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Molecular Phylogenetic Analysis of the Hawaiian Endemics *Schiedea* and *Alsinidendron* (Caryophyllaceae)

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ABSTRACT. *Schiedea* and *Alsinidendron* (Caryophyllaceae), which represent the fifth or sixth largest endemic radiation of species in the angiosperm flora of the Hawaiian Islands, exhibit striking diversity in morphology, breeding system, and habitat. To gain a historical perspective on this diversity, we conducted a phylogenetic analysis using restriction site variation in chloroplast DNA and nuclear ribosomal DNA. In addition, we compared, and ultimately combined, the molecular data with a recently published morphological data set. Within the *Schiedea-Alsinidendron* lineage, DNA variation is limited, and relationships are generally poorly resolved. These results raise the possibility that, following the initial colonization of the Hawaiian archipelago and the early diversification of the complex, much of the complex radiated rapidly and relatively recently. Phylogenetic analyses of DNA data revealed three clades within the complex (the *S. membranacea*, *S. nuttallii*, and *S. adamantis* clades), in agreement with results of a morphologically-based analysis. Molecular data do not, however, support the *S. globosa* clade, a weakly-supported clade based on morphology. A combined analysis of morphological and molecular data provided both greater resolution and stronger internal support than either data set did individually. The molecular and combined topologies suggest nearly identical patterns of the evolution of sexual dimorphism, habitat shifts, and biogeography within the complex. However, the greater resolution in trees derived from the combined analysis suggests simpler patterns of breeding-system evolution and habitat shifts. Sexual dimorphism may have evolved twice in the complex, with a single reversal to hermaphroditism in one species, or perhaps only once, with three reversals to hermaphroditism. Although the habitat occupied by the ancestor of the complex remains uncertain, it appears that a single shift to dry habitats more or less accompanied the evolution of dimorphic breeding systems, followed by a single shift back to a mesic environment in one species. Alternatively, two parallel shifts to dry habitats may have occurred. Molecular data are consistent with an origin on Kaua'i of the *S. membranacea*, *S. adamantis*, and *S. nuttallii* clades, as suggested by previous morphological analyses. However, both the molecular and combined trees suggest it is equally likely that the complex originated on O'ahu.

Schiedea and *Alsinidendron* (Caryophyllaceae: Alsinoideae), genera endemic to the Hawaiian Islands, exhibit a wide diversity of breeding systems and morphology and occur in a broad array of habitats (Wagner et al. 1995; Weller et al. 1995). *Schiedea* and *Alsinidendron* comprise 25 and four species, respectively, and form the fifth or sixth largest endemic radiation of species in the Hawaiian angiosperm flora (Wagner et al. 1995). Despite their diversity in morphology, breeding system, and habitat, they constitute a monophyletic group based on a suite of unusual morphological characters (Weller et al. 1990, 1995; Wagner et al. 1995). The variation in both habit and habitat in these two genera is among the most striking in Caryophyllaceae; the species vary from large vines of wet forests to compact shrubs, subshrubs, and perennial herbs of dry, exposed sites.

Schiedea and *Alsinidendron* therefore pose a series of intriguing evolutionary questions (Wagner et al. 1995). One of the most noteworthy phylogenetic problems is the possibility that *Schiedea* may be paraphyletic, with *Alsinidendron* derived from within *Schiedea* (Wagner et al. 1995). *Schiedea* and *Alsinidendron* also represent useful models for addressing patterns of morphological and ecological diversification. For example, breeding-system diversity is correlated with habitat in these genera.

Ten species of *Schiedea* have dioecious, subdioecious, or gynodioecious breeding systems (Table 1). All species having separate sexes occur in dry habitats, whereas hermaphroditic species are largely restricted to mesic areas (Weller et al. 1990, 1995). In addition, most species with separate sexes are wind-pollinated whereas those species that are hermaphroditic are either insect-pollinated or autogamous (Weller and Sakai 1990). Weller and Sakai (1990) therefore suggested that a scarcity of pollinators in dry, windy environments may have resulted in increased selfing rates, inbreeding depression, and the spread of male-sterile forms. Most recently, Weller et al. (1995) examined patterns of breeding-system evolution in *Schiedea* and *Alsinidendron* through a phylogenetic analysis of morphological characters; however, relationships based on morphological characters were generally weakly supported, and patterns of breeding-system evolution were not entirely resolved.

To resolve further the phylogenetic relationships in *Schiedea* and *Alsinidendron* and to explore patterns of diversification in this lineage, we employed analyses of restriction site variation in both the chloroplast genome (cpDNA) and the nuclear 18S-26S ribosomal RNA genes (rDNA). We also compared the DNA-based topologies with the morphologically-based trees of Weller et al. (1995). Furthermore, we combined the molecular and morphological data sets and conducted additional phylogenetic analyses. Our specific objectives were: 1) to elucidate phylogenetic relationships in the *Schiedea-Alsinidendron* lineage; and 2) to test recent hypotheses of breeding-system evolution, habitat diversification, and biogeography (Wagner et al. 1995; Weller et al. 1995).

Materials and Methods

Plant Samples. Included in the DNA analyses were three of the four species of *Alsinidendron* and 17 of the 23 extant species of *Schiedea* (Table 1). Two species, *S. amplexicaulis* H. Mann and *S. implexa* (Hillebr.) Sherff, are considered extinct. Of the six extant species of *Schiedea* not included, five were either unknown, not recognized taxonomically, or thought to be extinct at the time of DNA analyses. *Moehringia lateriflora*, also of subfamily Alsinoideae, and *Silene struthioloides* of subfamily Silenoideae were used as outgroups.

cpDNA and rDNA Restriction Site Analysis. For most species investigated, seeds were collected from plants in the field and subsequently germinated and cultured in the greenhouses at the University of California, Irvine. For a few taxa, leaves were collected in the field and mailed directly to the laboratory (Washington State University) for isolation of DNA. We isolated high-molecular-weight total DNA's using several procedures. For some taxa, modifications (Soltis et al. 1991) of the mini-prep protocol of Doyle and Doyle (1987) worked successfully. However, these modified mini-prep methods for DNA isolation did not provide suitable quantities of high-molecular-weight DNA for many of the taxa investigated. Therefore, for most species a large-scale DNA isolation procedure (Rieseberg et al. 1988; Soltis et al. 1991) requiring 10–20 grams (fresh weight) of leaf material was used.

DNA's were digested with the following 28 endonucleases using the specifications of the suppliers: *Acc*I, *Apa*I, *Ava*I, *Ava*II, *Ban*I, *Ban*II, *Bgl*I, *Bgl*II, *Bst*EII, *Bst*NI, *Bst*XI, *Cla*I, *Cfo*I, *Eco*RI, *Eco*RV, *Hae*II, *Hind*III, *Hpa*II, *Nci*I, *Pvu*II, *Pst*I, *Smn*I, *Ssp*I, *Sac*I, *Sac*II, *Sal*I, *Xba*I, and *Xho*I. DNA fragments were separated in 1.0% agarose gels, denatured, and transferred to nylon membranes (ZETABIND, Cuno Laboratory Products, Meriden, Connecticut) following the general methods of Palmer (1986).

Heterologous cpDNA probes from lettuce (Jansen and Palmer 1987) and petunia (used in place of the 22-kb inversion present in the chloroplast genome of lettuce) were labeled using the Random Primed DNA Labeling Kit (U. S. Biochemical Corporation, Cleveland, Ohio) and hybridized to the membrane-bound DNA fragments. cpDNA probes were kindly provided by J. D. Palmer and R. K. Jansen. To analyze rDNA variation, filters were probed with pGMr-1, a plasmid containing a single 18S-26S rDNA repeat from *Glycine max* L., kindly provided by E. A. Zimmer. Restriction sites were scored as present (1) or absent (0). Missing data were scored as question marks; 2.5% of the data matrix cells were scored as missing.

Phylogenetic Analysis of Molecular Data Set. Restriction site data were analyzed with various options of PAUP 3.1.1 (Swofford 1991). To evaluate the nonrandom structure of both the cpDNA data set and the combined cpDNA and rDNA data set, the skewness test (Hillis 1991; Huelsenbeck 1991; Hillis and Huelsenbeck 1992) was performed. For the skewness analyses, the RANDOM TREES feature of PAUP was used to generate 10,000 random trees and to calculate the g_1 statistic based on the distribution of the lengths of these trees.

TABLE 1. Breeding systems of species analyzed and islands where samples were collected. Except where noted, collection numbers are those of Weller and Sakai, and vouchers are at US.

Species	Sample	Island	Breeding system
Alsinidendron lychnoides (Hillebr.) Sherff	867	Kaua'i	Hermaphroditic
A. obovatum Sherff	868	O'ahu	Hermaphroditic
A. trinerve H. Mann	Perlman 5448 (BISH)	O'ahu	Hermaphroditic
Schiedea adamantis St. John	847	O'ahu	Gynodioecious
S. apokremnos St. John	865	Kaua'i	Gynodioecious
S. diffusa A. Gray	848	Maui	Hermaphroditic
S. globosa H. Mann	844	O'ahu	Subdioecious
S. globosa	850	Maui	Subdioecious
S. globosa	852	Maui	Subdioecious
S. haleakalensis Degener & Sherff	851	Maui	Dioecious
S. hookeri A. Gray	794	O'ahu	Hermaphroditic
S. kaalae Wawra	881	O'ahu	Hermaphroditic
S. kealiae Caum & Hosaka	791	O'ahu	Subdioecious
S. kealiae	862	O'ahu	Subdioecious
S. ligustrina Cham. & Schlechtend.	873	O'ahu	Dioecious
S. lydgatei Hillebr.	870	Moloka'i	Hermaphroditic
S. mannii St. John	793	O'ahu	Subdioecious
S. membranacea St. John	864	Kaua'i	Hermaphroditic
S. menziesii Hook.	849	Maui	Hermaphroditic
S. nuttallii Hook.	861	O'ahu	Hermaphroditic
S. pubescens Hillebr.	796	O'ahu	Hermaphroditic
S. salicaria Hillebr.	842	Maui	Gynodioecious
S. spergulina A. Gray	863	Kaua'i	Dioecious
Moehringia lateriflora (L.) Fenzl.	886	Japan	Hermaphroditic
Silene struthioloides A. Gray	882 (no voucher)	Maui	Hermaphroditic

Phylogenetic analyses were conducted with MULPARS, TBR branch-swapping, and "unweighted" character-state changes (in which gains and losses are weighted equally). To test for multiple islands of most parsimonious trees (Maddison 1991), 100 replicate tree searches with RANDOM taxon addition were conducted. To obtain estimates of reliability for monophyletic groups, bootstrap analysis (Felsenstein 1985) using 100 replicates and decay analysis (Bremer 1988) using the converse constraint method (Baum et al. 1994) were conducted. We first analyzed the cpDNA restriction site data set, then repeated the analysis with the rDNA restriction site changes added.

Reanalysis of Morphological Data Set. The cladistic analysis of morphological data (Weller et al. 1995) involved additional taxa not analyzed for DNA variation because herbarium specimens of rare or extinct taxa could be studied for morphological characters. Adequate amounts of leaf material of most of these rare and/or extinct taxa could not be obtained for restriction site analysis. We therefore removed those taxa for which molecular data were not available from the morphological data set (Weller et al. 1995) and reanalyzed this abridged data set. All characters used by Weller et al. (1995) were included in this reanalysis; as in Weller et al. (1995), breeding-system characters were excluded from the analysis because of the likely parallel evolution of sexual dimorphism within the *Schiedea-Alsinidendron* complex. The morphological data were analyzed with PAUP 3.1.1 as described above.

Phylogenetic Analysis of Combined Morphological and Molecular Data Set. We also combined the molecular (cpDNA and rDNA restriction sites) and abridged morphological data sets and conducted phylogenetic analyses as above, except using 1,000 bootstrap replicates and saving up to 1,000 trees per replicate.

RESULTS

Phylogenetic Analysis of Molecular Data. Forty-six cpDNA restriction site mutations were detected, 31 of which were shared by two or more species (including the outgroups) and were potentially parsimony-informative (Table 2, Appendix 1). However, 13 of these 31 informative restriction site mutations differentiate the outgroup species from

TABLE 2. Restriction site mutations detected in the *Schiedea-Alsinidendron* complex. The restriction enzymes for which mutations were observed and the locations of the mutations in the chloroplast genome or nuclear ribosomal DNA, along with the probes used to detect them, are provided. Regions of the chloroplast genome are designated as: LSC = Large Single-Copy Region; SSC = Small Single-Copy Region; IR = Inverted Repeat. cpDNA probes are from *Lactuca* and are labelled *Lac* with the fragment size (Jansen and Palmer 1987) or *Petunia* (labelled *Pet*); all cpDNA probes were provided by B. Jansen and J. Palmer. Mutations 47–49 occur in the nuclear rDNA and are so designated. The rDNA probe is labelled *Glycine* rDNA and was provided by L. Zimmer. Fragment sizes for each restriction site are given, with the larger fragment listed first (site absent) and the smaller fragments (site present) following. Character states (presence or absence) for these restriction sites are given for all taxa in the Appendix.

Character	Restriction enzyme	Location of mutation; probe	Restriction site
1	*Apa*I	SSC; *Lac* 18.8	7.9–6.7 + 1.2
2	*Ban*II	SSC; *Lac* 18.8	10.0–5.5 + 4.5
3	*Ava*II	SSC; *Lac* 18.8	5.1–2.8 + 2.3
4	*Bgl*II	SSC; *Lac* 18.8	11–3.5 + 7.0
5	*Bst*EII	SSC; *Lac* 18.8	6.6–3.3 + 3.3
6	*Bst*NI	SSC; *Lac* 18.8	3.2–2.4 + 0.8
7	*Bst*XI	SSC; *Lac* 18.8	11.5–6.2 + 5.3
8	*Eco*RI	SSC; *Lac* 18.8	2.7–2.2 + 0.5
9	*Eco*RI	SSC; *Lac* 18.8	4.4–2.7 + 1.7
10	*Hae*II	SSC; *Lac* 18.8	4.4–2.7 + 1.7
11	*Pvu*II	SSC; *Lac* 18.8	4.6–3.5 + 1.1
12	*Xba*I	SSC; *Lac* 18.8	8.2–2.7 + 5.5
13	*Ban*I	IR; *Lac* 12.3, 9.9, 3.5, 1.8	1.3–0.8 + 0.5
14	*Ban*I	IR; *Lac* 12.3, 9.9, 3.5, 1.8	13.6–6.8 + 6.8
15	*Ban*I	IR; *Lac* 12.3, 9.9, 3.5, 1.8	7.3–5.2 + 2.1
16	*Ban*II	IR; *Lac* 12.3, 9.9, 3.5, 1.8	12.0–11.5 + 0.5
17	*Ban*II	IR; *Lac* 12.3, 9.9, 3.5, 1.8	1.5–1.0 + 0.5
18	*Cfo*I	IR; *Lac* 12.3, 9.9, 3.5, 1.8	10.0–9.0 + 1.0
19	*Ava*II	IR; *Lac* 12.3, 9.9, 3.5, 1.8	5.0–4.0 + 1.0
20	*Bgl*II	IR; *Lac* 12.3, 9.9, 3.5, 1.8	6.0–4.0 + 2.0
21	*Bst*XI	IR; *Lac* 12.3, 9.9, 3.5, 1.8	15.0–10.0 + 5.0
22	*Bst*NI	IR; *Lac* 12.3, 9.9, 3.5, 1.8	5.0–3.5 + 1.5
23	*Xmn*I	IR; *Lac* 12.3, 9.9, 3.5, 1.8	12.0–11.0 + 1.0
24	*Xmn*I	LSC; *Pet* 9.0, 9.2, 15.3	6.0–5.0 + 1.0
25	*Hind*III	LSC; *Pet* 9.0, 9.2, 15.3	6.6–4.1 + 2.5
26	*Sac*II	LSC; *Pet* 9.0, 9.2, 15.3	15.0–10.0 + 5.0
27	*Sac*II	LSC; *Pet* 9.0, 9.2, 15.3	13.0–6.0 + 7.0
28	*Eco*RI	LSC; *Pet* 9.0, 9.2, 15.3	9.0–5.0 + 4.0
29	*Ban*I	LSC; *Pet* 9.0, 9.2, 15.3	3.2–2.5 + 0.7
30	*Ban*II	LSC; *Pet* 9.0, 9.2, 15.3	11.7–4.2 + 7.5
31	*Ava*II	LSC; *Sac* 3.8, 6.9, 7.7	4.4–3.5 + 0.8
32	*Bst*EII	LSC; *Sac* 3.8, 6.9, 7.7	15.0–12.2 + 2.8
33	*Bst*EII	LSC; *Sac* 3.8, 6.9, 7.7	15.0–7.0 + 8.0
34	*Bst*XI	LSC; *Sac* 3.8, 6.9, 7.7	8.5–7.0 + 1.5
35	*Bst*XI	LSC; *Sac* 3.8, 6.9, 7.7	8.5–4.5 + 4.0
36	*Bst*XI	LSC; *Sac* 3.8, 6.9, 7.7	10.0–8.5 + 1.5
37	*Bgl*I	LSC; *Sac* 10.6, 4.6, 5.4, 6.3	20.0–12.0 + 8.0
38	*Ban*II	LSC; *Sac* 10.6, 4.6, 5.4, 6.3	4.0–2.5 + 1.5
39	*Eco*RV	LSC; *Sac* 10.6, 4.6, 5.4, 6.3	2.0–1.8 + 0.2
40	*Eco*RI	LSC; *Sac* 10.6, 4.6, 5.4, 6.3	5.0–2.4 + 2.6
41	*Bst*XI	LSC; *Sac* 10.6, 4.6, 5.4, 6.3	12.0–10.5 + 1.5
42	*Ava*II	LSC; *Sac* 10.6, 4.6, 5.4, 6.3	4.3–1.8 + 1.6 + 0.9
43	*Ban*II	LSC; *Sac* 10.6, 4.6, 5.4, 6.3	18.0–12.0 + 6.0
44	*Ava*I	LSC; *Sac* 3.8, 6.9, 7.7	7.0–5.5 + 1.5
45	*Eco*RI	LSC; *Pet* 9.0, 9.2, 15.3	5.8–5.0 + 0.8
46	*Hae*II	LSC; *Sac* 3.8, 6.9	1.7–1.0 + 0.7
47	*Eco*RI	rDNA	8.0–6.0 + 2.0
48	*Eco*RI	rDNA	8.0–7.0 + 1.0
49	*Bst*EII	rDNA	11.0–8.0 + 3.0

the *Schiedea-Alsinidendron* complex; only 18 cpDNA restriction site mutations were parsimony-informative within *Schiedea* and *Alsinidendron*. The remaining restriction site mutations were autapomorphies. Three restriction site changes in rDNA were also detected, all of which were parsimony-informative within the *Schiedea-Alsinidendron* complex (Appendix 1). Results of the skewness test conducted on the cpDNA restriction site data suggest considerable nonrandom structure of the data. The g_1 value for the cpDNA data set is -1.617 ($p < 0.01$; Hillis and Huelsenbeck 1992). Parsimony analysis of only the cpDNA restriction site data resulted in 2,014 most parsimonious trees, each of 50 steps, with a consistency index (CI), excluding uninformative characters, of 0.886 and a retention index (RI) of 0.943.

The rDNA mutations do not conflict with the cpDNA restriction site data, but rather further resolve or support relationships suggested by the cpDNA analysis. One of the rDNA mutations supports the strong relationship between *A. trinerve* and *A. obovatum* suggested by cpDNA data, and a second rDNA mutation further supports the monophyly of *Alsinidendron* (see Fig. 1). The third rDNA mutation suggests a close relationship among *S. pubescens*, *S. nuttallii*, *S. diffusa*, and *S. kaalae*, whose relationships were unresolved based on cpDNA data. The skewness test on the combined cpDNA and rDNA data set also indicated significant nonrandom structure in the data ($g_1 = -1.690$; $p < 0.01$; Hillis and Huelsenbeck 1992). Phylogenetic analysis of the molecular data produced 870 shortest trees, each of 53 steps, with a CI of 0.895 (excluding uninformative characters) and a RI of 0.947.

The 50% majority-rule tree for the combined cpDNA/rDNA data set (Fig. 1) supports (100% bootstrap value, decay value of 13) the monophyly of the *Schiedea-Alsinidendron lineage*, relative to the outgroups used, with several clades present within this assemblage. One clade, hereafter referred to as the *S. membranacea* clade (terminology of Wagner et al. 1995; Weller et al. 1995), comprises *S. membranacea*, as well as the three species of *Alsinidendron* (Fig. 1). This clade appears as the sister to all other species of *Schiedea*. Within the *S. membranacea* clade, the monophyly of the three species of *Alsinidendron* is well-supported (bootstrap value of 97%, decay value of 3), as is the sister-group status of *A. obovatum* and *A. trinerve* (bootstrap value of 96%, decay value of 3). However, only one cpDNA mutation links *S. membranacea* with the three *Alsinidendron* species; half of the trees show *S. membranacea* as sister to all other species of *Schiedea* (i.e., *Schiedea* is monophyletic) because of a single cpDNA mutation shared by *S. membranacea* and all other *Schiedea* species.

With the exception of *S. membranacea*, all species of *Schiedea* consistently form a well-supported monophyletic group (bootstrap value of 100%, decay value of 4), with *S. spergulina* appearing as the sister to the remaining species, although this sister-group relationship is not supported by all most parsimonious trees. Relationships within this large clade are poorly resolved due to the small number of restriction site mutations detected within the complex, but two clades are noteworthy. Although present in only 63% of the shortest trees and weakly supported by the bootstrap analysis (value of 22%), one clade comprises *S. ligustrina*, *S. adamantis*, *S. salicaria*, *S. lydgatei*, *S. kealiae*, and *S. apokremnos*. This clade is similar to the morphologically-defined *S. adamantis* clade of Wagner et al. (1995) and Weller et al. (1995), lacking only *S. haleakalensis* and including *S. kealiae*, and will hereafter be referred to as the *S. adamantis* clade. Within the *S. adamantis* clade, a close relationship is suggested between *S. salicaria* and *S. lydgatei*, with a bootstrap value of 60% and a decay value of 1. A second clade, marked by one rDNA restriction site mutation, is composed of *S. pubescens*, *S. nuttallii*, *S. diffusa*, and *S. kaalae*. This clade is comparable to the *S. nuttallii* clade of Wagner et al. (1995) and Weller et al. (1995), differing only in the addition of *S. implexa* and *S. sp. nov.* in the morphologically-based trees (Fig. 2).

Phylogenetic Analysis of Abridged Morphological Data Set. To obtain phylogenetic trees based on morphological data, we used the data of Weller et al. (1995) and omitted those taxa for which DNA data were not available. The majority-rule consensus tree for the abridged morphological data set (tree not shown) is topologically nearly identical to that obtained by Weller et al. (1995; Fig. 2), differing only in the placement of the nine species omitted from the abridged tree. The four major clades noted previously are still present: the *S. membranacea*, *S. adamantis*, *S. nuttallii*, and *S. globosa* clades.

Phylogenetic Analysis of Combined Molecular/Morphological Data Set. The skewness test indicated significant nonrandom structure in the combined molecular and morphological data set ($g_1 = -1.245$; $p < 0.01$; Hillis and Huelsenbeck 1992). Phylogenetic analysis of this data set resulted in 20 shortest trees, each of 166 steps (CI = 0.610 exclud-

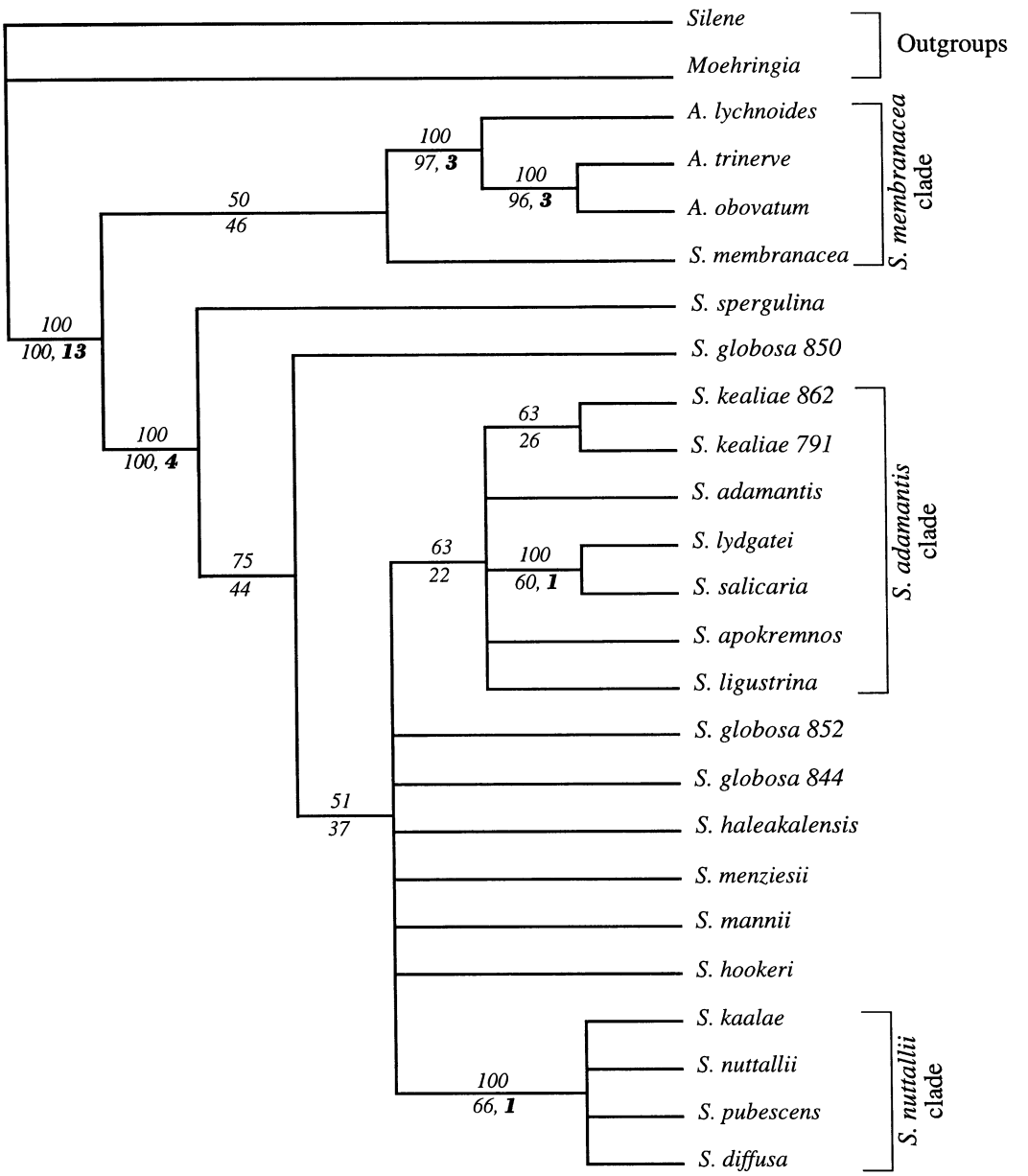


FIG. 1. Majority-rule consensus of 870 most parsimonious trees resulting from analysis of cpDNA and rDNA restriction site data. The analysis of cpDNA data alone produced the identical topology except that the *S. nuttallii* clade is not recognized (see text). Numbers above branches are the percentage of these 870 trees that support the branches; numbers below branches are bootstrap percentages and decay values (bold), respectively. Clades not present in all shortest trees have a decay value of 0.

ing uninformative characters; RI = 0.725). The strict consensus tree (Fig. 3) is topologically very similar to those obtained in the separate analyses of DNA and morphological data but is more fully resolved than the DNA strict consensus tree. Furthermore, all but four of the branches appear in all of the shortest trees. The *S. membranacea*, *S. adamantis*, and *S. nuttallii* clades are again present; however, as in the DNA analysis, members of the morphologically-based *S. globosa* clade (*S. kealiae*, *S. globosa*, *S. hookeri*,

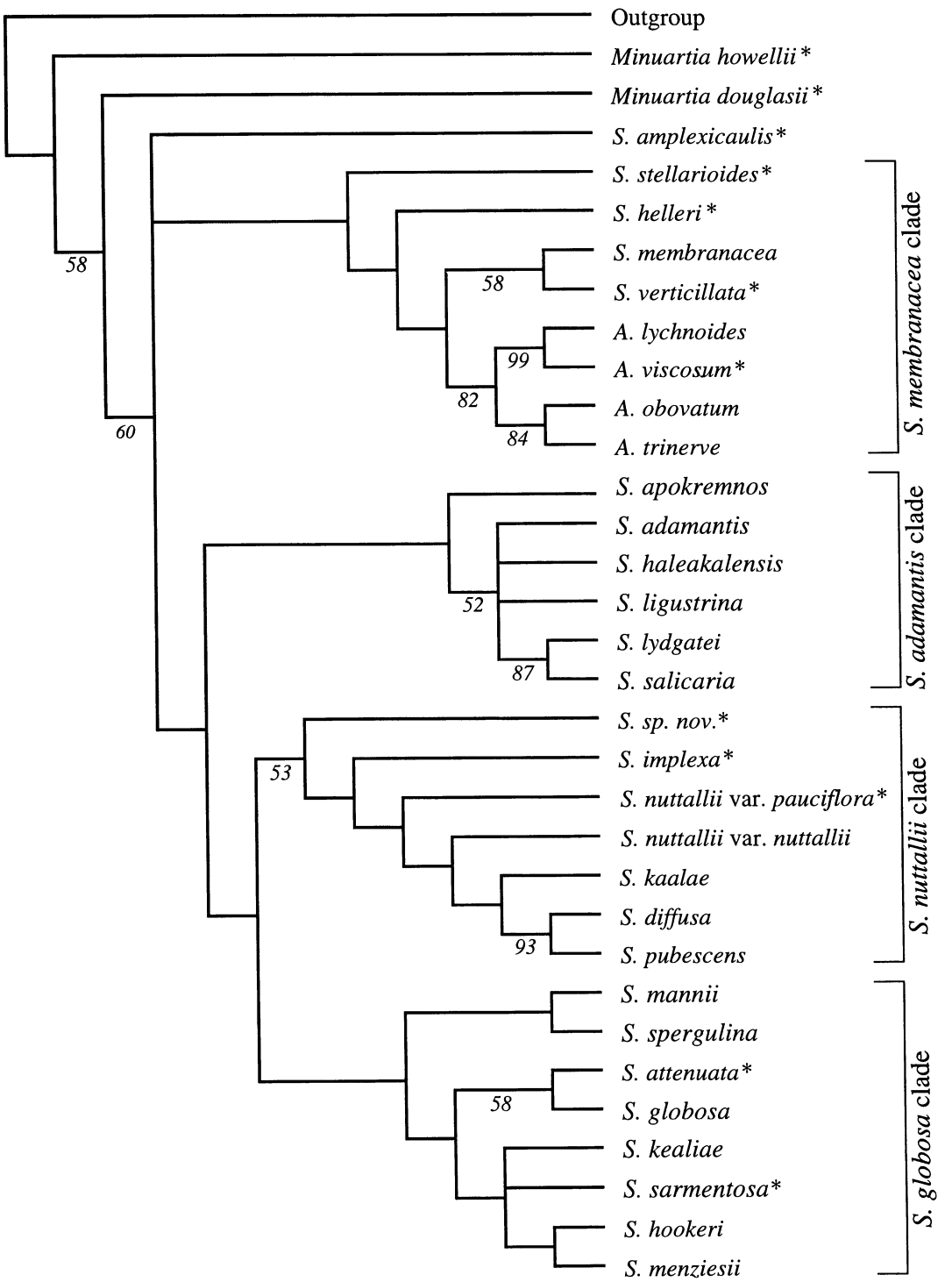


FIG. 2. Strict consensus of six most parsimonious trees resulting from analysis of morphological data (from Weller et al. 1995). Numbers below branches are bootstrap percentages; nodes lacking bootstrap values received < 50% bootstrap support. Asterisks designate those species not included in DNA analyses.

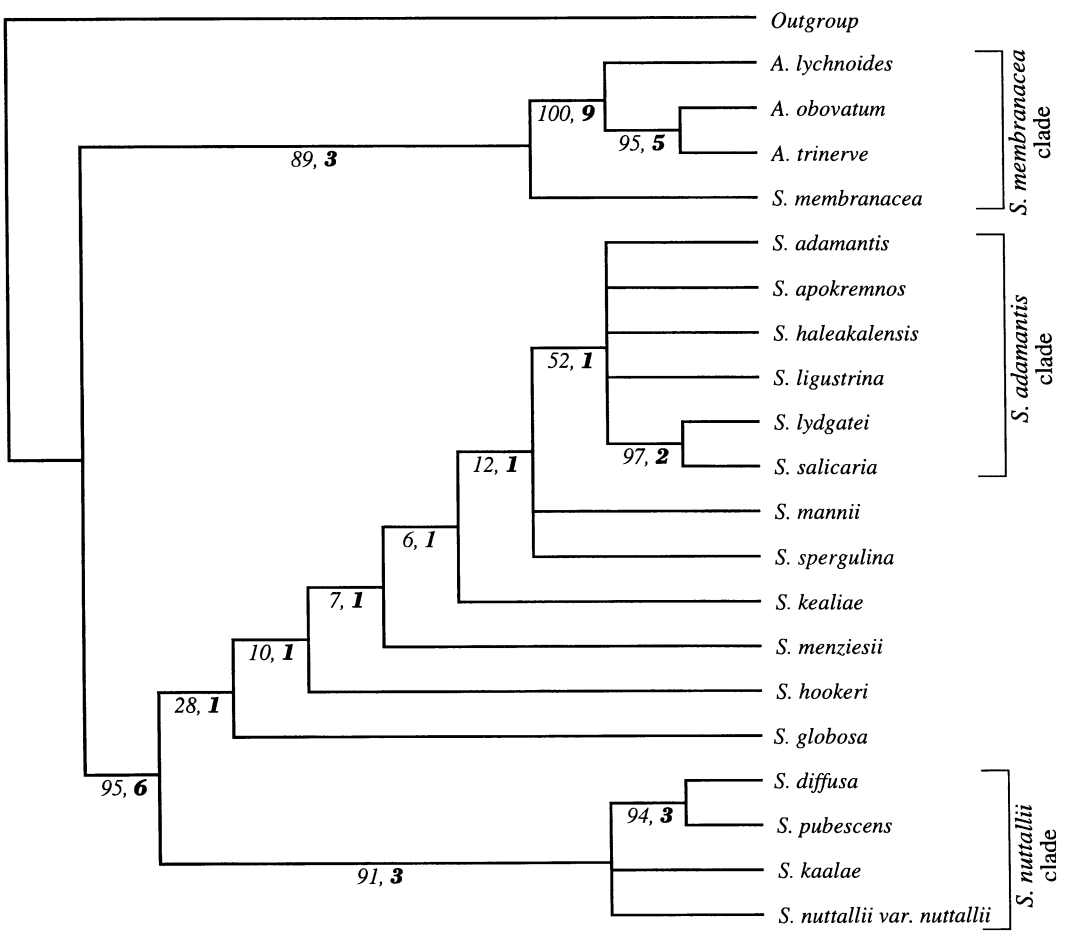


FIG. 3. Strict consensus of 20 most parsimonious trees resulting from analysis of a combined molecular and morphological data set. Numbers below branches are bootstrap percentages and decay values (bold), respectively.

S. mannii, *S. menziesii*, and *S. spergulina*) do not form a monophyletic group. In the combined analysis, they are part of a large clade within which is nested the *S. adamantis* clade.

DISCUSSION

Phylogenetic Relationships Based on DNA Data. Phylogenetic analyses of cpDNA restriction site data alone (tree not shown) and of the combined cpDNA/rDNA restriction site data (Fig. 1) produced congruent hypotheses of relationships in the *Schiedea-Alsinidendron* complex. Because the three rDNA mutations do not contradict, but rather agree with or complement, the cpDNA data, we will limit our discussion to the most parsimonious trees resulting from the analysis of the combined cpDNA/rDNA data (Fig. 1).

Molecular data suggest a well-supported *Schiedea-Alsinidendron* clade relative to the outgroups *Moehringia lateriflora* and *Silene struthioloides*. The monophyly of the *Schiedea-Alsinidendron* lineage should be further tested, however, as part of a more comprehensive molecular study of genera of Caryophyllaceae, including *Minuartia*, now believed on morphological grounds to be most closely related to the *Schiedea-Alsinidendron* complex (Wagner et al. 1995). Nonetheless, these results are in agreement with morphological data (Weller et al. 1990, 1995) that similarly indicate that *Alsinidendron* and *Schiedea* form a well-supported monophyletic group. Morphologically, these two genera are unusual in Caryophyllaceae in that they possess specialized floral nectaries and lack petals (Wagner et al. 1995).

Within the *Schiedea-Alsinidendron* complex, relationships are generally poorly resolved based on

molecular data due in part to a paucity of re-striction site mutations. Despite the small number of informative restriction site mutations, several lineages are apparent within the complex, and these agree in large part with clades recovered by phylogenetic analysis of morphological characters (Fig. 2; Weller et al. 1995). The three species of *Alsinidendron* form a well-supported lineage based on molecular data. These three species of *Alsinidendron*, together with *S. helleri* Sherff, *S. membranacea*, *S. stellarioides* H. Mann, and *S. verticillata* F. Brown, compose the *S. membranacea* clade in the morphologically-based cladistic analysis (Fig. 2; Wagner et al. 1995; Weller et al. 1995). Of the five *Schiedea* species present in the *S. membranacea* clade based on morphology, leaf material of only *S. membranacea* and *S. verticillata* was available at the time of this study, and DNA of *S. verticillata* proved intractable. The position of *S. membranacea* is uncertain, however, based on DNA data. One cpDNA restriction site mutation unites *S. membranacea* with species of *Alsinidendron*, whereas a second cpDNA mutation unites *S. membranacea* with all other species of *Schiedea*. A *S. membranacea* clade comparable to that of Weller et al. occurs in 50% of the shortest trees, making *Schiedea* paraphyletic, whereas the second topology suggests that *Alsinidendron* and *Schiedea* are each monophyletic and sister taxa. Thus, at this point, molecular data are inconclusive regarding the proposed paraphyly of *Schiedea* based on morphological data. Although the placement of *S. membranacea* is problematic in the DNA trees, *S. membranacea* clearly possesses many symplesiomorphic restriction sites also found in *Alsinidendron* and lacks the five synapomorphies that define the monophyletic remainder of *Schiedea*. Hence, molecular and morphological data agree in the general composition and phylogenetic position of the *S. membranacea* clade, although the exact placement of *S. membranacea* in the molecular analysis is uncertain. The position of *S. membranacea* could perhaps be stabilized in the molecular analysis with the inclusion of additional species (i.e., *S. stellarioides*, *S. helleri*, and *S. verticillata*) shown in the morphological study (Weller et al. 1995) to be part of the *S. membranacea* clade, along with *S. membranacea* and *Alsinidendron*.

A second weakly-supported lineage based on DNA data consists of *S. ligustrina*, *S. adamantis*, *S. salicaria*, *S. lydgatei*, *S. kealiae*, and *S. apokremnos*, a clade nearly identical to the *S. adamantis* clade of Weller et al. (1995). The DNA-based *S. adamantis* clade differs from its morphologically-based counterpart only in the absence of *S. haleakalensis* and the inclusion of *S. kealiae* in this clade in the molecular tree. The morphologically-based cladistic analysis (Weller et al. 1995) places *S. kealiae* in what Weller et al. term the *S. globosa* clade (*S. globosa*, *S. attenuata* W. L. Wagner, Weller & Sakai, *S. hookeri*, *S. menziesii*, *S. sarmentosa* Degener & Sherff, *S. kealiae*, *S. spergulina*, and *S. mannii*). Because both the DNA-based *S. adamantis* clade and morphologically-based *S. globosa* clade are only weakly supported, the discrepancy in the placement of *S. kealiae* could easily be the result of homoplasy in either the molecular or morphological data set. The combined analysis places *S. haleakalensis* in the *S. adamantis* clade and excludes *S. kealiae*, recovering a clade that closely resembles that found in the morphological analysis.

Although the morphologically-based *S. globosa* clade is weakly supported, morphological data suggest a close relationship between *S. kealiae* and *S. sarmentosa*, *S. hookeri*, and *S. menziesii*. The discrepancy in the placement of *S. kealiae* in the morphological and molecular trees may not reflect real conflict between the data sets because in neither analysis is the position of *S. kealiae* strongly supported. However, the difference may result from different nuclear and organellar histories in *S. kealiae*. The chloroplast genome of *S. kealiae* may have been obtained from a member of the *S. adamantis* clade. Both *S. kealiae* and *S. ligustrina* (of the *S. adamantis* clade) occur in the Waianae Mountains on O'ahu, providing the opportunity for the transfer of the *S. ligustrina* chloroplast genome to *S. kealiae*. Hybridization in the complex is known for two other species pairs (S. Weller and A. Sakai, unpubl. data), although hybridization involving *S. kealiae* has not been reported.

The third DNA-based clade (*S. pubescens*, *S. nuttallii*, *S. diffusa*, and *S. kaalae*) is identical to the morphologically-based *S. nuttallii* clade of Weller et al. (1995) with two exceptions. *Schiedea implexa*, an extinct taxon that could not be sampled for DNA variation, is also a member of the morphological *S. nuttallii* clade. In addition, the *S. nuttallii* clade inferred from morphology also contains a recently discovered and currently undescribed species, for which leaf material was not available for DNA analysis.

One of the most obvious differences between the DNA-based and morphologically-based phylogenetic trees is that the former do not reveal a *S.*

globosa clade (*S. globosa*, *S. attenuata*, *S. hookeri*, *S. menziesii*, *S. sarmentosa*, *S. kealiae*, *S. spergulina*, and *S. mannii*). These species are part of a large polytomy in trees based on DNA data (Fig. 1) and are united by only a single character, the presence of long, attenuate leaf tips, in the morphologically-based trees. It is, therefore, the most weakly-supported alliance based on morphology (Wagner et al. 1995; Weller et al. 1995). Furthermore, *S. globosa* itself does not appear monophyletic in the DNA tree, with population 850 appearing as the sister to the large clade of *Schiedea* species that includes two other populations of *S. globosa*. Although this placement of *S. globosa* is weakly supported, the three populations of *S. globosa* fail to form a monophyletic group in the strict consensus of all shortest molecular-based tress; they form part of a 13-chotomy. However, populations 850 and 844 appear in the same small clade in a phylogenetic analysis of sequences of the internal transcribed spacers of nuclear rDNA (P. Soltis et al., unpubl. data).

Phylogenetic Analysis of the Combined Morphological and Molecular Data Set. Phylogenetic analysis of a combined morphological and molecular data set (Fig. 3) provided results similar to those obtained via the analysis of cpDNA and rDNA restriction sites (Fig. 1) in that three of the four major clades of Weller et al. (1995) are again present: the *S. membranacea* clade, the *S. adamantis* clade, and the *S. nuttallii* clade. Members of the *S. globosa* clade (Weller et al. 1995) do not form a monophyletic group in the combined analysis, but instead are part of a clade out of which the *S. adamantis* clade is derived. In the combined analysis (Fig. 3), the problematic *S. kealiae* (part of the *S. globosa* clade based on morphology, but a member of the *S. adamantis* clade in the DNA analyses) is also part of this large clade.

Both the *S. nuttallii* and *S. membranacea* clades are strongly supported in the combined morphological and molecular analysis, with bootstrap values of 91% and 89%, respectively, and each with a decay value of 3. In contrast, the monophyly of the *S. adamantis* clade is only weakly supported (bootstrap value of 52%, decay value of 1). Strongly-supported relationships are also suggested for: 1) the three species of *Alsinidendron* (bootstrap value of 100%, decay value of 9); 2) *A. obovatum* and *A. trinerve* (95%, 5), and 3) *S. diffusa* and *S. pubescens* (94%, 3). Also strongly supported is the monophyly of *Schiedea*, minus *S. membranacea* (bootstrap value of 95%, decay value of 6).

Evolution of Breeding Systems. In the sections that follow, we will address questions of breeding-system evolution, habitat shifts, and biogeography based on the phylogenetic trees for the *Schiedea-Alsinidendron* complex. The trees based on molecular data only and those derived from the combined molecular and morphological data set are largely congruent. Because of this congruence and because the trees from the combined analysis are more fully resolved and have higher internal support than those based only on DNA data, we will base these discussions on the strict consensus tree resulting from the combined analysis (Fig. 3).

Mapping breeding-system diversity onto the morphologically-based tree indicates that sexual dimorphism (gynodioecy, subdioecy, and dioecy) evolved from one to six times, depending on whether breeding systems are coded as monomorphic vs. dimorphic or, alternatively, as hermaphroditic, gynodioecious, subdioecious, or dioecious (Weller et al. 1995). Although breeding-system characters were not included in the morphological analyses, subsequent inclusion of these characters had little effect on topology or the interpretation of breeding-system evolution. Weller et al. (1995) concluded that two transitions to sexual dimorphism are most likely and that one to several reversals from dimorphism to hermaphroditism also occurred.

Mapping the breeding systems, as summarized in Table 1, onto the molecular tree (Fig. 1) provides an equivocal interpretation of the evolution of sexual dimorphism but does not contradict the interpretations based only on morphological data (Weller et al. 1995). Scoring breeding systems as sexually monomorphic vs. dimorphic (i.e., scoring gynodioecy, subdioecy, and dioecy as a single state; see Weller et al. 1995), the combined morphological and molecular analysis implies two origins of sexual dimorphism, once in *S. globosa* and once in the ancestor of the large clade that comprises the remaining eight sexually dimorphic species. This interpretation requires a single reversal to hermaphroditism in *S. lydgatei*. An interpretation that is one step longer involves a single origin of sexual dimorphism in the ancestor of the sister group of the *S. nuttallii* clade, with independent reversals to hermaphroditism in *S. hookeri*, *S. menziesii*, and *S. lydgatei*. Multi-state coding of breeding systems and ordered transitions from hermaphroditism to dioecy, with gynodioecy and subdioecy as intermediates (Charlesworth and Charlesworth 1978), results in a more complex picture of breeding-system

evolution, with changes to hermaphroditism, gynodioecy, and dioecy from a subdioecious ancestor all taking place within the *S. adamantis* clade.

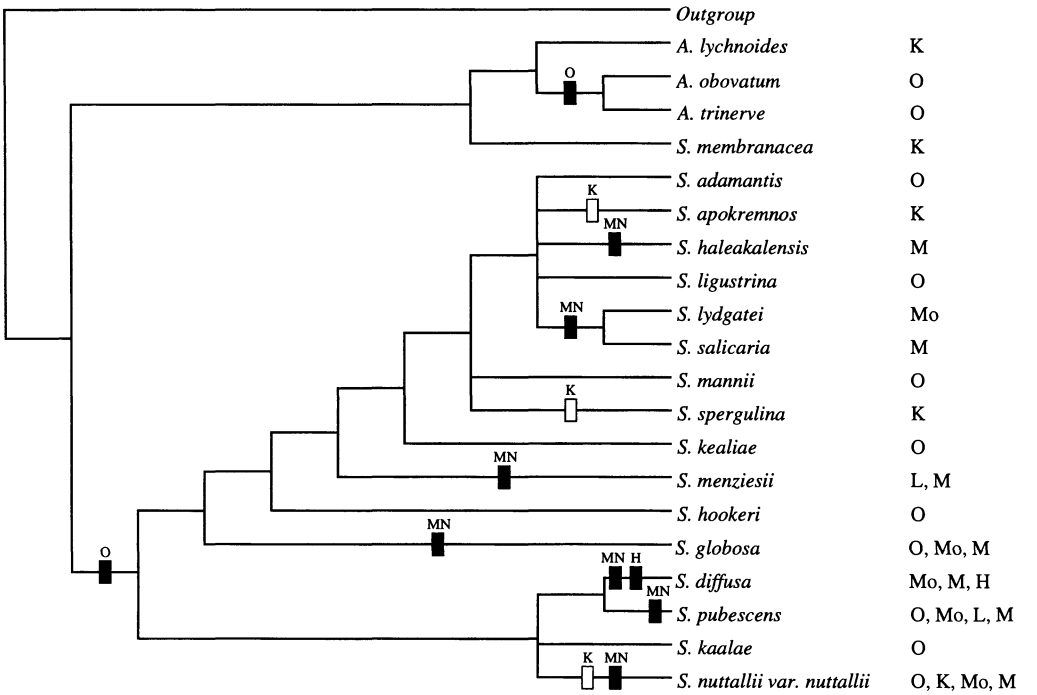
Habitat Shifts. Trees based on the combined analysis imply a simpler pattern of habitat diversification in the *Schiedea-Alsinidendron* complex than do trees based on either morphological or molecular data alone. All of the combined trees suggest a single shift to dry habitats in the ancestor of the large clade comprising the *S. adamantis* clade and the members of the *S. globosa* clade, with a single shift back to a mesic habitat in *S. hookeri*. The habitat occupied by the ancestral members of the complex is uncertain. The closest relatives of *Schiedea* and *Alsinidendron*, based on morphology, occur in relatively dry habitats, suggesting that the ancestor of the *Schiedea-Alsinidendron* lineage may have also occupied dry habitats. This would require a shift to mesic habitats early in the history of the lineage, before the divergence of the *S. membranacea* clade from the remainder of *Schiedea*. Alternatively, if the *S. membranacea* and *S. nuttallii* clades are truly successive sister groups to the remainder of the complex, then the ancestor may have occupied a mesic habitat because both basal lineages occur in mesic or wet habitats.

Biogeography. Using the most parsimonious trees for *Schiedea* and *Alsinidendron* based on cladistic analyses of morphological characters, Wagner et al. (1995) suggested that the *S. membranacea*, *S. adamantis*, and *S. nuttallii* clades all originated on Kaua'i, the oldest of the current main Hawaiian Islands. Furthermore, if we consider only these three clades, analyses of both the molecular data and the combined molecular and morphological data do not contradict this hypothesis, and molecular data generally support the conclusion (Wagner et al. 1995) that colonization events in *Schiedea* and *Alsinidendron* have proceeded from older to younger islands. *Schiedea membranacea* and *Alsinidendron lychnoides* are the basal members of the *S. membranacea* clade and are restricted to Kaua'i, and the *S. membranacea* clade is the sister to all other taxa in the complex. However, relationships within both the *S. nuttallii* and *S. adamantis* clades are too poorly resolved to permit analysis of the patterns of colonization in these clades.

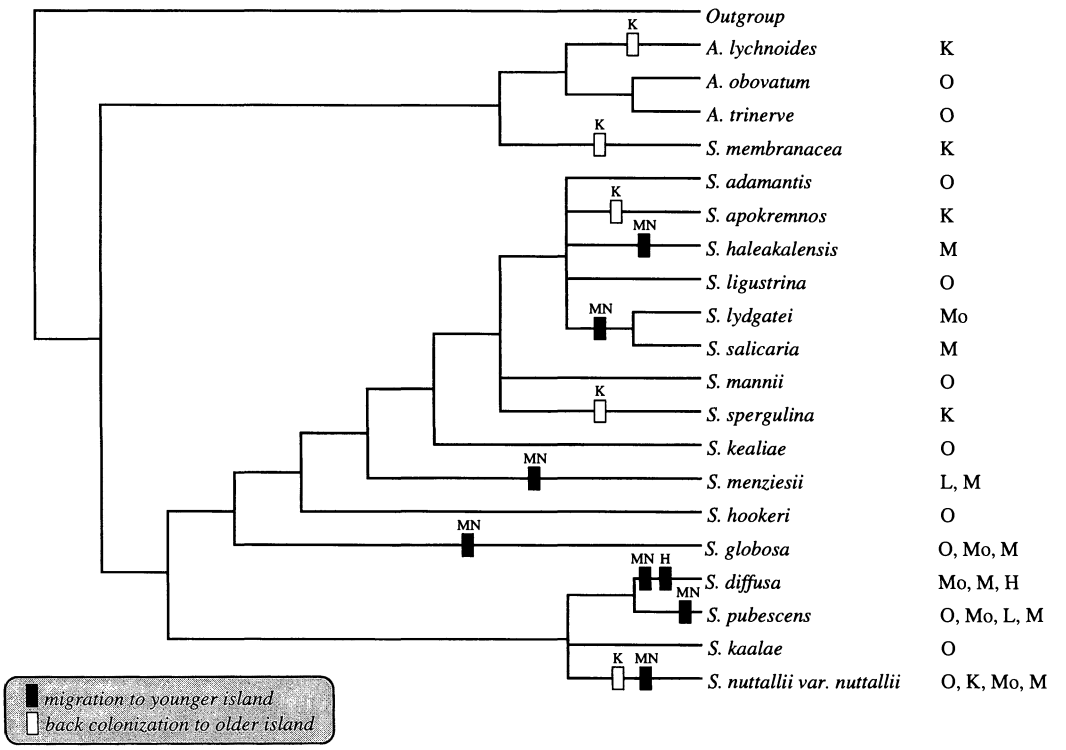
Although molecular analyses generally support an origin of the *S. membranacea*, *S. adamantis*, and *S. nuttallii* clades on Kaua'i (Fig. 4A), more complex scenarios are required when the phytogeography of the entire complex is considered. The distributions of the remaining species (members of the morphologically-based *S. globosa* clade) raise the possibility of an origin for the complex on O'ahu (Fig. 4B), rather than on Kaua'i or even older islands as suggested by Wagner et al. (1995). Based on the strict consensus tree from the combined morphological and molecular analysis, an origin for the complex on Kaua'i (Fig. 4A) requires perhaps a single migration to O'ahu in the ancestor of the sister group to the *S. membranacea* clade, with subsequent shifts to other islands to account for the distributions of the remaining species. This scenario involves fewer steps (13) than independent radiations from Kaua'i for the *S. membranacea*, *S. nuttallii*, and *S. adamantis* clades (17 steps, not shown). An origin of the complex on O'ahu, followed by several independent migrations to Kaua'i and to other islands (13 steps; Fig. 4B), is equally parsimonious to an origin on Kaua'i followed by an early migration to O'ahu (Fig. 4A). However, an origin of the complex on O'ahu requires four or five "back colonizations" to older islands (i.e., Kaua'i) whereas an origin on Kaua'i (followed by a migration to O'ahu in the ancestor of the sister group of the *S. membranacea* clade) suggests only three, for *S. nuttallii*, *S. spergulina*, and *S. apokremnos*. Finally, because the morphologically-based *S. globosa* clade was not recovered in either the molecular or combined analyses (perhaps because of undersampling of the potential members of this clade in the latter studies), inferred patterns of colonization, particularly of O'ahu, differ between the morphological analysis and the molecular and combined analyses. Expanded molecular and combined analyses might resolve more fully the relationships in this portion of the tree and provide a clearer picture of patterns of dispersal and colonization.

Wagner et al. (1995) further suggested that the origin of the *Schiedea-Alsinidendron* complex was a relatively old event, with the original colonization of the archipelago occurring on islands that are now severely eroded and subsided. The *S. membranacea* clade may therefore represent the remnants of this original diversification within the complex. One striking feature, however, of the molecular analysis is the paucity of restriction site mutations for both cpDNA and rDNA. Similarly, ITS sequence analysis (P. Soltis et al., unpubl. data) reveals very few base substitutions among these species. These results are in contrast to the results of both cpDNA restriction site surveys and ITS sequence data for Hawaiian silverswords (Baldwin et al. 1990; Baldwin 1992), a group of endemics typified by higher levels of

A. Origin on Kaua'i



B. Origin on O'ahu



molecular diversity. Most of the restriction site mutations detected herein actually differentiate species of *Alsinidendron* from species of *Schiedea*. Even the position of *S. membranacea* as sister to *Alsinidendron* in the molecular analysis is equivocal, with half of the shortest trees also showing *Schiedea* as monophyletic. Thus, assuming a monophyletic *Schiedea* (this alternative is *not* depicted in Fig. 1) and a rough molecular clock, molecular data support Wagner et al.'s (1995) hypothesis that species of *Alsinidendron*, with their accumulated restriction site mutations, represent an older lineage and perhaps are remnants of the original diversification of the complex on the Hawaiian Islands. The paucity of restriction site mutations outside of *Alsinidendron* might then suggest that the remainder of the complex (i.e., most species of *Schiedea*) is the result of a relatively recent and rapid diversification. However, the combined morphological/molecular analysis strongly supports the *S. membranacea* clade as the sister to all other species of *Schiedea*, making *Schiedea* paraphyletic. If *Schiedea* is indeed paraphyletic (as shown in Figs. 1, 3), *Alsinidendron* cannot represent an ancient lineage separate from *S. membranacea* (and perhaps other species as shown in the morphological analysis alone; Fig. 2). Age alone could therefore not be responsible for the greater cpDNA divergence of *Alsinidendron*. Alternatively, species of *Alsinidendron* may experience accelerated rates of cpDNA evolution. All species of *Alsinidendron* have autogamous breeding systems, a trait that may lead to rapid generation time and increased rates of molecular evolution (e.g., Britten 1986; Gaut et al. 1992). In contrast, species of *Schiedea* appear to have longer reproductive cycles characterized by outcrossing. If rates of molecular evolution are dependent on generation time, the longer life cycles of these *Schiedea* species may reduce the rate of molecular evolution, as reflected in the lower molecular diversity observed outside the *S. membranacea* clade. Finally, limited sampling of key taxa may also affect our inferences of rates and patterns of molecular divergence in *Schiedea* and *Alsinidendron*. For example, several key species of the morphologically-based *S. membranacea* clade (*S. stellarioides*, *S. helleri*, and *S. verticillata*) could not be included in the molecular analysis. The relationships of these species must be ascertained prior to further assessments of rates of molecular evolution or the patterns and timing of speciation in the *Schiedea-Alsinidendron* complex.

Conclusions. Although few restriction site mutations were identified in either the cpDNA or rDNA of *Schiedea* and *Alsinidendron*, the resulting phylogenetic trees were largely consistent with those based on morphology. The combined analysis of morphological and molecular characters can often provide further resolution and stronger support for some of the internal branches than either data set did alone (cf. Barrett et al. 1991; reviewed by de Queiroz et al. 1995). Given the greater resolution of the combined tree, patterns of breeding-system evolution and habitat shifts are much simpler than those proposed by Wagner et al. (1995) and Weller et al. (1995). The combined analysis implies that sexual dimorphism arose either twice in the complex, with one reversal to hermaphroditism in *S. lydgatei*, or only once, with three reversals to hermaphroditism. A single shift to dry habitats more or less accompanied this change in breeding system, with a single shift back to a mesic habitat in *S. hookeri*. Patterns of colonization remain uncertain, with origins for the complex on Kaua'i and O'ahu equally likely.

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FIG. 4. Possible biogeographic scenarios based on strict consensus tree shown in Fig. 3. 4A. Beginning with origin on Kaua'i; requires 13 steps after the origin of the complex. 4B. Beginning with origin on O'ahu; requires 13 steps after the origin of the complex. Note that the migration pattern depicted in the *S. membranacea* clade is one of two possibilities. Dark rectangles indicate shifts to younger islands (H = shift to Hawai'i; K = shift to Kaua'i; MN = colonization of some or all islands of Maui Nui; O = shift to O'ahu). Open rectangles represent "back colonizations" to older islands. Letters to the right of the species names designate the distributions of the species (H = Hawai'i; K = Kaua'i; L = Lana'i; M = Maui; Mo = Moloka'i; O = O'ahu).

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APPENDIX. Data matrix used for molecular phylogenetic analysis of *Schiedea*, *Alsinidendron*, and outgroups. Data for characters 1 through 49 are listed from left to right, with 1 and 0 designating presence and absence of restriction sites, respectively. The first 46 characters are cpDNA restriction site mutations; the last three are rDNA restriction site mutations. More information on characters 1–49 is given in Table 2.

A. lychnoides	0 0 1 0 1 0 0 1 0 0 0 0 0 1 0 0 0 1 1 0 0 0 1 1 1 0 0 1 0 0 1 0 0 0 1 1 0 0 0 1 1 1 0 0 1 1 0 1 1
A. obovatum	0 0 1 0 1 1 0 0 0 0 1 0 0 1 1 0 0 1 0 0 0 0 1 1 1 0 0 1 0 0 1 0 0 0 0 1 0 0 0 1 1 1 0 0 0 1 0 1 0
A. trinerve	0 0 1 0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 0 0 0 0 1 1 1 0 0 1 0 0 1 0 0 0 0 1 0 0 0 1 1 1 0 0 1 1 0 1 0
S. adamantis	0 0 ? 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 0 ? 0 0 1 1 0 0 ? 0 1 0 1 0 0 0 1 0 1 0 ? 1 1 0 0 1
S. apokremnos	1 0 ? 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 ? ? 0 0 1 1 0 0 1 0 1 0 ? 0 0 0 1 0 1 0 ? 1 1 0 0 1
S. diffusa	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 1 ? 1
S. globosa 844	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 ? 1 1 0 0 1 1 0 0 1 1 1 0 1 0 0 0 1 0 1 0 ? 1 1 0 0 1
S. globosa 850	0 0 1 0 ? 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 ? 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. globosa 852	0 0 ? 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. haleakalensis	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. hookeri	0 0 1 0 0 0 0 0 0 0 0 ? 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. kaalae	0 0 1 0 0 0 0 0 0 0 0 ? 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 0 0 0 0 1 0 1 0 1 1 0 1 0 1
S. kealiae 791	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 0 0 0 1 1 0 0 1 0 1 0 ? 0 0 0 1 0 1 0 0 1 1 0 0 1
S. kealiae 862	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 ? 1 0 0 0 1 1 0 0 1 0 ? 0 ? 0 0 0 1 0 1 0 0 1 1 0 0 1
S. ligustrina	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 0 0 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. lydgatei	0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 0 0 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. mannii	0 0 1 0 0 0 0 0 0 1 0 0 1 0 0 1 1 1 1 ? 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. membranacea	0 0 1 0 1 0 0 0 0 0 0 0 0 1 0 0 1 1 1 0 0 0 1 1 1 0 1 1 1 0 0 0 0 1 0 1 0 0 0 1 1 1 0 0 1 1 0 0 1
S. menziesii	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. nuttallii	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 1 1 1 0 0 1
S. pubescens	0 0 1 0 0 0 0 0 0 0 0 ? 1 0 0 1 1 1 1 0 1 1 1 1 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 1 0 1
S. salicaria	0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 0 0 1 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 0 1
S. spergulina	0 0 ? 0 1 0 0 0 0 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 ? 0 0 1 1 0 0 1 0 1 0 1 0 0 0 1 0 1 0 ? 1 1 0 0 1
Silene struthioloides	0 1 1 1 1 0 1 0 1 0 0 1 0 0 0 ? 1 0 1 1 ? 0 1 1 1 1 0 0 1 1 1 0 0 0 0 1 0 1 1 0 0 0 1 0 1 1 0 0 1
M. lateriflora	0 1 1 1 1 0 ? 0 1 1 0 ? 0 0 0 0 1 0 1 1 0 0 1 1 1 1 0 0 1 1 1 0 0 0 0 1 1 1 1 0 0 ? 1 0 1 1 0 0 1