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Hybrid Origin and Genomic Mosaicism of *Dubautia scabra* (Hawaiian Silversword Alliance; Asteraceae, Madiinae)

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Abstract—Incongruence among different estimates of species relationships in plants, from different molecules, cytogenetic data, biogeographic data, morphological/anatomical data or other sources, has been used frequently as an indication of introgression, hybrid species origin, or chloroplast (cp) capture. In plants, these incongruences are most often seen between data derived from the nuclear vs. the cp genomes and the nuclear markers used for comparison usually have been from the nuclear ribosomal (nr) internal transcribed spacer region (ITS). The amount of genomic material shared between introgressing species can be highly variable. In some of these cases, other nuclear genomic regions have moved between species without leaving a signature on the nrITS. An example of well-supported phylogenetic incongruence is the placement of *Dubautia scabra* (DC.) D. D. Keck in the Hawaiian silversword alliance (HSA); evolutionary hypotheses for *D. scabra* based on molecular as opposed to cytogenetic data are strongly discordant. In this paper, we test these two conflicting phylogenetic hypotheses regarding the evolutionary relationships of *Dubautia scabra* using evidence from six low-copy nuclear genes, as well as multiple chloroplast noncoding regions and nrITS. The nrITS region is also examined for the presence of multiple copy types. Incongruence between inferred relationships based on nuclear chromosomal arrangements and molecular phylogenetic data from chloroplast DNA and nrITS is resolved in favor of a hypothesis of ancient hybridization rather than cytogenetic homoplasy involving dysploidy. Most single-copy nuclear genes track histories of *D. scabra* compatible with cytogenetic data whereas chloroplast and nrITS data track a common, different history that appears to reflect hybridization with a chromosomally distinct lineage that also occurs on Maui Nui and Hawai'i (the Big Island).

Keywords—*Dubautia*, genome mosaic, Hawaiian silversword alliance, hybridization, introgression, phylogenetic incongruence.

Incongruence among different estimates of species relationships in plants, from different molecules, cytogenetic data, biogeographic data, morphological/anatomical data or other sources, has been used frequently as an indication of introgression, hybrid species origin, or chloroplast (cp) capture (Rieseberg and Wendel 1993; Mason-Gamer and Kellogg 1996; Maddison 1997). In plants, these differences are most often reported for data derived from the nuclear vs. the cp genomes (Soltis and Kuzoff 1995; Soltis et al. 1996; Baum et al. 1998; Setoguchi and Watanabe 2000; Yoo et al. 2002; Okuyama et al. 2005) and the nuclear markers used for comparison usually have been from the 18S–26S nuclear ribosomal internal transcribed spacer region (nrITS; but see Doyle et al. 2004).

In most plants, the plastid genomes are maternally inherited, while the nuclear genome is biparentally inherited (Corriveau and Coleman 1988; Rieseberg and Soltis 1991). Thus, one might expect that a plant genome resulting from a hybridization event would have maternal chloroplast and mitochondrial genomes, but a nuclear genome with a complement or mosaic of genes from both parental genomes. However, because the nuclear ribosomal (nr) gene family is subject to concerted evolution, it may have different evolutionary fates over time (Wendel 2000; Alvarez and Wendel 2003); both suites of parental copies may be retained, the different sequences may recombine to form chimeric repeats, or only one of the parental copy-types may be maintained (Zimmer et al. 1980; Wendel et al. 1995; Alvarez and Wendel 2003). Thus, if concerted evolution homogenizes nr repeats to a paternal copy-type or if recombination yields nr repeats with a strongly paternal influence, a disparity will arise between phylogenies derived from nr and maternally-inherited chloroplast datasets (Wendel et al. 1995). However, if a maternal nr copy-type is fixed in the genome, then there may be no difference between the chloroplast and nr-derived phylogenies. This could lead to erroneous conclusions in systems where hybridization and/or recombination have been important evolutionary factors (Wendel et al. 1995; Ainouche and Bayer 1997; Alvarez and Wendel 2003).

The amount of genomic material shared between introgressing species can be highly variable (Rieseberg et al. 1995, 1996, 1999). Strictly speaking, in chloroplast capture, only the chloroplast genome is shared between species (Wolfe and Elisens 1995; Van Raamsdonck et al. 1997; Jackson et al. 1999; Kornkven et al. 1999). In some of these cases, other nuclear genomic regions may have been transferred between species, i.e. introgressed, without leaving a signature on the nrITS. Genetic mapping studies have shown that introgression may be highly biased, with significantly more gene flow in one direction than another (Cruzan and Arnold 1994; Petit et al. 2004; Bouck et al. 2005; Martin et al. 2005). Most previous analyses of introgressive hybridization have used only one or two nuclear genic regions, which does not allow for analysis of the extent of mosaicism in the introgressed genome (Wendel et al. 1995; Ainouche and Bayer 1997; Alvarez and Wendel 2003).

An example of well-supported phylogenetic incongruence is the placement of *Dubautia scabra* (DC.) D. D. Keck in the Hawaiian silversword alliance (HSA), where the molecular and cytogenetic data suggest different evolutionary histories. *Dubautia scabra* comprises two subspecies found on Maui and Hawai'i, with *D. scabra* subsp. *leiophylla* endemic to wet forest areas and *D. scabra* subsp. *scabra* on young lava flows. This species is unusual in the HSA because of its white flowers and self-compatible breeding system. *Dubautia scabra* can be

an early successional species and is typically one of the first angiosperms to colonize recent lava flows (Carr 1985).

In both nrITS and chloroplast restriction-site phylogenies, *Dubautia scabra* is placed in a well supported clade that is nested within a deeper clade corresponding to *D.* section *Railliardia* (sensu Carr 1985; Baldwin et al. 1990; Baldwin and Robichaux 1995; Baldwin 1997). Section *Railliardia* is centered on the younger islands in the Hawaiian archipelago and appears to represent a recent adaptive radiation within the HSA (Carr 1985; Carr et al. 1989; Baldwin and Robichaux 1995). In this paper, we will restrict our discussion to a monophyletic subgroup of the section that includes only the Maui Nui (the once contiguous land mass that includes the modern islands of Maui, Molokai, and Lanai) and Hawai'i endemic species, which includes *D. arborea* (A. Gray) D. D. Keck, *D. ciliolata* (DC.) D. D. Keck, *D. linearis* (Gaudich.) D. D. Keck, *D. menziesii* (A. Gray) D. D. Keck, *D. platyphylla* (A. Gray) D. D. Keck, *D. reticulata* (Sherff) D. D. Keck, *D. waianapanapaensis* G. D. Carr, and possibly *D. scabra*.

In many Hawaiian species radiations, including the HSA, the ages of clades often follow the age-structure of the Hawaiian archipelago, with the earliest diverging clades occurring on the oldest island, Kaua'i, and the most highly nested clades occurring on the youngest islands, Maui Nui and Hawai'i (Baldwin and Robichaux 1995; Funk and Wagner 1995; Baldwin 1997). Thus, the placement of *Dubautia scabra* in the clade containing the young-island endemic species within *D.* section *Railliardia* makes biogeographic sense.

However, the cytogenetic data provide a different estimate of relationships. The HSA is allopolyploid, with progenitor lineages from the California Floristic Province that either both had $n = 7$ or had $n = 7$ and $n = 8$ (Carr and Kyhos 1981, 1986; Carr 1985; Barrier et al. 1999). Thus, a chromosome number no lower than $n = 14$ is considered the basal state in the HSA; $n = 14$ is shared by all taxa of the HSA except for *Dubautia* section *Railliardia* exclusive of *D. scabra* (which has $n = 14$). The other species in section *Railliardia* share a derived dysploid condition ($n = 13$) based on an unequal reciprocal (Robertsonian) translocation and loss of a centric fragment (Carr and Kyhos 1981, 1986; Carr 1985; Baldwin et al. 1990; Baldwin and Robichaux 1995), with the exception of *D. scabra*, which has $n = 14$. All $n = 13$ species appear to have the same cytogenetic arrangement, leading to the assumption that they have had a single origin (Carr 1985). In contrast, the $n = 14$ species show considerable cytogenetic variation, with at least six different cytotypes differentiated by reciprocal translocations (Carr and Kyhos 1981, 1986; Carr 1985). Within the $n = 14$ species, *D. scabra* shares a chromosomal arrangement with *D. latifolia* (Carr 2003a), a Kaua'i-endemic species that is placed in an earlier diverging clade within the HSA based on nrITS data (Baldwin and Robichaux 1995; Baldwin and Sanderson 1998).

Thus, there is a conflict in implied species relationships between the cp and nrITS molecular data and biogeographic data on the one hand and the cytogenetic data on the other hand. This conflict could indicate cytogenetic homoplasy, with independent derivations of the same $n = 13$ cytotype or a reversion to the $n = 14$ state and independent origin of the *Dubautia latifolia* cytogenetic arrangement in *D. scabra*. As discussed by Baldwin (1997), such homoplasy in chromosome arrangements would be unlikely, on the one hand, because such structural mutations involve two simultaneous breakages and reunions of chromosome arms (see Carson 1992); on the other hand, the two relevant chromosomes have undergone all three possible whole-arm rearrangements during the history of the HSA, suggesting that the group may be prone to such chromosomal rearrangements (Carr and Kyhos 1986). Dysploid reductions are often, but not always, considered to be irreversible traits, and should thus be excellent as synapomorphies (Bull and Charnov 1985).

Alternatively, a homoploid hybrid origin of *Dubautia scabra* involving a taxon with the *D. latifolia* genome (*Dubautia* Genome 4; Carr and Kyhos 1986) and an $n = 13$ member of *D.* section *Railliardia* could explain the conflicts (Baldwin 1997). As noted above, the difference in chromosome number between *D. scabra* and the other species of section *Railliardia* is the result of a Robertsonian translocation and does not represent a major intrinsic barrier to gene flow; natural and artificial hybrids of that combination have higher than expected (i.e. > 50%) pollen stainability (≈ fertility), as do hybrids between *D. latifolia*, which shares the same genomic arrangement as *D. scabra*, and $n = 13$ *Dubautia* species (Carr and Kyhos 1981, 1986). In his 1985 monograph of the HSA, Carr noted that *D. scabra* appears to be "transitional" between the mesophytic older-island endemic $n = 14$ taxa and the mostly xerophytic young-island endemic $n = 13$ taxa, combining characteristics of both groups.

Thus, despite congruence between the nrITS and chloroplast phylogenies, there are two strongly supported inferences of species relationship for *Dubautia scabra* (the chloroplast and nrITS data on the one hand, and the cytogenetic data on the other) and these hypotheses of relationship are mutually exclusive unless cytogenetic homoplasy or hybridization are invoked to reconcile them.

In this paper, we tested these two conflicting phylogenetic hypotheses regarding the evolutionary relationships and history of *Dubautia scabra*. We used phylogenetic evidence from multiple single-copy nuclear genes, as well as multiple chloroplast noncoding regions and nrITS to test these conflicting hypotheses (Fig. 1). The nrITS region was also examined for the presence of multiple copy types. We then used the results to generate a scenario for the evolution of *D. scabra*.

Materials and Methods

***Taxa Examined*—**In order to address the conflicting hypotheses of the placement of *Dubautia scabra* relative to the rest of *D.* section *Railliardia*, a total of 13 samples representing ten species from major clades of sect. *Railliardia* were sampled, including three samples of *D. scabra* representing both subspecies. All species of section *Railliardia* from Maui and Hawai'i were sampled. One sample of *D. laxa* subsp. *hirsuta* was used as an exemplar of the older-island species. Samples used and their provenance are listed in Table 1. Two samples from the genus *Argyroxiphium* were used as the outgroup for all analyses, based on previous phylogenetic analyses (Baldwin et al. 1990; Baldwin and Robichaux 1995; Baldwin 1997).

***DNA Extraction and Amplification*—**DNAs were extracted using either the large prep or the Nucleon Phytopure (GE Biosciences/Amersham, Piscataway, New Jersey) protocol, both as described in Friar (2005).

All nuclear single-copy gene-specific primers used in this study were developed specifically for *Dubautia* species and their close relatives, including the rest of the HSA and the California tarweeds. As the HSA is allotetraploid, each nuclear gene is represented by at least two loci. *Asap*1 and *Asap*3 were studied previously; both loci have A and B copies in the HSA (Barrier et al. 1999; Lawton-Rauh et al. 2003). Both homoeologues of *Asap*3, *Asap*3A and *Asap*3B, were used, but only *Asap*1A was included in this study because of limited variation at the *Asap*1B locus. The *Adh* loci (*Dadh*, for *Dubautia Adh*) were developed for this project. Three loci (A, B, and C) were found (Friar et al. in prep), but only *Dadh*A and *Dadh*B were used in this study because of limited sampling at the *Dadh*C locus. Copy specific primers for each single-copy nuclear locus (*Dadh*A, *Dadh*B, *Asap*1A, *Asap*3A, and *Asap*3B) were used. General-use plastid and nrITS

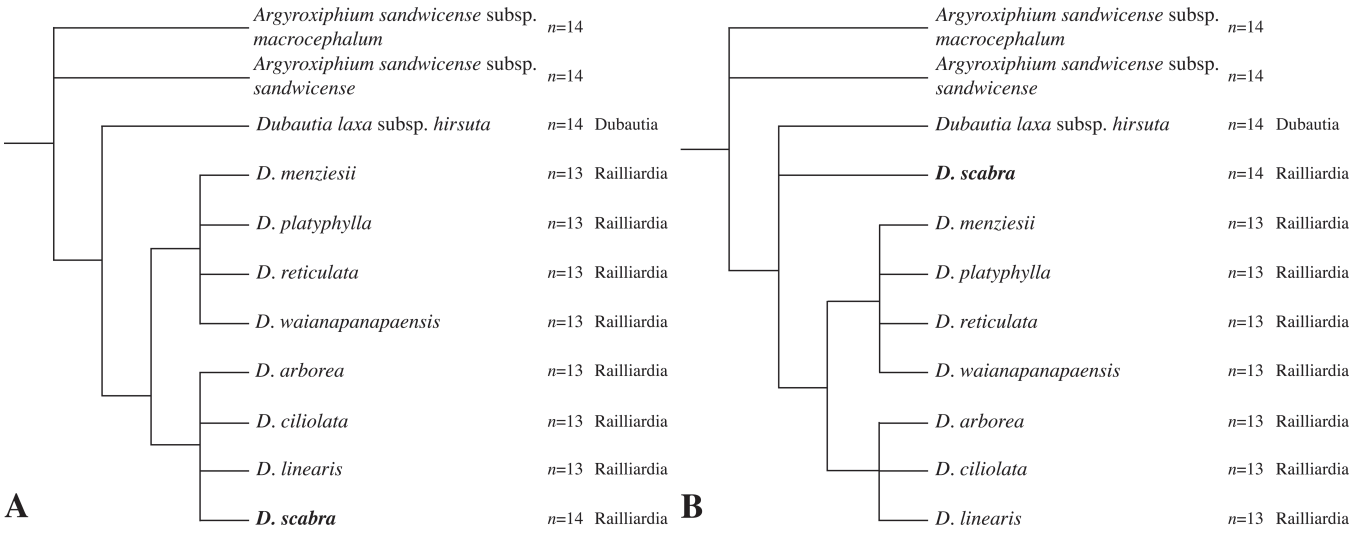

FIG. 1. Constraint trees used for tests of phylogenetic topology. Chromosome number is indicated for all species. For species of *Dubautia*, their sectional placement is also indicated. (A) Constraint tree 1, with *Dubautia scabra* placed within the Maui Nui–Big Island clade of *D*. section *Railliardia*, nested among young-island endemic species with $n = 13$. (B) Constraint tree 2, with *D. scabra* placed outside a clade containing the sampled $n = 13$ species of *D*. section *Railliardia*.

primers were synthesized from descriptions in the literature. All primers used and their sources are listed in Table 2.

Amplifications were performed with an annealing temperature of 56–64° in 10 μL reactions. *Pfu* DNA polymerase (Stratagene, La Jolla, California) was used to minimize polymerase error (summarized at http://micro.nwfsc.noaa.gov/protocols/taq-errors.html) with an ammonium sulfate PCR buffer for single-copy nuclear gene regions. Some reactions also included up to 5% DMSO, which improved product yield and specificity. PCR products were cleaned using an abbreviated version of the PEG-precipitation protocol (Johnson and Soltis 1995). For cloned loci, amplicons were cloned using the Zero-Blunt Topo TA Kit (Invitrogen Life Technologies, Carlsbad, California). Minipreps of liquid cultures were cleaned using Eppendorf Plasmid MiniPrep Kits (Westbury, New York), quantified using a Biospec-1601 spectrophotometer (Shimadzu Biotech, Columbia, Maryland), and cycle-sequenced using 1/8 concentration ABI BigDye III (Applied Biosystems, Inc., Foster City, California) on PTC-100 thermalcyclers (MJ Research Inc., Reno, Nevada). A large number of internal primers were used in addition to M13F and M13R primers for sequencing, as indicated in Table 2. Samples were visualized on an ABI 3100 platform (Applied Biosystems, Inc.) at Rancho Santa Ana Botanic Garden (Claremont, California).

***Sequence Alignment and Analysis*—**Vector fragments and primer regions were trimmed and sequences were edited in Sequencher v. 4.2.1 (Gene Codes Corp., Ann Arbor, Michigan). Edited sequences were aligned manually using Se-Al v. 2.0a11 (Rambaut 2001) and exported into a large, concatenated (interleaved) NEXUS file for analysis in PAUP*4.0b10 (as below) (Swofford 2002). Ambiguously aligned regions (including microsatellites) were identified in the *rps16*, *Asap1*A, *Asap3*A, and *Asap3*B matrices, and were excluded from all analyses. Gaps, present in the *psbA–trnH* IGS, *rps16*, *Dadh*A, *Dadh*B, *Asap1*A, *Asap3*A, and *Asap3*B matrices were coded separately as multistate characters at the end of the concatenated matrix. GenBank accession numbers for each dataset are as follows *Dadh*A EU349217–EU349229; *Dadh*B EU341838–EU341852; *Asap1*A EU341929–EU341944; *Asap3*A EU341853–EU341866; *Asap3*B EU341867–EU341876; nrITS EU341945–EU341967; *psbA–trnH* IGS EU341877–EU341889; *rpl16* intron EU341890–EU341902; *rps16* intron EU341903–EU241915; *trnK–matK* 5′ IGS EU341916–EU341928. Aligned datasets and resulting phylogenies have been submitted to TreeBASE (study number S1959).

Each single-copy nuclear dataset (*Dadh*A, *Dadh*B, *Asap1*A, *Asap3*A, *Asap3*B), the nrITS matrix, and the concatenated chloroplast dataset were analyzed independently. Two different nrITS datasets were used. The first was direct-sequenced nrITS from the species sampled in this study (the "species" nrITS dataset). The second included cloned copies of nrITS from the three samples of *Dubautia scabra* (the "clone" dataset). The optimal model of evolution for each was determined using Modeltest version 3.7 (Posada and Crandall 1998). Gene trees were generated using the optimal model of evolution for each gene using Maximum Likelihood analysis as implemented in PAUP* 4.0b10 (Swofford 2002), employing TBR branch-swapping, steepest descent with 100 random-addition sequences, and holding 10 trees per step. Statistical support for each node was assessed using likelihood bootstrapping with 100 bootstrap pseudoreplicates, each with 10 random-addition sequences and holding 10 trees per step. Significant differences between each gene tree and two different constraint trees were assessed using Shimodaira-Hasegawa (SH) tests using full optimization and 1,000 bootstrap pseudoreplicates (Shimodaira and Hasegawa 1999). The constraint trees are shown in Fig. 1. Constraint tree 1 represents the hypothesis of *D. scabra* being placed within the Maui Nui-Hawai'i clade, nested among species of *D*. section *Railliardia* with $n = 13$, as in the nr and chloroplast DNA trees. Constraint tree 2 represents the hypothesis that *D. scabra* should be placed outside the section *Railliardia* clade comprised of all $n = 13$ species. To evaluate the role of the different accessions of *D. scabra*, each accession was deleted individually, and the phylogenetic analyses rerun. This was done for each dataset separately.

RESULTS

Number of aligned nucleotides, number of variable sites, and the model chosen for likelihood analyses for each dataset are shown in Table 3. Maximum-likelihood (ML) trees for the single-copy nuclear and chloroplast datasets are shown in Fig. 2, with likelihood bootstrap support shown over each node. ML trees for the "species" nrITS and cloned nrITS datasets are shown in Fig. 3. Likelihood values for the ML tree and the two constraint trees for each dataset are listed in Table 3.

All five single-copy nuclear gene trees (Fig. 2) showed significant differences in tree topology from constraint tree 1 (Fig. 1). Three of the single-copy nuclear gene trees (*Dadh*B, *Asap*1A, and *Asap*3A), as well as the chloroplast gene tree, also displayed significant topological differences from constraint tree 2. It appears that the three datasets (*Dadh*B, *Asap*1A, and *Asap*3A) that produce topologies that differ significantly from both constraint trees do so, at least in part, because the three sampled populations of *Dubautia scabra* show different affinities. In the *Dadh*B tree, the samples from the *D. scabra* OL and PH populations group together in a clade with the rest of section *Railliardia*, while *D. scabra* HV is placed at the base of the tree with the outgroups.

TABLE 1. Primers Used to Sequence *Dubautia scabra* and Allied Species

Locus and Primer name	Application	Primer sequence (5′ to 3′)	Reference
Nuclear regions			
Dadh			
*Dadh*A-95F	PCR and sequencing	GCTTTAACAAGAATCCGCATTCG	This paper
*Dadh*B-86F	PCR and sequencing	AAGTTTTCGACTTTACAAGAATCCG	This paper
*Dadh*C-61F	PCR and sequencing	GTTTAGTAGCTGTTGGCCTTGCA	This paper
Dadh-E10R	PCR and sequencing	CAAAGGGCTTATTGATCTCTGAAAA	This paper
Dadh-568F	Sequencing	CAGGGGTATTATTGGTCTTGT	This paper
Dadh-568R	Sequencing	ACAAGACCAATAATACCCCTG	This paper
Dadh-IF	Sequencing	TGATCTCTGCCTTTGAATGTG	This paper
Dadh-IR	Sequencing	CCAGACCCCAACCCTACAAA	This paper
Asap1A			
Asap1-F	PCR and sequencing	GGTGCAGTGGGTATCTTTTAATTC	This paper
Asap1-R	PCR and sequencing	ATCGGCTGCAGACTCAGGTC	Lawton-Rauh et al. 1999
Asap1-500F	Sequencing	TTGCGTGTTATYGTTTTTGC	This paper
Asap1-502Ra	Sequencing	TAGCAAAAACAATAAKACGC	This paper
Asap1-993F	Sequencing	AACGGGATTACACACGAAGG	This paper
Asap1-996Ra	Sequencing	AAACCTTCGTGTGTAWTCYY	This paper
Asap1-1256R	Sequencing	AGTAAAAAGCCACAATGACG	This paper
Asap1-1258F	Sequencing	TCATTGTGGCTTTTTACTCG	This paper
Asap3			
Asap3-F	PCR and sequencing	TACAAACAGGCAGGTGACATACTC	Lawton-Rauh et al. 1999
*Asap3*A-1067R	PCR and sequencing	TTGCCTGCAAACCCATTCARGTT	This paper
*Asap3*B-1284R	PCR and sequencing	GCACACCCATTCAAATTAAGTTTATCA	This paper
Asap3-335F	Sequencing	TCTCTGGACCTCCCACTATGAGG	Lawton-Rauh et al. 1999
Asap3-335R	Sequencing	CCTCATAGTGGGAGGTCCAGAGA	Lawton-Rauh et al. 1999
Asap3-630F	Sequencing	TGGTTAATTCGTTAATAGCAAC	Lawton-Rauh et al. 1999
Asap3-630R	Sequencing	GTTGCTATTAACGAATTAACCA	Lawton-Rauh et al. 1999
Asap3-1000F	Sequencing	GCTTTAACATTTTATACATAAT	Lawton-Rauh et al. 1999
Asap3-1000R	Sequencing	ATTATGTATAAAATGTTAAAGC	Lawton-Rauh et al. 1999
nrITS			
241R	PCR and Sequencing	CAGTGCCTCGTGGTGGCGACA	Prince and Kress 2006
5a	PCR and sequencing	TTATCATTTAGAGGAAGGAGAAGTCG	Prince and Kress 2006
5.8S-F	Sequencing	TCACGGCAACGGATATCTCGG	Prince and Kress 2006
5.8S-R	Sequencing	ACGGGATTCTGCAATTCACAC	Prince and Kress 2006
Chloroplast regions			
psbA–trnH IGS			
psbA-F	PCR and sequencing	GTTATCGATGAACGTAATGCTC	Modified from Sang et al. 1997
trnH-R	PCR and sequencing	CGCGCATGGTGGATTCACAATC	Modified from Prince and Kress 2006
rpl16 intron			
rpl16-F	PCR and sequencing	GGAGTATTAGGAGTTAAAATTTGG	Prince and Kress, 2006
rpl16-1516R	PCR and sequencing	CCCTTCATTCTTCCTCTATGTTG	Kelchner and Clark 1997
rpl16-IR-Z	Sequencing	ATTAATGGAGAAGCTATGGG	Prince and Kress 2006
rpl16-543F	Sequencing	TCAAGAAGCGATGGGAACGATGG	Butterworth et al. 2002
rps16 intron			
*rps*F	PCR and sequencing	GTGGTAGAAAGCAACGTGCGACTT	Oxelman et al. 1997
*rps*R	PCR and sequencing	TCGGGATCGAACATCAATTGCAAC	Oxelman et al. 1997
*rps*16-DubIF	Sequencing	GAAGTAATGTCTAAACCCAA	This paper
*rps*16-DubIR	Sequencing	CCCTTTTTCGTCTCGTTTAA	This paper
trnK–*mat*K 5′ IGS			
*trnK*1F	PCR and sequencing	CTCAACGGTAGAGTACTCG	Steele and Vilgalys 1994
Dub5′ IGS-1R	PCR and sequencing	GAAAACGAACATAAATACT	This paper
Dub5′ IGS-2R	Sequencing	CAGTCAAAACAAGGTATTC	This paper
Dub5′ IGS-1F	Sequencing	GAAAACGAACATAAATACT	This paper

In the *Asap*1A tree, *D. scabra* PH groups with *D. laxa* subsp. *hirsuta*, while *D. scabra* HV and *D. scabra* OL are placed sister to the rest of section *Railliardia*. In the *Asap*3A tree, *D. scabra* HV and OL form a clade in the multifurcation at the base of the tree, while *D. scabra* PH groups with some of the members of section *Railliardia*, though the base of this tree is largely unresolved (and the displaced position of *D. reticulata* and *D. waianapanapaensis* relative to the other $n = 13$ taxa contributes to the incongruence with constraint tree 2). The chloroplast (Fig. 2F) and nrITS (Fig. 3A) gene trees are congruent with constraint tree 1 (*D. scabra* placed within the Maui Nui–Hawai'i clade of section *Railliardia*). Sequential deletion of the different accessions of *D. scabra* did not change the results appreciably, though it generally reduced the resolution of the trees to some extent.

For the nrITS "clone" gene tree, 20 clones were sequenced from each of the three samples of *D. scabra* to determine if multiple repeat-types are maintained in the genome. An ML tree incorporating the cloned sequences is presented in Fig. 3. Identical sequences were removed from the analysis. All clones from *D. scabra* group together in the same clade with *D. arborea*, *D. ciliolata*, and *D. linearis*, just as the direct sequenced samples in the "species" tree do. Thus, there is no evidence that multiple nrITS copies inherited through hybridization between different lineages of the HSA are being maintained in the *D. scabra* genome.

TABLE 2. Length, number of variable and phylogenetically informative sites, and likelihood model chosen for each locus

Locus	Aligned length	Number of variable sites	Number of phylogenetically informative sites	Likelihood model chosen
*Dadh*A	790	22	4	F81
*Dadh*B	781	15	6	F81
*Asap*1A	1763	80	48	TVM+G
*Asap*3A	1063	26	13	HKY
*Asap*3B	1389	33	15	F81uF+I
nrITS	644	32	17	K80
Chloroplast regions	3215	25	18	GTR+I

DISCUSSION

In many cases, phylogenetic incongruence has been considered to be a sign of failure of one of the datasets to reflect evolutionary history, leading to an oversimplified "gene tree vs. species tree" perspective (Maddison 1997). However, it has become increasingly clear that differences in phylogenetic reconstructions may result from differences in evolutionary history for different genomes or genes (Mason-Gamer and Kellogg 1996; Rieseberg et al. 1996; Van Raamsdonck et al. 1997; Baum et al. 1998; Jackson et al. 1999; Martinsen et al. 2001; Chan and Levin 2005). Allelic sorting in the single-copy nuclear genes may account for some of the detected incongruence; e.g. in the single-copy nuclear gene trees that did not resolve *D. scabra* alleles as monophyletic, some other topological disparities with cytogenetic, nrITS, and/or chloroplast results are evident. However, strong support for a sister-group relationship between *D. scabra* and a clade comprising all $n = 13$ *Railliardia* taxa was obtained in a nuclear gene tree (*Asap3*B; Fig. 2E) that resolved all alleles of *D. scabra* as monophyletic and is wholly congruent with cytogenetic data (and with ITS and chloroplast trees except for the position of *D. scabra*). Although differences in topology between chloroplast and nrITS trees may represent a history of hybridization or hybrid origin in plants (see above), comparisons among multiple single-copy nuclear gene trees can provide more lines of evidence for examining complex histories, including instances of lineage sorting or hybrid speciation in insular lineages (Howarth and Baum 2005; Pollard et al. 2006).

In this paper, we tested phylogenies generated from multiple single-copy nuclear genes, over 3kb of chloroplast DNA sequence, and nrITS, against two hypotheses of relationship for *Dubautia scabra*. Both hypotheses have been strongly supported by different, but equally convincing datasets in the past. The nrITS and chloroplast datasets, along with biogeographic inferences, support a placement of *D. scabra* within the Maui Nui–Big Island clade of *D.* section *Railliardia*, nested among the $n = 13$ species, with either the $n = 14$ state or $n = 13$ state being homoplastic. The cytogenetic data, on the other hand, support the placement of *D. scabra* outside a clade including all $n = 13$ species.

TABLE 3. Likelihood values for each gene tree for the most likely tree and both constraint trees. [1]Shimodaira-Hasegawa test results in parentheses.

Locus	Maximum Likelihood Tree	Constraint tree 1[1]	Constraint tree 2[1]
*Dadh*A	1229.60	1275.88 (p = 0.039)*	1247.64 (p = 0.062)
*Dadh*B	1184.10	1236.68 (p = 0.021)*	1229.56 (p = 0.036)*
*Asap*1A	2981.17	3148.81 (p < 0.000)*	3116.10 (p < 0.000)*
*Asap*3A	1600.78	1772.99 (p = 0.003)*	1707.72 (p = 0.002)*
*Asap*3B	1965.33	2136.92 (p < 0.000)*	1984.23 (p = 0.052)
nrITS	1098.85	1098.85 (p = 1.000)	1122.46 (p = 0.040)*
Chloroplast regions	4373.77	4386.96 (p = 0.162)	4454.07 (p = 0.001)*

We found that both hypotheses are supported to greater or lesser degrees in the various datasets. The chloroplast and nrITS phylogenies presented here are concordant with those published previously (Baldwin et al. 1990; Baldwin and Robichaux 1995; Baldwin 1997). However, the single-copy nuclear genes show a variety of placements for the different populations of *Dubautia scabra*, though none are consistent with *D. scabra* as a whole nested among $n = 13$ species in *D.* section *Railliardia*. The genome of *D. scabra* therefore appears to be a mosaic of at least two genomes; one of an $n = 13$ member of section *Railliardia*, and another of an $n = 14$ *Dubautia* lineage. The actual hybridizing taxa may not be extant species, but rather ancestors of current species, and there may have been multiple hybridization events, potentially with different species, that gave rise to *D. scabra*.

The single-copy nuclear gene trees thus provide molecular evidence for the hypothesis that hybridization rather than cytogenetic homoplasy involving dysploidy accounts for the previously detected incongruities between relationships of *Dubautia scabra* inferred from nuclear chromosomal arrangements (Carr and Kyhos 1986; Carr 2003a) as opposed to nrITS and chloroplast DNA (Baldwin et al. 1990; Baldwin and Robichaux 1995). As discussed by Baldwin (1997), recent introgressive hybridization with the Maui Nui–Big Island clade of *D.* section *Railliardia* is unlikely to account for these incongruities because nrITS sequences of *D. scabra* from both Maui Nui and the Hawai'i constitute a clade within the $n = 13$ group (see Fig. 3) and cpDNA sequences are similarly nested among $n = 13$ species (Fig. 3F); in other words, subspecific divergence and dispersal of *D. scabra* between Maui Nui and the Big Island must have postdated the inferred hybridization. Given that *D. scabra* has been documented as a parent in more instances of natural hybridization, both in terms of different species combinations and actual events, than any other member of the HSA (Carr 1985; 2003b), the degree of cytoplasmic and nrITS uniformity in that species is surprisingly high.

As noted by Howarth and Baum (2005), the traditional distinction between introgression and hybrid speciation is often unclear at the homoploid level. In *Dubautia scabra*, pervasive genetic contribution from $n = 13$ ancestors in nrITS and chloroplast DNA and perhaps morphological traits shared with other members of *D.* section *Railliardia* (e.g. pappus plumosity) combined with an $n = 14$ genomic arrangement and single-copy nuclear genes not belonging to the $n = 13$ species is consistent with either extensive ancient introgression or homoploid hybrid speciation.

As in many reported cases of chloroplast capture (Rieseberg and Soltis 1991; Wolfe and Elisens 1995; Rieseberg et al. 1996; Soltis et al. 1996; Baum et al. 1998; Jackson et al. 1999; Yoo et al. 2002; Okuyama et al. 2005), in this study, the chloroplast genome appears to have been incorporated into *Dubautia scabra* from its young-island relatives more readily than the nuclear single-copy genes; the chloroplast genes together uniformly support the placement of *D. scabra* with the young-island species, while the single-copy nuclear genes retain some of the contrasting signal in some populations

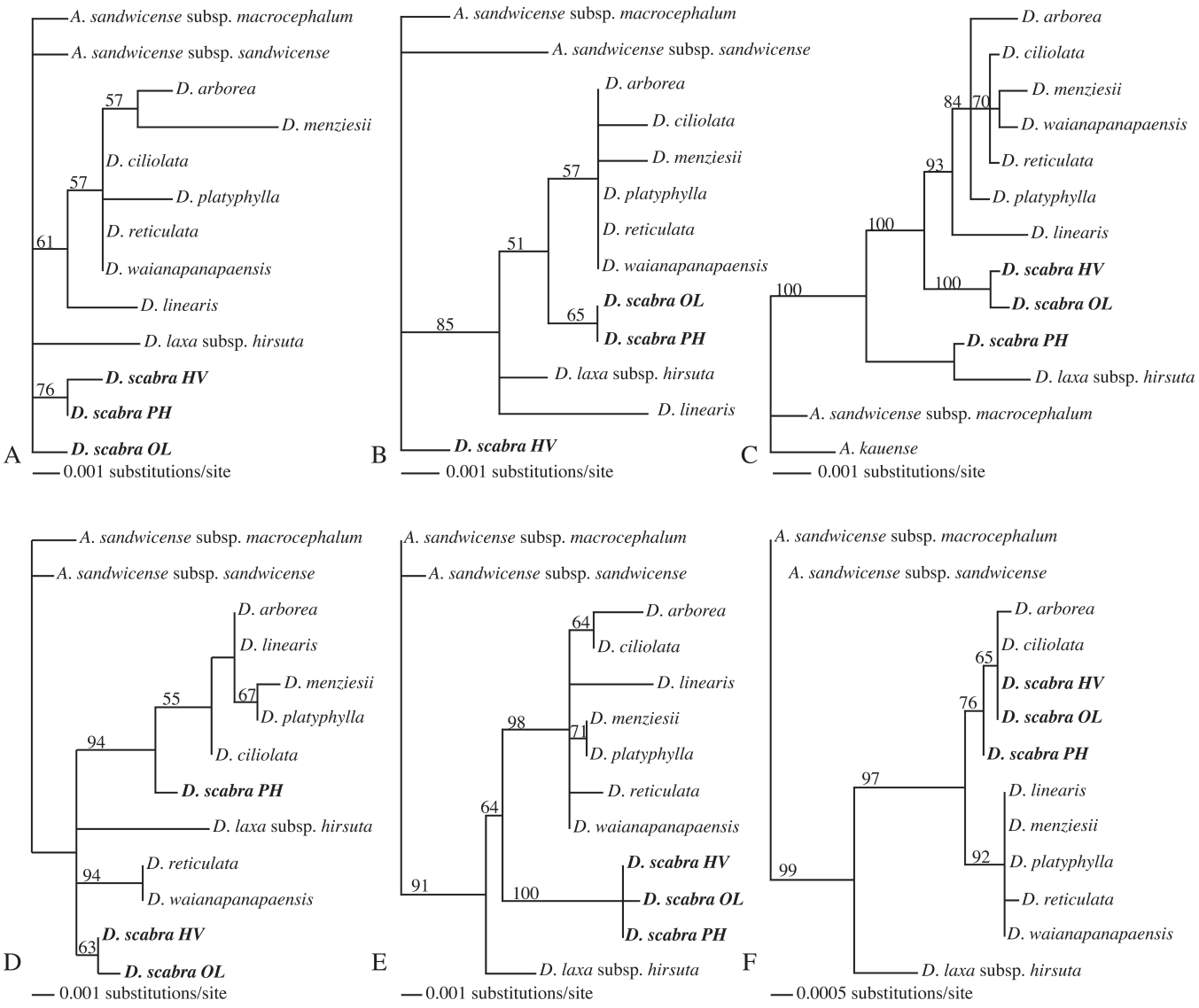

FIG. 2. Maximum likelihood phylogenies for five nuclear genes and a concatenated chloroplast dataset. Numbers above nodes represent likelihood bootstrap support. (A) *Dadh*A, (B) *Dadh*B, (C) *Asap1*A, (D) *Asap3*A, (E) *Asap3*B, (F) concatenated chloroplast regions.

(e.g. *Asap1*A, *Asap1*B; Fig. 1). At least three hypotheses have been proposed to explain the ease of cytoplasmic capture through hybridization compared to such incorporation of nuclear markers; the selection-linkage hypothesis, the minority hypothesis, and cytonuclear incompatibilities (Martinsen et al. 2001; Tsitrone et al. 2003; Chan and Levin 2005). The selection-linkage hypothesis postulates that "speciation genes" reside primarily on the nuclear chromosomes, which therefore have a higher potential for deleterious phenotypic consequences in recombinant or alien genetic backgrounds compared to the cytoplasmic genomes (Martinsen et al. 2001; Funk and Omland 2003). By contrast, the "minority hypothesis" suggests that female flowers of the less abundant taxon are more likely to be pollinated with majority pollen, potentially resulting in the replacement of nuclear genes in the minority taxon by those of the more abundant taxon, while retaining the original (minority-taxon) cytoplasmic background (Rieseberg et al. 1996). This process has been documented dramatically in European oaks (Petit et al. 2004). The cytonuclear incompatibility model, proposed by Tsitrone et al. (2003) suggested that chloroplast capture may result from cytonuclear incompatibilities between the nuclear and chloroplast genomes. As noted above, the *D. scabra* case is not one of strict chloroplast capture; nrDNA (especially) and some of the single-copy nuclear genes (i.e. *Asap1*A and perhaps *Dadh*B) also show evidence of ancestry from the younger-island $n = 13$ *Dubautia* species.

Although the origin of *Dubautia scabra* through homoploid hybrid speciation is highly plausible, given the above evidence, exploration of possible introgression scenarios is warranted. Several aspects of the biology of modern *D. scabra* could either facilitate or prevent the acquisition of genes or chloroplast genomes through hybridization depending upon the model of introgression. First, *D. scabra* can be an early successional species, and the first angiosperm to colonize new lava flows on Hawai'i (Carr 1985). Also, *D. scabra* is one of the few self-compatible species known in the HSA (Carr 1985; Carr et al. 1986), perhaps as a result of selection for its colonizing ability in new habitats (Jain 1976; Lloyd 1992). Partial selfing can theoretically increase the rate and probability of chloroplast capture under a model of cytonuclear

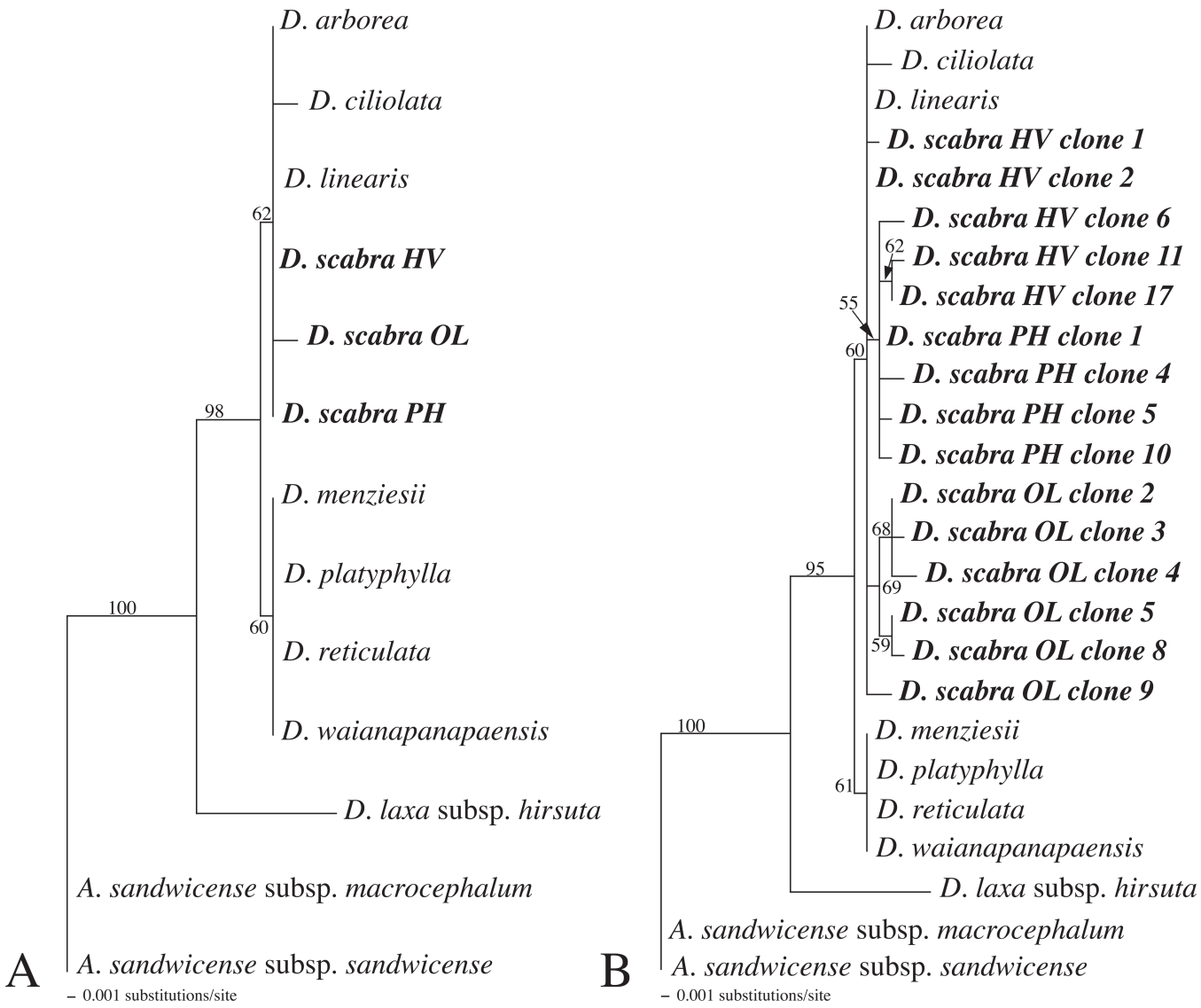

FIG. 3. Maximum likelihood phylogenies of the nrITS region in *Dubautia*. Numbers above nodes represent likelihood bootstrap support. (A) Phylogeny from direct sequences of PCR products, (B) phylogeny derived from multiple cloned copies of nrITS for *D. scabra*.

interaction, assuming female fitness is thereby elevated (Tsitrone et al. 2003).

The same biological attributes of *Dubautia scabra* may mitigate against capture of a chloroplast genome from an $n = 13$ species of the Maui Nui–Big Island clade of *D.* section *Railliardia* under the "minority" hypothesis. Given that *D. scabra* is invasive, one would expect that it would frequently be the minority taxon in hybrid zones with other species, and that it would therefore be the maternal parent of hybrids, providing the chloroplast genome. This study found the opposite pattern, in which the chloroplast genome of other *Railliardia* clade species has been incorporated into *D. scabra*. From an alternative perspective, *D. scabra*, though it invades new lava habitats, usually occurs in fairly dense populations, and thus may not remain the minority taxon for long following a dispersal event. Being self-compatible, it does not require pollination to reproduce and might therefore resist being swamped by nuclear genes from other taxa. In contrast, the other species of section *Railliardia* are mostly self-incompatible, and thus, under pollen-limiting conditions, may be more likely to set seed from interspecific crosses and consequently be subject to displacement of most nuclear genes by a species such as *D. scabra*.

Presence of nrITS repeats of the Maui Nui–Big Island $n = 13$ *Railliardia* clade in *Dubautia scabra* could well be the result of rapid fixation to a maternal ($n = 13$) copy type in the same hybridization episode that led to incorporation of the $n = 13$ *Railliardia* chloroplast in *D. scabra*. The loss of one parental repeat type in otherwise additive hybrids or allopolyploids has been documented in a wide variety of systems (reviewed in Alvarez and Wendel 2003) and has been observed experimentally within a few generations following initial hybridization between species of *Armeria* (Fuertes Aquilar et al. 1999). Lack of evidence of nrITS recombination or multiple copy types attributable to allopolyploidy of the HSA (Baldwin and Sanderson 1998; Barrier et al. 1999) may provide other evidence of rapid, unidirectional concerted evolution of nrITS sequences in the same Hawaiian lineage.

Given these findings, it seems likely that a progenitor of *Dubautia scabra* arose on Kauai, where the only other species known to share the same chromosomal arrangement (*D. latifolia*) is endemic. As is common in a wide diversity of Ha-

waiian lineages (Funk and Wagner 1995), including the HSA (Baldwin and Robichaux 1995), the same ancestor of *D. scabra* probably underwent interisland dispersal to the younger islands, where it was involved in hybridization that resulted in modern *D. scabra*, prior to widespread dispersal of *D. scabra* across Maui Nui and Hawai'i. Whether the ancient hybridization responsible for genomic mosaicism in *D. scabra* had major or subtle phenotypic effects remains uncertain; however, long-recognized, shared morphological attributes between *D. scabra* and $n = 13$ young-island species, which together constitute section *Railliardia*, may well reflect that history of hybridization. Genomic dissection of *D. scabra* in comparison with other species of the HSA should provide further insights into the role of hybridization during evolutionary radiation of these fascinating plants.

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APPENDIX 1. Samples of *Dubautia* and *Argyroxiphium* used in this study and their source, voucher, and herbarium housing the voucher. BISH, Bishop Museum; HAW, University of Hawai'i, Manoa; RSA, Rancho Santa Ana Botanic Garden. Note: samples marked with '†' indicate that we do not have collecting permits for flowering material from these rare taxa.

Argyroxiphium sandwicense DC. subsp. *macrocephalum* (A. Gray) Meyrat, Haleakala Crater, Maui, *Meyrat et al. MK21* (HAW)†, *Argyroxiphium sandwicense* DC. subsp. *sandwicense,* Mauna Kea, Hawai'i, *Meyrat et al. SL1* (HAW)†, *Dubautia arborea* (A. Gray) D. D. Keck, Mauna Kea, Hawai'i, *Columbus 4001* (RSA), *D. ciliolata* (DC.) D. D. Keck subsp. *ciliolata,* Mauna Kea, Hawai'i, *Columbus 3998* (RSA), *D. ciliolata* (DC.) D. D. Keck subsp. *glutinosa* G. D. Carr, Kilauea, Hawai'i, *Columbus 4005* (RSA), *D. laxa* Hook. & Arn. subsp. *hirsuta* (Hillebr.) G. D. Carr, O'ahu, *McGlaughlin 1* (RSA), *D. linearis* (Gaudich.) D. D. Keck subsp. *linearis,* Haleakala, Maui, *McGlaughlin 10* (RSA), *D. menziesii* (A. Gray) D. D. Keck, Haleakala, Maui, *McGlaughlin 12* (RSA), *D. platyphylla* (A. Gray) D. D. Keck, Haleakala, Maui, *McGlaughlin 13* (RSA), *D. reticulata* (Sherff) D. D. Keck, Haleakala, Maui, *McGlaughlin 11* (RSA), *D. scabra* (DC.) D. D. Keck subsp. *leiophylla* (A. Gray) G. D. Carr OL, Haleakala, Maui, *Columbus 4683* (RSA), *D. scabra* (DC.) D. D. Keck subsp. *scabra* HV, Kilauea, Hawai'i, *Columbus 3998* (RSA), *D. scabra* (DC.) D. D. Keck subsp. *scabra* PH, Mauna Kea, Hawai'i, *Columbus 4004* (RSA), *D. waianapanapaensis* G. D. Carr (formerly treated as *D. dolosa* O. Deg. & Sherff) G. D. Carr, Haleakala, Maui, *Carr et al. 1017* (BISH)†.