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GENEALOGICAL EVIDENCE OF HOMOPLOID HYBRID SPECIATION IN AN ADAPTIVE RADIATION OF *SCAEVOLA* (GOODENIACEAE) IN THE HAWAIIAN ISLANDS

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Abstract.—Although homoploid hybrid speciation is increasingly recognized as an important phenomenon in plant evolution, its role in adaptive radiations is poorly documented. We studied a clade of seven extant species of *Scaevola* that are endemic to the Hawaiian Islands and show substantial ecological and morphological diversity. We estimated the genealogies for alleles isolated from multiple accessions of each species at four nuclear loci: the ITS region, and the introns of three nuclear genes, *LEAFY* (*LFY*), *NITRATE REDUCTASE* (*NIA*), and *GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE* (*G3PDH*). For five of the seven species, there was complete concordance among the genealogies estimated from the four loci and, when all four regions were combined, the relationships among these five species were fully resolved. Inclusion of alleles from the remaining two species, *S. procera* and *S. kilaueae*, resulted in incongruence among loci, which appears to reflect a history of hybridization. Based on the distribution of alleles, we infer that *S. procera* is the result of a homoploid hybrid speciation event between *S. gaudichaudii* and *S. mollis* and that *S. kilaueae* is probably the result of hybrid speciation between *S. coriacea* and *S. chamissoniana*. In each case the inferred hybridization is consistent with morphological, ecological, and geographic information. We conclude that homoploid hybrid speciation may be more common than is perceived and may play a role in generating novel combinations of adaptive traits that arise during island radiations.

Key words.—Adaptive radiation, Hawaiian Islands, homoploid, hybridization, nuclear introns, *Scaevola*, speciation.

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Hybridization has been argued to have diverse evolutionary effects such as loss of species diversity (Seehausen et al. 1997), formation of stable clines at species overlaps (Barton and Hewitt 1985), and the reinforcement of species boundaries (Noor 1995). Additionally, hybridization can lead to novel hybrid species (Harrison 1990; Arnold 1997; Rieseberg 1997). Hybridization has long been considered to play an important role in plant evolution (Kerner 1894–1895; Lotsy 1916; Anderson 1949; Heiser 1949; Stebbins 1950, 1959; Baker 1951), although the study of hybrid speciation has historically focused on allopolyploidy as a mechanism for generating new lineages that are reproductively isolated from their parental species (Stebbins 1959; Lewis 1979; Grant 1981). The emphasis on allopolyploidy reflects, among other things, the relative ease with which allopolyploid hybrid species can be identified based on chromosomal data. In contrast, homoploid hybrid species are not easily detected without detailed molecular analysis. Thus, it is only recently that homoploid hybrid speciation has received much attention, with a small but increasing number of documented cases (see Rieseberg 1997). It has been shown that homoploid hybrid species can acquire novel combinations of traits, and sometimes also manifest new, ''transgressive'' phenotypes (McDade 1990; Schwarzback et al. 2001), and that, in consequence, they may be able to invade different habitats from those occupied by either parent (Rieseberg et al. 2003). This interpretation suggests a possible role of homoploid hybridization in island radiations, which frequently entail the rapid diversification of species into new habitats without the formation of intrinsic postmating reproductive isolation (Givnish and Sytsma 1997) or extensive polyploidy (Kyhos and Carr 1994).

The Hawaiian Archipelago is a classic system for the study of speciation and adaptive radiation given its density of vastly different habitats in close proximity and the opportunity to increase variation rapidly within bottlenecked dispersers (Carlquist 1974, 1980; Carson and Templeton 1984; Wagner and Funk 1995). The Hawaiian flora is characterized by a high degree of obligate outcrossing (including the highest incidence of dioecy of any flora) and a lack of postzygotic isolating mechanisms (Carr 1998; Sakai et al. 1995). However, Hawaiian radiations generally show a lack of accumulated molecular sequence variation, making it difficult to reconstruct the phylogenetic relationships with commonly used markers (Carlquist 1980; Baldwin and Robichaux 1995; Givnish et al. 1995; Soltis et al. 1996; Baldwin et al. 1998; Ganders et al. 2000). Given a possible lack of genetic isolating mechanisms coupled with a patchwork of divergent novel habitats, how common is hybrid speciation in the Hawaiian flora? We provide a model to answer this question in an endemic diploid clade of Hawaiian *Scaevola* (Goodeniaceae), a group of interfertile (Gillett 1966; 1972), diploid species ($n = 8$; Carr 1998), in which all the species are found in different habitat types.

There are 10 species of *Scaevola* in the Hawaiian Archipelago (Patterson 1990; Wagner 1996), derived from three separate dispersal events (Howarth et al. 2003). Two of these introductions account for a single species each. The third, which we focus on here, resulted in a radiation that gave rise to eight endemic, diploid species: *S. chamissoniana*, *S. coriacea*, *S. gaudichaudiana*, *S. gaudichaudii*, *S. hobdyi*, *S. kilaueae*, *S. mollis*, and *S. procera*. However, *S. hobdyi* was not included in this study (but see Howarth et al. 2003) because it is known only from the holotype and is presumed extinct

(Wagner 1996). Each of the remaining seven species is found in a distinct habitat zone (see Patterson 1990). *Scaevola coriacea* is found on coastal sand dunes. Historically, it inhabited all islands, but human disturbance has led to extirpation in most localities, with one remaining significant population from Waiheʻe, Maui, and a few relict populations on Maui and Mokuhoʻoniki islet east of Molokaʻi. *Scaevola gaudichaudii* is a lowland (75–800 m altitude) dry forest species inhabiting all the major islands. *Scaevola kilaueae* is found only on young lava flows and is presently confined to the active volcanoes of Kilauea and Mauna Loa, on Hawaiʻi. *Scaevola chamissoniana* and *S. gaudichaudiana* are found in mesic mid-elevation (200–800 m) wet forests on different islands, with *S. chamissoniana* on Hawaiʻi and Maui Nui (Maui, Molokaʻi, and Lanaʻi) and *S. gaudichaudiana* on Kauaʻi and Oʻahu. *Scaevola procera* and *S. mollis* are both higher-elevation (600–1400 m) wet forest species, with *S. mollis* on Kauaʻi, Oʻahu, and rarely Molokaʻi and *S. procera* on Kauaʻi and Molokaʻi. On the islands where these two species co-exist, *S. procera* grows at higher elevations than *S. mollis*.

Morphological traits that vary among these *Scaevola* species are not distributed in a clearly hierarchical manner, obscuring phylogenetic relationships among the species (Patterson 1995). However, it has been suggested that *S. coriacea* is sister to the rest of the species due to its overall phenotypic similarity with *S. plumieri* (Carlquist 1969), an outgroup species that is closely related to this Hawaiian clade (Howarth et al. 2003). Additionally, *S. procera* has been suggested to be a hybrid species derived from *S. mollis* and *S. gaudichaudiana* (Carlquist 1974, 1980; Patterson 1990), two species that are known to hybridize in sympatry (Gillett 1966). Phylogenetic analysis of internal transcribed spacer (ITS) sequence data has elucidated the pattern of dispersal of *Scaevola* into the Hawaiian Islands (Howarth et al. 2003), but did not produce a resolved phylogeny among the eight endemic diploid species. In the current study, sequence data from intron regions in three low-copy nuclear genes and new data from ITS were analyzed and combined with previously published ITS data. The nuclear genes studied were *FLORICAULA/LEAFY* (*LFY*), *NITRATE REDUCTASE* (*NIA*; Howarth and Baum 2002), and *GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE* (*G3PDH*; Strand et al. 1997). *LFY* is a DNA-binding transcription factor that controls the identity of the floral meristem in a variety of species and is directly involved in the activation of several floral organ identity genes in *Arabidopsis* (Parcy et al. 1998). It contains two large introns in conserved positions in seed plants (Frohlich and Meyerowitz 1997) and ferns (Hoot and Taylor 2001). The second intron of *LFY* varies in length from 83 bases to 4.6 kb, although most of the taxa examined across vascular plants have introns ranging from 0.5 kb to 2 kb (Frohlich and Meyerowitz 1997; Hoot and Taylor 2001). The utility of the *LFY* intron 2 for low-level phylogenetic analysis had not previously been evaluated, except for a recent study in Araceae (Grob et al. 2004). *NIA* contains three large introns in the same location in all higher plants examined (Salanoubat and Ha 1993). The third intron is usually large (up to 1.6 kb; Jensen et al. 1994). We previously demonstrated that this intron had an appropriate amount of variation for examining species and population-level questions (Howarth and Baum 2002). *G3PDH* contains a region of five exons and four introns (Strand et al. 1997) that has been useful in interspecies and intraspecies phylogenetic reconstruction (Olsen and Schaal 1999).

We reasoned that the patterns of alleles from four separate nuclear regions (*LFY, NIA, G3PDH,* and ITS) would shed light on reticulation during the radiation of diploid *Scaevola* species in the Hawaiian Islands. From an adaptive radiation such as this clade of *Scaevola*, we expect a potential combination of dichotomously branching species, species of hybrid origin, recent hybridization, and possible confounding incomplete lineage sorting. If there is extensive incomplete lineage sorting, it is unlikely that there would be a subset of species for which all gene regions agreed on relationships. Homoploid hybrid speciation, on the other hand, predicts that the incongruence would be confined to specific nodes among the gene trees. Within potential hybrid species, where lineage sorting is complete, we expect the alleles to be monophyletic, but closely related to different parental species when analyzed with different genes. Alternatively, where lineage sorting is not complete, we expect to find related alleles from both parental species still extant in the hybrid species. Therefore, hybrid speciation is potentially distinguished from incomplete lineage sorting because it predicts that a sizable subset of species (those that lack hybrid ancestry) would show a consistent pattern of relationships across all gene regions. Additionally, there could potentially be very recent hybridization (e.g., F_1s), resulting in heterozygosity for both parental alleles (and most likely identical to those alleles) at most or all loci. Although more complicated patterns can potentially arise if introgression and/or lineage sorting are sufficiently abundant, we expect that by studying four independent nuclear markers we may elucidate the extent to which hybridization has contributed to the radiation of *Scaevola* in the Hawaiian Islands.

Materials and Methods

Plant Collection and DNA Extraction

A total of 20 *Scaevola* individuals, including two or three accessions from each of the seven studied species, were sampled (Table 1). Three species, *S. mollis, S. gaudichaudiana,* and *S. kilaueae*, were each collected from two separate populations from a single island. DNA was extracted from fresh material or silica gel samples. The fresh material was extracted and purified with a modification of the cetyltrimethyl ammonium bromide isolation method (Doyle and Doyle 1987). These samples were accessioned into the Hawaiian Plant DNA Library, Honolulu (Morden et al. 1996; Randell and Morden 1999). Silica gel samples were extracted with Qiagen Plant Extraction kits (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Amplification

To amplify the second intron of *LFY*, a protein alignment was produced across angiosperms, and degenerate primers (LFY. F2: 5′-CGTGGSAAAAAGAAYGGYYTDGATTA-3′ and LFY. R1: 5′-CATTTTDGGYTTGTTKATGTA-3′) were designed

TABLE 1. List of taxa used in this study, with collection location. H, Hawai'i; K, Kaua'i; M, Maui; O, O'ahu. Available vouchers from Hawaiian Plant Database Library (HPDL) are housed at BISH (Bishop Museum Herbarium). The others are housed at A (Harvard University Herbaria). GenBank numbers range from AY894427–AY894520; GenBank numbers in the table include only the last three digits.

Species	Accession	Location, Area	GenBank			
			LFY	*NIA*	*G3PDH*	ITS
Scaevola chamissoniana	Howarth 47, HPDL 253	H, Volcano	427, 428	455	481	502
S. chamissoniana	Howarth 49, HPDL 255	H, Volcano	429	456, 457	482	503
S. coriacea	Goldstein sn., HPDL 50	M, Waihe'e	430	458	483	504
S. coriacea	Goldstein sn., HPDL 56	M, Waihe'e	431	459	484	505
S. coriacea	Goldstein sn., HPDL 61	M, Waihe'e	432, 433	460	485, 486	506
S. gaudichaudiana	Howarth 11, HPDL 219	O, Lanipo Trail	434, 435	461	487	507
S. gaudichaudiana	Howarth 14, HPDL 222	O, Lanipo Trail	436	462	488	508
S. gaudichaudiana	Howarth 27, HPDL 234	O, Tantalus Trail	437	463	489	509
S. gaudichaudii	Howarth 31, HPDL 237	K, Kokee	438, 439	464, 465	490	510
S. gaudichaudii	Howarth 32, HPDL 238	K, Kokee	440	466, 467	491	511
S. gaudichaudii	Howarth 1–96, HPDL 995	K, Kokee	441, 442	468	492	512
S. kilaueae	Morden 1141, HPDL 178	H, Kilauea	443	469	493	513
S. kilaueae	Howarth and Stone 1	H, Kaimu, Puna	444	470	494	514
S. kilaueae	Howarth and Stone 2	H, Kaimu, Puna	445, 446	471	495	515
S. mollis	Howarth 10, HPDL 218	O, Lanipo Trail	447	472, 473	496	516
S. mollis	Howarth 20, HPDL 227	O, Tantalus Trail	448, 449	474, 475	497, 498	517
S. procera	Howarth 36, HPDL 242	K, Alaka'i Swamp	450	476	499	518
S. procera	Howarth 2-1	K, Alaka'i Swamp	451, 552	477, 478	500	519
S. procera	Howarth 2-2	K, Alaka'i Swamp	553, 554	479, 480	501	520

from conserved regions of the second and third exons. Initial amplification and sequencing allowed the design of *Scaevola*-specific primers (LFY.F3: 5′-ATTACCTCTTCCATCTCTACG AGC-3′ and LFY.R4: 5′-AACCGAAACACTTGGTTCGTCA CC-3′), which were used for all subsequent amplifications. Amplifications used the following cycling program: 95°C, 50 sec; 58°C, 1 min; 72°C, 1 min 30 sec; repeat 38 cycles.

The third intron of *NIA* was amplified as described in Howarth and Baum (2002). *Scaevola*-specific primers were also produced for subsequent amplifications (NIA3.intF: 5′-CACTCAACCC GAGAAGCTCATTTGG-3′ and NIA3.R3: 5′-CCCTGCAGA ATCAACCAACCATAA-3′). Amplifications used a step-down cycling program with the annealing temperature dropping from 65°C to 50°C with the following cycles: 94°C, 1 min; 65–50°C, 1 min; 72°C, 1 min 30 sec; 38 cycles.

The genomic region of *G3PDH*, including four introns and five exons, was amplified from primers designed by Strand et al. (1997), with the same polymerase chain reaction (PCR) conditions as used for *LFY*. The ITS region was amplified as in Howarth et al. (2003). Amplifications used the following cycling program: 95°C, 50 sec; 60°C, 50 sec; 72°C, 1 min 50 sec; repeat 30 cycles.

All PCRs were performed using *Taq* DNA Polymerase (Boehringer Mannheim, Indianapolis, IN or Qiagen Inc.) or *Pfu* Turbo (Stratagene, La Jolla, CA) in 25 μl, with final concentrations of 2.5 mM $MgCl_2$, 0.5 μM of each primer, 0.8 mM dNTPs, and 0.5X Q solution (Qiagen Inc.). The samples were gel purified and cleaned with the Qiagen PCR cleanup kit (Qiagen Inc.). The amplified products from *LFY*, *NIA*, and *G3PDH* were then cloned using the Promega (Madison, WI) T-EZ TA cloning kit or the Invitrogen TOPO TA Cloning Kit for Sequencing (Carlsbad, CA), with blue/white colony selection. Five to eight white colonies were picked for each individual to screen for multiple loci and to increase the likelihood of sequencing both alleles of heterozygotes (if both alleles amplify and clone equally, the chance of finding both alleles from five clones in a heterozygote is 93.75%). The colonies were grown in 2 ml of LB, and plasmid DNA was extracted with the Sigma Miniprep kit (Sigma, St. Louis, MO). In most cases, ITS samples were sequenced directly from the original PCR amplifications. The product was then sequenced using the BigDye system (PE Applied Biosystems, Foster City, CA) according to manufacturer's instructions and electrophoresed with an ABI 377 or ABI 3100. Sequencing reactions used the same primers as for PCR except that internal primers were needed for sequencing through the *LFY* intron and some of the *NIA* clones. These primers were: LFY.intF: 5′-TTTGTTCTTTACTAGAATTAAC-3′, LFY.intR: 5′-GTTCA TAGTCCATATACAGTAC-3′, NIA3.int2F: 5′-TAGAAGAA TTAGACTTGGAGTAAGG-3′, and NIA3.int2R: 5′-GAAAA GGACTAATCAAAATGGTTTTG-3′.

Phylogenetic Analyses

Initially the clones from each individual at each separate locus were analyzed, and consensus sequences were created in homozygous individuals. Heterozygous individuals were determined by the presence of shared indels and base changes among different clones, and a consensus sequence for each separate allele was created. Recombinant PCR sequences (PCR hybrids) were occasionally detected in heterozygotes through comparing clones from a single individual (Paabo et al. 1990) and were removed from the analyses. Allele sequences were aligned by eye. All of the conspecific, completely identical alleles were condensed into one sequence. This resulted in four separate matrices (one for each locus), each including all the distinct alleles that were found within each species. These data can be obtained from TreeBASE (www.treebase.org).

Each matrix was analyzed separately using the maximum parsimony criterion with PAUP* 4.0b10 (Swofford 2001). Characters were treated as unordered and equally weighted,

TABLE 2. Comparative information about each locus. Heterozygotes means the number of collected individuals that had two alleles. CI, consistency index.

	Aligned matrix	Variable		Parsimony-informative		Hetero-zygotes	Coded indels	% AT	Trees (branch length)	CI all species (w/o *procera* and *kilaueae*)
		no.	%	no.	%					
LFY	1845	98	5.3	64	3.5	9	8	71.1	2 (114)	0.9035 (0.9419)
NIA	1370	44	3.2	37	2.7	7	4	73.2	2 (56)	0.7857 (1.000)
region 1	349	18		16		—-	1	73.3	3 (19)	0.9474 (1.000)
region 2	1022	26		21		—-	3	73.1	17 (28)	0.9286 (1.000)
G3PDH	931	12	1.3	5	0.5	2	4	60.6	1 (12)	1.000 (1.000)
ITS	795	13	1.6	10	1.3	0	0	47.8	1 (13)	1.000 (1.000)

and gaps were coded as missing. Indels were coded as binary characters excluding those that consisted of A and T repeats in which homology assessment was weak. Branches were collapsed if the minimum length was zero. Branch-and-bound searches were performed. Bootstrap analysis used 1000 pseudoreplicates subject to branch-and-bound searches.

To conduct combined analyses across the four loci, we generated concatenated matrices at the (1) allele, (2) individual, and (3) species levels. For each of these combined analyses, indel data were included (as binary characters) and were treated similarly to sequence data. At the allelic level we coded two alleles for each individual (using identical alleles in homozygotes). We then generated combined matrices, concatenating the different alleles from heterozygotes in all combinations, and retained the concatenated matrix with the highest and lowest consistency index when subject to simple maximum-parsimony searches. At the individual level, we produced a consensus sequence for each individual across both alleles, coding polymorphisms with standard ambiguity codes but including insertion sequences when deletions occurred in the other allele. At the species level we used a similar procedure, producing a consensus sequence for each species across all alleles found.

The three combined matrices were divided into four character partitions (for each locus, including the indels) and then compared using the partition homogeneity test (Farris et al. 1994) as performed in PAUP* under parsimony with branch-and-bound searches. We conducted combined branch-and-bound parsimony analyses on a subset of taxa, which were determined by observing species that did not conflict with monophyly across loci and comparing significant discordance based on the partition homogeneity test. The combined matrices were rooted with *S. plumieri* (data only from ITS; Howarth et al. 2003). Bootstrap analysis used 1000 pseudoreplicates subject to branch-and-bound searches.

RESULTS

Individual Loci Analyses

Each individual locus was initially analyzed separately. The alignment lengths and percent variation for each locus are given in Table 2. *LFY* and *NIA* contained considerably more parsimony-informative variation than the other two loci, with 3.5% and 2.7% informative sites, respectively, compared to 0.5% (*G3PDH*) and 1.3% (ITS). Nonetheless, each region provided information about relationships (Fig. 1). The trees were polarized with *S. coriacea* for uniformity, although the true root is unknown. A broader analysis of *Scaevola* with ITS suggested that the root lies between the clade containing *S. coriacea* and *S. gaudichaudii* and the clade containing the rest of the species (Howarth et al. 2003).

Resolution of LFY

The aligned *LFY* matrix contained 21 different alleles, 1845 characters, including eight indels, and resulted in two trees with 114 steps (Fig. 1A). The tree is relatively well resolved and includes several consistent indel characters. These data indicate a long branch between the monophyletic *S. coriacea* and the rest of the taxa. Each species shows monophyly of its sampled accessions except *S. procera* and *S. kilaueae*.

Resolution of NIA

The aligned *NIA* matrix included 20 different alleles, with 1370 characters, including four indels, resulting in two trees with 56 steps (Fig. 1B). *Scaevola coriacea* is the only species for which all alleles form a monophyletic group. However, the consensus trees do not contradict exclusivity for three other species (*S. gaudichaudiana*, *S. kilaueae*, and *S. mollis*). Additionally, removal of either *S. gaudichaudii* or *S. procera*, which are intermingled, would leave the other species as potentially monophyletic. The same is true for *S. chamissoniana* and *S. kilaueae*.

Visual inspection of the *NIA* locus showed that it could be divided into two ''miniloci'' separated by a zone of apparent recombination. Each minilocus supported differing relationships across alleles, while positions within each locus tended to show a consistent pattern of relationships. The 5′ minilocus comprises bases 1–348, and the 3′ minilocus comprises bases 349–1370. Interestingly, the region containing bases 349–398 is a repetitive, uncharacteristically GC-rich area with multiple indel events ranging from 9 to 29 bases, and could possibly be a recombination hotspot. To identify possible recombinant alleles, we analyzed the two miniloci separately. Analysis of the 5′ minilocus supports a divergent clade with one allele of *S. procera* and three of *S. gaudichaudii* (Fig. 2A). Analysis of the 3′ minilocus (Fig. 2B) resulted in a tree with a similarly long branch isolating three different alleles of *S. procera* and four alleles of *S. gaudichaudii*. Comparing the two trees, one *S. gaudichaudii* haplotype (2B) and four *S. procera* haplotypes (1A, 2A, 2B, 3AB) appear to be recombinants.

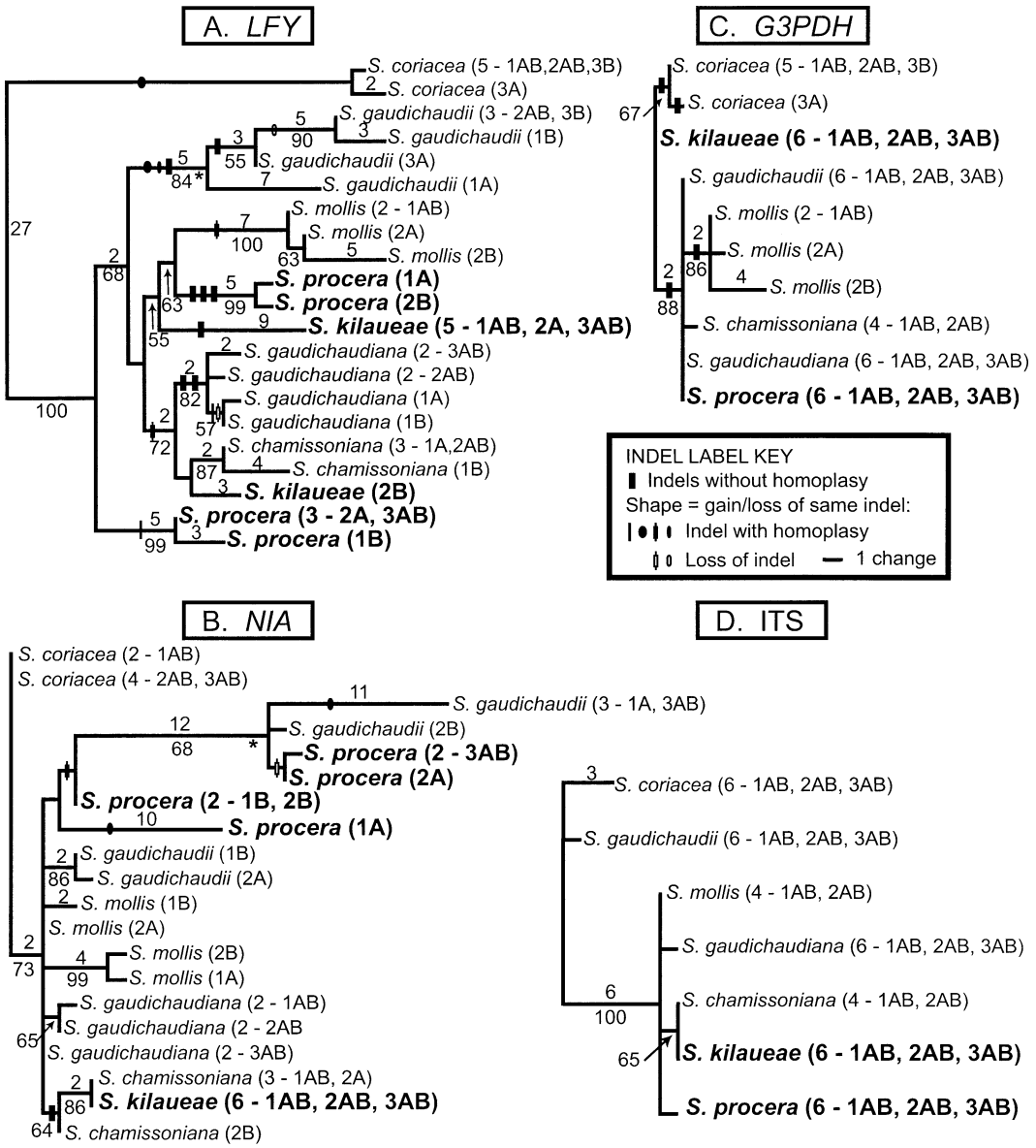

FIG. 1. Most parsimonious phylograms for each locus with all differing alleles. All trees are polarized with *Scaevola coriacea* to simplify comparisons. (A) *LFY*, (B) *NIA*, (C) *G3PDH*, and (D) ITS. The loci ITS and *G3PDH* had only one most parsimonious tree. The loci *LFY* and *NIA* are displayed with one of two most parsimonious trees, with the only changes among trees within the clade labeled with an asterisk. Numbers above lines are branch lengths (branch lengths of 1 are not labeled), and numbers below lines are bootstrap support of ≥50%. Branch lengths included coded indels, which are marked on branch (see key). Species names are followed by, in parentheses, number of alleles, and individual (number) and allele (AB). Individuals are numbered in the order they appear in Table 1.

Resolution of G3PDH

The aligned *G3PDH* matrix contained 10 different alleles, 931 characters including four indels, and yielded one tree with 12 steps. The variation within *G3PDH* was minimal (Fig. 1C, Table 2), and all of the alleles of *S. gaudichaudiana*, *S. gaudichaudii*, and *S. procera* are identical to each other. Nonetheless, the data did not conflict with the exclusivity (sensu Baum and Shaw 1995) of any species. *Scaevola coriacea* and all six of the *S. kilaueae* alleles are separated from the remaining species by a single base change and a one-base indel. Additionally, all of the *S. coriacea* alleles contain an eight-base deletion, and all of the *S. chamissoniana* alleles are in a clade supported by a single base. Finally, all the alleles of *S. mollis* are in a clade supported by one base pair and an eight-base insertion (separate from that of *S. coriacea*).

Resolution of the ITS

The aligned ITS matrix included seven different alleles with 795 characters resulting in one tree with 13 steps. Unlike the other loci, no intraspecific variation was found (Fig. 1D). All of the species appear monophyletic, except *S. mollis*, which appears to be fixed for an ancestral allele. There was no evidence of multiple peaks in the electropherograms for any taxa. In contrast, double peaks were found in recent

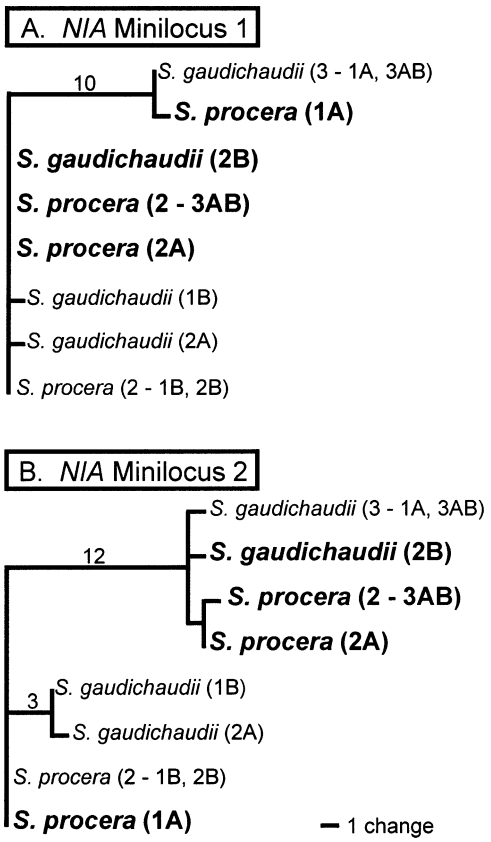

FIG. 2. Recombination in *NIA*, showing a phylogram of one of three most parsimonious trees in region 1 (A), and one of 17 most parsimonious trees in region 2 (B). Region one included characters 1–348 and region 2 included characters 349–1370. Recombinant alleles are bold.

hybrid individuals, *S. chamissoniana* × *gaudichaudii* and *S. gaudichaudiana* × *mollis* (D. G. Howarth, unpubl. data).

Combined Analyses

Partition homogeneity tests for the three concatenated matrices all indicated that the regions have significantly different phylogenetic signals (Table 3). Results were similar for each of the three methods, therefore we here report the results for the second concatenated matrix, which generated a single consensus sequence for heterozygote individuals.

Based on the analysis of the single-locus trees, it appeared that the discordance could be attributed primarily to alleles from two species, *S. procera* and *S. kilaueae* (Fig. 1). When sequences from all the individuals of these two species are removed, the remaining data no longer show significant discordance across the four loci ($P = 0.538$; Table 3), and each locus analyzed independently suggested compatible species relationships. Thus, although there is some discordance within species, as expected because of recombination (Avise and Ball 1990), the overall pattern is one of genealogical concordance in the relationships of these five traditionally recognized species. When any single individual was used from each species (excluding *S. kilaueae* and *S. procera*), the partition homogeneity test indicated that the signal was identical across loci ($P = 1.00$), implying that recombination among the five putatively exclusive species was occurring within species.

TABLE 3. Trees generated by individual consensus sequences (see analysis 2, Materials and Methods) with all individuals from different species deleted. Number of trees, length, and consistency index (CI) of the combined matrix with different taxa excluded. Partition homogeneity test result across all four regions given with *P*-value (asterisk denotes significance).

Excluded species	Trees	Length	CI	*P*
None	12	293	0.9352	0.001*
Scaevola coriacea	2	246	0.9715	0.008*
S. gaudichaudii	2	202	0.9653	0.125
S. mollis	12	259	0.9305	0.001*
S. gaudichaudiana	12	282	0.9433	0.001*
S. chamissoniana	4	280	0.9464	0.001*
S. kilaueae	4	267	0.9476	0.001*
S. procera	4	227	0.9648	0.004*
S. procera and *S. kilaueae*	4	201	0.9851	0.538

The combined matrix across individual consensus sequences from the five species was 4944 characters (including 15 coded indels). The ingroup taxa contained 147 variable sites, of which 75 were parsimony informative. Maximum parsimony searches, including the outgroup taxon, *S. plumieri*, yielded four trees with 228 steps (CI = 0.9868, RI = 0.9639; Fig. 3). Maximum parsimony analyses of the ingroup taxa alone yielded four trees of length 201 (CI = 0.9851, RI = 0.9799). Species clades were supported with bootstraps ≥94% (Fig. 3). Thus, removal of *S. procera* and *S. kilaueae* resulted not only in concordant species relationships, but also in apparent species exclusivity.

The root of this Hawaiian endemic diploid clade, based on ITS data alone, is situated between a clade containing *S. coriacea* and *S. gaudichaudii* and a clade containing the remaining included species (Fig. 3; Howarth et al. 2003). Given such a rooting, the combined data of all four regions support the existence of two primary clades among the Hawaiian diploid *Scaevola* species. The first group contains the two species of drier habitats, *S. coriacea* and *S. gaudichaudii*, which are well supported as sister to each other (bootstrap = 94%). The other clade (bootstrap = 87%) contains the species from wetter habitats. Within this latter clade, *S. mollis* is sister to a clade composed of *S. gaudichaudiana* and *S. chamissoniana* (bootstrap = 78%).

Alleles of Scaevola procera and Scaevola kilaueae

At each single-copy locus, alleles of *S. procera* tend to cluster with either *S. gaudichaudii* or *S. mollis*. In ITS, the *S. procera* sequences are monophyletic and are most similar to *S. mollis* (Fig. 1D). In *LFY*, the *S. procera* alleles appear in two separate clades with two alleles sister to the *S. mollis* clade and four alleles sister to alleles of all of the species except *S. coriacea*. However, one of these latter alleles does share a substitution (homoplasious in this phylogeny, G → A, base 202) with only *S. gaudichaudii*. In *G3PDH*, *S. procera* is identical to *S. gaudichaudii* although variation in this region is minimal. Finally, at *NIA*, the *S. procera* alleles appear to share recombinant alleles with *S. gaudichaudii* (see above; Fig. 2).

The *S. kilaueae* alleles tend to cluster with *S. chamissoniana*

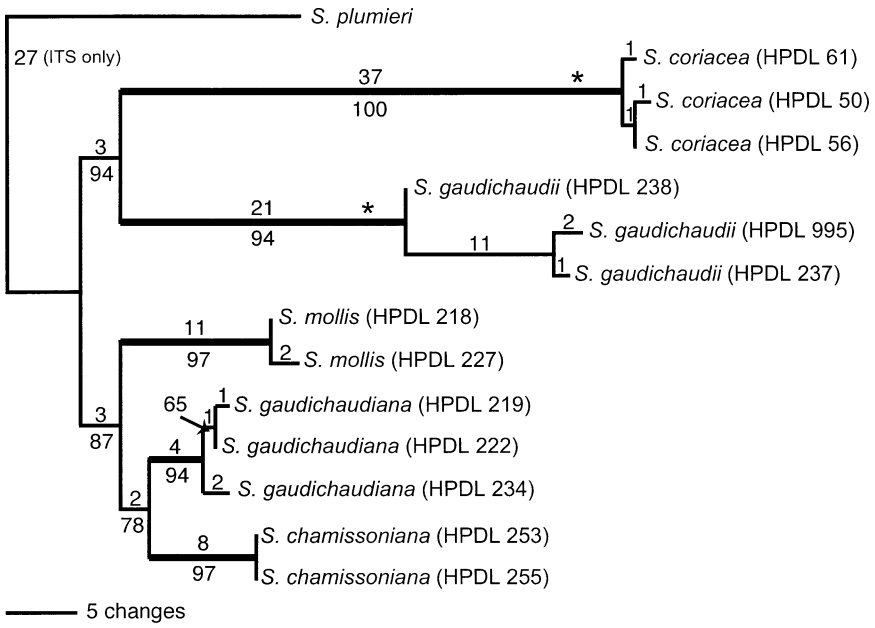

FIG. 3. Phylogram of one of four most parsimonious trees in the combined search (excluding *Scaevola procera* and *S. kilaueae* alleles) with analysis 2 (see Materials and Methods). Differences among trees were within clades labeled with an asterisk. Numbers above the lines are branch lengths, and numbers below are bootstrap support of ≥50%. Branch length of outgroup is based only on the ITS region.

or *S. coriacea*. In ITS and *NIA*, all the *S. kilaueae* alleles are monophyletic and identical to alleles from *S. chamissoniana*. At *LFY*, one allele is sister to the *S. chamissoniana* clade, and five identical alleles are sister to the *S. mollis*/*S. procera* clade (Fig. 1A). The latter five identical alleles are on a long branch that is supported as sister to the *S. mollis*/*S. procera* clade by one base, but additionally share a single-base polymorphism with the *S. chamissoniana* alleles (C → A, base 397). Thus, it is plausible that these alleles are actually more closely related to *S. chamissoniana* than they appear on the most parsimonious tree. In contrast, at *G3PDH*, all the *S. kilaueae* alleles are identical in nucleotide sequence to those of *S. coriacea*, the species to which *S. kilaueae* is most similar morphologically (D. G. Howarth, pers. obs.). Although the variation supporting the placement of *S. kilaueae* in the *G3PDH* tree is minimal (one base change and one indel), this placement is strengthened by the fixation of all three *S. kilaueae* individuals for one shared allele with *S. coriacea*.

DISCUSSION

Intron Utility in Phylogenetics

Nuclear introns have become increasingly popular for elucidating phylogenetic relationships in closely related plant groups (Sang et al. 1997; Mason-Gamer et al. 1998; Bailey and Doyle 1999; Evens et al. 2000; Tank and Sang 2001; Walsh and Hoot 2001; Howarth and Baum 2002; Malcomber 2002; Caicedo and Schaal 2004). Here we describe the phylogenetic relationships of a clade of Hawaiian *Scaevola*, using multiple intron regions, which had remained not fully resolved using other analyses (morphology, flavonoids: Patterson 1984; morphological cladistics: Patterson 1995; and ITS sequencing: Howarth et al. 2003). The problematic resolution of this clade appears to have stemmed from a combination of minimal molecular variation in commonly used marker regions and hybrid speciation events. Therefore, we are optimistic that a similar scheme of combined analysis of multiple nuclear regions will help resolve other, similarly complicated groups.

Larger (>1 kb) nuclear introns, in particular, may contain the most potentially informative variation, as observed in this study. Additionally, plant genes fall into two distinct classes based on GC content (Carels and Bernardi 2000), with the GC-poor genes frequently having larger introns. Our observations fit their hypothesis with the larger, more variable introns containing high AT richness for both *LFY* (71%) and *NIA* (73%) (Table 2). Nonetheless, it is clear that large introns such as those in *NIA* and *LFY* contain more variation than either ITS or *G3PDH*.

This paper is, to our knowledge, the first effort to use the second intron of *LFY* for low-level phylogenetic analysis in eudicots where it is often considerably larger than in monocots. This gene has the advantage of usually existing as a single copy and having two large introns in conserved positions (Frohlich and Meyerowitz 1997; Hoot and Taylor 2001). Furthermore, exons 2 and 3 are highly conserved, facilitating the design of conserved primers. Finally, the second intron of *LFY* has been useful in examining species-level relationships in *Isoetes* (Hoot and Taylor 2001) and *Amorphophallus* (Grob et al. 2004). Thus, *LFY* intron 2 is an excellent candidate for phylogenetic analysis of closely related plant species and even, potentially, for intraspecific studies. Indeed, we found that *LFY* intron 2 had abundant sequence variation among the Hawaiian endemic *Scaevola* and detected 21 alleles, more than any of the other markers sampled. This

gene region provided the most resolved phylogeny of any of the four gene regions studied.

Relationships and Ecology of Scaevola

Scaevola colonized the Hawaiian Islands three separate times (Howarth et al. 2003), resulting in the establishment of a widespread tropical Pacific strand species, *Scaevola taccada*; an endemic tetraploid, *Scaevola glabra*; and the clade of endemic diploid ($n = 8$; Carr 1998) species examined in this study. The latter group is part of a clade that includes endemic taxa from Cuba and Socotra and a widespread tropical strand species, *Scaevola plumieri* Vahl. *Scaevola plumieri* exists on coasts of tropical South America, the Caribbean, and Africa (including Madagascar and Socotra; van Balgooy 1975), but not in the Hawaiian Islands. *Scaevola coriacea* shares substantial morphological similarity with *S. plumieri*, leading to the hypothesis that an ancestor of *S. plumieri* may have given rise to these Hawaiian endemic diploids (Carlquist 1969), a hypothesis that is consistent with analysis of ITS data (Howarth et al. 2003).

Based on the four-gene combined phylogenetic analysis (excluding *S. procera* and *S. kilaueae*) rooted with *S. plumieri*, the basal split in the endemic diploid clade leads to two dryland species (*S. coriacea* and *S. gaudichaudii*) on the one hand and three species of wetter environments on the other (*S. chamissoniana*, *S. gaudichaudiana*, and *S. mollis*; Fig. 3). *Scaevola coriacea* is a prostrate, sprawling coastal dune species with short white flowers. It is highly endangered with only one significant population, which occurs on Maui. *Scaevola gaudichaudii* is a small shrub (<1 m), growing on dry ridges less than 800 m, with small yellow flowers that nearly lack the petal wing. Together, these two species share the characteristics of glandular hairs with pseudostellate heads, shorter stature, succulent leaves, few flowers in a cyme (solitary to three), and historical occurrence on all of the high Hawaiian Islands. In contrast, the three wet-forest species are characterized by glandular single-celled hairs, taller stature, and larger leaves.

Based on our combined analyses, *S. mollis* is sister to the other two mesic species and is a small shrub (1–2.5 m tall) that occurs on the tops of ridges (800–1100 m). It is distinguishable by its purple flowers, dense pubescence, and usually three- or fewer-flowered cymes. *Scaevola gaudichaudiana* and *S. chamissoniana* both have white flowers, are much less pubescent, can have more than 18 flowers per cyme, and grow at lower elevations. These two species are distinguished by range (*S. gaudichaudiana* occurs on Kaua‘i and O‘ahu, whereas *S. chamissoniana* inhabits all the younger islands of Maui, Moloka‘i, Lana‘i, and Hawai‘i), as well as by *S. chamissoniana* being larger and having more teeth on the leaves. Thus, each of these species grows in a different habitat zone, separated from the other species by rainfall and/or elevation, with the only significant overlap in habitat type (*S. gaudichaudiana* and *S. chamissoniana*) being separated by island. Given that many native Hawaiian bees, which are the only documented pollinator sightings on Hawaiian *Scaevola* (Perkins 1913; Zimmerman 1948), are specific to confined climatological zones, these separate habitats could readily have conferred reproductive isolation.

All of the five species discussed above were supported as being exclusive groups (Baum and Shaw 1995): alleles from a single species were sometimes found to form clades in individual gene genealogies, and the monophyly of their alleles was never contradicted. Although multiple islands were sampled in this analysis and some species were sampled from multiple populations, each species was only collected from a single island (Table 1), raising the possibility that we might be missing confounding infraspecific variation. However, additional data (Howarth 2002) lead us to believe that the haplotype clades truly capture the phylogenetic affinities of the entire species (Table 4). One sample that was sequenced for all loci except *G3PDH*, a *S. gaudichaudiana* from Kaua‘i (all *S. gaudichaudiana* accessions in this study were from O‘ahu) clustered with conspecifics in the other loci. Likewise, two accessions of *S. chamissoniana* from Maui (vs. Hawai‘i) contained alleles resembling previously identified *S. chamissoniana* alleles at ITS and *NIA*, although, amplification was unsuccessful in *LFY* and *G3PDH*. Finally, samples from Maui taken from a zone of apparent hybridization between *S. chamissoniana* and *S. gaudichaudii* indicated that those alleles clustered with one or the other parental species alleles despite the fact that our previous accessions from the parental species came from Hawai‘i and Kaua‘i respectively (Table 4). Thus, despite our narrow geographic sampling from some species, our combined analysis probably accurately reflects the exclusivity and phylogenetic relationships of the five nonreticulate species.

Hybrid Scaevola Species

The five species discussed above show genealogical exclusivity (Baum and Shaw 1995) and appear to be related via a single divergent species tree. In contrast, *S. procera* and *S. kilaueae* alleles appear in different places in different gene trees and frequently do not form clades even at a single locus. We suggest here that these data can be explained as a product of homoploid hybrid speciation.

There are generally two explanations for discordance among gene trees: lineage-sorting (retention of alleles through one or more speciation events and subsequent sorting) and horizontal transfer. In this case, the alleles from the five aforementioned species show concordance within and among loci, and only two species show discordant patterns—with alleles falling in quite disparate parts of the tree. Such a pattern is hard to explain by lineage sorting because when this phenomenon affects an internal branch, discordant patterns are expected for different descendants of that node. Here we found strict concordance for five species, with all discordance being assignable to *S. procera* and *S. kilaueae*. Furthermore, these two species have the smallest historical ranges (apart from the presumed extinct *Scaevola hobdyi*), making it highly unlikely that they alone would maintain ancient alleles. Therefore these data clearly suggest horizontal gene transfer in the parentage of *S. procera* and *S. kilaueae*, presumably via historic hybridization.

Traditionally biologists have distinguished introgressive hybridization, where some genes cross a species barrier via hybridization and backcrossing, and hybrid speciation, where two lineages merge to give rise to a descendent species that

TABLE 4. Other amplified taxa not used in the analyses due to lack of a complete dataset or hybrid status. HPDL, Hawaiian Plant Database Library (Morden et al. 1996); Opp, collections by H. Oppenheimer (specimens deposited at A; Harvard University Herbaria). The species affiliations are given for each locus with the variation given in parentheses; apo, apomorphy(-ies). When the allele had no clear affinities (was situated along the major polytomy of the tree) we identified the placement as "backbone."

Taxon identification	Island	*LFY*	*NIA*	*G3PDH*	ITS
S. procera, HPDL 246	Kauai	—	—	—	*procera* (identical)
S. gaudichaudiana, HPDL 247	Kauai	*gaudichaudiana* (in clade)	*gaudichaudiana* (shares indel)	—	*gaudichaudiana* (identical)
S. chamissoniana, Opp. 19804	Maui	—	backbone (1 apo)	—	*chamissoniana* and *kilaueae* (1 apo)
S. chamissoniana, Opp 69904	Maui	—	backbone (identical)	—	*chamissoniana* and *kilaueae* (identical)
S. chamissoniana × *gaudichaudii*, Opp 49918					
Allele A	Maui	*chamissoniana* (in clade)	backbone (identical)	backbone (2 apo)	*chamissoniana* and *kilaueae* (identical)
Allele B	Maui	*gaudichaudii* (in clade)	backbone (2 apo, indel)	—	*gaudichaudii* (identical)

is an admixture of both parental lineages (Rieseberg 1997). In the case of polyploid hybrid speciation, the distinction is clear because the hybrid species, at least initially, has a genome made up of the entirety of both parental genomes. However, the distinction between introgression and hybrid speciation is less clear when there is no chromosome doubling. Rather, homoploid hybrid speciation tends to be invoked in cases where there is a significant genetic contribution to the descendant species from both parental species, where the ecological success of the descendant can be attributed, at least partly, to the acquisition of traits from both parents, and where the descendant has subsequently become reproductively isolated from both parents. These attributes fit the description for *S. procera* and *S. kilaueae*.

Evolution of Scaevola procera

Carlquist (1974, 1980) hypothesized that *S. procera* was a hybrid between *S. gaudichaudiana* and *S. mollis* because these two species are known to hybridize and form swarms consisting of clines of intermediate morphologies wherever their habitats abut (Gillett 1966; D. G. Howarth unpubl. data) and because *S. procera* shares purple flowers with *S. mollis* and slightly longer peduncles with *S. gaudichaudiana*. However, we did not find alleles from *S. procera* that are shared with *S. gaudichaudiana* at any of the loci (Fig. 1). Additionally, *S. procera* shares more characteristics with *S. gaudichaudii* than it does with *S. gaudichaudiana* (the common hybridization partner of *S. mollis*).

Our data suggest that the alleles of *S. procera* tend to be most similar to alleles from *S. mollis* and *S. gaudichaudii*, which leads us to hypothesize that *S. procera* is a homoploid hybrid species derived from these two parental species. In support of this claim, *S. procera* exists on islands where both *S. mollis* and *S. gaudichaudii* currently grow, meaning there has been potential for contact. Additionally, *S. procera* combines traits found only in the putative parents: with *S. gaudichaudii* it shares a near lack of petal wings, and with *S. mollis* it shares large, hairy leaves and occurrence in high-elevation, wet habitats. Interestingly, *S. procera* leaves have interspersed pseudostellate hairs (dry clade) and simple hairs (wet clade), and its flowers are quite variable ranging from yellowish (*S. gaudichaudii* is the only other yellow-flowered species in the clade) to purple (*S. mollis* is the only other purple-flowered species in the clade). The mix of morphological characters seen in *S. procera* is fully supportive of the hypothesis that it is derived from a genetic fusion of populations closely related to extant *S. gaudichaudii* and *S. mollis*.

Notwithstanding its apparent hybrid ancestry, *S. procera* appears to have been quite genetically isolated (excluding current hybrids probably due to human disturbance through habitat restriction [Burney et al. 2001] and significant pollinator shifts [Kato and Kawakita 2004]) as evidenced by the possession of unique characteristics. It grows at a higher elevation than any other taxon, is taller than any of the other species, and has curled bracteoles. Further, at three of the four loci studied, all of the *S. procera* alleles differ from any of the sampled *S. gaudichaudii* and *S. mollis* alleles, and for ITS, *S. procera* is fixed for a novel haplotype. Thus, we

conclude that *S. procera* is now an established species of homoploid hybrid origin that has been reproductively isolated from other *Scaevola* species for a considerable time period.

Evolution of Scaevola kilaueae

In the decades since its discovery (Degener 1933), *S. kilaueae* has never been hypothesized to be a hybrid. We found that, whereas it predominantly shares alleles with *S. chamissoniana*, it is fixed for an allele that is shared with *S. coriacea* at *G3PDH*, leading us to hypothesize that *S. kilaueae* is a homoploid hybrid species derived from these two parental species. Morphologically, *S. kilaueae* shares many characteristics with *S. coriacea* (D. G. Howarth, pers. obs.) and is closely related to *S. coriacea* in a morphological cladistic analysis (Patterson 1995). These two species share, for example, a sprawling habit, succulent leaves, a pubescent indusium (on the stigma), and sepals connate without distinct lobes. Equally, however, the flower morphology of *S. kilaueae* resembles that of *S. chamissoniana*, with white flowers (also in *S. coriacea*), wide petal wings, petals longer than the corolla tube, and a style at least as long as the corolla tube. As in *S. procera*, *S. kilaueae* is also additive for hair type having interspersed pseudostellate and simple hairs on its leaves. Additionally, in *S. kilaueae*, the number of flowers in a cyme (Fig. 4) and the length of the peduncles are intermediate between *S. coriacea* and *S. chamissoniana*. *Scaevola kilaueae* is the most restricted species, growing only on relatively young lava flows only on the youngest island of Hawai‘i. *Scaevola chamissoniana* is found on this island, and *S. coriacea* historically grew there as well. The fact that *S. kilaueae* is established on lava flows on the youngest island (0.5 to 1 million years old) and is nearly identical or identical in sequence to one of the putative parental species in three of the four genes examined suggests that this species may have resulted from fairly recent hybridization. This is also supported by the fact that most of the characteristics of *S. kilaueae* are intermediate between the two putative parental species. We hypothesize that this is a stable homoploid hybrid species between *S. chamissoniana* and *S. coriacea* given that it is fixed at three of the four loci, has a derived novel allele at the *LFY* locus, and it grows in a narrow, specialized habitat. As in the case of *S. procera*, the ecological attributes of *S. kilaueae* most likely arose through the combination of genetic material from the two putative parents.

Ecological Novelty and Adaptive Radiation

Natural hybridization is frequent in many plant groups (Ellstrand et al. 1996; Arnold 1997) and has been touted as a potential source of evolutionary novelties. For example, all studied examples of homoploid hybrid species tend to occupy novel (and often marginal) habitats from their parents (Rieseberg 1997; Wolfe et al. 1998; Wang et al. 2001). Indeed, it has been suggested that hybridization could be the quickest way to create new variation, allowing a plant to invade a novel habitat (and possibly have higher fitness than either parent in the new environment; Arnold and Hodges 1995). Furthermore, because such habitat shifts can reduce gene flow between the hybrids and their parent species, ecological shifts may increase the potential for homoploid hybrid species to persist (Buerkle et al. 2000). It is striking, therefore, that the two putative hybrid *Scaevola* species do not live in the same habitats as their inferred parents, but rather they occupy relatively extreme environments. *Scaevola kilaueae* only grows in small populations in novel habitats of young lava flows (Fig. 3), whereas *S. procera* occurs in wetter forests, at higher elevations than either hypothesized parent (Patterson 1990). It is tempting to speculate, therefore, that the novel gene combinations generated during hybrid speciation were necessary for *Scaevola* to establish in these environments.

We hypothesize that unlike the work in *Helianthus* (Rieseberg et al. 1995), the generation of novel gene complexes through hybridization that occurs in many of the Hawaiian plant groups does not cause genomic reorganization. The current data suggest that there may be maintenance of the ability to intercross among any species originating from a single dispersal event (and sometimes even with sister species outside of the Hawaiian Islands; Rabakonandrianina and Carr 1981; Carr et al. 1996; Randell et al. 2004), perhaps due to the use of hybridization as a survival mechanism in dynamic habitats (proposed by Carr 1995). These hypotheses are likely broadly applicable to other oceanic island ecosystems with similar patterns of crossability and chromosomal stasis (Stuessy and Crawford 1998). It appears that most of the reproductive isolation that occurs in the Hawaiian flora is through habitat specificity, and thus prezygotic mechanisms.

Our combined analysis of *Scaevola* led us to conclude that the earliest divergence subsequent to the invasion of the Hawaiian Islands involved habitat specialization, with one clade specializing in dryer areas (*S. coriacea* and *S. gaudichaudii*) and the other in more mesic environments (*S. chamissoniana*, *S. gaudichaudiana*, and *S. mollis*; Fig. 4). Therefore, it is intriguing that both hybrid speciation events happened across this deep divide, involving representatives of these two ecologically divergent clades (Fig. 4). Given the sample size and the possibility that we overlooked hybridization within the dry and mesic-adapted clades, it would be premature to conclude that successful homoploid hybrid speciation required that the parental species be ecologically divergent. Nonetheless, the pattern suggests that hybridization involving ecologically similar species is less likely to result in a stable, reproductively isolated descendant than is hybridization between ecologically distinct species (with ecological specialization into a third, novel environment).

How Common is Hybrid Speciation?

Polyploid speciation has played a significant role in plant evolution (Grant 1981), but homoploid hybrid speciation is thought to be quite rare. However, the perceived scarcity of homoploid hybrid speciation might partially reflect the fact that it is more difficult to detect (Rieseberg 1997). To identify homoploid hybrid species, incongruence among multiple independent genetic loci needs to be demonstrated (Rieseberg et al. 1990; Arnold 1993; Rieseberg 1995), but among recently diverged or slowly evolving plant species, it may be difficult to obtain fully resolved phylogenies for even one locus let alone the two or more needed to have a high likelihood of identifying hybridization events. As a consequence, it is likely that the role of homoploid hybridization has been

A.

species	island[a]	habitat[a]	flowers per cyme[a]	hair type[b]
S. coriacea	all	coastal strand	1-3	pseudostellate
S. gaudichaudii	all	dry ridges and scrubland	1-3	pseudostellate
S. procera	K, Mo	wet forests	1-3	pseudostellate and simple
S. mollis	K, O, and Mo	wet forests	1-3	simple
S. gaudichaudiana	K, O	wet forests	7-18	simple
S. chamissoniana	Mo, L, M, and H	wet forests	7-15	simple
S. kilaueae	H	open forests and scrubland	3-7	pseudostellate and simple

B.

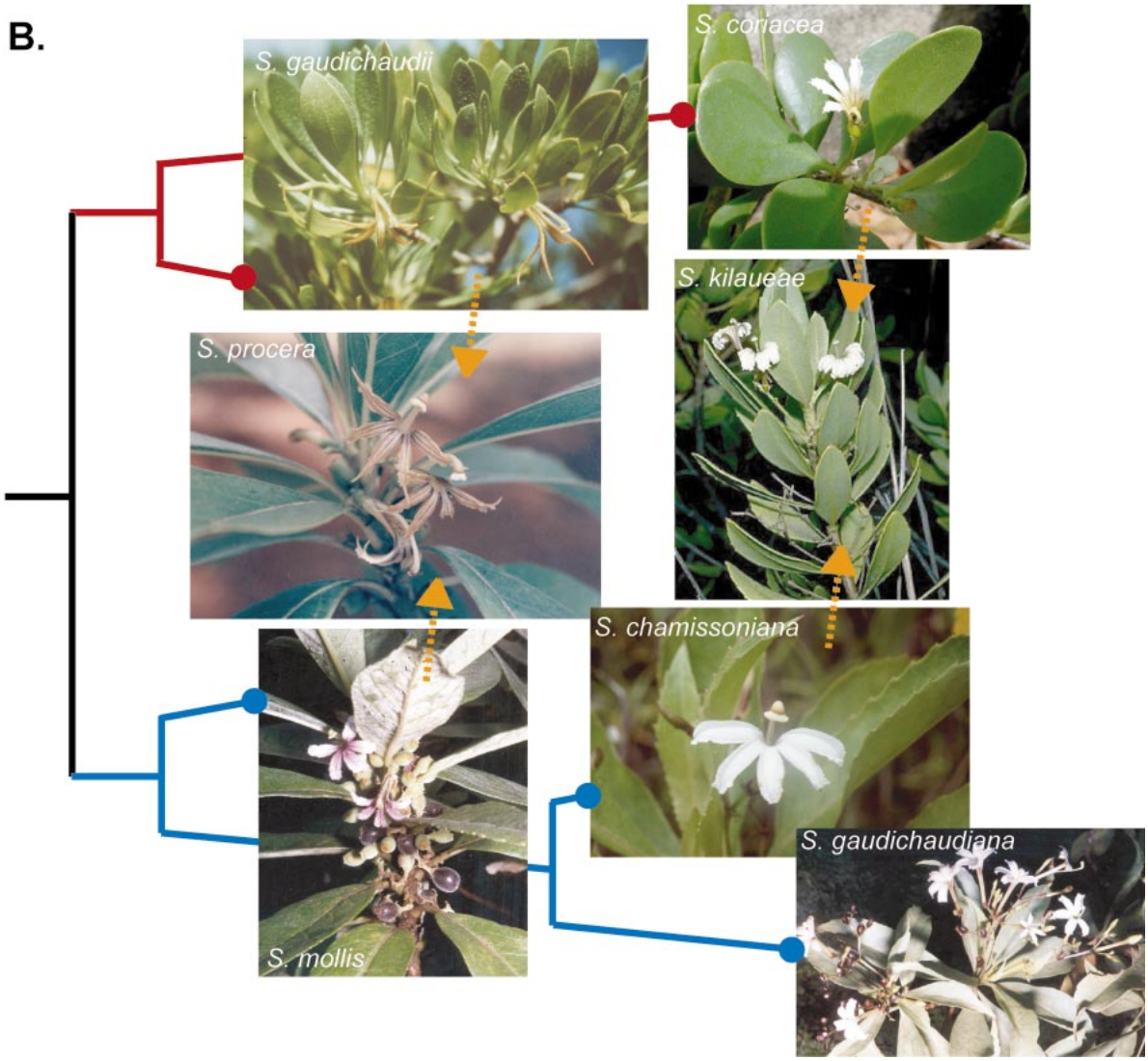

FIG. 4. Hypothesized evolutionary history of all seven extant Hawaiian endemic diploids. (A) Character list and geographical location of species. (B) Images of each species. H, Hawai'i; K, Kaua'i; L, Lana'i; M, Maui; Mo, Moloka'i; O, O'ahu. Photographs of *Scaevola coriacea*, *S. gaudichaudii*, and *S. kilaueae* are all reprinted with permission from the Smithsonian Institution, Department of Botany. Photograph of *S. gaudichaudii* by J. K. Obata; photographs of *S. coriacea* and *S. kilaueae* by Warren L. Wagner; photographs of remaining species by F. G. Howarth. [a]Patterson 1990; Carolin 1990. [b]D. G. Howarth, pers. obs.

dramatically underestimated. This is especially likely among the endemic plants of islands (such as Bonin, Juan Fernandez, and Macaronesian Islands; Crawford et al. 1993; Francisco-Ortega et al. 1996; Setoguchi and Watanabe 2000; Mort et al. 2002). A tantalizing number of hybridization events have been hypothesized, from large clades of island species with only partially resolved phylogenies, suggesting exciting possibilities for an increase in that number when the groups are fully resolved (e.g., Hawaiian Islands [Baldwin et al. 1990; Lindqvist et al. 2003] and Canary Islands [Francisco-Ortega et al. 1997; Mort et al. 2002]). These considerations raise the intriguing possibility that homoploid hybrid speciation is not just an important contributor to adaptive radiation, but, by facilitating dramatic ecological shifts, it may be an engine of adaptive radiation on islands such as those of the Hawaiian Archipelago.

Here, we report the first study using multiple variable nuclear introns from multiple individuals to examine a closely related plant clade and find that homoploid hybrid speciation has played a significant role in the origin of two of seven species. This finding suggests that homoploid hybrid speciation may be much more common than currently thought, and the discovery and use of additional molecular tools for studying closely related species should pave the way for a much deeper understanding of evolution.

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