

Artificial and Natural Hybridization in *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae): the Importance of Phylogeny, Genetic Divergence, Breeding System, and Population Size

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ABSTRACT. Artificial hybridizations of *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae), genera endemic to the Hawaiian Islands, were used to study the effects of phylogenetic relationship, genetic divergence, breeding system, and population size on the ability of species to cross and produce fertile F_1 hybrids. Using 17 taxa, all crosses attempted produced vigorous F_1 hybrids, although pollen fertility of hybrids varied substantially. Fertility of F_1 hybrids was positively related to genetic identity measured using allozyme variability. Fertility of hybrids was not predicted by phylogenetic relationships, estimated from morphological and molecular data. Hybrids between species with dimorphic breeding systems had significantly higher pollen fertility than other combinations, although this effect was not significant when population size was controlled in the analysis. The association of dimorphism and ability to produce fertile F_1 hybrids is probably indirect: dimorphic species of *Schiedea* occur in large populations and have high genetic identities. Although artificial hybrids are easily produced in the greenhouse, natural hybridization in *Schiedea* and *Alsinidendron* is limited, apparently because instances of sympatry are rare and autogamy is often found in one of the two species occurring sympatrically.

The ability of species to hybridize and produce fertile offspring is often interpreted as indicative of close evolutionary relationship, with high fertility of hybrids resulting from genetic similarity among parental species (Clausen et al. 1939; Stebbins 1950; Raven 1977; Grant 1981; Coyne and Orr 1989, 1997). The ability to produce fertile hybrids has been used to infer relationships among plants for over a century (Raven 1980).

From a phylogenetic perspective, the ability to hybridize has been hypothesized as a plesiomorphic or ancestral trait (Vickery 1978; Rosen 1979; Funk 1985; Smith 1992). Acquisition of a post zygotic barrier to hybridization represents an evolutionarily derived trait resulting from the accumulation of genetic differences. Subsequent speciation events may result in clades of closely related, interfertile species, until new barriers to hybridization evolve within these clades. Eventually, lineages may consist of clades of interfertile species, isolated by hybrid sterility from other clades. Post-zygotic crossing barriers may not evolve in clades, and there are many genera, and even families of flowering plants, where few barriers to hybridization are known (Solbrig 1970; Grant 1981; Hodges 1997). In cases of sympatry, the occurrence of post-zygotic isolating

mechanisms associated with reduced fitness of hybrids might result in selection for pre-zygotic barriers to hybridization, because parents that do not produce any hybrids would have the highest fitness.

In lineages where barriers to hybridization occur, barriers might follow patterns related to phylogeny, or instead be related to overall genetic similarity (e.g., phenetic patterns based on allozyme similarity; Nei 1978), which often differ substantially from phylogenetic patterns. The amount of genetic similarity retained by diverging populations would be affected by many factors, including population size and breeding system. In species with large populations characterized by outbreeding, fixation or loss of genetic variation would be less common than in small or inbreeding populations. Retention of genetic similarity in large populations is more likely and should be associated with potentially greater interfertility. In contrast, in small populations genomic divergence associated with random processes and inbreeding should result in reduced potential for production of fertile hybrids.

To address these predictions about the potential effects of phylogeny and overall genetic similarity on hybrid fertility, we studied artificial hybrids in

Schiedea and *Alsinidendron* (Caryophyllaceae: Alsinoideae), two closely related Hawaiian genera that demonstrate extraordinary variation in breeding systems as well as population size. A central goal of the study was to determine whether sterility barriers between species in this lineage follow patterns related to phylogeny, or are better explained by factors associated with population size and breeding system. A second goal was to examine patterns of sympatry and putative cases of natural hybridization in *Schiedea* and *Alsinidendron*, and the role hybridization may have played in the evolution of this diverse lineage.

MATERIALS AND METHODS

Study Organisms. *Schiedea* and *Alsinidendron* (Caryophyllaceae: subfam. Alsinoideae), represent the fifth or sixth largest adaptive radiation in the Hawaiian Islands, and together the genera are considered monophyletic because of specialized extensions of the nectaries (Wagner et al. 1995; Weller et al. 1995). The 29 species of *Schiedea* and 4 species of *Alsinidendron* occupy a wide range of habitats and possess diverse morphology and breeding systems (Wagner et al. 1995; Weller et al. 1995; Sakai et al. 1997; Wagner and Herbst 1999). Species occurring in wet or mesic forests are large-leaved hermaphroditic vines, herbs, or shrubs. Species occupying dry habitats are often narrow-leaved woody shrubs that are typically gynodioecious (i.e., female and hermaphroditic individuals present in populations), subdioecious (female, male, and hermaphroditic individuals in populations), or dioecious (female and male individuals in populations); gynodioecy, subdioecy, and dioecy are collectively termed dimorphic breeding systems. All dimorphic species in this lineage are wind pollinated (Weller et al., 1998). Phylogenetic analysis based on morphological traits indicates that there are four major clades within the endemic Hawaiian Alsinoideae (Fig. 1; Wagner et al. 1995; Weller et al. 1995); results from cpDNA studies, which yield less resolved trees, also support the existence of three of the four clades (Soltis et al. 1996). Allozyme studies indicate that high genetic identities are positively associated with large population size (Weller et al. 1996).

Artificial Hybridization. Crosses of *Schiedea* and *Alsinidendron* were made in the greenhouse under pollinator-free conditions. Plants were grown from seeds or cuttings collected in the field. Sixteen of twenty-nine species of *Schiedea* and one species

of *Alsinidendron* were used in crosses. Of the 136 non-reciprocal combinations possible for 17 taxa, 98 different, usually non-reciprocal hybrid combinations were made. Sixteen species of *Schiedea* and *Alsinidendron* were not crossed because species were extinct (two species), considered extinct at the time (three species), were discovered and described as new to science after the crosses were completed (two species), or were not in cultivation (nine cases, including five species that were not recognized as distinct at the time the crosses were made).

For crosses between sexually dimorphic species and hermaphroditic species, female plants were generally used as seed parents to avoid the necessity of emasculating hermaphroditic flowers. When both species pairs were hermaphroditic, flowers of individuals used as female parents were emasculated on the first day of anthesis, prior to anther dehiscence. Pollinations were carried out two to three days later, after stigmas had become receptive. Recently dehisced anthers were used to coat all stigmas with pollen. Wind-pollinated species were isolated in different areas of the greenhouse to prevent contamination. Because females were used whenever possible as seed parents, very few reciprocal crosses were made.

In the first of two sets of crosses, we investigated the effects of male parentage on seed production, seed germination, and pollen fertility of F_1 hybrids. For these crosses we used the same female individuals as parents in crosses to males or hermaphrodites of different species as well as individuals of the same species. Two individuals each of *S. globosa* and *S. kealiae*, and one individual of *S. adamantis* were used as female parents. Seed production was recorded for an average of 25 capsules per cross. Seeds were germinated in the greenhouse following a required six-month period of dormancy. We planted eight to nine two-inch pots with 25 seeds per pot and scored germination after all seedlings had emerged. Essentially no mortality occurred prior to transplanting, and survival was not analyzed separately from germination. For each plant used as a seed parent, we made intraspecific crosses within populations for comparison to the effects of interspecific hybridization.

To gain a more complete understanding of patterns of F_1 hybrid sterility, we used many more species in the second set of crosses. These crosses were made at different times than those used to detect the effect of male parentage and no attempt was made to compare seed production or percent germination between the two sets of crosses.

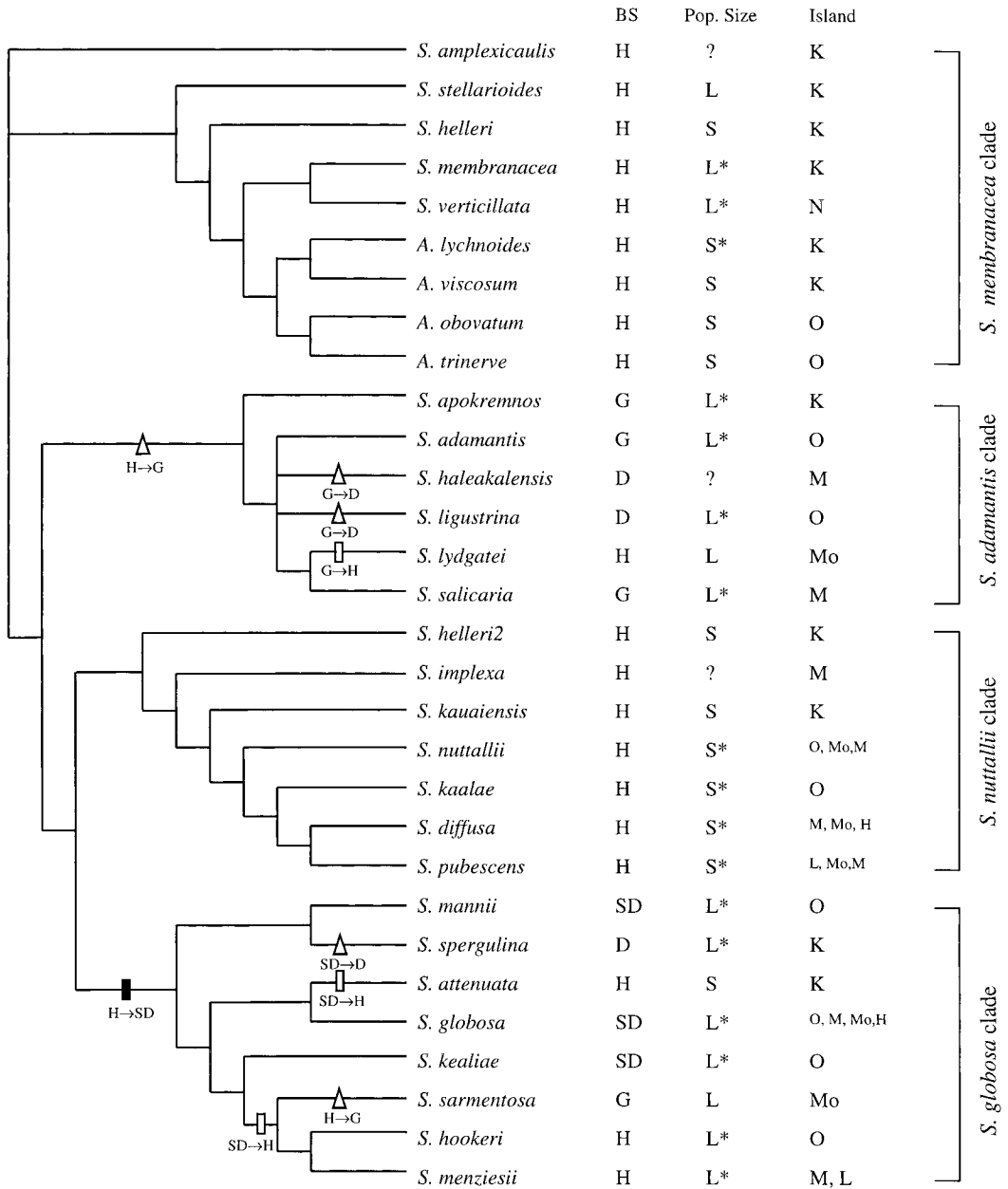


FIG. 1. Phylogeny of *Schiedea* and *Alsinidendron* (Weller et al. 1995), based on morphological data. Breeding system transitions are shown on branches of phylogeny, which represents one of six equally parsimonious trees and differs only minimally from the strict consensus. Molecular data support results shown here, although the phylogeny is less resolved (Soltis et al. 1996). Unique transitions are indicated by solid bars, homoplasious transitions by open triangles, and reversals by open bars (for bootstrap values of the strict consensus tree and details on character mapping, see Weller et al. 1995). Taxonomy reflects updates outlined by Wagner and Herbst (1999; the occurrence of *S. helleri2* in the phylogeny reflects errors in coding characters in Wagner et al. [1995] and Weller et al. [1995]). Not all currently recognized species are shown in the phylogeny. Breeding systems (BS), population size (Pop), and island distributions (Island) indicated to right of species (hermaphroditic = H, gynodioecious = G, subdioecious = SD, dioecious = D; L = populations with more than 100 individuals, S = populations with less than 30 individuals [see Weller et al., 1996 for details of how population size was assessed]; Hawai'i = H, Lana'i = L, Maui = M, Moloka'i = Mo, Nihoa = N, Kaua'i = K, O'ahu = O). Asterisks by population size indicate species included in crossing program.

For both sets of crosses, we raised approximately 30 progeny from each cross to maturity. Pollen fertility was measured for five F_1 hybrids, chosen haphazardly from each group of progeny. One anther from each of five different flowers from each plant was placed on a separate slide with 0.1 % aniline blue in lactophenol (1:1:1:1 lactic acid, phenol, glycerin, and water by weight); 200 pollen grains per anther were scored for fertility. Fully expanded pollen grains filled to the cell wall with darkly staining cytoplasm were considered fertile. Shrunken pollen grains lacking cytoplasm or with reduced quantities of cytoplasm were scored as infertile. Average fertility for each individual was used in statistical analyses, where individuals served as replicates for the effects of hybridization on pollen fertility. Temporal variation in pollen fertility was compared for some F_1 hybrids and parents by measuring the pollen fertility of the same individuals at intervals ranging from six months to one year.

Data Analysis. Analysis of variance was used to detect the effects of clade (the *S. adamantis*, *S. globosa*, *S. membranacea*, and *S. nuttallii* clades; Fig. 1) on fertility of F_1 hybrids among species. Arcsine-transformed pollen fertility was the dependent variable in the ANOVA. Crosses were largely restricted to clades dominated by dimorphic species (the *S. adamantis* and *S. globosa* clades; Weller et al. 1995) due to the relatively small number of crosses between hermaphroditic species that were attempted. Because population size of parental species might affect hybrid fertility, these analyses were also carried out for species occurring in large populations only. Populations consisting of more than 100 individuals were classified as large; those with fewer than 30 individuals were considered small (Weller et al. 1996). An analysis using species occurring in small populations could not be performed because of limited numbers of crosses. All analyses were carried out using SAS (SAS Institute 1989). When necessary, significance levels were adjusted using the sequential Bonferroni method for multiple contrasts (Sokal and Rohlf, 1995). Because not all contrasts in analysis of variance are phylogenetically independent, results should be interpreted with caution.

Fertility of F_1 hybrids was also interpreted with respect to Nei's (1978) unbiased genetic identities, derived from allozyme data (Weller et al. 1996). Arcsine-transformed pollen fertility of F_1 hybrids was regressed against genetic identity. For this analysis pollen fertility was averaged for crosses involving the same parental species. The data were

corrected for phylogenetic relationships using Felsenstein's (1985) method (cf. Coyne and Orr 1989, 1997).

The effects of breeding system on fertility of F_1 hybrids between species were also investigated using ANOVA. The four categories of crosses used in the analysis were dimorphic \times dimorphic crosses, dimorphic \times hermaphroditic crosses, hermaphroditic \times dimorphic crosses, and hermaphroditic \times hermaphroditic crosses. The hermaphroditic category refers to those species where populations consist only of hermaphroditic individuals.

Effects of Geographic Separation. The potential effects of geographic separation on fertility of F_1 hybrids were investigated by coding crosses according to whether parental species occur on the same island, adjacent islands (e.g., Kaua'i and O'ahu), or are separated by one or two islands (e.g., Kaua'i and Maui or Kaua'i and the island of Hawai'i). Analysis of variance was used to detect differences in arcsine-transformed pollen fertility.

Natural Hybridization. Potential natural hybridization was detected through morphological intermediacy of putative hybrids in the field and in collections from more than 30 herbaria. The potential occurrence of hybrids among progeny of species occurring sympatrically was also investigated.

RESULTS

Artificial Hybridization. All crosses of *Schiedea* and *Alsiniidendron* produced seeds that germinated and yielded vigorous F_1 hybrids. In the smaller set of crosses with female parentage held constant, there were no obvious patterns to differences in hybrid seed production. Several individuals of *S. globosa* and *S. kealiae* produced significantly different numbers of seeds per capsule when different species, or even different individuals of the same species were used as male parents (Table 1). Intraspecific crosses did not show consistently higher seed production than crosses between species. Significant differences in seed production related to use of different species as male parents were also found for *S. adamantis* ($P < 0.0001$, data not shown).

Germination was very high for all inter- and intraspecific crosses using *S. adamantis*, *S. globosa*, and *S. kealiae* as seed parents. Variation depending on male parentage occurred when *S. adamantis* and *S. kealiae* were used as seed parents (Table 2). In both species, interspecific crosses had higher germination than intraspecific crosses. There were no other obvious patterns for these species. No differences

TABLE 1. Inter- and intraspecific seed production per capsule in crosses of *Schiedea* and *Alsinidendron* where female parentage is controlled. Island of origin and population size are shown for each species used in crosses (N = Nihoa, K = Kaua'i, O = O'ahu, and M = Maui; L = large population size, S = small population size). Species listed twice as female or male parents are different genotypes. Sample size (N) is the number of crosses. Values for seed production per capsule with the same superscript within each maternal parent are not significantly different. For all crosses, seed production varied significantly ($P < 0.0001$).

Female parent	Male parent	Seeds per capsule (mean)	Standard deviation	N	
<i>S. globosa</i> (O-L)	<i>S. kealiae</i> (O-L)	37.7 ^a	16.34	23	
	<i>S. nuttallii</i> (O-S)	37.6 ^a	9.65	27	
	<i>S. membranacea</i> (K-L)	37.0 ^a	10.38	25	
	<i>S. ligustrina</i> (O-L)	35.7 ^{ab}	11.29	16	
	<i>S. menziesii</i> (M-L)	34.2 ^{ab}	10.50	26	
	<i>S. adamantis</i> (O-L)	34.1 ^{ab}	9.90	30	
	<i>S. adamantis</i> (O-L)	32.9 ^{ab}	9.65	35	
	<i>S. globosa</i> (O-L)	32.2 ^{ab}	8.56	29	
	<i>S. verticillata</i> (N-L)	32.2 ^{ab}	9.86	25	
	<i>S. salicaria</i> (M-L)	31.7 ^{ab}	14.57	26	
	<i>S. apokremnos</i> (K-L)	27.0 ^{ab}	9.15	26	
	<i>A. lychnoides</i> (K-S)	25.1 ^b	14.28	50	
	<i>S. adamantis</i> (O-L)	13.8 ^c	10.96	32	
	<i>A. lychnoides</i> (K-S)	13.3 ^c	7.28	12	
	<i>S. globosa</i> (O-L)	<i>S. adamantis</i> (O-L)	27.2 ^a	6.51	47
		<i>S. kealiae</i> (O-L)	25.9 ^{ab}	8.85	40
		<i>S. salicaria</i> (M-L)	25.5 ^{ab}	4.72	59
		<i>S. globosa</i> (O-L)	25.5 ^{ab}	6.63	48
		<i>S. hookeri</i> (O-L)	24.3 ^{abc}	9.65	5
<i>S. globosa</i> (O-L)		23.3 ^{abc}	4.12	49	
<i>S. adamantis</i> (O-L)		23.3 ^{abc}	4.57	45	
<i>S. spergulina</i> (K-L)		21.7 ^{abcd}	5.87	65	
<i>S. nuttallii</i> (O-S)		20.8 ^{abcd}	6.96	63	
<i>S. menziesii</i> (M-L)		19.8 ^{abcd}	6.44	61	
<i>S. kaalae</i> (O-S)		19.1 ^{abcde}	8.15	66	
<i>S. adamantis</i> (O-L)		18.8 ^{bcde}	6.59	46	
<i>S. ligustrina</i> (O-L)		18.0 ^{bcde}	7.96	50	
<i>S. kealiae</i> (O-L)		16.2 ^{cde}	6.74	1	
<i>S. adamantis</i> (O-L)		14.5 ^{de}	6.39	4	
<i>S. apokremnos</i> (K-L)		14.4 ^{de}	7.98	60	
<i>A. lychnoides</i> (K-S)		11.3 ^e	6.16	62	
<i>S. kealiae</i> (O-L)		<i>S. kealiae</i> (O-L)	17.9 ^a	4.18	30
		<i>S. apokremnos</i> (K-L)	15.9 ^{ab}	3.69	21
	<i>S. adamantis</i> (O-L)	15.1 ^{ab}	4.23	15	
	<i>S. salicaria</i> (M-L)	15.0 ^{ab}	3.20	34	
	<i>S. ligustrina</i> (O-L)	14.9 ^{ab}	5.13	15	
	<i>S. spergulina</i> (K-L)	14.8 ^{ab}	3.32	25	
	<i>S. hookeri</i> (O-L)	14.4 ^{abc}	5.68	30	
	<i>S. adamantis</i> (O-L)	13.7 ^{abcd}	4.61	15	
	<i>S. menziesii</i> (M-L)	11.6 ^{bcde}	5.11	25	
	<i>S. apokremnos</i> (K-L)	10.0 ^{cde}	4.78	25	
	<i>S. nuttallii</i> (O-S)	9.5 ^{de}	4.65	19	
	<i>S. kaalae</i> (O-S)	8.4 ^e	5.19	19	
	<i>S. kealiae</i> (O-L)	<i>S. ligustrina</i> (O-L)	17.4 ^a	3.84	18
		<i>s. globosa</i> (O-L)	17.3 ^a	3.55	35
		<i>S. globosa</i> (O-L)	16.7 ^a	2.46	24
		<i>S. kealiae</i> (O-L)	16.2 ^a	7.16	9
		<i>S. globosa</i> (O-L)	14.1 ^a	3.61	22
		<i>S. globosa</i> (O-L)	14.1 ^a	2.32	29
		<i>S. hookeri</i> (O-L)	13.6 ^a	4.07	25
<i>S. adamantis</i> (O-L)		13.6 ^a	3.53	30	
<i>S. salicaria</i> (M-L)		13.6 ^a	2.66	30	

TABLE 2. Germination of seeds produced from inter- and intraspecific crosses of *Schiedea*. Island of origin and population size are shown for each species used in crosses (O = O'ahu, M = Maui; L = large population, S = small population). Values for germination with the same superscript are not significantly different. Species listed twice are different genotypes. Sample size is number of pots with 25 seeds. For *S. adamantis* and *S. kealiae* as seed parents, $P < 0.0001$; for *S. globosa*, $P = 0.2987$.

Female parent	Male parent	Percent germination (mean)	Standard deviation	N
<i>S. adamantis</i> (O-L)	<i>S. kealiae</i> (O-L)	96 ^a	4	10
	<i>S. hookeri</i> (O-L)	92 ^a	13	10
	<i>S. kealiae</i> (O-L)	87 ^{abc}	15	10
	<i>S. ligustrina</i> (O-L)	91 ^{abc}	5	10
	<i>S. salicaria</i> (M-L)	91 ^{abc}	16	10
	<i>S. globosa</i> (O-L)	88 ^{bc}	7	10
	<i>S. globosa</i> (O-L)	76 ^{bc}	12	5
	<i>S. mannii</i> (O-L)	66 ^c	13	8
	<i>S. adamantis</i> (O-L)	66 ^c	14	2
	<i>S. globosa</i> (O-L)	<i>S. ligustrina</i> (O)	93 ^a	6
<i>S. globosa</i> (O-L)		88 ^a	8	10
<i>S. kealiae</i> (O-L)		86 ^a	12	10
<i>S. adamantis</i> (O-L)		84 ^a	10	9
<i>S. hookeri</i> (O-L)		80 ^a	10	10
<i>S. kealiae</i> (O-L)		<i>S. adamantis</i> (O-L)	82 ^a	17
	<i>S. mannii</i> (O-L)	76 ^a	11	3
	<i>S. globosa</i> (O-L)	75 ^a	13	5
	<i>S. hookeri</i> (O-L)	73 ^a	13	10
	<i>S. salicaria</i> (M-L)	63 ^a	16	10
	<i>S. ligustrina</i> (O-L)	60 ^a	17	7
	<i>S. kealiae</i> (O-L)	15 ^b	18	11

in germination related to male parentage were observed for *S. globosa*.

Pollen fertility for all hybrids resulting from this subset of crosses using *S. adamantis*, *S. globosa*, and *S. kealiae* as seed parents was very high, ranging from 80–99% (Table 3). Pollen fertility measured at different times for eight hybrid and five parental individuals varied significantly in some cases (Table 4). Differences, when they occurred, were less than those found among categories based on the population size, breeding system, or degree of geographic separation.

In the larger set of crosses using 17 taxa of *Schiedea* and *Alsinidendron*, crosses within clade (includes as one category clade 1 × clade 1 crosses, clade 2 × clade 2 crosses, etc.) produced hybrids with an average pollen fertility of 76.2%, compared to an average of 49.1% for hybrids among species belonging to different clades (includes clade 1 × clade 2 crosses, clade 1 × clade 3 crosses, etc.). This difference was significant using ANOVA ($F=12.24$; $df=1,93$; $P=0.0007$; $P=0.034$ using the sequential Bonferroni test). When the analysis was restricted to hybrids with parents only from large popula-

tions, there were no significant differences in average pollen fertility related to clade membership (within clade crosses=76.6%; between clade crosses=70.1%; $F=0.79$, $df=1,53$; $P=0.377$).

When the four categories of within-clade crosses (e.g., all clade 1 × clade 1 crosses vs. all clade 2 × clade 2 crosses, etc.) were compared to the between-clade crosses grouped as a single category (e.g., all clade 1 × clade 2 crosses, all clade 1 × clade 3 crosses, etc.), there were also significant differences in pollen fertility ($F=3.71$, $df=4,90$; $P=0.0076$; $P=0.025$ using the Bonferroni adjustment). Hybrids of crosses within the *S. globosa* clade had the highest pollen fertility (86.2%), compared to fertilities of 48.7–69.2 for hybrids within the other clades, and 49.1% for hybrids between species in different clades. Most crosses were either within the two clades containing primarily dimorphic species, or between clades. When the analysis was repeated using only those species occurring in large populations, differences were no longer significant ($F=1.62$, $df=3,51$; $P=0.1965$).

Genetic identity (Nei 1978), calculated from allozyme data (Weller et al. 1996), was strongly cor-

TABLE 3. Pollen fertility of F_1 hybrids and progeny of intraspecific crosses of *Schiedea*. Island of origin and population size are shown for each species used in crosses (N = Nihoa, K = Kaua'i, O = O'ahu, and M = Maui; L = large population size, S = small population size). Species listed twice are different genotypes. Sample size (N) is the number of plants used to estimate pollen fertility. Values for seed production per capsule with the same superscript are not significantly different. For crosses to *S. adamantis* $P = 0.012$; $P = 0.0042$ for crosses to *S. globosa*, and for crosses to *S. kealiae* $P = 0.3562$.

Female parent	Male parent	Pollen fertility (mean)	Standard deviation	N
<i>S. adamantis</i> (O-L)	<i>S. salicaria</i> (M-L)	99.1 ^a	0.07	5
	<i>S. kealiae</i> (O-L)	97.8 ^{ab}	0.09	5
	<i>S. ligustrina</i> (O-L)	97.8 ^{ab}	0.70	5
	<i>S. hookeri</i> (O-L)	94.1 ^{ab}	2.67	5
	<i>S. kealiae</i> (O-L)	92.1 ^{ab}	1.94	5
	<i>S. adamantis</i> (O-L)	92.1 ^{ab}	1.89	5
	<i>S. globosa</i> (O-L)	90.5 ^{ab}	0.42	5
	<i>S. mannii</i> (O-L)	89.1 ^b	2.08	5
<i>S. globosa</i> (O-L)	<i>S. hookeri</i> (O-L)	98.6 ^a	0.09	5
	<i>S. kealiae</i> (O-L)	98.6 ^a	0.18	5
	<i>S. adamantis</i> (O-L)	93.5 ^{ab}	0.08	5
	<i>S. ligustrina</i> (O-L)	93.5 ^{ab}	1.13	5
	<i>S. globosa</i> (O-L)	89.8 ^b	1.84	5
<i>S. kealiae</i> (O-L)	<i>S. kealiae</i> (O-L)	93.9 ^a	2.81	5
	<i>S. globosa</i> (O-L)	93.0 ^a	0.56	5
	<i>S. hookeri</i> (O-L)	92.7 ^a	5.61	3
	<i>S. ligustrina</i> (O-L)	92.2 ^a	0.85	5
	<i>S. adamantis</i> (O-L)	91.5 ^a	1.32	5
	<i>S. salicaria</i> (M-L)	91.2 ^a	1.89	5
	<i>S. mannii</i> (O-L)	80.0 ^a	2.30	4

related with pollen fertility of F_1 hybrids (no correction for phylogeny: $r^2 = 0.35$; $F = 26.4$, $df = 1,50$; $P = 0.0001$; all contrasts phylogenetically independent [fig. 2]: $r^2 = 0.706$; $F = 14.4$, $df = 1,7$; $P = 0.009$;

Felsenstein 1985; Coyne and Orr 1989, 1997). Species with high genetic identities also occurred in large populations (Weller et al. 1996), indicating that high fertility of F_1 hybrids is positively asso-

TABLE 4. Variation in pollen fertility over time for parents and F_1 hybrids of *Schiedea*, and the probability that fertilities do not differ by date.

Parent or hybrid combination	Mean pollen fertility, date 1	Mean pollen fertility, date 2	Probability
Parents:			
<i>S. adamantis</i>	98.5	98.3	0.2847
<i>S. globosa</i>	98.1	98.9	0.5749
<i>S. hookeri</i>	97.6	99.9	0.0138
<i>S. ligustrina</i>	97.3	98.0	0.5182
<i>S. salicaria</i>	99.2	99.5	0.6571
Hybrids:			
<i>S. adamantis</i> × <i>S. hookeri</i>	75.8	74.2	0.5805
<i>S. adamantis</i> × <i>S. salicaria</i>	97.4	96.4	0.4307
<i>S. adamantis</i> × <i>S. salicaria</i>	98.6	99.0	0.2990
<i>S. adamantis</i> × <i>S. kealiae</i>	77.7	95.4	0.0018
<i>S. adamantis</i> × <i>S. mannii</i>	82.4	95.0	0.0049
<i>S. diffusa</i> × <i>S. globosa</i>	28.4	29.3	0.7296
<i>S. diffusa</i> × <i>S. kealiae</i>	11.6	6.2	0.0174
<i>S. hookeri</i> × <i>S. salicaria</i>	99.6	99.8	0.4763

ciated not only with high genetic identity but also with large population size of parental species.

When crosses were categorized by breeding systems, the F_1 hybrids of crosses between dimorphic species ($D \times D$ crosses) had significantly higher pollen fertility (Tukey's posthoc comparison), with over twice the pollen fertility of crosses involving hermaphroditic species ($D \times H$, $H \times D$, and $H \times H$ crosses; $F=11.5$, $df=3,90$; $P<0.0001$; $P=0.01$ using the Bonferroni adjustment). The F_1 hybrids with hermaphroditic parents did not differ significantly in pollen fertility. When only those parental species occurring in large populations were included in the analysis, breeding system had no significant effect ($F=2.03$; $df=3,50$; $P=0.122$).

Geographic separation had no effect on fertility of F_1 hybrids ($F=1.83$, $df=3,92$, $P=0.1467$), although average pollen fertility declined monotonically as geographic distance between the species used as parents increased.

Natural Hybridization. Because sympatry in *Schiedea* and *Alsinidendron* is rare, there are few opportunities for production of natural hybrids. Nine of the eleven known cases of sympatry occur on the oldest main island of Kaua'i and the Wai'anae Mts. of O'ahu; two additional cases of sympatry are known from the younger islands of Moloka'i and West Maui. Putative hybrids have been observed in five of these ten instances of current sympatry; past sympatry and hybridization may have occurred between *S. kealiae* and *S. ligustrina* (Table 5). In three of the five cases of current sympatry where no hybrids have been found, at least one of the species in the sympatric pair is facultatively or obligately selfing (Table 5).

DISCUSSION

All crosses attempted between species of *Schiedea* and *Alsinidendron* were successful in producing F_1 hybrids. These results, in combination with over two decades of studies of experimental crosses of other Hawaiian plant species, leave little doubt that production of vigorous hybrids is possible within monophyletic lineages, even when the parental species differ dramatically in morphology, habitat, and physiology (e.g., *Bidens* [Asteraceae, Gillett and Lim 1970; Ganders and Nagata 1984], *Lipochaeta* [Asteraceae, Gardner 1979; Rabakonandrianina 1981], *Portulaca* [Portulacaceae, Kim and Carr 1990], the silversword alliance [Asteraceae, Robichaux et al. 1990; Carr 1995], *Tetramolopium* [Asteraceae, Lowrey 1986; Lowrey 1995], and *Wikstroemia* [Thy-

melaeeaceae, Mayer 1991]). In these genera, artificially produced hybrids often have high pollen fertility, although in *Tetramolopium* molecular evidence indicates that post-mating isolating mechanisms may explain reduced pollen fertility following interspecific crosses (Lowrey 1986; Whitkus 1998). For most lineages, there appear to be few barriers to genetic exchange. In those few cases where fertility of artificially produced F_1 hybrids was low, differences in chromosome number occurred (i.e., *Portulaca*, Kim and Carr 1990; the silversword alliance, Carr 1995).

Although F_1 hybrids within Hawaiian lineages are often easily obtained, whether this feature is more pronounced in the Hawaiian Islands than elsewhere is debatable. The potential for hybrid production, especially among woody species, is very common among flowering plants (Mayer 1991; Carr 1995), and may be equally common in continental areas. The widespread potential for hybridization in the Hawaiian Islands may be a function of the predominance of the woody habit, rather than the result of features related to insularity (Ellstrand et al. 1996). Additionally, the Asteraceae are strongly represented among taxa studied in artificial crossing programs, and this may provide a biased view of the extent of barriers to crossing within Hawaiian lineages.

In contrast to many other Hawaiian lineages, in *Schiedea* and *Alsinidendron* we found there were striking differences in hybrid pollen fertility, which ranged from almost 100% to near zero. The ability to produce fertile hybrids may result from genetic similarity retained from the ancestral species; if so, then the potential to produce fertile hybrids is a plesiomorphic trait (Rosen 1979; Funk, 1985; Smith, 1992). If this argument is correct, within clades of closely related species with little genetic differentiation, pollen fertility of hybrids should be high because genomes retain features of the ancestral genome for the clade. As species differentiate genetically because of selection or drift, reductions in hybrid fertility are likely (although not always found, cf. Hodges 1997). New species derived from a genetically isolated species would be expected to show similar patterns of low hybrid fertility when hybridized to related species, suggesting that the inability to hybridize is a derived, or apomorphic trait (Rosen 1979; Funk 1985).

Evidence from biosystematic studies, although not framed in phylogenetic terms, is consistent with these general predictions: when levels of hy-

TABLE 5. Cases of current sympatry or hypothesized past sympatry in *Schiedea* and *Alsinidendron*, and the extent of putative natural hybridization.

Sympatric species pair and breeding systems	Location of region of sympatry	Extent of hybridization	Method of detection	
<i>S. hookeri</i> (hermaphroditic, outcrossing)	<i>S. ligustrina</i> (dioecious, outcrossing)	Mokule'ia, Wai'anae Mts., O'ahu	Minimal, one collection at interface of habitat of the parental species	Morphological intermediacy
<i>S. hookeri</i> (hermaphroditic, outcrossing)	<i>S. mannii</i> (subdioecious, outcrossing)	Wai'anae Kai Forest Reserve, Wai'anae Mts., O'ahu	Minimal, two instances known	Morphological intermediacy
<i>S. kauaiensis</i> (hermaphroditic, outcrossing)	<i>S. membranacea</i> (hermaphroditic, outcrossing)	Kuia Valley, Kaua'i	No hybrids detected	
<i>S. ligustrina</i> (dioecious, outcrossing)	<i>S. kealiae</i> (subdioecious, outcrossing)	Probably in Wai'anae Mts., O'ahu	Past hybridization with presumed introgression	cpDNA
<i>S. ligustrina</i> (dioecious, outcrossing)	<i>S. mannii</i> (subdioecious, outcrossing)	'Ohikilolo Ridge, west of Wai'anae Kai Forest Reserve, Wai'anae Mts., O'ahu	Hybrids common in one locality with most individuals of <i>S. mannii</i> exhibiting influence of hybridization	Morphological intermediacy
<i>S. lydgatei</i> (hermaphroditic, outcrossing)	<i>S. sarmentosa</i> (gynodioecious, outcrossing)	Makolelau Gulch, Molo'ka'i	Hybrids present at interface of shrublands (<i>S. lydgatei</i> habitat) and cliffs (<i>S. sarmentosa</i> habitat)	Hybrids inferred from morphological intermediacy
<i>S. menziesii</i> (hermaphroditic, intermediate selfing rates)	<i>S. salicaria</i> (gynodioecious, females highly outcrossed, hermaphrodites with intermediate selfing rates)	Ridge between Pu'u Ula-ula and Pu'u Ko'ai, West Maui	Apparent hybrid swarm, very few parental types observed in population	Morphological intermediacy
<i>S. nuttallii</i> (hermaphroditic, outcrossing)	<i>Alsinidendron obovatum</i> (hermaphroditic, facultative selfer)	Two known instances of sympatry, Pahole Natural Area Reserve, Wai'anae Mts., O'ahu	No hybrids detected	
<i>S. pentandra</i> (hermaphroditic, outcrossing)	<i>Alsinidendron trinerve</i> (hermaphroditic, cleistogamous)	One known instance of sympatry on slopes of Mt. Ka'ala, O'ahu	No hybrids detected	
<i>S. spergulina</i> (hermaphroditic, outcrossing)	<i>S. stellarioides</i> (hermaphroditic, partially selfing)	Wai'alae Valley, Kaua'i	No hybrids detected	
<i>S. stellarioides</i> (hermaphroditic, partially outcrossing)	<i>Alsinidendron viscosum</i> (hermaphroditic, facultative autogamy)	Wai'alae Valley, Kaua'i	No hybrids detected	

brid sterility in artificially produced hybrids have been investigated, species within sections of large genera are more likely to produce fertile hybrids than species occurring in different sections (Solbrig 1970; Grant 1981). Hybrid sterility may help substantiate phylogenetic relationships, but could be

misleading because it is likely to evolve repeatedly, whenever a newly derived species undergoes substantial genetic differentiation or when there is selection for isolating mechanisms. As a result, it is the ability to produce fertile hybrids within lineages (a plesiomorphic trait), combined with the fail-

ure to produce hybrids among lineages that helps in understanding evolutionary relationships.

Patterns of hybrid sterility in *Schiedea* and *Alsinidendron* do not appear to reflect phylogenetic relationships based on morphological or molecular data (Wagner et al. 1995; Weller et al. 1995; Soltis et al. 1996; Sakai et al. 1997). The high average fertility of all crosses within clades, relative to the average for all crosses among clades, is probably an artifact of the clades represented in crosses. The clades most heavily represented in within- and between-clade crosses were the *S. adamantis* and *S. globosa* clades (Weller et al. 1995), which are characterized by species occurring in large populations. In contrast, species in the *S. membranacea* and *S. nuttallii* clades, characterized by small population size, were used extensively for between-clade crosses, but not for within-clade crosses.

When the potentially confounding effects of population size were removed by analyzing hybrids of species occurring in large populations only, no significant differences were evident for the within- and between-clade categories, suggesting that population size may play a significant role in determining the level of hybrid fertility. Unfortunately, a parallel analysis, using only species occurring in small populations, could not be carried out because of the limited number of hybrid combinations using species with small population size. More crosses among species occurring in small populations would be useful for separating the effects of clade from differences in population size, but no single analysis can separate these effects when all four clades are considered together because clade identity is confounded with population size.

Differences in chromosome number do not appear to influence production of hybrids or patterns of hybrid fertility. Only two chromosome counts have been published, with $2n=60$ for *Alsinidendron trinerve* (Skottsberg 1953) and *Schiedea verticillata* (Carr 1985). A broader survey of chromosome numbers would be of interest.

Patterns of hybrid fertility in *Schiedea* and *Alsinidendron* based on clade membership and population size are confounded by the lack of phylogenetic independence for many crosses. In contrast, the striking relationship between degree of fertility of F_1 hybrids and genetic identity (Fig. 2), when corrected for phylogeny, suggests a possible mechanism for the patterns of hybrid fertility observed in *Schiedea* and *Alsinidendron*. High genetic identities in this lineage are strongly associated with large population size (Weller et al. 1996). Presumably,

large population size results in retention of genetic similarity (the plesiomorphic condition) among species. Species occurring in large populations may also show the greatest similarity in loci that determine the ability to produce fertile hybrids. In small populations, fixation and loss of alleles due to drift may lead to genetic divergence at loci associated with production of fertile hybrid offspring, and eventually to sterility of hybrids.

Similar patterns of postzygotic isolating mechanisms and genetic distance have been shown in *Drosophila* (Coayne and Orr 1989, 1997). In *Drosophila*, as in the case of *Schiedea* and *Alsinidendron*, the relationship held for data corrected for lack of phylogenetic independence using Felsenstein's (1985) approach, as well as for the analysis where each species pair was considered phylogenetically independent.

Fixation and loss of allozyme variants, and presumably other genes throughout the genome including those associated with ability to produce fertile hybrids, are especially evident in species occurring in small populations. This pattern of low genetic identity can be seen within both *Alsinidendron* and *Schiedea* (Weller et al. 1996). The monophyletic *Alsinidendron* clade, consisting of four facultatively or obligately selfing species occurring in small populations, is characterized by several fixed, unique allozyme variants (Weller et al. 1996). A similar pattern of fixation of unique alleles occurs in the *S. nuttallii* clade (Weller et al. 1996), also characterized by small population size and frequent autogamy.

Large population size, the factor most strongly associated with production of fertile hybrids, may be associated with the evolution of dimorphism because all dimorphic species are wind pollinated (Weller et al. 1998) and wind pollinated species usually occur in large, dense populations (Levin and Kerster 1974). In contrast, most hermaphroditic *Schiedea* species and all *Alsinidendron* species occur in low densities in small populations scattered throughout mesic or wet forest (Sakai et al. 1997). The six hermaphroditic species occurring in large populations generally have high average genetic identities (Weller et al. 1996).

In addition to population size, the degree of morphological divergence in species of *Schiedea* and *Alsinidendron* may influence fertility of hybrids. The highly divergent herbaceous species (*A. lychnoides*, *S. kaalae*, *S. membranacea*, and *S. verticillata*) produced moderately to highly sterile hybrids in most combinations with other species, even though two of these species occur in large populations and would

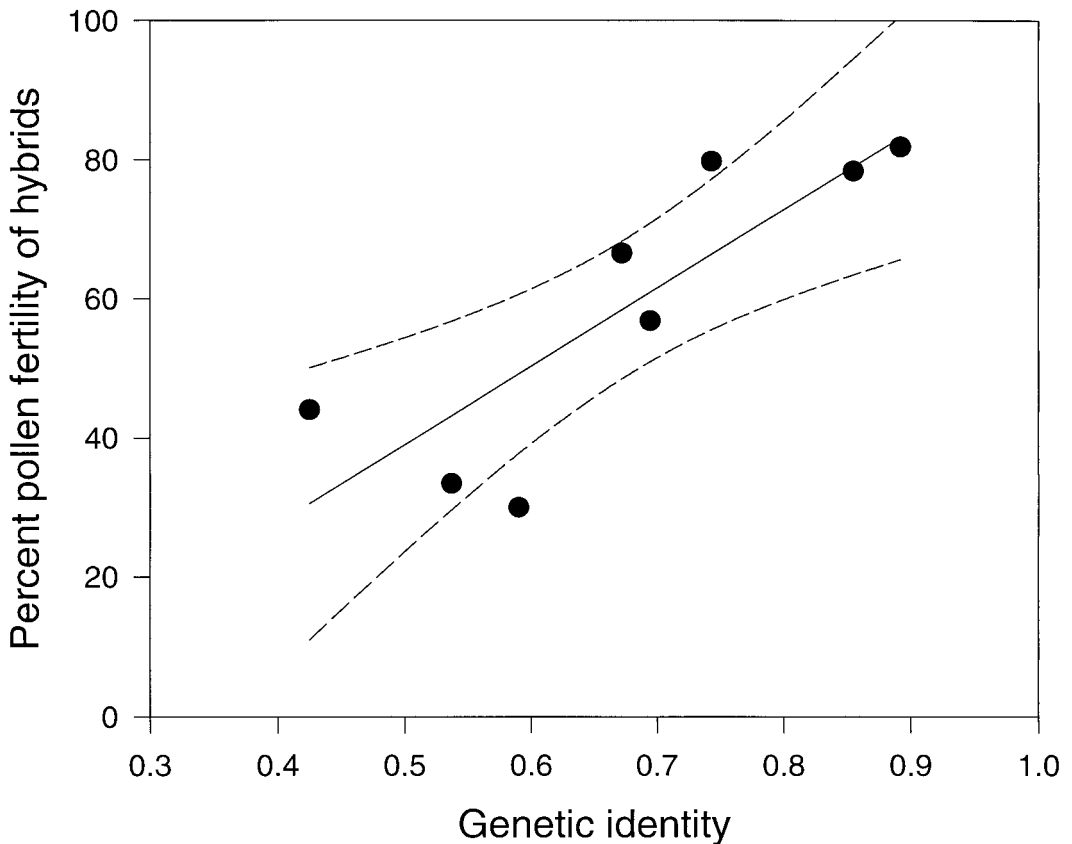


FIG. 2. Regression of percent pollen fertility of F_1 hybrids against genetic identity, based on allozyme variability (Weller et al. 1996). Only phylogenetically independent contrasts are included. Equation for the regression is $y = 112.65x - 17.34$. Dotted lines are 95% confidence intervals.

otherwise be expected to produce fertile hybrids. The general association of woodiness and potential for hybridization is well known (Mayer 1991; Carr 1995; Ellstrand et al. 1996), and consistent with the tendency for woody species of *Schiedea* to produce fertile hybrids. In *Schiedea* woody species are derived from herbaceous ancestors (Carlquist 1994), suggesting that herbaceous ancestry has not limited the potential for hybridization.

The significance of natural hybridization appears to be limited by the rarity of sympatry, although in the few cases of sympatry, putative natural hybrids are often present (Table 5). The most extensive cases of hybridization occur between species that are known or are likely to have fertile F_1 hybrids following artificial hybridization. The extent of current hybridization suggests that reticulate evolution has played a minor role in the adaptive radiation of *Schiedea* and *Alsinidendron*. Like many other line-

ages in the Hawaiian Islands (Wagner and Funk 1995), the endemic Hawaiian Alsinoideae have colonized from the older to younger Hawaiian Islands (Wagner et al. 1995). Rare colonization events, the narrow, non-overlapping ranges of most species, specialized habitats, and occurrence of facultative or obligate autogamy in a number of species have probably reduced instances of sympatry and limited opportunities for hybridization in the rare cases of sympatry. The few cases of current widespread hybridization may in part result from recent disturbance, although in one of the two cases of extensive hybridization there is evidence of a stable hybrid zone that existed when the earliest collections were made in the Hawaiian Islands.

Little evidence exists for extensive natural hybridization in the past. Chloroplast DNA data (Soltis et al. 1996) indicate that *Schiedea kealiae* (placed in the *S. globosa* clade on the basis of morphological

evidence [Weller et al. 1995]), is instead part of the *S. adamantis* clade (Soltis et al. 1996). *Schiedea kealiae* could have obtained its chloroplast genome through hybridization with a species in the *S. adamantis* clade; *S. ligustrina* is a likely candidate because the species co-occur in the Wai'anae Mts. on O'ahu. No other molecular evidence indicates past hybridization events in *Schiedea* or *Alsinidendron*.

Hybridization can be important in the evolution of new species (Arnold 1997), and is especially significant in the evolution of island lineages (Rattenbury 1962; Raven 1973; Carlquist 1974), although views on the extent of natural hybridization in the Hawaiian Islands vary (Gillett and Lim 1970; Gillett 1972; Ganders and Nagata 1984; Ganders 1989). Data compiled from Wagner et al. (1999) indicate hybridization, or more rarely taxa of hybrid origin occur in 34 of 216 (16%) native genera. The list includes essentially all of the larger genera, although in many of these genera hybrids are uncommon.

Hybridization in the silversword alliance has probably resulted in transfer of genetic material between genera in several cases, and may also have resulted in production of new, stable taxa (Carr 1995). Baldwin (Baldwin et al. 1995; Baldwin 1997, 1998) provided compelling evidence for ancient hybridization in the silversword alliance on Kaua'i. The most extensive interspecific hybridization in the Hawaiian flora occurs in *Cyrtandra* (Smith et al. 1996; Wagner et al. 1999).

Genomic differentiation in the Hawaiian Alsinoideae, which appears greatest among those species occurring in small populations and in those species with facultative or obligate selfing, has probably reduced opportunities for gene exchange in this lineage. Among species of *Schiedea* and *Alsinidendron*, reduced pollen fertility in hybrids, a postzygotic isolating mechanism, limits gene exchange. Artificial hybridization demonstrates that species have the potential for production of vigorous hybrid offspring when they come into contact, indicating that selection for prezygotic barriers to gene exchange has not occurred within the lineage. The extensive radiation in the Hawaiian Alsinoideae, and the modest evidence for natural hybridization, indicate that evolution in the lineage has occurred as colonists have reached new habitats and new islands, and that the limited occurrence of these events has prevented significant hybridization during this process.

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