The genetic basis of male sterility in *Schiedea* (Caryophyllaceae), an endemic Hawaiian genus

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Interspecific crosses of *Schiedea* and *Alsinidendron* (Caryophyllaceae), endemic Hawaiian genera, produced segregation patterns of females, males, and hermaphrodites consistent with nuclear control of male sterility. Female excesses, typical in hybrids when mismatches occur between cytoplasmic factors controlling male sterility and nuclear factors restoring fertility, were not observed in any crosses. The nuclear gene controlling expression of the male (or hermaphroditic) sex in *Schiedea* is dominant; females are homozygous recessives. Results from intraspecific crosses and segregations among progeny raised from field-collected seeds were largely consistent with results obtained from interspecific and intergeneric crosses. In *Schiedea* gynodioecy is likely to represent an early stage in the evolution of dioecy. The transition from gynodioecy to full dioecy may be a relatively rapid event favoured by nuclear control of male sterility, a view supported by the occurrence of gynodioecy, subdioecy, and dioecy in *Schiedea*.

Keywords: dioecy, gynodioecy, Hawaii, male sterility, Schiedea, subdioecy.

Introduction

Gynodioecy, the occurrence of females and hermaphrodites in a population, may be an early stage in the evolution of dioecy (Lloyd, 1976) but the argument has been difficult to frame in genetic terms. The difficulty arises because of the complex and poorly understood genetic mechanisms that underlie sex expression in most of the gynodioecious species that have been studied (Charlesworth, 1981). Available studies have usually demonstrated complex interactions between cytoplasmic factors leading to male sterility and nuclear genes that restore male fertility (Frank, 1989). The widespread occurrence of cytoplasmic factors among gynodioecious species has led to the view that nuclear control of male sterility is rare or non-existent (Gouyon & Couvet, 1985), although there have been a few demonstrations of nuclear control of male sterility (Kohn, 1988; Connor & Charlesworth, 1989).

Theoretical arguments have demonstrated the importance of cytoplasmic versus nuclear control of male sterility in the evolution of gynodioecy and dioecy. Cytoplasmically induced male sterility is favourable for the spread of females in populations but not the evolution of full dioecy (Charlesworth & Ganders, 1979; Charlesworth, 1981; Charlesworth, 1984). The rarity of dioecy in some plant families where gynodioecy is widespread and under partial cytoplasmic control (e.g. Lamiaceae; Darwin, 1877; Khevr-Pour, 1981; Couvet et al., 1986) provides circumstantial evidence for the stability of gynodioecy. In contrast, if control of male sterility is nuclear, the frequency of females is likely to increase rapidly (Ross, 1978; Charlesworth, 1981). In these populations, high female frequency will lead to selection for increased male function in hermaphrodites, and eventually the evolution of subdioecy (males, females, and hermaphrodites) and full dioecy (males and females only; Charlesworth & Charlesworth, 1978). When control of male sterility is through nuclear genes, gynodioecy and subdioecy may be transitory phases rapidly supplanted by full dioecy (Charlesworth, 1984).

The endemic Hawaiian genus *Schiedea* (Caryophyllaceae) provides a good opportunity to study the genetic basis of male sterility, and the role of nuclear versus cytoplasmic control in the evolution of full dioecy. Dicliny (including gynodioecy, subdioecy, and dioecy) is found in eight of the 22 species of *Schiedea* (Weller *et al.*, 1990). The four species in the closely related *Alsinidendron* are hermaphroditic. All

apparent stages in the evolution of dioecy are found in Schiedea, ranging from gynodioecious species with as low as 12-13 per cent females in populations, to species that are fully dioecious. The occurrence of gynodioecy in the same genus as dioecy strongly suggests that the dioecious species went through gynodioecious stages in their evolutionary past. The pattern of breeding system diversity in Schiedea also suggests that control of male sterility is likely to be through nuclear genes, as two species are fully dioecious. The variability of diclinous breeding systems in Schiedea may result from the apparently recent evolution of dicliny (Weller et al., 1990). As a consequence, Schiedea may represent a unique window on evolutionary processes associated with the spread of male sterility in populations.

In order to detect the presence of cytoplasmic factors, the crossing strategy included intergeneric, interspecific, interpopulational, and intrapopulational crosses. Crosses among populations often reveal the presence of cytoplasmic factors that cannot be detected using intrapopulational crosses (Kheyr-Pour, 1981; Gouyon & Couvet, 1985). Interspecific and intergeneric crosses among Schiedea and the closely related Alsinidendron produce vigorous, and in many cases highly fertile hybrids, which allow tests for the occurrence of differentiated cytoplasm among divergent populations. Reciprocal crosses were carried out in a large number of cases because of their value for detecting cytoplasmic differences among populations (Kheyr-Pour, 1981). Crosses included hermaphroditic and diclinous species, to identify the heterogametic sex dominance relationships. Intrapopulational and crosses and self-pollinations were carried out to confirm interpretations based on interspecific crosses. Segregations from naturally pollinated gynodioecious and subdioecious species were evaluated in light of hypotheses based on controlled crosses.

Materials and methods

All control led crosses were made under greenhouse conditions. Seventeen of the 21 extant species of *Schiedea* and *Alsinidendron* were used for interspecific and intergeneric crosses, including all eight diclinous species of *Schiedea* (Tables 1 and 2). Plants used as maternal parents in interspecific crosses were usually females (plants with pistillate flowers) from diclinous populations. These plants were isolated from potential pollen donors before crosses were made. When hermaphroditic individuals (plants with perfect flowers) were used as maternal parents flowers were emasculated before another dehiscence. Crosses were carried out with fine forceps; when paternal parents were diclinous species with compact inflorescences (S. globosa, S. kealiae, and S. apokremnos), the entire inflorescence was rubbed against the receptive stigmas of female flowers. In several cases crosses were repeated using different individuals of the same species. Reciprocal (bi-directional) crosses were made to determine whether the direction of cross had any effect on segregations: however, dicliny prevented use of the same individuals as female and male parents in most crosses. For several species intraspecific crosses were made for comparison with interspecific crosses. Seeds were collected when ripe, approximately 1 month after pollination, and planted approximately 6 months later following a period of dormancy. Approximately 30 individuals were raised to maturity for most crosses and scored for sex expression. Sexes were scored as female versus males + hermaphrodites. No attempt was made to distinguish between hermaphrodites and males of interspecific hybrids because of the difficulty in determining visually whether stigmas were functional.

Intraspecific crosses and self pollinations were carried out for Schiedea salicaria and S. adamantis, two gynodioecious species. Plants used for crosses of S. salicaria were collected from the field, or raised from seed collected from naturally pollinated females and hermaphrodites found in two natural populations occurring about 1 km apart on West Maui. Females occur at a frequency of 12-13 per cent in these populations (Weller et al., 1990). Eleven progeny (1.5 per cent) failed to flower by the end of the experiment and were discarded. Because of indications of sex lability among female progeny of S. salicaria, questionable individuals were saved and rescored for sex at least once. Individuals of S. adamantis grown from cuttings collected at Diamond Head Crater on O'ahu, the only known population of this species, were used in crosses and self pollinations. The frequency of females in this population is 41 per cent.

Sex expression was investigated among the progeny of naturally pollinated individuals of gynodioecious *S. salicaria*, subdioecious *S. globosa*, and hermaphroditic *S. lydgatei*. Sex of parental individuals was determined in the field, and sex ratios among the progeny were interpreted in light of results from controlled crosses. Progeny were raised from seeds collected from 2 years for *S. salicaria*, and a single year for *S. globosa* and *S. lydgatei*. Progeny of gynodioecious *S. salicaria* were scored as females of hermaphrodites. Because of the sex lability noted among progeny from controlled crosses of *S. salicaria*, individuals raised from the second year of seed collections were saved and rescored for sex at least once. Progeny of subdioecious *S. globosa* were scored as females, males, or hermaTable 1 Sex ratios in progeny of intra- and interspecific crosses using gynodioecious (G_i) , subdioecious (SubD), and dioecious (D) species. The sex of the individual used in the cross (F, M, or H) follows the breeding system designation. Identification numbers following species names refer to specific individuals in populations, except where only three digits are shown. In this case, two to three individuals from a population were used as male parents. Totals are shown for progeny having the same maternal parent

	× Male parent	Sex of offspring	
Female parent		Female	Male + hermaphrodite
S. adamantis 847-B (G-H	5) × S. adamantis 847-5 (G-H)	10	21
S. adamantis 847-B (G-I	S. globosa 844-12-3 (SubD-M)	14	15
S. adamantis 847-B (G-I	F) S. globosa 844-12-4 (SubD-M)	22	9
S. adamantis 847-B (G-I	5) S. kealiae 791-14 (SubD-M)	14	16
S. adamantis 847-B (G-I	S) S. kealiae 791-16 (SubD-H)	17	14
S. adamantis 847-B (G-I	S) S. ligustrina 846 (D-M)	12	14
S. adamantis 847-B (G-H	5) S. mannii 793-1 (SubD-M)	13	14
Total		102	103
S. elohosa 844-4-59 (Sul	D-F)×S. adamantis 847-1 (G-H)	19	12
$S_{alobosa} = 844-4-59$ (Sul	D-F) S. apokremnos 865 (G-H)	4	4
S. globosa 844-4-59 (Sul	(D-F) S globosa 844 12-11 (SubD-M)	15	16
S. globosa 844-4-59 (Sul	D-F) S kealige 791-1 (SubD-H)	16	13
S. globora 844-4-59 (Sul	$(D-F) = S_{ij} i gustring 846 (D-M)$	7	7
<i>S. globosa</i> 844-4-59 (Sul	pD-F) S. spergulina 863 (D-M)	3	6
Total		64	58
S. hogling 701-7 (SubD-)	$E_{\rm A} = \times S_{\rm adamontis} 847-1 (G-H)$	9	21
S. keeling 701.7 (SubD-1)	(f) = S. uuunuuuus 0+7 1 (O M) (G) = S. uuunuuus 0+7 1 (O M)	17	9
S. keallas 201 7 (SubD-)	$S_{1} = S_{2} = \frac{14}{5} (S_{1} + 12) (S_{$	11	13
5. $keattae 791-7 (SubD-)$	$E_{1} = \sum_{i=1}^{n} \sum_{j=1}^{n} \frac{1}{2} \left(\frac{3}{2} \frac{1}{2} \frac$	15	10
5. Keattae 791-7 (SubD-	$ \begin{array}{c} \text{S. ingustitini 040 (D-M)} \\ \text{S. ingustitini 703-1 (SubD-M)} \end{array} $	2	3
<i>S. kealiae</i> 791-7 (SubD-) <i>S. kealiae</i> 791-7 (SubD-)	F = S, spergulina 863-6 (D-M)	3	2
Total		55	65

phrodites. Hermaphrodites, which for S. globosa are defined as individuals producing staminate and pistillate flowers, possess small numbers of pistillate flowers, and were detected by rescoring at least once. Progeny of hermaphroditic S. lydgatei were either pistillate or hermaphroditic. Potential sex lability was detected by scoring for sex on two occasions. Expected and observed segregations were compared using G tests; $G_{\rm H}$ and $G_{\rm P}$ refer to G tests for heterogeneity and pooled data, respectively (Sokal & Rohlf, 1981). G values with a probability of 0.05 or less were considered significant. For interspecific crosses using S. globosa and S. kealiae as seed parents, intraspecific crosses of female and heterozygous hermaphrodites of S. salicaria, and selfs of heterozygous hermaphrodites of S. salicaria, progeny were not included in the statistical analysis when the expected results were less than 5.

Results

Sex expression in progeny of interspecific crosses

Interspecific crosses produced two segregation patterns. In the first of these patterns (Table 1), females were produced in the same numbers as males and hermaphrodites combined among progeny of three diclinous species used as female parents in crosses (*S. adamantis*, $G_{\rm H} = 10.2$, d.f. = 6, ns; $G_{\rm P} = 0.005$, d.f. = 1, ns; *S. globosa*, $G_{\rm H} = 1.16$, d.f. = 3, ns; $G_{\rm P} = 0.764$, d.f. = 1, ns; *S. kealiae*, $G_{\rm H} = 8.60$, d.f. = 3, P < 0.05; **Table 2** Sex ratios in progeny of interspecific or intergeneric crosses using hermaphroditic individuals of gynodioecious (*S. salicaria*) or hermaphroditic species as one parent. Breeding system designations are followed by sex of individuals of diclinous species, as in Table 1. Identification numbers following species names refer to specific individuals in populations, except where only three digits are shown. In this case, two to three individuals from a population were used as either female or male parents

		Sex of offspring	
Female parent	× Male parent	Female	Male + hermaphrodite
A. lychonoides 867-16 (H)	× S. globosa 844-12-4 (SubD-M)	0	11
A. lychnoides 867-31 (H)	× S. globosa 844-12-4 (SubD-M)	0	18
S. adamantis 847-B (G-F)	× S. hookeri 794-3 (H)	0	27
S. adamantis 847-B (G-F)	S. salicaria 842-1 (G-H)	0	29
S. diffusa 848-1 (H)	× S. globosa 844-5-11 (SubD-H)	0	33
S. diffusa 848-1 (H)	S. globosa 844-8-4 (SubD-M)	0	7
S. diffusa 848-1 (H)	S. adamantis 847-C (G-H)	0	5
S. globosa 844-4-59 (SubD-F)	$\times A.$ lychnoides 867-31 (H)	0	14
S. globosa 844-4-59 (SubD-F)	A. lychnoides Alkali (H)	0	14
S. globosa 844-4-59 (SubD-F)	S. hookeri 794-1 (H)	ŏ	21
S. globosa 844-4-59 (SubD-F)	S. kaalae 881-1 (H)	Õ	22
S. globosa 844-4-59 (SubD-F)	S. menziesii 849 (H)	õ	24
S. globosa 844-4-59 (SubD-F)	S. nuttallii 861-12 (H)	Ô	27
S. globosa 844-4-59 (SubD-F)	S. salicaria 842-1 (G-H)	Ň	46
S. globosa 844-7-129 (SubD-F)	$\times A$, lychnoides 867-31 (H)	Õ	72
S. globosa 844-7-129 (SubD-F)	A. lychnoides Alkali (H)	Õ	24
S. globosa 844-7-129 (SubD-F)	S. kaalae 881-1 (H)	1	12
S. globosa 844-7-129 (SubD-F)	S. membranacea 864 (H)	Î.	21
S. globosa 844-7-129 (SubD-F)	S. menziesii 849 (H)	Ď	21
S. globosa 844-7-129 (SubD-F)	S_i nuttallii 861-17 (H)	ů Ú	16
S. globosa 844-7-129 (SubD-F)	S. verticillata 880-1 (H)	0	20
S. globosa 844-91-2-23 (SubD-F)	$\times A$ trinerve Perlmon 5448 (H)	Û Û	20
S. hookeri 794 (H)	$\times S$ salicaria 842 (G-H)	0	13
S. kaalae 881-1 (H)	\times S membranacea 864-0 (H)	0	10
S. kealiae 791-6 (SubD-F)	× S. verticillata 880-1 (H)	0	8
S. kealiae 791-7 (SubD-F)	\times S hookeri 704-3 (H)	1	22
S. kealiae 791-7 (SubD-F)	$\lesssim kaalaa 881.1 / U$	1	24
S. kealiae 791-7 (SubD-F)	$\int mantiacii 840/U$	0	10
S. kealiae 791-7 (SubD-F)	S metalli $861_{-}17$ (U)	0	17
S. kealiae 791-7 (SubD-F)	S. salicaria $842-2$ (C. II)	0	8
S. verticillata 880-1 (H)	$\sum Summa 042-2 (U-H)$	0	29
S. verticillata 880-1 (H)	$\sim 5.$ Runne 001-1 (Π) X S globosa 844-12 5 (Sul D II)	U	15
S. verticillata 880-1 (H)	$\sim 0.500000000000000000000000000000000000$	U	20
S. verticillata 880-1 (H)	× S. salicaria 842-8 (G-H)	0	24 24

 $G_p = 0.009$, d.f. = 1, ns). Female parents in these crosses have either gynodioecious or subdioecious breeding systems; species used as male parents were gynodioecious, subdioecious or dioecious. The gynodioecious species used as male parents were *S. apokremnos*, where the frequency of females among progeny raised in the greenhouse was 41 per cent, and *S. adamantis*, where the frequency of females in the field is also 41 per cent. The segregation of equal numbers of females and males + hermaphrodites indicates that one of the parents used in each cross was heterozygous at the sexdetermining locus.

Based on segregation patterns in progeny of hermaphroditic species, the male (or hermaphroditic) sex in diclinous species is likely to be heterogametic. When at least one parent in a cross was a hermaphroditic species, or a hermaphrodite of gynodioecious *S. salicaria*, only hermaphrodites + males were produced in the progeny (Table 2, note the appearance of one female in each of two crosses, interpreted as contamination). Exclusive production of hermaphrodites + males occurred regardless of the direction of the cross, and also carried across generic boundaries, i.e. hybrids of *S. globosa* with *A. lychnoides* and *A. tri*- *nerve* produced only hermaphrodites. The results are consistent with the hypothesis that the allele that determines hermaphroditism is homozygous in hermaphroditic species (and the hermaphroditic individual of *S. salicaria* used as a male parent), and dominant to the allele that governs expression of male sterility.

Interspecific crosses provided no evidence for cytoplasmic control of male sterility in *Schiedea*. Different cytotypes are characteristic of species with cytoplasmic control of male sterility (Frank, 1989), and crosses among populations are likely to lead to female excesses when mismatches of cytotypes and male fertilityrestoring factors occur. Despite the large number of species used in crosses of *Schiedea* and *Alsinidendron*, no female excesses were observed (Tables 1 and 2). Furthermore, no differences were detected in reciprocal crosses between populations (Tables 1 and 2), a result consistent with the presence of uniform cytoplasm.

Sex expression in progeny resulting from intraspecific crosses

Results obtained from intraspecific crosses of *S. salicaria* were generally consistent with nuclear control of sex expression. Females crossed with hermaphroditic progeny raised from field-collected seed of female *S. salicaria* segregated females and hermaphrodites in approximately equal numbers ($G_{\rm H} = 0.211$, d.f. = 1, ns; $G_{\rm P} = 2.02$, d.f. = 1, ns, Table 3a), the expected result

Table 3 Sex ratios among progeny from controlled crosses of gynodioecious *S. salicaria*. Crosses are categorized by the most likely genetic categories of the parents, based on the progeny ratios

	Sex of of	Sex of offspring		
Female parent × Male parent	Female	Hermophrodite	Mortality	Total
(a) Female × heterozygous he	ermaphrodite			
842-65-5 ×842-4	10	7	1	18
842-103-15×842-4	10	5	4	19
853-65-5 × 853-102-2	5	1	2	8
(b) Heterozygous hermaphro	dite × homoz	ygous hermaphrodi	te (or reciproc	cal)
842-57-12 ×853-102-1	0	77	9	86
842-57-12 × 853-33-3	1	7	0	8
853-33-20 × 842-57-12	0	35	11	46
853-102-1 ×842-57-12	0	73	11	84
(c) Homozygous hermaphro	dite selfed			
842-57-12 × self	0	8	4	12
(d) Female \times homozygous he	rmaphrodite			
842-65-5 × 842-5	4	26	9	39
842-65-5 × 842-2	1	24	5	30
842-9-17 ×842-2	0	5	0	5
853-103-15×842-5	1	15	10	26
853-12-7 ×842-5	0	13	11	24
(e) Heterozygous hermaphro	odite selfed			
842-46-24 × self	7	8	11	26
$853-3-9 \times \text{self}$	3	6	3	12
853-33-1 × self	1	3	1	5
853-33-20 × self	6	1	7	14
853-46-23 × self	3	9	17	29
853-102-2 × self	30	30	18	78
853-102-11 × self	24	27	47	98
(f) Heterozygous hermaphr	odite × heter	ozygous hermaphrod	lite	
853-25-12 ×853-102-1	6	7	2	15

because hermaphroditic progeny of females should be heterozygous.

A second category of hermaphrodites segregated very few females (7/290 surviving offspring; Table 3b-d); these hermaphrodites are presumed to be homozygous at the sex-determining locus. One hermaphrodite (842-57-12) segregated virtually no females (1/193, Table 3b), with the direction of the cross having no effect on segregation pattern. Self-pollination of 842-57-12 produced no females, although only eight progeny survived to flowering (Table 3c). Two additional hermaphrodites (842-2 and 842-5) segregated very few females when crossed to females (Table 3d); these individuals also are presumed to be homozygous at the sex-determining locus. Six of the seven unexpected females that appeared were produced among the progeny of 842-5, which suggests sex lability or contamination largely restricted to this family.

When selfed, hermaphroditic offspring of females segregated females and hermaphrodites, as expected if these individuals are heterozygotes (Table 3e). A similar result was obtained for one cross of two different heterozygous hermaphrodites (Table 3f: the small number of progeny precluded statistical analysis of the sex ratio). Selfs or crosses involving only heterozygotes should produce 3:1 ratios of hermaphrodites: females, an expectation clearly at variance with the results obtained for pooled data from selfs of S. salicaria ($G_{\rm H} = 0.101$, d.f. = 1, ns; $G_{\rm P} = 28.7$, d.f. = 1, P < 0.005). The mortality of seedlings produced through self-pollination was twice that from seedlings produced through outcrossing (38 per cent mortality for selfs, n = 286; 19 per cent for outcrosses, n = 440; G=32.2, d.f. = 1, P < 0.005), which may have contributed to the female bias.

Self-pollinations and crosses of hermaphrodites of S. adamantis yielded females and hermaphrodites, again indicating that the hermaphroditic sex is heterozygous (Table 4). Crosses between females and hermaphrodites also yielded females and hermaphrodites in an approximate 1:1 ratio (Table 4; G=1.60, d.f. = 1; P > 0.05).

 Table 4 Sex ratios among progeny from controlled crosses of gynodioecious S. adamantis

	Sex of offspring		
Type of cross	Female	Hermaphrodite	
Self-pollinations and crosses among hermaphrodites	4	8	
Female × hermaphrodite	12	19	

Sex ratios in progeny of naturally pollinated S. salicaria, S. globosa, and S. lydgatei

Progeny raised from 11 female individuals of S. salicaria yielded 24 per cent females (n = 293 progeny)Table 5). Fewer females were produced in the progeny of hermaphrodites (15 per cent, n = 670 progeny). The majority (29/34) of hermaphroditic individuals of S. salicaria produced some females in their progeny (Table 5), which suggests that these hermaphrodites were heterozygous for male sterility. In most cases, however, the number of females was quite small (e.g. seven families had a single female among the progeny, and another seven families had two females in each set of progeny). Similar results were obtained for progeny raised from seeds collected 2 years later in the same population. There was no indication of sex lability in the second set of progeny, which was scored for sex on two occasions separated by 2-3 months. The low frequency of females in S. salicaria in the field (12-13 per cent) indicates that most hermaphrodites should be homozygous at the sex-determining locus. The high frequency of apparently heterozygous hermaphrodites in the population is therefore surprising.

Among progeny of female S. globosa, 44.6 per cent of the offspring were female, 38.2 per cent were male, and 17.3 per cent were hermaphroditic (Table 6). The sex ratio is significantly biased towards males + hermaphrodites combined (G=9.33, d.f. = 1, P < 0.005, Table 6). No attempt was made to test for heterogeneity because many families contained few hermaphrodites. All six hermaphroditic families produced females and were heterozygous at the sex-determining locus. Hermaphrodites yielded an approximate 3:1 ratio of hermaphrodites + males:females (G=1.73, d.f.=1; P>0.05), a result consistent with either 100 per cent selfing of heterozygous hermaphrodites or outcrossing to other heterozygous males or hermaphrodites. The actual selfing rate of hermaphrodites is intermediate between complete selfing and complete outcrossing (A. K. Sakai & S. G. Weller, unpublished data). Heterozygous males (or hermaphrodites) are expected to predominate in S. globosa, because hermaphrodites are rare under field conditions and most offspring are produced from females (Weller & Sakai, 1990).

The occurrence of hermaphroditic function in some heterozygous individuals of *S. globosa* is under environmental and genetic control. Under uniform greenhouse conditions there was significant heterogeneity in the expression of hermaphroditism among maternal families grown from field-collected seed, which indicates that the degree of female function in hermaphrodites may be a quantitative trait (Weller & **Table 5** Sex ratios in progeny raised in the greenhouse fromseeds of naturally pollinated S. salicaria (population 842)collected in 1987. Identification numbers of maternalparents are for permanently marked plants growing at thefield site

Table 6 Sex ratios of progeny raised in the greenhouse fromseeds of naturally pollinated S. globosa (population 844).Identification numbers of maternal parents are for permanently marked plants growing at the field site

	Sex of offspring			
Family	Female	Male	Hermaphroditic	
Progeny ra	ised from femal	e parents		
1	7	8	0	
2	7	8	0	
5	15	13	6	
18	5	9	2	
25	8	5	8	
27	6	9	4	
30	29	15	14	
43	42	15	0	
46	3	5	5	
49	8	6	5	
51	10	2	4	
62	27	18	10	
64	7	7	5	
68	26	32	24	
70	8	6	2	
76	7	5	4	
105	8	8	14	
118	9	8	1	
138	19	17	5	
150	29	31	3	
170	27	32	5	
172	9	11	4	
173	18	9	5	
202	13	15	6	
240	7	9	1	
Total	354	303	137	
Progeny ra	ised from herm	aphroditic pa	rents	
12	22	33	36	
19	4	3	13	
28	27	14	21	
91	20	52	23	
187	4	4	3	
212	8	2	11	
Total	85	108	107	

Sakai, 1990). Fifty per cent of the individuals scored as males in the field became hermaphroditic when grown in the greenhouse, which shows that sex expression also has a strong environmental component (Weller & Sakai, 1990).

S. lydgatei, a strictly hermaphroditic species under field conditions, produced 1-4 female progeny in five of the 23 families raised from field-collected seed; average family size was 11.1. All but one female were consistent in sex across scoring dates separated by 6 months.

Discussion

Segregation patterns in interspecific and intergeneric crosses of *Schiedea* and *Alsinidendron* are largely consistent with nuclear control of sex expression, male heterogamety, and dominance of the allele that determines the male or hermaphroditic sex. Female-biased sex ratios, often characteristic when cytoplasmic factors control male sterility, were not found in any of the interspecific or intergeneric crosses. Because the use of the same female in combination with male or hermaphroditic individuals from many other populations increases the probability of detecting cytoplasmic factors, it seems apparent that *Schiedea* and *Alsini-dendron* lack cytoplasmic variability.

Results from intraspecific crosses of S. salicaria conformed for the most part with those obtained from interspecific and intergeneric crosses. The appearance of small numbers of females in several of the controlled crosses, and in many of the progeny of hermaphrodites raised from field-collected seeds was puzzling, because many hermaphrodites should be homozygous at the sex-determining locus given the low frequency of females. Labile sex was noted in several cases but does not account for the appearance of females among the progeny of the majority of hermaphrodites in the field, assuming the single locus model is correct. The presence of these females may result from developmental instability associated with inbreeding (Schemske, 1983; Dudash, 1990). In Ipomopsis aggregata, which is normally hermaphroditic, inbreeding results in the production of pistillate individuals (Benjamin & Hainsworth, 1986); in Nemophila menziesii selfing results in individuals with partial pollen abortion and incomplete anther dehiscence (Ganders, 1978). Selfing rates (~ 50 per cent; A. K. Sakai & S. G. Weller, unpublished data) and inbreeding depression (Sakai et al., 1989) in S. salicaria are high enough to have a similar effect on sex expression, beyond the effect of the hypothesized single Mendelian locus that controls sex expression.

A second possibility that accounts for the appearance of a few females in the progeny of most hermaphrodites of *S. salicaria* is a mutation that results in male sterility at loci other than the main male sterility locus (D. Charlesworth, personal communication). The low fitness associated with the majority of these mutations may prevent their spread in the population. Mutations that result in male sterile individuals with low fitness may occur in *S. lydgatei* as well, which would explain the presence of male sterile individuals among greenhouse progeny but not in the field population.

The distribution of breeding systems in Schiedea and Alsinidendron is also suggestive of nuclear control of male sterility. Theoretical studies (Ross, 1978;

Charlesworth, 1981; Charlesworth, 1984) have shown that nuclear control should lead to the rapid evolution of dioecy, whereas cytoplasmic control of male sterility results in stable gynodioecy. Subdioecy and full dioecy are more common than gynodioecy in Schiedea. Of the eight diclinous species in the genus, three are gynodioecious (Weller et al., 1990), and the remaining diclinous species are either subdioecious or fully dioecious. Among the three gynodioecious species, the breeding system of S. adamantis, where females occur at a frequency of 41 per cent, shows features similar to subdioecy. Females of S. adamantis produce more capsules than hermaphrodites, and seeds of females weigh more and have higher germination rates than those of hermaphrodites (A. K. Sakai & S. G. Weller, unpublished data). In contrast, hermaphrodites of S. salicaria, where the frequency of females is 12-13 per cent, show little evidence of reduced female function (Weller & Sakai, 1990). Gynodioecious breeding systems may represent a brief transitory phase in breeding system evolution in Schiedea, with full dioecy representing a stable end-point of the trajectory. Detection of the intermediate stages in Schiedea is probably fortuitous, resulting from the relatively recent adaptive radiation in the genus (Weller et al., 1990). In contrast to Schiedea, the occurrence of stable gynodioecy is typified by the Hawaiian Bidens, where 13 of the 27 taxa are gynodioecious, and control of male sterility is partially cytoplasmic (Sun, 1987). Among the Hawaiian Bidens, there is no indication that full dioecy may be evolving.

The genetic system that controls male sterility in Schiedea is considerably simpler than those that have been hypothesized for many other gynodioecious species (Ganders, 1978; Kheyr-Pour, 1981; Van Damme & Van Delden, 1982; Van Damme, 1983; Sun, 1987). In these studies the interaction of cytoplasmic and nuclear-restoring factors (Couvet et al., 1986) undoubtedly contributes to the complexity of the gene interactions. In Phacelia linearis, a single nuclear factor appears to control male sterility, although cytoplasmic factors may vary among populations (Eckhart, 1990). A single nuclear factor appears to govern sex expression in Cucurbita foetidissima (Kohn, 1988), although in contrast to Schiedea, females of C. foetidissima are heterogametic. Connor and Charlesworth's (1989) conclusion that three nuclear loci govern male sterility in Cortaderia represents another study demonstrating nuclear control of male sterility in a gynodioecious species. As more studies become available, for species in genera where gynodioecy appears to be evolving toward dioecy (sensu Ross, 1978; Charlesworth, 1981), simpler nuclear systems may predominate.

Interspecific crosses of diclinous Schiedea species all

resulted in segregation of females, which indicates that the genes controlling male sterility are allelic. Based on interspecific crosses, Sun (1987) reached a similar conclusion for the Hawaiian Bidens, and suggested that the 13 gynodioecious species of Bidens evolved from a single ancestral gynodioecious species. In contrast to Bidens, biogeographic evidence indicates that dicliny has evolved independently on three to six occasions in Schiedea (Weller et al., 1990). In each case, it appears that a mutation at the same locus is responsible for male sterility. Diclinous species of Schiedea all occur in dry habitats in Hawaii: hermaphroditic species for the most part are found in mesic or wet forest (Weller et al., 1990). This ecological correlation suggests that higher selfing rates and the subsequent expression of inbreeding depression, perhaps associated with loss of pollinators in dry habitats, may have led to the spread of male sterility in these populations.

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