows the number of umbels pollinated. In all cases, five flowers were pollinated in each umbel. Numbered progeny have been rearranged into incompatibility types, based upon crossing success. The compatibility of each cross was categorized according to the criteria outlined in Table 2, and the shading reflects this categorization.

to be incompatible. Thus, only one pair of crosses displayed inexplicable reciprocal differences: all three pollinated umbels of the cross 11 × 24 matured fruits, but only one of five umbels of the reciprocal cross 24 × 11 matured fruits.

Specific results from D2 and D3: Figures 5 and 6 show the proportion of umbels setting fruit for D2 and D3. Inspection of the unpooled data shows that, despite the fact that D2 and D3 comprised some 4720 and 4215 hand-pollinations, respectively, the compatibility of many crosses remains unresolved because of inadequate sample sizes. For this reason, the crosses are categorized based on the results from pooled data, although values for unpooled data are shown. Importantly, apart from the exceptions noted below, we found little evidence for reciprocal differences between crosses in these data sets or for crosses in the much larger data set of D1 (7415 hand-pollinations).

Several plants included in D2 and D3 warrant special attention. Plant 10 in D3 appeared to be largely female sterile, though not male sterile. Fruit-set following cross-pollination of this plant was only 4.0% ($N = 125$), which is much less than the average outcross fruit-set of 30.7% ($N = 75$) for all other plants in the 6P × 4P family (Table 1). To account for this female sterility, we have

coded crosses 10 × 11 and 10 × 50 as “compatible” based on the male function of plant 10, even though they would otherwise fall into the “ambiguous” category. Plant 12 from D2 and plant 50 from D3 also produced many more fruits as females than as males. Plant 12 matured fruits pollinated from most plants, including parental plants 2P and 6P, except 27, 6, and itself. Only plants 35, 31, 40, 25, and 5, however, matured fruits pollinated by plant 12. Similarly, plant 50 from D3 matured fruits pollinated by every plant except 9, including its parents, but only plants 3, 6, 7, and 52 regularly matured fruits pollinated by plant 50.

Genetic interpretation of D1, D2, and D3: Plants from D1–D3 related as full siblings segregated into a limited number of intraincompatible classes, and some of these classes were cross-compatible (Figures 4–6). The ratio of compatible to incompatible full-sib crosses was 1:2.45 (Table 5). Four plants occasionally set fruits following self-pollinations, but in all cases, these fruits contained small numbers of seeds, all of which were inviable. Most backcrosses to the parental plants were incompatible, but a few (<10%) were apparently compatible.

A single-locus model of SI can account for most of the data from D2 and D3, but it only partially explains the results from D1. First, we assume that the parental

plants for each diallel were heterozygous for different alleles at the SI locus (S), such that one parent was S_1S_2 and the second parent was S_3S_4 (see Figure 7). Then, as shown in Table 6, the full-sib progeny can be assigned to the four genotypic classes that would result from segregation of those four alleles. Crosses should be incompatible whenever two plants share one or both alleles at the S-locus (Figure 7).

The one-locus model fits the data from D2 remarkably well. If the atypical plant 12 is ignored for now, then according to the classification scheme shown in Table 6, no crosses were falsely categorized as compatible and only four reciprocal crosses were falsely categorized as incompatible (36×25 , 36×5 , 6×25 , and 6×5 ; Figure 5). Two of these (36×25 and 36×5) involved only two pollinated umbels. The other two crosses (6×25 and 6×5) set some fruit, albeit on only 30.0 and 20.0% of the pollinated umbels, and these fruits had seed numbers that were about average for compatible pollinations of this family (98 filled, 2 unfilled; 67 filled, 0 unfilled; 48 filled, 3 unfilled). The false incompatibility of these crosses might therefore be attributed to sampling error, especially since this family displayed the lowest rate of outcross fruit-set (Table 1). Moreover, as we pointed out earlier, it would not be surprising if

some compatible, full-sib crosses failed to mature fruits because of inbreeding depression. Plant 12 can also be added into this one-locus scheme; it can be assigned to class I, because it was reciprocally cross-incompatible with the other plants in class I and reciprocally cross-compatible with plants in class IV. It was abnormal, however, in that it matured fruits when pollinated by plants in classes II and III, with which it presumably shares one allele at the S-locus. Similarly, it matured fruits from backcross pollinations to its parents, with which it also shares one S-allele. Thus, whereas one shared allele was sufficient to confer incompatibility for all other plants, plant 12 required two shared alleles.

The one-locus model fits the data from D3 nearly as well as it fits the data from D2. Again, if we ignore the atypical plant 50, we find no full-sib crosses falsely categorized as compatible or as incompatible (Figure 6). There are, however, two backcrosses categorized as probably compatible ($3 \times 6P$, $12 \times 4P$), but each involves only a single pollinated umbel. Additionally, 10 reciprocal pairs of full-sib crosses and backcrosses that, based on the one-locus model, should be incompatible, set fruit often enough that they were assigned to the ambiguous category. With one exception, however, these ambiguous crosses involved only two or three pollinated

		MALE PLANT															
		7	10	16	15	5	8	24	23	11	9	3	6	1	32	17	4P
FEMALE PLANT	7	0 12	0 11	0 19	0 13	0 15	.09 32	.07 28	.12 25	.05 21	0 9	0 11	.8 5	.67 3	.83 6	.73 15	.04 23
	10		0 12	0 12	0 10	0 15	.04 27	0 11	0 13	0 6	0 10	.1 6	.67 2	1 10	.6 7	.71 10	0 10
	16			0 22	.06 16	.04 23	.11 18	.16 25	0 22	.06 18	0 9	.17 18	.75 8	1 3	.6 15	.67 9	0 11
	15				.2 10	.28 18	.36 11	.18 11	.13 15	0 11	.33 6	.8 5	1 4		1 5	.67 6	.18 17
	5					0 20	0 21	.08 13	.04 23	1 8	.67 6	.75 4	.83 6	1 2	1 8	.64 14	0 10
	8						0 34	.03 29	.13 30	.83 6	1 2	.75 4	1 5	1 2	1 7	.83 6	0 16
	24							0 16	0 15	.5 8	.75 4	.33 6	.11 19	0 4	.08 12	.05 19	.25 8
	23								0 10	.64 14	.5 4	1 5	.29 17	0 3	0 17	.1 29	0 15
	11									0 12	0 8	0 15	0 16	0 1	0 21	0 16	0 9
	9										0 6	0 8	.2 5	0 3	0 7	0 5	0 6
	3											0 10	.5 6	.33 3	.11 19	0 11	.25 4
	6												.1 20	0 2	.06 17	0 13	.14 7
	1													0 4	0 3	0 4	0 4
	32														0 24	0 20	0 14
	17															0 16	0 11
	4P																a

compatible, probably compatible, ambiguous, probably incompatible, incompatible, insufficient data

^a See Figure 1

Figure 4.—Plants of *A. exaltata* related as full-sibs were crossed in a diallel (D1), including self-pollinations and backcross-pollinations to the female parent (4P). The top number shows the proportion of umbels that matured fruit, and the bottom number shows the number of umbels pollinated, after data from reciprocal pairs were pooled. In all cases, five flowers were pollinated in each umbel. Numbered progeny have been rearranged into incompatibility types, based upon crossing success. The compatibility of each cross was categorized according to the criteria outlined in Table 2, and the shading reflects this categorization.

		MALE PLANT																	
		12	27	36	6	16	2	18	34	3	32	23	35	31	40	25	5	2P	6P
FEMALE PLANT	12	0	0	.33	0	1	1	.33	1	1	1	1	.33	1	1	1	1	1	1
		12	3	3	9	2	2	3	1	2	1	2	3	1	1	2	1	1	2
	27	0	0	0	0	.13	0	0	0	0	0	0	.25	.6	.67	1	.5	2	0
		6	16	3	8	8	2	6	4	5	2	4	5	3	1	2	2	1	9
	36	0	0	0	0	0	0	0	0	0	0	0	0	.66	.33	1	0	0	0
		3	4	8	2	2	3	2	2	3	1	1	3	3	1	2	1	1	2
	6	0	0	0	0	0	0	0	0	.5	0	0	0	1	1	1	.5	1	.5
		5	7	3	10	5	2	4	4	5	3	4	2	1	2	6	1	2	6
	16	0	0	0	0	0	0	0	0	1	.83	.66	1	0	0	0	0	0	0
		8	8	5	7	16	3	4	1	6	3	1	8	7	3	8	4	2	8
	2	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0
		2	1	1	1	2	6	1	1	4	5	3	4	2	1	2	2	2	3
	18	0	0	0	0	0	0	0	0	1	.5	1	1	0	0	0	0	0	0
		8	5	4	6	5	3	8	1	2	1	1	4	4	1	3	4	2	4
	34	0	.33	0	0	.5	1	1	0	0	0	0	0	0	0	0	0	0	0
		4	3	0	1	2	1	2	4	3	0	3	3	3	4	1	2	3	0
	3	0	0	.5	0	.33	1	1	0	0	0	0	0	0	0	0	0	0	0
		7	8	2	3	3	2	2	8	16	3	3	10	7	0	9	4	2	9
	32	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
		0	0	2	1	1	1	0	1	6	2	1	2	0	1	2	1	2	1
23	0	0	0	1	.67	1	1	0	0	0	0	0	0	0	0	0	0	.5	
	0	3	3	1	3	2	1	2	4	2	8	3	4	2	3	2	1	2	
35	.67	1	.5	.66	0	0	0	0	.14	0	0	0	0	0	0	0	0	.14	
	3	3	4	3	7	3	7	5	7	0	2	8	6	1	7	4	3	7	
31	1	.67	1	1	0	0	0	0	0	0	0	0	0	0	.33	0	0	0	
	2	3	2	1	8	3	5	5	7	3	3	7	10	3	7	4	1	9	
40	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1	2	1	1	1	1	0	1	1	1	1	2	3	4	1	2	3	2	
25	1	.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1	2	0	4	0	0	3	1	3	2	3	2	3	1	6	3	0	3	
5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	2	1	4	3	2	2	1	2	1	2	1	3	1	3	6	1	3	
2P	0	0	0	.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	0	1	0	4	1	1	0	0	1	1	2	0	2	0	0	0	0	0	
6P	0	0	0	0	0	0	0	0	.14	0	0	0	0	0	0	0	0	0	
	4	2	1	3	6	3	1	4	7	2	2	6	7	1	4	3		see Figure 1	

■ compatible,
 ■ probably compatible,
 ■ ambiguous,
 ■ probably incompatible,
 □ incompatible,
 ■ insufficient data

Figure 5.—Plants of *A. exaltata* related as full-sibs were crossed in a diallel (D2), including self-pollinations and backcross-pollinations to the female (2P) and male (6P) parents. The top number shows the proportion of umbels that matured fruit, and the bottom number shows the number of umbels pollinated. In all cases, five flowers were pollinated in each umbel. Numbered progeny have been rearranged into incompatibility types, based upon crossing success. Pooled data for each pair of reciprocal crosses (not shown) were used to categorize the crosses according to the criteria outlined in Table 2, and the shading reflects this categorization.

umbels. Nevertheless, in D2, only four reciprocal pairs of incompatible crosses were falsely categorized as ambiguous, and it therefore appears that the SI system was much leakier in D3 than in D2. Finally, the behavior of plant 50 was similar to that of plant 12 in D2. It has been assigned to class IV, because it was reciprocally cross-compatible with plants in class I. It set fruit when pollinated by plants in classes II and III, but those same plants failed to set fruit following pollination by plant 50. Thus, like plant 12, one shared incompatibility allele appears to be insufficient to confer incompatibility in plant 50.

The data from D1, however, fail to conform precisely to the one-locus model. The model does hold for the nine plants assigned to genotypic classes in Table 6. Inclusion of plants 11, 9, and 3 in class III and plants 15, 5, and 8 in class I leads, however, to the formation of eight pairs of falsely compatible crosses (15 × 3, 5 × 11, 5 × 9, 5 × 3, 8 × 11, 8 × 9, 8 × 3, 3 × 6, and reciprocals). Any other classification scheme, however, results in more crosses that fail to behave as predicted. Furthermore, the fruits from the apparently miscategorized crosses cannot be discounted as leaks because the

crosses had high overall fruit- and seed-set. For example, all eight umbels pollinated for the reciprocal cross 5 × 11 set fruit, and these umbels averaged 2.75 fruits, each of which contained a mean of 75.5 filled seeds with a mean proportion of filled seeds of 0.95.

Thus, to fit the data from D1 into the framework of a one-locus model, the action of modifier alleles at other genes must be invoked. In this case, a modifier that weakens the functioning of the S_i allele can explain many of the anomalies in the data. Plants 15, 5, and 8 (genotype S_iS_j), but not plants 7, 10, and 16 (also genotype S_iS_j), might possess this modifier, rendering crosses between plants 15, 5, and 8 and plants 11, 9, and 3 (genotype S_iS_i) compatible. Depending on the specific action of such a modifier, plants 11, 9, and 3 might or might not also express it. If this modifier allele is included in the one-locus model, only 2 of the 98 pairs of reciprocal full-sib crosses categorized as compatible, incompatible, and probably incompatible in Figure 4 remain unexplained. The modifier allele hypothesis predicts that incompatible cross 15 × 11 will be compatible; it also cannot account for the compatibility of cross 3 × 6.

		MALE PLANT															
		52	6	7	10	1	3	12	14	9	5	2	4	11	50	4P	6P
FEMALE PLANT	52	.25 4	0 2	0 1	0 2	.5 2	0 2	1 1	1 1	0 1	1 2	0 2	.5 2	1 1	1 1	0 2	0 2
	6	0 3	0 20	0 8	0 9	0 8	0 3	0 7	.14 7	1 1	0 2	0 7	0 7	.6 5	1 1	0 8	.1 10
	7	0 3	0 9	0 14	0 6	0 8	0 3	0 6	0 3	0 3	0 2	0 6	0 7	.75 4	1 2	.1 10	0 8
	10	0 4	0 8	0 6	0 6	0 6	0 3	0 4	0 4	0 1	0 1	0 6	0 5	a 7	a 6	0 7	0 4
	1	0 3	0 6	.13 8	0 5	0 14	0 2	0 5	0 2	0 5	0 2	.5 2	.75 4	0 7	.2 5	0 8	.67 9
	3	1 1	0 3	0 2	0 3	0 3	0 8	.2 3	0 4	0 3	1 1	1 1	1 3	0 1	0 1	.5 2	1 1
	12	0 2	0 3	.33 3	.33 3	0 5	.5 2	0 8	0 3	0 3	1 1	1 1	1 1	0 4	0 5	1 1	.5 2
	14	0 1	.33 3	0 2	0 2	1 1	0 2	0 0	0 4	0 1	0 1	0 1	0 1	.5 2	0 3	.5 4	0 2
	9	0 1	0 4	0 2	0 2	1 1	0 3	0 3	0 3	0 6	0 3	0 3	0 1	1 1	.5 2	0 3	0 2
	5	0 3	0 1	0 2	0 1	0 3	0 1	0 2	0 2	0 2	0 12	0 0	0 0	0 0	0 2	0 3	0 5
	2	0 3	0 2	0 3	0 3	1 2	1 1	.67 3	1 1	1 1	1 1	0 6	0 2	0 3	0 4	0 5	0 3
	4	0 3	0 8	0 4	0 6	1 3	.5 2	1 2	1 1	1 1	1 1	0 6	0 10	0 6	0 6	0 5	0 7
	11	1 1	1 6	1 6	1 4	0 6	1 1	1 1	0 4	0 2	0 0	0 6	0 9	0 8	0 6	0 7	0 6
	50	1 2	.67 3	1 3	1 3	1 3	.5 2	.5 2	.5 2	0 4	1 1	.5 2	.3 10	.33 6	.13 16	.2 10	1 3
	4P	0 1	0 0	0 2	0 1	0 3	0 1	0 0	0 4	0 0	0 0	0 0	0 0	0 0	0 0	0 3	0 0
	6P	0 2	0 5	0 5	0 5	0 9	0 0	0 1	0 0	0 0	0 0	0 2	0 6	0 4	0 5	0 0	0 0

■ compatible, ■ probably compatible, ■ ambiguous, ■ probably incompatible, □ incompatible, ▨ insufficient data
^a Indicates female-sterile plant (see RESULTS)

Figure 6.—Plants of *A. exaltata* related as full-sibs were crossed in a diallel (D3), including self-pollinations and backcross-pollinations to the female (6P) and male (4P) parents. The top number shows the proportion of umbels that matured fruit, and the bottom number shows the number of umbels pollinated. In all cases, five flowers were pollinated in each umbel. Numbered progeny have been rearranged into incompatibility types, based upon crossing success. Pooled data for each pair of reciprocal crosses (not shown) were used to categorize the crosses according to the criteria outlined in Table 2, and the shading reflects this categorization.

Because a one-locus model of SI cannot account exactly for all of the crossing results in D1, we also considered two-locus models. We evaluated numerous two-locus models, but were unable to identify one that provided a better fit to the data than the proposed one-locus model (Lipow 1998).

Specific results from D4: The progeny included in D4 resulted from crossing of a self-sterile (2P) to a self-fertile plant (3P). Of the 16 plants studied, 5 were self-compatible, with fruits maturing on at least half of the

pollinated umbels, whereas 11 were largely or entirely self-incompatible, with <20% of umbels maturing fruit (Figure 8). Moreover, the selfed umbels that set fruit on the largely self-incompatible plants matured fewer fruits than did those on the self-compatible plants: 1.143 ± 0.415 (7) vs. 1.786 ± 0.138 (14); mean \pm SD (*N*). These fruits also had fewer filled seeds [22.0 ± 16.10 (4) vs. 33.75 ± 13.8 (8)] and a smaller proportion of filled seeds [0.487 ± 0.415 (4) vs. 0.663 ± 0.138 (8)]. Sample sizes were too small, however, to permit testing for significant differences. Most plants appeared to be capable of backcrossing with their self-fertile parent, and at least some could be backcrossed to their self-sterile parent, although the latter parent died before all of the planned backcrosses with it could be completed.

The results from full-sib crosses in D4 did not fall into a clear bimodal distribution, as they had done for D1–D3. For 48.9% of the crosses, fruits matured on only some pollinated umbels (Figure 9). Of the remaining crosses, 32.9% always matured fruit and 18.3% never matured fruit. We decided not to pool pairs of reciprocal crosses, since unexplained reciprocal differences existed for nine pairs (10 \times 20, 11 \times 5, 12 \times 8, 16 \times 5, 26 \times 5, 26 \times 8, 26 \times 21, 5 \times 8, 8 \times 21, and reciprocals). Instead, a total of 226 of the 240 possible individual full-

TABLE 5

Summary of the proportion of crosses from D1–D4 categorized as compatible and incompatible according to the criteria outlined in Table 2

Diallel	Compatible ^a	Incompatible ^b	Ambiguous	N
D1	0.346	0.625	0.029	208
D2	0.235	0.722	0.043	230
D3	0.242	0.670	0.088	182
D4	0.645	0.197	0.158	228

^aIncludes crosses categorized as compatible and probably compatible.

^bIncludes crosses categorized as incompatible and probably incompatible.

		MALE PLANT			
		S_1S_3	S_1S_4	S_2S_3	S_2S_4
FEMALE PLANT	S_1S_3				■
	S_1S_4			■	
	S_2S_3		■		
	S_2S_4	■			

■ compatible, □ incompatible

Figure 7.—The model for control of postzygotic self-incompatibility in *A. exaltata* by a single S-locus. Two unrelated parental plants were assumed to be heterozygous for different alleles at the S-gene and to have genotypes S_1S_2 and S_3S_4 . These plants were crossed to generate arrays of full-sib progeny with genotypes S_1S_3 , S_1S_4 , S_2S_3 , and S_2S_4 . The full-sibs were subsequently crossed in diallels, and all crosses between plants sharing one or both alleles at the S-locus were incompatible.

sib crosses were categorized according to the criteria outlined in Table 2: 64.5% as compatible or probably compatible, 19.7% as incompatible or probably incompatible, and 15.8% as ambiguous.

The full-sibs in D4 roughly segregated into two classes. Class I consisted of self-incompatible plants 10, 11, 12, 14, and 20, whereas class II consisted of self-incompatible plants 16, 26, 17, and 27 and self-compatible plants 5, 8, 21, 15, and 22. No differences in full-sib crossing behavior were apparent between the self-incompatible and self-compatible members of class II. We compared the average proportion of umbels maturing at least one fruit on each plant for full-sib crosses (Table 7) both within and between classes using two-sample Z-tests for all pairwise combinations. For the between-class crosses, the proportion of umbels maturing fruit was high, and it was not influenced by which class of plants served as the female or as the male parent ($Z = 1.23$; $P > 0.05$). Most crosses within class II were compatible also, but

the average proportion of umbels maturing fruit (0.689) was significantly less for these crosses than it was for crosses between class I and II ($Z = 1.93$ and 3.31 ; $P = 0.55$ and $P < 0.001$). Within class I most crosses were incompatible; consequently, the proportion was significantly less for these crosses than for crosses within class II ($Z = 9.22$; $P < 0.0001$). Despite the significant differences, however, several individual crosses violated this two-class scheme. For example, within class I, the cross 10×20 appeared to be compatible, while within class II, the cross 21×8 was incompatible. There were also several cases of apparent incompatibility between plants in class I and class II (e.g., 12×8).

Genetic interpretation of D4: Two mechanisms can bring about self-fertility in otherwise self-incompatible species (reviewed by Levin 1996). True self-fertility results from replacement of incompatibility alleles by alleles conferring self-fertility at the S-locus. Alternatively, modifier alleles at genes other than the S-locus can inhibit the activity of functional S-alleles and cause pseudo-self-fertility. Pseudo-self-fertility is more common than true self-fertility, and its expression is much more variable (Levin 1996). It is characterized by a continuous distribution of self-fertility levels in progeny and by self-fertility that depends on the environment. The results from D4 are consistent with a model of pseudo-self-fertility, but not with a model of true self-fertility.

Although both mechanisms of self-fertility can produce two classes among full-sib progeny from the cross of a self-incompatible and a self-fertile plant, the pseudo-self-fertility model better fits the compatibility patterns observed in D4. First, assume that the self-fertile parental plant has a functional S-locus but is heterozygous for a dominant pseudo-self-fertility allele. Half of its progeny (class I) will not receive this allele and, therefore, will be self-incompatible. Within this class, progeny differing in both alleles at the S-locus will be compatible. Progeny in class II will be heterozygous for the pseudo-self-fertility allele and, thus, self-fertile and cross-fertile with all other plants. Likewise, with true self-fertility, progeny of a self-fertile plant heterozygous for a true self-fertility allele will group into self-incompatible and self-fertile classes. In this case, however, all progeny in

TABLE 6

Plants from D1–D3 segregated into presumed incompatibility classes and were assigned genotypes

Diallel	I (S_1S_3)	II (S_2S_3)	III (S_1S_4)	IV (S_2S_4)	Not placed
D1	7, 10, 16	24, 23		6, ^a 1, 32, 17	11, 9, 3, 15, ^a 5, 8
D2	27, 36, 6, 12 ^a	16, 2, 18	34, 3, 32, 23	35, 31, 40, 25, 5	
D3	52, 6, 7, 10	1, 3, 12, 14, 9, 5	2, 4	11, 50 ^a	

The classification scheme assumes that SI is controlled by a single gene and that the parental plants had genotypes S_1S_2 and S_3S_4 . The full-sib progeny therefore have genotypes S_1S_3 , S_1S_4 , S_2S_3 , and S_2S_4 . Only crosses between plants with no alleles in common are compatible.

^a Indicates exceptional plants referred to in results.

TABLE 7

The plants from D4 roughly segregated into two-compatibility classes and the average proportion of umbels that matured at least one fruit was calculated for crosses within and among these classes

Female × male class	Proportion of umbels that matured fruit	No. of crosses
I × I	0.214	40
II × II	0.690	72
I × II	0.776	62
II × I	0.689	62

the self-incompatible class should be intrincompatible, because these plants share at least one S-allele. The observation that some plants in class I of D4 are compatible fits the model of pseudo-self-fertility, but is contrary to that of true self-fertility.

There are several other reasons why pseudo-self-fertility is the mostly likely cause of self-fertility in D4. As expected, the expression of self-fertility among plants and cross-fertility between plants in class II was highly variable. Additionally, for about half of all full-sib crosses in D4, fruits were produced on some umbels, strongly suggesting that the self-fertility and cross-compatibility were environment dependent. Thus, plants in D4 display the characteristics typically associated with pseudo-self-fertility.

DISCUSSION

We have shown that *A. exaltata* expresses an SI system, controlled primarily by a single S-gene. Incompatibility occurs whenever two plants share one or more alleles at the S-gene. Earlier studies have demonstrated that the stage of rejection of self-fertilized ovules is highly uniform across individuals within species and across species of *Asclepias* (Sparrow and Pearson 1948; Sage and Williams 1991). Thus, there is little doubt that the active and specific rejection following self-pollination, which is the hallmark of SI, occurs in milkweeds. Our study appears to be the first rigorous demonstration that late-acting SI is a genetically based S-locus phenomenon in *Asclepias* (cf. Seavey and Bawa 1986; Sage and Williams 1994).

Several unique features of late-acting SI and of milkweed floral morphology prevent the single-locus SI system described here from being pigeonholed as either gametophytic or sporophytic. The male incompatibility phenotype is determined by the haploid genotype of the pollen tube in gametophytic SI, but the diploid genotype of the pollen parent determines the male phenotype in sporophytic SI (de Nettancourt 1977, 1997). In *A. exaltata*, one shared allele was sufficient to elicit an incompatibility response; therefore, self-recognition appears to involve the diploid male genotype.

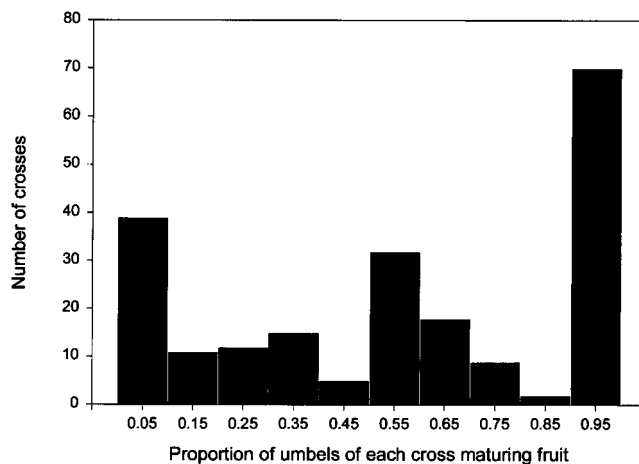


Figure 8.—Frequency histogram showing the proportion of umbels that matured fruit for crosses among plants of *A. exaltata* related as full siblings. The histogram includes data from D4 (see materials and methods).

This implies sporophytic control. Most sporophytic systems, however, show dominance hierarchies among SI alleles (de Nettancourt 1977), but such hierarchies were not detected in this study. Moreover, if the recognition and rejection steps of SI are decoupled, then recognition is not necessarily under diploid control. Suppose that the haploid genotype of individual pollen tubes determines the recognition step in milkweeds (*i.e.*, gametophytic control). Then, crosses of plants sharing one incompatibility allele result in ovaries with a 1:1 ratio of compatibly to incompatibly fertilized ovules. If more than 50% of ovules must be fertilized with compatible pollen in order for the ovary to mature into a fruit, then this cross would be rejected. Thus, either sporophytic or gametophytic self-recognition could explain the observed crossing patterns.

Self-incompatibility is not universal in *A. exaltata*, as one of eight field-collected plants and some of its progeny were entirely self-fertile. We have also identified pseudo-self-fertile individuals in several other natural populations of *A. exaltata*. Populations in the southernmost portions of the species range, including the northern Georgia population examined here, contain a higher frequency of these individuals (0–34%) than populations located toward the center of the species range in Virginia (3–22%; Lipow *et al.* 1999). The more southern populations tend to be more isolated and to contain fewer individuals. This could have created conditions under which the reproductive assurance provided by pseudo-self-fertility has been favored by selection.

It is not surprising that pseudo-self-fertility modifiers exist in *A. exaltata*. Partially self-fertile plants have been observed in hundreds of species that are normally self-sterile (Lloyd and Schoen 1992), and genes conferring pseudo-self-fertility have been described for taxa with

		MALE PLANT																		
		10	11	12	13	14	20	28	16	26	17	27	5	8	21	15	22	2P	3P	
FEMALE PLANT	10	.1 18	.3 3	.7 3	0 8	.3 3	.7 3		.8 5	1 2	1 3	.3 3	1 2	1 2	1 4	1 3	1 3		1 2	
	11	.3 4	.1 11	.2 5	.3 3	0 6	0 7	0 2	0 2	1 3	1 3	.5 2	.2 5	1 2	1 1	1 1	1 1		1 0	
	12	.3 3	0 5	0 8	0 4	0 5	0 8		1 2	1 2	.5 4	.5 2	.4 11	0 8	.4 5	1 2	1 3		0 2	
	13	.3 3	0 2	0 0	.2 6	.5 2	0 1	0 1	1 3	1 2	1 1	1 2	1 1	1 1	1 1	1 0	1 1		0 1	
	14	1 1	0 1	0 1	0 1	0 2	0 3	0 1	1 2	1 1	0 1	1 1	0 3	.5 1	1 2	1 1	1 1		0 1	
	20	.2 6	0 8	0 8	.3 7	0 0	0 11	0 4	0 3	1 3	.3 6	.3 3	1 2	.4 9	.4 5	1 2	1 3		0 1	
	28	.3 3	0 4	.5 2	0 2	0 2	.3 3	0 4	.5 2	1 1	1 1	.5 2	1 1	1 1	1 1	.7 3	1 1		1 2	
	16	.7 6	.6 8	1 2	.5 4	.5 4	.7 6	1 14	.1 7	.1 2	1 2	1 8	0 10	.3 11	1 2	0 6	.7 6		8 4	
	26	1 3	1 2	1 3	.7 3	.7 3	1 2	1 4	.3 4	.1 13	.3 6	.4 5	.2 6	0 8	.2 11	.5 2	.4 5		0 1	
	17	.7 3	1 1	.8 4		0 1	.5 2	1 2	.7 3	0 1	0 4	1 4	0 6	.5 2	0 2	1 2	.5 0		3 3	
	27	1 1	1 1	1 2	1 2	1 3	1 1	1 1	1 1	1 1	1 2	1 6	1 2	.5 1	1 2	1 1	1 1		1 1	
	5	1 2	.6 7	1 2	.8 4	.1 7	.8 4	1 1	1 3	1 2	.3 4	.3 4	1 3	.1 9	.3 8	.5 2	.6 5		1 0	
	8	1 2	.6 5	.8 4	1 4	1 3	.7 1	1 3	.7 3	1 2	.8 4	.3 4	.7 3	1 3	1 2	1 2	.6 7		1 1	
	21	1 2	.5 2	.8 4	1 2	.5 2	.1 7	1 1	1 2	.7 3	1 1	1 1	.2 6	0 6	.5 4	1 1	1 1		.7 0	
	15	1 2	1 1	1 1	1 1	1 1	1 2	1 3	1 1	1 3	1 2	0 3	.3 3	1 5	1 1	1 1	1 1		1 2	
	22	.6 5	.5 2	1 5	1 3	1 2	1 5	1 3	.7 6	1 2	.7 3	.2 13	.6 7	1 2	1 2	1 1	.7 10		1 1	
	2P		0 2	0 0	0 1	0 0	0 1	0 0	0 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0		see Figure 1	
	3P	1 1	1 1	1 1	1 1	1 1	.5 2	1 2	.8 4	1 2	.5 2	1 2	1 1	1 3	1 1	1 2	.5 2		1 2	

■ compatible,
 probably compatible,
 ambiguous,
 probably incompatible,
 insufficient data,
 incompatible,
 insufficient data

Figure 9.—Plants of *A. exaltata* related as full-sibs were crossed in a diallel (D4), including self-pollinations and backcross-pollinations. The female parent (2P) of these plants was self-sterile and the male parent (3P) was self-fertile. The top number shows the proportion of umbels that matured fruit, and the bottom number shows the number of umbels pollinated. In all cases, five flowers were pollinated per umbel. Numbered progeny have been rearranged into incompatibility types, based upon crossing relationships. The compatibility of each cross was categorized according to the criteria outlined in Table 2, and the shading reflects this categorization.

many different types of SI (Levin 1996). Moreover, the shift from SI to self-compatibility, which has occurred at least twice in the genus *Asclepias* (Kephart 1981; Wyatt and Broyles 1997), most often entails a serial increase in the level of pseudo-self-fertility (Mulcahy 1984; Latta and Ritland 1993; Levin 1996).

Other types of modifiers of S-gene function were also discovered in our diallels. In D1, some plants appeared to possess a modifier that altered the function of the *S_i* allele only, possibly because the modifier was linked to *S_i*. Additionally, two plants (12 from D2 and 50 from D3) were identified as having normal male, but abnormal female, expression of SI, suggesting that genetic control of the S-locus is gender specific. In gametophytic (Flaschenriem and Ascher 1979; Clark *et al.* 1990) and sporophytic (Nasrallah *et al.* 1992) systems, different S-locus sequences are thought to be expressed in pollen and pistil, based on descriptions of similar mutants exhibiting gender-specific breakdown of SI.

Despite the existence of modifiers, late-acting SI is an effective barrier to fruit-set following self-pollinations in *A. exaltata*. All plants included in this study, excluding

self-fertile 3P and its progeny, were entirely self-sterile. The SI system occasionally leaked, but the frequency of leaks was extremely rare following self-pollinations (<0.5% of selfed flowers matured fruit), and only slightly more common following full-sib cross-pollinations. Additionally, all populations of *A. exaltata*, regardless of their geographical range, appear to be entirely outcrossed, as determined indirectly by fixation indices of allozyme loci (Broyles and Wyatt 1993; Broyles 1998) and directly by paternity analysis (Broyles and Wyatt 1990; Broyles *et al.* 1994).

The S-locus of *A. exaltata* is probably highly polymorphic. Extremely high allelic diversity at the S-locus is typical for all types of homomorphic SI and is maintained by negative frequency-dependent selection (reviewed by Richman and Kohn 1996). Furthermore, in the parental diallel involving eight field-collected plants of *A. exaltata*, all but 2 of 25 pairs of reciprocal crosses were compatible. These plants must contain between 10 and 14 unique S-alleles, since one or both alleles must be shared in the 2 incompatible crosses and since the S-genotype of self-fertile 3P cannot be determined.

It seems likely that postzygotic self-incompatibility is widespread in *Asclepias*. At least seven species of *Asclepias* are predominantly self-sterile (reviewed by Wyatt and Broyles 1994; Wyatt *et al.* 1996, 1998), and ovarian rejection of self-pollen has been demonstrated in four of these (Sparrow and Pearson 1948; Kephart 1981; Sage and Williams 1991). Indeed, it now seems likely that postzygotic self-incompatibility is basal within the Asclepiadaceae. Our recent crossing and microscopic studies have shown that *G. suberosus* (Lipow and Wyatt 1998), *P. aphylla* (Lipow 1998), and *A. cannabinum* (Lipow and Wyatt 1999) are also self-sterile and lack barriers to self-fertilization. *P. aphylla* belongs to the Periplocoideae, the most basal subfamily within the Asclepiadaceae, whereas *A. cannabinum* belongs to the Apocynoideae, the subfamily of the Apocynaceae that gave rise to the Asclepiadaceae (Judd *et al.* 1994; Struwe *et al.* 1994; Liede 1996). Thus, the evolution of postzygotic self-incompatibility probably antedates the split of the Asclepiadaceae from the Apocynaceae.

We thank M. Asmussen, C. Ivey, J. Hamrick, and R. Price for comments on earlier versions of this manuscript. A. Tull, K. Davis, M. Zimmerman, and M. Smith provided excellent greenhouse care. Funding was provided by a grant-in-aid from the Department of Botany at the University of Georgia and by National Science Foundation grant DEB-9623925. This article represents a portion of a Ph.D. dissertation submitted to the Department of Botany at the University of Georgia.

LITERATURE CITED

- Ascher, P. D., 1984 Self-incompatibility, pp. 92–110 in *Petunia: Monographs on Theoretical and Applied Genetics 9*, edited by K. C. Sink. Springer Verlag, New York.
- Bateman, A. J., 1954 Self-incompatibility systems in angiosperms. II. *Iberis amara*. *Heredity* **8**: 305–332.
- Bookman, S. S., 1983a Effects of pollination timing on fruiting in *Asclepias speciosa* Torr. (Asclepiadaceae). *Am. J. Bot.* **70**: 897–905.
- Bookman, S. S., 1983b Costs and benefits of flower abscission and fruit abortion in *Asclepias speciosa*. *Ecology* **64**: 264–273.
- Bookman, S. S., 1984 Evidence for selective fruit production in *Asclepias*. *Evolution* **38**: 72–86.
- Brandham, P. E., and S. J. Owens, 1978 The genetic control of self-incompatibility in the genus *Gasteria* (Liliaceae). *Heredity* **40**: 165–169.
- Brewbaker, J. L., and D. D. Gores, 1967 Genetics of self-incompatibility in the monocot genera *Ananas* (pineapple) and *Gasteria*. *Am. J. Bot.* **54**: 611–616.
- Broyles, S. B., 1998 Postglacial migration and loss of allozyme variation in northern populations of *Asclepias exaltata* (Asclepiadaceae). *Am. J. Bot.* **85**: 1091–1097.
- Broyles, S. B., and R. Wyatt, 1990 Paternity analysis in a natural population of *Asclepias exaltata*: multiple paternity, functional gender, and the “pollen donation hypothesis.” *Evolution* **44**: 1454–1468.
- Broyles, S. B., and R. Wyatt, 1993 The consequences of self-pollination in *Asclepias exaltata*, a self-incompatible milkweed. *Am. J. Bot.* **80**: 41–44.
- Broyles, S. B., A. Schnabel and R. Wyatt, 1994 Evidence for long-distance pollen dispersal in milkweeds (*Asclepias exaltata*). *Evolution* **48**: 1032–1040.
- Chaplin, S. J., and J. L. Walker, 1982 Energetic constraints and adaptive significance of the floral display of a forest milkweed. *Ecology* **63**: 1857–1870.
- Charlesworth, D., 1985 Distribution of dioecy and self-incompatibility in angiosperms, pp. 237–268 in *Evolution—Essays in Honour of John Maynard Smith*, edited by P. J. Greenwood and M. Slatkin. Cambridge University Press, Cambridge.
- Charlesworth, D., and B. Charlesworth, 1987 Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**: 237–268.
- Clark, K. R., J. J. Okuley, P. D. Collins and T. L. Sims, 1990 Sequence variability and developmental expression of S-alleles in self-incompatible and pseudo-self-compatible *Petunia*. *Plant Cell* **2**: 815–826.
- Cope, F. W., 1962 The mechanism of pollen incompatibility in *Theobroma cacao* L. *Heredity* **17**: 157–182.
- Cornish, M. A., M. D. Hayward and M. J. Lawrence, 1979 Self-incompatibility in ryegrass. I. Genetic control in diploid *Lolium perenne* L. *Heredity* **43**: 95–106.
- de Nettancourt, D., 1977 *Incompatibility in Angiosperms*. Springer-Verlag, New York.
- de Nettancourt, D., 1997 Incompatibility in angiosperms. *Sex. Plant Reprod.* **10**: 185–199.
- Flaschenriem, D. R., and P. D. Ascher, 1979 Pollen tube expression of pseudo-self-compatibility (PSC) in *Petunia hybrida*. *Theor. Appl. Genet.* **54**: 97–101.
- Franklin, F. C. H., M. J. Lawrence and V. E. Franklin-Tong, 1995 Cell and molecular biology of self-incompatibility in flowering plants. *Int. Rev. Cytol.* **158**: 1–64.
- Gibbs, P. E., and M. Bianchi, 1993 Post-pollination events in species of *Chorisia* (Bombacaceae) and *Tabebuia* (Bignoniaceae) with late-acting self-incompatibility. *Bot. Acta* **106**: 64–71.
- Hughes, M. B., and E. B. Babcock, 1950 Self-incompatibility in *Crepis foetida* (L.) subsp. *rheoadifolia* (Bieb.) Schniz et Keller. *Genetics* **35**: 570–588.
- Judd, W. S., R. W. Sanders and M. J. Donoghue, 1994 Angiosperm family pairs: preliminary phylogenetic analysis. *Harv. Pap. Bot.* **5**: 3–51.
- Kahn, A. P., and D. H. Morse, 1991 Pollinium germination and putative ovule penetration in self and cross pollinated common milkweed *Asclepias syriaca*. *Am. Midl. Nat.* **126**: 61–67.
- Kephart, S. R., 1981 Breeding systems in *Asclepias incarnata* L., *A. syriaca* L., and *A. verticillata* L. *Am. J. Bot.* **68**: 226–232.
- Klekowski, E. J., 1988 *Mutation, Developmental Selection, and Plant Evolution*. Columbia University Press, New York.
- Knight, R., and H. H. Rogers, 1955 Incompatibility in *Theobroma cacao*. *Heredity* **9**: 69–77.
- Krebs, S. L., and J. F. Hancock, 1990 Early-acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum*. *Theor. Appl. Genet.* **79**: 825–832.
- Latta, R., and K. Ritland, 1993 Models for the evolution of selfing under alternative modes of inheritance. *Heredity* **71**: 1–10.
- Levin, D. A., 1989 Inbreeding depression in partially self-fertilizing *Phlox*. *Evolution* **43**: 1417–1423.
- Levin, D. A., 1996 The evolutionary significance of pseudo-self-fertility. *Am. Nat.* **148**: 321–332.
- Liede, S., 1996 Anther differentiation in the Asclepiadaceae–Asclepiadaceae: form and function, pp. 221–235 in *The Anther: Form, Function, and Phylogeny*, edited by W. G. D’Arcy and R. C. Keating. Cambridge University Press, New York.
- Lipow, S. R., 1998 Postzygotic self-incompatibility and self-fertility in the Apocynaceae and Asclepiadaceae. Ph.D. Dissertation, University of Georgia, Athens, GA.
- Lipow, S. R., and R. Wyatt, 1998 Reproductive biology and breeding system of *Gonolobus suberosus* (Asclepiadaceae). *J. Torrey Bot. Soc.* **125**: 183–193.
- Lipow, S. R., and R. Wyatt, 1999 Floral morphology and late-acting self-incompatibility in *Apocynum cannabinum* (Apocynaceae). *Plant Syst. Evol.* **219**: 99–109.
- Lipow, S. R., S. B. Broyles and R. Wyatt, 1999 Population differences in self-fertility in the “self-incompatible” milkweed *Asclepias exaltata* (Asclepiadaceae). *Am. J. Bot.* **99**: 1114–1120.
- Littell, R. C., G. A. Milliken, W. W. Stroup and R. D. Wolfinger, 1996 *SAS System for Mixed Models*. SAS Institute Inc., Cary, NC.
- Lloyd, D. G., and D. J. Schoen, 1992 Self and cross-fertilization in plants. I. Functional dimensions. *Int. J. Plant Sci.* **153**: 358–369.
- Mulcahy, D. L., 1984 The relationships between self-incompatibility, pseudo-compatibility and self-compatibility, pp. 229–235 in *Plant Biosystematics*, edited by W. F. Grant. Academic Press, Toronto.
- Naaborgh, A. T., and M. T. M. Willemse, 1992 The ovular incom-

- patibility system in *Gasteria verrucos* (Mill) H. Duval. *Euphytica* **58**: 231–240.
- Nasrallah, M. E., M. K. Kandasamy and J. B. Nasrallah, 1992 A genetically defined trans-acting locus regulates S-locus function in *Brassica*. *Plant J.* **2**: 497–506.
- Queller, D. C., 1985 Proximate and ultimate causes of low fruit production in *Asclepias exaltata*. *Oikos* **44**: 373–381.
- Richman, A. D., and J. R. Kohn, 1996 Learning from rejection: the evolutionary biology of single locus incompatibility. *TREE* **11**: 497–502.
- Sage, T. L., and E. G. Williams, 1991 Self-incompatibility in *Asclepias*. *Plant Cell Incomp. Newsl.* **23**: 55–57.
- Sage, T. L., and E. G. Williams, 1994 Ovarian and other late-acting self-incompatibility systems, pp. 116–140 in *Genetic Control of Self-Incompatibility and Reproductive Development in Flowering Plants*, edited by E. G. Williams, A. E. Clarke and R. B. Knox. Kluwer Academic Publishers, Boston.
- SAS Institute, 1985 *SAS User's Guide: Basics*, version 5. SAS Institute Inc., Cary, NC.
- Seavey, S. R., and K. S. Bawa, 1986 Late-acting self-incompatibility in angiosperms. *Bot. Rev.* **52**: 195–219.
- Seavey, S. R., and S. K. Carter, 1994 Self-sterility in *Epilobium obcordatum* (Onagraceae). *Am. J. Bot.* **81**: 331–338.
- Shannon, T. R., and R. Wyatt, 1986 Reproductive biology of *Asclepias exaltata*. *Am. J. Bot.* **73**: 11–20.
- Sparrow, F. K., and N. L. Pearson, 1948 Pollen compatibility in *Asclepias syriaca*. *J. Agric. Res.* **77**: 187–199.
- Struwe, L., V. A. Albert and B. Bremer, 1994 Cladistics and family level classification of the Gentianales. *Cladistics* **10**: 175–206.
- Waser, N. M., and M. V. Price, 1991 Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules usurped? *Am. J. Bot.* **78**: 1036–1043.
- Wiens, D., C. L. Calvin, C. I. Davern, D. Frank and S. R. Seavey, 1987 Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia* **71**: 501–509.
- Wilbur, H. M., 1976 Life history evolution in seven milkweeds of the genus *Asclepias*. *J. Ecol.* **64**: 223–240.
- Woodson, R. E., Jr., 1954 The North American species of *Asclepias*. *L. Ann. Missouri Bot. Gard.* **41**: 1–211.
- Wyatt, R., 1976 Pollination and fruit-set in *Asclepias*: a reappraisal. *Am. J. Bot.* **63**: 845–851.
- Wyatt, R., and S. B. Broyles, 1994 Ecology and evolution of reproduction in milkweeds. *Annu. Rev. Ecol. Syst.* **25**: 423–441.
- Wyatt, R., and S. B. Broyles, 1997 The weedy tropical milkweeds *Asclepias curassavica* and *A. fruticosa* are self-compatible. *Biotropica* **29**: 232–234.
- Wyatt, R., C. T. Ivey and S. R. Lipow, 1996 The breeding system of desert milkweed, *Asclepias subulata*. *Bull. Torrey Bot. Club* **123**: 180–183.
- Wyatt, R., A. L. Edwards, S. R. Lipow and C. I. Ivey, 1998 The rare *Asclepias texana* and its widespread sister species, *A. perennis*, are self-incompatible and interfertile. *Syst. Bot.* **23**: 151–156.

Communicating editor: M. K. Uyenoyama