Evolutionary relationships, interisland biogeography, and molecular evolution in the Hawaiian violets (*Viola*: Violaceae)[1]

J. Christopher Havran,[2,5] Kenneth J. Sytsma,[3] and Harvey E. Ballard Jr.[4]

[2]Department of Biology, Campbell University, Buies Creek, North Carolina 27506 USA; [3]Laboratory of Vascular Plant Systematics and Evolution, Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701 USA; and [4]Department of Botany, University of Wisconsin, Madison, Wisconsin 53706 USA

The endemic Hawaiian flora offers remarkable opportunities to study the patterns of plant morphological and molecular evolution. The Hawaiian violets are a monophyletic lineage of nine taxa distributed across six main islands of the Hawaiian archipelago. To describe the evolutionary relationships, biogeography, and molecular evolution rates of the Hawaiian violets, we conducted a phylogenetic study using nuclear rDNA internal transcribed spacer sequences from specimens of each species. Parsimony, maximum likelihood (ML), and Bayesian inference reconstructions of island colonization and radiation strongly suggest that the Hawaiian violets first colonized the Maui Nui Complex, quickly radiated to Kaua'i and O'ahu, and recently dispersed to Hawai'i. The lineage consists of "wet" and "dry" clades restricted to distinct precipitation regimes. The ML and Bayesian inference reconstructions of shifts in habitat, habit, and leaf shape indicate that ecologically analogous taxa have undergone parallel evolution in leaf morphology and habit. This parallel evolution correlates with shifts to specialized habitats. Relative rate tests showed that woody and herbaceous sister species possess equal molecular evolution rates. The incongruity of molecular evolution rates in taxa on younger islands suggests that these rates may not be determined by growth form (or lifespan) alone, but may be influenced by complex dispersal events.

Key words: adaptive radiation; biogeography; character evolution; dispersal; Hawaiian Islands ITS; relative rate; *Viola*; Violaceae.

The endemic Hawaiian flora provides many stunning examples of adaptive radiation (Givnish, 1997; Schluter, 2000)—two examples being the tarweeds (Baldwin et al., 1990; Baldwin and Robichaux, 1995; Baldwin, 1997; Carlquist et al., 2003) and lobeliads (Givnish et al., 1995, 2004, 2009). In the context of the physical geography of the Hawaiian Islands, this flora provides many opportunities to investigate the patterns and processes of plant morphological and molecular evolution. Allopatry (through interisland dispersal events) and parapatry have been shown to contribute to the majority of speciation events in endemic Hawaiian angiosperm lineages (Funk and Wagner, 1995; Price and Wagner, 2004). The Hawaiian Islands represent a chronosequence of islands formed along a classic "volcanic conveyor belt", with older islands in the northwest Pacific (Kure Atoll) and younger, currently building islands in the southeast (Hawai'i) (Carson and Clague, 1995). The well-documented chronology of the Hawaiian archipelago (Carson and

Clague, 1995; Price and Elliott-Fisk, 2004) provides a framework to elucidate the establishment, interisland dispersal, and diversification of lineages across the main islands of the Hawaiian archipelago (Funk and Wagner, 1995).

Each Hawaiian island was subjected to similar climatic and erosional forces during its development (Mueller-Dombois and Fosberg, 1998; Wagner et al., 1999; Vitousek, 2004). Habitat regimes are typically arranged along an elevation and precipitation gradient (Mueller-Dombois and Fosberg, 1998). High-elevation bogs and cloud forests are found on Kaua'i (e.g., Alaka'i Swamp), Moloka'i, Maui, (e.g., Pepe'opae and Pu'u Kukui), and Hawai'i, while lower-elevation leeward regions are typically dry to mesic (Mueller-Dombois and Fosberg, 1998). The islands of Maui, Moloka'i, Lana'i, and Kaho'olawe were connected continuously until 600 kya and were connected intermittently until their separation about 20 kya (Carson and Clague, 1995). Due to the shared geological history between these islands, they are typically represented as a single biogeographic unit: Maui Nui. The younger island of Hawai'i currently lacks well developed high-elevation bogs and dry cliffs, which are found on older, more weathered islands. The iterative habitat regimes across the archipelago would be expected to facilitate the parallel evolution of congeneric taxa in similar habitats on different islands, establishing morphologically similar but phylogenetically distinct species across islands and morphologically divergent sister species in different intraisland habitats (Givnish and Sytsma, 1997; Losos et al., 1998, 2006; Givnish et al., 2009). Comparison of trait parallelisms in similar habitat regimes can potentially provide insight into the processes of functional (and adaptive) trait evolution (Ackerly and Monson, 2003; Ackerly et al., 2000; Webb et al., 2002, 2006).

The Hawaiian violets (*Viola*: Violaceae) (Fig. 1) are a monophyletic lineage of mostly woody angiosperms that have undergone a putative adaptive radiation on the Hawaiian Islands

Fig. 1. The Hawaiian violets. (A) *Viola kauaensis*: Alaka'i Swamp, Kaua'i; (B) *V. wailenalenae*: Koke'e State Park, Kaua'i; (C) *V. chamissoniana*: Waianae Mountains, O'ahu; (D) *V. maviensis*: Pepeopa'e Bog, Moloka'i; (E) *V. robusta*: Kamakou Preserve, Moloka'i; (F) *V. lanaiensis*: Lanaihale, Lana'i; (G) *V. tracheliifolia*: Waimea Canyon, Kaua'i; (H) *V. oahuensis*: Ko'olau Mountains, O'ahu; (I) *V. helenae*: Wahiawa Drainage: Kaua'i. *Photo credits*—A, B, D, and E: J. Christopher Havran; C and H: O'ahu Army Natural Resources Program; F: Hank Oppenheimer; G: Michelle Clark; I: R. Gustafson.

(Ballard et al., 1998; Ballard and Sytsma, 2000). The lineage consists of nine species (Ballard et al., 1998) distributed on six islands of the Hawaiian archipelago: Kaua'i, O'ahu, Maui Nui (Moloka'i, Maui, and Lana'i), and Hawai'i (Fig. 1). The lineage was most likely established through a single colonization event, probably facilitated by migratory birds (Ballard and Sytsma, 2000). The sister species to the lineage is *V. langsdorffii* Ging., a rhizomatous violet that ranges from northern California, USA to Hokkaido, Japan (Ballard et al., 1998; Ballard and Sytsma, 2000) across the northern Pacific boreal region called Beringia. Only one species of Hawaiian violet has a herbaceous growth form similar to *V. langsdorffii*.

Differences in molecular evolution rates between species have often been attributed to differences in duration or growth form (i.e., herbaceous vs. woody) (Sanderson, 1998). An herbaceous growth form is expected to have a greater number of generations over unit time than a woody growth form, therefore contributing to a higher rate of mutation and molecular evolution (Eriksson and Bremer, 1992; Dodd et al., 1999). For many island radiations, dispersal to neighboring or newly established islands is most often associated with a speciation event and/or a unique radiation on that new island (Funk and Wagner, 1995).

We conducted a phylogenetic study of the Hawaiian violets using 16 nuclear rDNA internal transcribed spacer (ITS) se-

quences (14 ingroup populations and two outgroup populations) to explore hypotheses of phylogenetic relationships, interisland biogeography, diversification, and rates of molecular evolution. We sampled each species of Hawaiian violet, and incorporated individuals of the same species on different islands. We asked five questions: (1) What are the phylogenetic relationships of the Hawaiian violets? (2) Where was the ancestor to the Hawaiian violets established? (3) What habitat did the ancestor to the Hawaiian violets first occupy? (3) What are the diversification and dispersal patterns of the species across the Hawaiian Islands? (4) Are there differences in the rates of molecular evolution between herbaceous and woody taxa as well as between conspecific populations on different islands?

MATERIALS AND METHODS

Species descriptions—Each species of Hawaiian violet is restricted to one of five vegetation regimes: dry forest, dry cliff, mesic forest, cloud forest, and montane bog. *Viola helenae* C. Forbes and Lydgate and *V. lanaiensis* Becker possess lanceolate leaves and are restricted to the mesic banks of streams in low or midelevation mesic forests of Kaua'i and Lana'i, respectively. *Viola waile-nalenae* (Rock) Skottsb., *V. oahuensis* Forbes, and *V. robusta* are restricted to the wet, high-elevation cloud forests of Kaua'i, O'ahu, and Moloka'i, respectively. *Viola wailenalenae* and *V. robusta* are monopodial subshrubs producing petiolate, broadly elliptical leaves with cuneate leaf bases. *Viola oahuensis* may occur as a single or multistemmed shrub producing obovate leaves with sessile bases. Herbaceous *V. kauaensis* and woody *V. maviensis* H. Mann possess short- to long-petiolate leaves, orbicular to reniform blades borne on reclining to creeping stems, and are restricted to the high-elevation, montane bogs. *Viola kauaensis* is abundant in the Alaka'i Swamp of Kaua'i, and rare on O'ahu. *Vi-ola maviensis* is abundant in the Pepe'opae Bog on Moloka'i, Pu'u Kapu'u on Maui, the Kohala Mountains of Hawai'i (BISH: C. N. Forbes 925.H), the windward slope of Mauna Loa (BISH: Forbes 929.H), and has been collected throughout the eastern portion of Hawai'i. *Viola tracheliifolia* is a highly branched shrub bearing long-petioled, long-pointed leaves with truncate to cordate bases, that occupies modal, narrowly specific habitats with dry soils in midelevation dry to mesic forests of Kaua'i, O'ahu, Moloka'i, and Maui. *Viola chamissoniana* is a short highly branched shrub with small leaves, restricted to the dry cliffs of central O'ahu.

On the basis of apparent leaf morphological intergradation and unclear taxonomic or ecological boundaries, Wagner et al. (1999) treated *V. tracheliifolia* Ging. and *V. robusta* Hbd. as subspecies of *V. chamissoniana* Ging. Intergrades between the three species have been reexamined and have proven to be either misidentified variants of one taxon or hybrid individuals. We are currently maintaining all three taxa at the species rank.

Taxa sampling—Sequences were obtained from 14 populations and taxon representatives of nine morphologically and ecologically distinct species. One representative from each island was sampled (Table 1). Ten sequences, representing taxa in Kaua'i, O'ahu, Hawai'i, Alaska, and Japan, based on previous studies (Ballard et al., 1998; Ballard and Sytsma, 2000), were obtained from GenBank. Four additional sequences (*V. maviensis*, *V. tracheliifolia*, and *V. robusta* from Moloka'i; and *V. maviensis* from Maui) were generated from material collected on Maui and Moloka'i in 2003 and 2006, respectively.

Sequencing and alignment—Genomic DNA was extracted with a Promega Wizard Genomic DNA Purification Kit (Madison, Wisconsin, USA) and purified using a 6% CTAB protocol (Doyle and Doyle, 1987). The polymerase chain reaction (PCR) (Mullis and Faloona, 1987) was used to amplify sequences with the primer sequence 5′-GTCCACTGAACCTTATCATTTAG-3′ ("ITS leu 1"; Ballard et al., 1998) and 5′-TCCTTCCGCTTATTGATATGC-3′ ("ITS 4"; White et al., 1990; Ballard et al., 1998), as described by Baldwin et al. (1995). PCR products were cleaned with a Promega Wizard PCR Preps DNA Purification System, quantified using a Nano-Drop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA), and sequenced on an Applied Biosystems 3130 × 1 Genetic Analyzer (Foster City, California, USA) using primer sequence 5′-GGAAGGAGAAGTCGTAACAAGG-3′ ("ITS 5"; White et al., 1990; Ballard and Sytsma, 2000) for ITS 1 and 5′-GCATCGATGAAGAACGTAGC-3′ ("ITS 3"; Baum et al., 1994; Ballard and

Sytsma, 2000) for ITS2 with substantial overlap of the two sequences in the middle of the gene region.

The ITS1 and ITS2 spacer boundaries were identified against sequences for previously published Hawaiian violet sequences (Ballard et al., 1998; Ballard and Sytsma, 2000). Sequences were aligned visually in the programs PAUP* version 4.01b (Swofford, 1998) and Sequencher version 4.7 (Gene Codes, Ann Arbor, Michigan, USA). Data for the 18S- and 26S-coding regions flanking the ITS region and the 5.8S-coding region between the ITS1 and ITS2 spacers were excluded due to the lack of informative characters (Ballard and Sytsma, 2000).

Phylogenetic analyses—Phylogenetic analyses of the ITS1 and ITS2 sequences included maximum likelihood (ML) (Felsenstein, 1981), parsimony (MP) (Fitch, 1971), and Bayesian analysis (BA) (Huelsenbeck and Ronquist, 2001; Posada and Buckley, 2004). Parsimony and maximum likelihood analysis were conducted in PAUP* v. 4.01b (Swofford, 1998). The appropriate model of sequence evolution (HKY+G), base frequencies (A = 0.1953, C = 0.3294, G = 0.3062, T = 0.1692), rate matrix (A-C = 0.3978, A-G = 2.6372, A-T = 0.3107, C-G = 0.7810, C-T = 2.9038), and transition : transversion ratio (1.2751) for the sequences was determined with the program Modeltest version 3.7 (Posada and Crandall, 1998). Parsimony analysis was conducted under the constraints of 10 000 random-addition sequences and tree-bisection-reconstruction (TBR) branch swapping. Internal support in the parsimony tree was measured from 10 000 replicates in bootstrap analysis (Felsenstein, 1985) and jackknife analyses (Farris et al., 1996) with 36.8% character deletion; consistency index (CI) (Kluge and Farris, 1969) and retention index (RI) (Archie, 1989; Farris, 1989) measured internal consistency of the Fitch parsimony analysis. Bayesian analysis was conducted in the program MrBayes version 3.0b4 (Ronquist and Huelsenbeck, 2003) using four Markov chains and 500 000 generations, sampling every 100 generations. Posterior probabilities were calculated using the final 4001 trees.

Patterns of island colonization and dispersal—Parsimony reconstructions of geographic distribution were carried out in the program MacClade version 3.0 (Maddison and Maddison, 1992). Although the Hawaiian island chain is comprised of eight high islands currently, geologically these comprise four island groups (Carson and Clague, 1995): (1) Kaua'i/Ni'ihau; (2) O'ahu; (3) Moloka'i, Lana'i, Maui, and Kaho'olawe ("Maui Nui") and (4) Hawai'i. Outgroup taxa were coded as non-Hawaiian. Island distributions (unordered) were traced onto the more resolved ML tree (also one of the two MP trees), which was nearly identical to the Bayesian inference tree (also the second of the MP trees). Accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN) options were used to select among the most parsimonious ancestral state reconstructions (Swofford and Maddison, 1987). Ancestral states of equivocal tracings were investigated to determine the fewest number of steps necessary to derive the current distribution of violet species across the Hawaiian archipelago.

Colonization and dispersal across the Hawaiian Islands were reconstructed under ML (Pagel, 1999) and Bayesian (Pagel et al., 2004) criteria using the BayesMultiState option (ML or MCMC modes of analysis) in the program BayesTraits version 1.0 (Pagel and Meade, 2007). To reduce the uncertainty and arbitrariness of choosing priors under MCMC, we used the hyperprior approach (the rjhp command) as recommended (Pagel et al., 2004; Pagel and Meade, 2007). Combinations of hyperprior values (exponential or gamma, mean and variance) and rate parameter values were explored to find acceptance rates when running the Markov chains of between 20 and 40% (as recommended by Pagel and Meade, 2007). All subsequent analyses used the reversible-jump hyperprior command (rjhp gamma 0 10 0 10) that seeded the mean and variance of the gamma prior from uniform hyperpriors both on the interval 0–10 and a rate parameter of 100 (ratedev 100). We explored the use of several possible fully resolved trees of the ML tree (involving *V. oahuensis*, *V. lanaiensis*, and the *V. maviensis* clade), with branch lengths retained, to determine if significant differences were generated in interpretation. Each analysis generated 20 transition rates (q_{01}, q_{02}…q_{42}, q_{43}) for all possible interisland and source area dispersal events (Beringia = 0, Kaua'i = 1, O'ahu = 2, Maui Nui = 3, Hawai'i = 4). Nonzero q transition rates should be interpreted in a relative manner, as the magnitude of the rate is dependent on parameter values and branch lengths of the ITS phylogeny (not scaled to the total length of the tree).

Patterns of habitat colonization and habit and leaf form evolution—Reconstruction of ancestral features relating to growth form and leaf shape in the first colonizing Hawaiian violet and its ancestral habitat was estimated under ML and Bayesian inference in BayesTraits using the same assumptions and ML

TABLE 1. Voucher and GenBank accession numbers for individuals sampled for ITS sequences of taxa of Hawaiian *Viola* and outgroups.

Taxon	Voucher / ITS1 and ITS2 GenBank accession numbers
V. langsdorffii Ging. (outgroup)	USA, Alaska, 20 July 1975, *Craighead s.n.* (WIS) [AF097259, AF097305]
V. langsdorffii Ging. (outgroup)	Japan, Hokkaido, Sorachi, *Murata, Koyama and Yahara 37863* (WIS) [FJ895309, FJ895318]
V. chamissoniana Ging.	USA, Hawai'i, O'ahu, *Wood 1833, Perlman and Obata* (US) [AF115955, AF115959]
V. helenae C. Forbes & Lydgate	USA, Hawai'i, Kaua'i, 30 June 1993, *Perlman s.n.* (PTBG) [AF097261, AF097307]
V. kauaensis A. Gray	USA, Hawai'i, Kaua'i, [no date], *Nepokroeff 786b* (WIS) [AF097262, AF097308]
V. kauaensis A. Gray	USA, Hawai'i, O'ahu, 12 March 1995, *Perlman and Garnett 14704* (PTBG) [AF115957, AF115961]
V. lanaiensis Becker	USA, Hawai'i, Lana'i, 12 February 1997, *Wood 6008 and Perlman* (PTBG 023123) [site voucher; FJ895310, FJ895319]
V. maviensis H. Mann	USA, Hawai'i, Moloka'i, 1 July 2007, *Havran 2001 and Sack* (BISH) [FJ895311, FJ895320]
V. maviensis H. Mann	USA, Hawai'i, Maui, [no date], *Nepokroeff 850* (BHO) [FJ895312, FJ895321]
V. maviensis H. Mann	USA, Hawai'i, Hawai'i, [no date], *Nepokroeff 910* (WIS) [FJ895313, FJ895322]
V. oahuensis Forbes	USA, Hawai'i, O'ahu, 12 October 1987, *Perlman 6586, Obata and Hill* (US 3235857) [FJ895314, FJ895323]
V. robusta Hbd.	USA, Hawai'i, Moloka'i, 1 July 2007 *Havran 2002 and Sack* (BISH) [FJ895315, FJ895324]
V. tracheliifolia Ging.	USA, Hawai'i, Kaua'i, 16 June 1993, *Nepokroeff 774* (WIS) [AF097261, AF097307]
V. tracheliifolia Ging.	USA, Hawai'i, O'ahu, 6 December 1987, *Perlman 7053 and Obata* (US 3235812) [FJ895316, FJ895325]
V. tracheliifolia Ging.	USA, Hawai'i, Moloka'i, 30 June 2007, *Havran 2004* (BISH) [FJ895317, FJ895326]
V. wailenalenae (Rock) Skottsb.	USA, Hawai'i, Kaua'i, 12 March 1981, *Warshauer 3383 and McEldowney* (BISH) [AF115958, AF115962]

trees as in the biogeographical analysis. Taxa were scored for the following habitat states: montane bog, cloud forest, mesic streambank, dry cliff, or dry forest. Habit or growth form states included rhizomatous herb, dwarf subshrub, few branched subshrub, shrub-treelet, or many-branched shrub. Leaf form states included petiolate orbicular, petiolate ovate, petiolate lanceolate, sessile obovate, petiolate short-pointed, or petiolate long-pointed cordate. All characters were treated as unordered.

Molecular evolution rates—Potential differences in molecular evolution rates were tested through the Tajima test (Tajima, 1993) using Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (Tamura et al., 2007). seven comparisons were made within the violet sublineages on Kaua'i and O'ahu (between *V. kauaensis*, *V. wailenalenae*, and *V. helenae*), and Maui Nui and Hawai'i (between *V. maviensis* and *V. robusta*). Within the Kaua'i and O'ahu group, three independent comparisons were made between *V. kauaensis* on Kaua'i and O'ahu and *V. wailenalenae* (Table 2). On Maui Nui and Hawai'i, four independent comparisons were made between *V. maviensis* on Maui, Moloka'i, and Hawai'i, and *V. robusta* on Moloka'i (Table 2). *P*-values less than 0.05 were used to reject the null hypothesis of equal molecular evolution rates between lineages (Tajima, 1993).

RESULTS

Sequence characteristics—Characteristics of sequences for the new accessions fall well within the values for unaligned length, and numbers of variable and phylogenetically informative characters, previously reported by Ballard and Sytsma (2000) for Hawaiian violets. In every instance, PCR amplification resulted in a single, sharp fragment, and sequences generated from these single products were clean and unambiguous. No multiple products were found during the study. While the Hawaiian violet lineage is presumed to be a relatively highly polyploid group, the ITS region provides no difficulties for sequencing, indicating that concerted evolution across multiple loci has proceeded to the extent that all loci have the same sequence. Issues of paralogy, often cited as a concern for ITS sequence utilization in other groups, are not apparent for the Hawaiian violets. Multiple island accessions for certain species always fall out together, suggesting that the ITS sequence includes only single copy loci and is providing a phylogenetically meaningful signal.

Phylogenetic analyses—The three phylogenetic analyses yielded trees with nearly or fully identical topologies (Fig. 2). Parsimony analysis resulted in two trees (topologies represented

in Fig. 2A and Fig. 2B) of length 75 with consistency index (CI) of 0.694 (excluding uninformative characters) and a retention index (RI) of 0.761. Of 470 characters, 24 were phylogenetically informative. The two topologies differ only in the collapse of one node in the dry forest/dry cliff "dry" clade. The ML and Bayesian analyses generated the trees shown in Fig. 2A and B, respectively. Branch length is very short in the one branch that differs between the two trees. Moderate to high bootstrap and jackknife values support most subclades and sister-species pairs. Bootstrap, jackknife, and posterior probabilities support the designation of two sister clades that show different habitat associations: *V. tracheliifolia* and *V. chamissoniana* fall in a dry clade, and the remainder group in a mesic forest-cloud forest-bog "wet" clade. These two clades do not receive strong support under MP or ML, but each receives moderate support under Bayesian inference (0.89 and 0.77 PP, respectively). All analyses indicate a trichotomy involving *V. oahuensis*, *V. lanaiensis*, and the *V. maviensis* clade (Fig. 2). In all analyses, populations of *V. tracheliifolia* from Kaua'i and O'ahu form a well-supported clade. Only in the ML tree and one of the MP trees (Fig. 2A) does *V. tracheliifolia* from Moloka'i represent the sister to the clade of *V. tracheliifolia* from Kaua'i and O'ahu and *V. chamissoniana* from O'ahu (Fig. 2A). In the Bayesian inference tree and in the second MP tree, this node collapses to a trichotomy.

Within the wet clade, there are two subclades, one on Kaua'i and another on O'ahu/Maui Nui/Hawai'i, each with representatives from the mesic forest, cloud forest, and montane bog habitats. *Viola helenae*, a mesic forest violet on Kaua'i, is sister to the rest of the wet clade. On Kaua'i, *V. kauaensis* and *V. wailenalenae* represent a sister species pair of bog and cloud forest species. *Viola robusta*, designated a subspecies of *V. chamissoniana* by Skottsberg (1940) but retained as a species by St. John (1989), emerges as a sister to *V. maviensis* on Moloka'i (Fig. 2). This placement of *V. robusta* is consistent across all three phylogenetic analyses, indicating that the taxon should not be recognized as a subspecies of *V. chamissoniana*. On Moloka'i, *V. maviensis* and *V. robusta* represent a sister species pair of bog and cloud forest species, morphologically and ecologically analogous to the pair on Kaua'i. *Viola lanaiensis*, is placed in an unresolved node with *V. oahuensis* and the *V. maviensis* clade (Fig. 2).

Patterns of island colonization and dispersal: Parsimony reconstruction—Using the ACCTRAN (or DELTRAN, not shown) option in MacClade, the ancestral island for the current
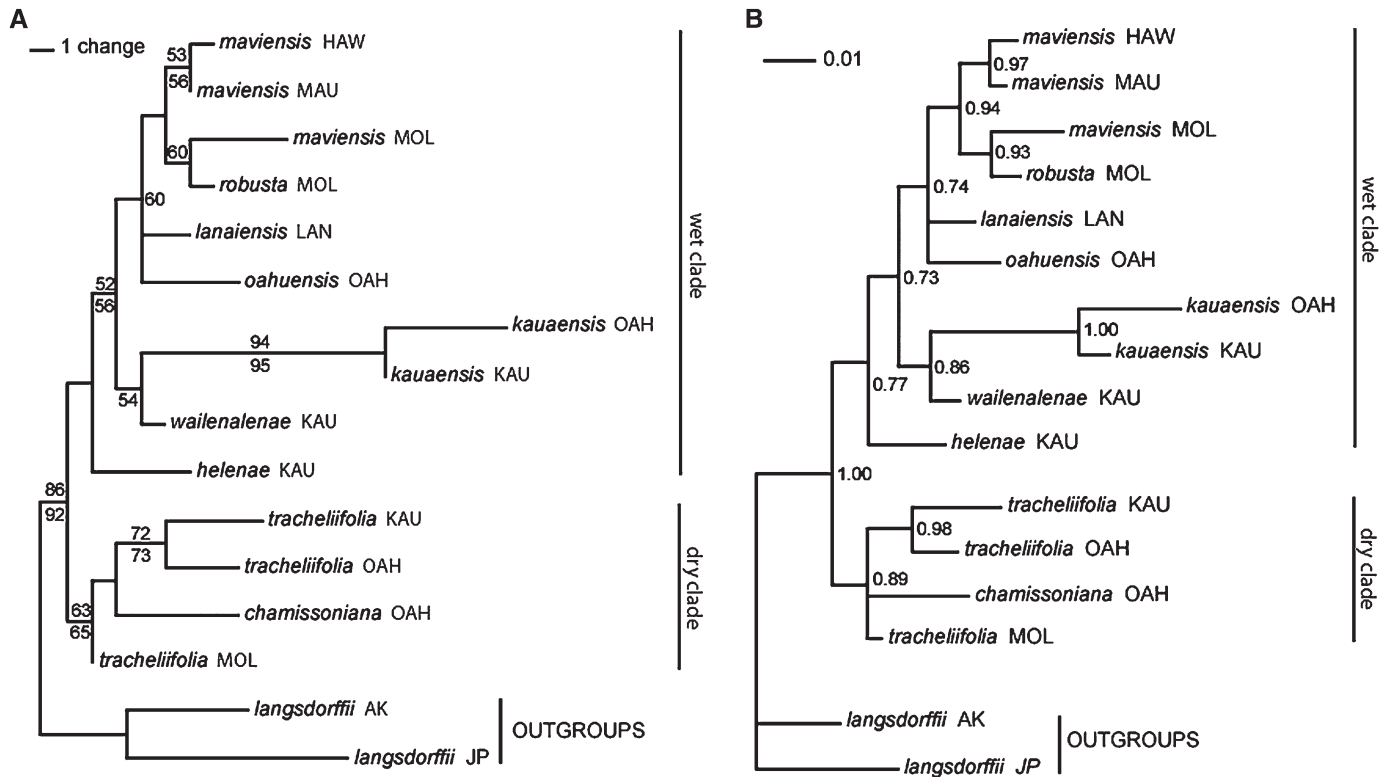
Fig. 2. Phylogenetic relationships among Hawaiian violets. (A) maximum likelihood (ML) tree of ITS 1 and ITS 2 sequences (ML and maximum parsimony reconstructions were congruent); CI = 0.694, CI = 0.849 with all characters, RI = 0.761. Bootstrap values > 50% noted above branches and jackknife values > 50% noted below branches. Solid arrow indicates unique 26-bp deletion in Hawaiian taxa. (B) Bayesian inference tree of Bayesian analysis of ITS1 and ITS2 sequences. Posterior probabilities are noted at branches. KAU = Kaua'i, OAH = O'ahu, MOL = Moloka'i, MAU = Maui, HAW = Hawai'i.

radiation of Hawaiian violets is unclear—Kaua'i, O'ahu, and Maui Nui are all possible (Fig. 3). Between the two main clades of Hawaiian violets, the wet clade, consisting of *V. maviensis*, *V. robusta*, *V. kauaensis*, *V. wailenalenae*, *V. oahuensis*, *V. helenae*, and *V. lanaiensis*, clearly has an origin on Kaua'i, whereas the origins of the dry clade are equivocal (Fig. 3). Within the wet clade, a dispersal event of *V. kauaensis* from Kaua'i to O'ahu has occurred. A Maui Nui origin for the *V. maviensis* clade is suggested. However, *V. oahuensis* and *V. lanaiensis* represent a polytomy, indicating that *V. oahuensis* may have had its origin on O'ahu or on Maui Nui followed by back-colonization to O'ahu. *Viola maviensis* from Hawai'i has dispersed from Maui Nui, most likely from Maui. In contrast, the DEL-TRAN option indicates an equivocal origin of the wet clade. *Viola kauaensis* and the *V. maviensis* clade have origins on Kaua'i and Maui Nui from an equivocal ancestor, respectively. A Maui Nui origin for the dry clade is suggested. Subsequent dispersals to O'ahu and Kaua'i for *V. tracheliifolia* are equivocal. Reconstruction of ancestral states in MacClade (Maddison and Maddison, 1992) revealed that the entire Hawaiian violet lineage most likely had an origin on Kaua'i, the oldest island in the archipelago. An origin on Kaua'i resulted in eight steps to the current distribution compared to nine steps for an origin on Maui Nui. Although the ACCTRAN and DELTRAN options indicated a putative origin of the dry clade on Maui Nui, manipulation of ancestral states indicated that the clade most likely originated on Kaua'i or O'ahu (eight steps each). The origin of *V. oahuensis* was not resolved. There are an equal number of

steps (eight) for an origin on O'ahu or an origin on Maui Nui followed by a back-colonization to O'ahu.

Patterns of island colonization and dispersal: ML and Bayesian inference reconstructions—Unlike parsimony reconstructions, both ML and Bayesian inference reconstructions use branch lengths in placing support for a given island at a node (see Appendices 1–4 for ML and Bayesian inference scores; selected significant scores are presented in brackets in this and subsequent paragraphs). ML and Bayesian inference support values for islands at each node were generally similar with significant differences seen only at two nodes (E, H) at the base of the wet clade (Fig. 4A). An origin of the extant Hawaiian violets on Maui Nui was implicated under both ML (0.9222) and Bayesian inference (0.7565) (Fig. 4A, node A). The dry clade then originated on Maui Nui (Fig. 4A, node B), with back colonizations to O'ahu and then to Kaua'i, as suggested under parsimony reconstruction (Fig. 3). The origination of the wet clade on Kaua'i, as suggested under parsimony reconstruction (Fig. 3), is not supported by either ML or Bayesian inference (Fig. 4A, node E). ML weakly places the origin of the wet clade (and the dry clade) on Maui Nui, although not strongly (0.6617). Bayesian inference is more ambiguous and places weak support for the origin of the wet clade on Maui Nui (0.3195) and Kaua'i (0.4195). A Mau Nui origin would entail diversification of the *V. maviensis* subclade (Fig. 4A, node I) on Maui Nui (with dispersals to O'ahu and Hawai'i) and also subsequent dispersals back to Kaua'i (and then forward to O'ahu) in the *V. kauaensis*
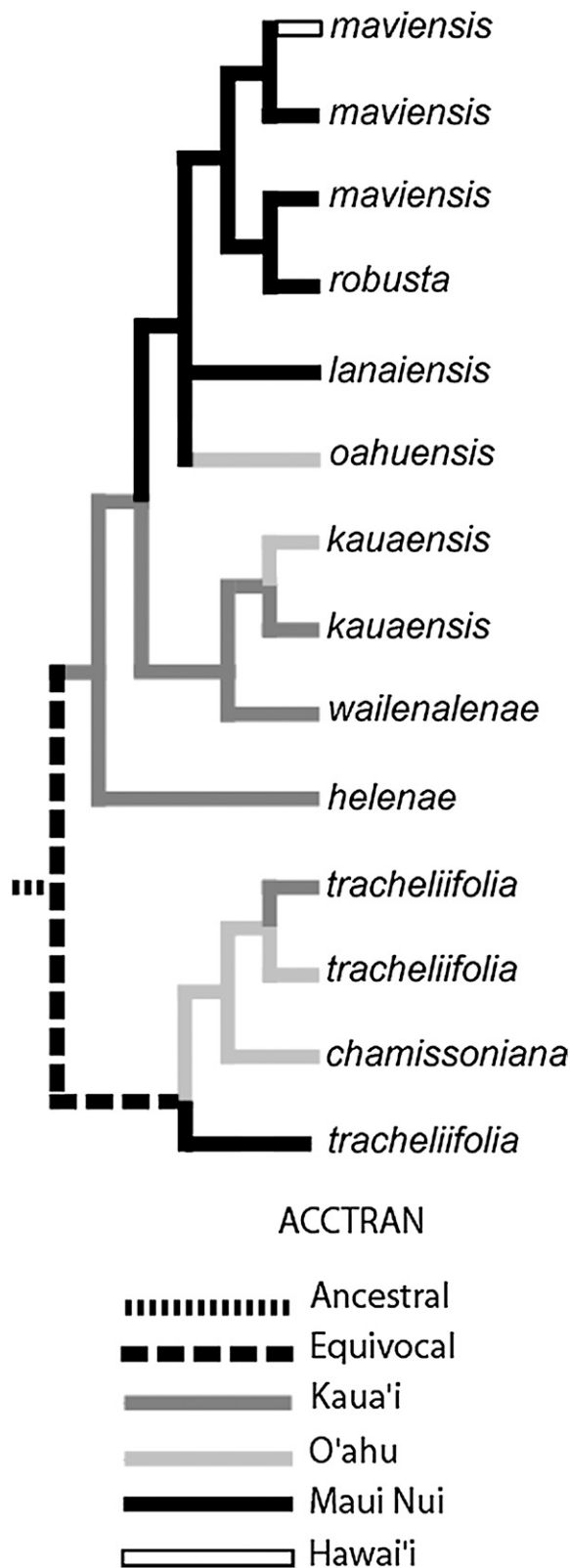
ACCTRAN

Fig. 3. Biogeographic hypothesis mapped onto the maximum likeli-hood tree using the ACCTRAN option in MacClade v. 3.0 (Maddison and Maddison 1992). Shadings indicate island tracings on each branch. The ancestral condition is seen leading into the Hawaiian clade.

subclade (Fig. 4A, nodes F, G). On the other hand, a Kaua'i ori-gin of the wet clade would in turn indicate that it was the *V. kauaensis* subclade that diversified in place with subsequent dispersals to Mau Nui and other islands giving rise to the *V. maviensis* subclade.

Interisland transition rates indicate the relative amount of di-rectional dispersal from one island to another and are dependent on the unit of branch lengths used in the ITS molecular phylog-eny (substitutions per nucleotide site). The pattern of transition rates supports Maui Nui as the island of colonization (Fig. 5A, B). All other nonzero rates indicate dispersal either (1) from Mau Nui to the other three main islands or (2) reciprocal disper-sals between Kaua'i and O'ahu.

Patterns of habitat colonization and habit and leaf form evolution—The original habitat occupied by ancestors of the Hawaiian violets can be either dry forest or cloud forest (Fig. 4B) (with the sister species of Hawaiian violets, *V. langsdorffii*, scored for "montane bog" although it occurs in ecologically similar, wet and cold, boggy tundra conditions). ML supports cloud forest (0.8920) and Bayesian inference indicates dry for-est (0.6090) at the base. Within the dry clade, subsequent diver-sification from dry forest to the dry cliff habitat has occurred once. Cloud forest and mesic streambank habitats are equally likely at the base of the wet clade with both ML and Bayesian inference (Fig. 4B, node E). Secondary shifts to montane bog from either cloud forests or mesic streambank conditions are more recent and occur in parallel in both the *V. kauaensis* and *V. maviensis* subclades (Fig. 4B, nodes F, J).

Growth-form evolution largely tracks habitat shifts (Fig. 4C). The basal condition within Hawaiian violets is somewhat unclear in terms of habit. ML provides strong support (0.9885) for the shrub-treelet condition at the base (Fig. 4C, node A), the habit common in the dry clade. Bayesian inference, how-ever, provides weaker support for the shrub-treelet state (0.5621) and some support (0.3369) for the few-branched sub-shrub state that is the ancestral state for the wet clade. Within the dry clade a single shift from shrub-treelets to a many-branched shrub coincides with the invasion of dry cliffs (Fig. 4C, node C). Within the wet clade, largely comprising few-branched subshrubs, the evolution of both dwarf subshrubs and rhizomatous herbs coincides with the invasion of high el-evation bogs (Fig. 4C, nodes K, F, respectively). The rhizom-atous herb habit of *V. kauaensis* is thus a reversal back to the habit seen in the outgroup species *V. langsdorffii* from boggy conditions of Beringia.

Leaf shape evolution, like growth form, is correlated with shifts in habitat (Fig. 4D). The ancestral condition within the Hawaiian violets is ambiguous (Fig. 4D, node A). ML moder-ately supports (0.7489) as the basal condition the petiolate-ovate leaves occurring in two wet clade species. Bayesian inference provides little support for this state (or most others) at the base of the Hawaiian violets, but weakly supports (0.5752) the common dry clade state of petiolate-cordate leaves with long tips. A minor but noticeable shift to petiolate, short-tipped leaves in *V. chamissoniana* occurs in concert with the shift to the dry cliff habitat (Fig. 4D, node C). Petiolate ovate or petio-late-lanceolate leaves represent the basal conditions within the wet clade. Sessile obovate leaves evolve in only *V. oahuensis* from one of these petiolate leaf types (Fig. 4D, node I). The shift in the wet clade from the ancestral petiolate-ovate or lan-ceolate leaves to petiolate-orbicular leaves occurs twice with the parallel shift to high elevation bogs in the *V. maviensis* and
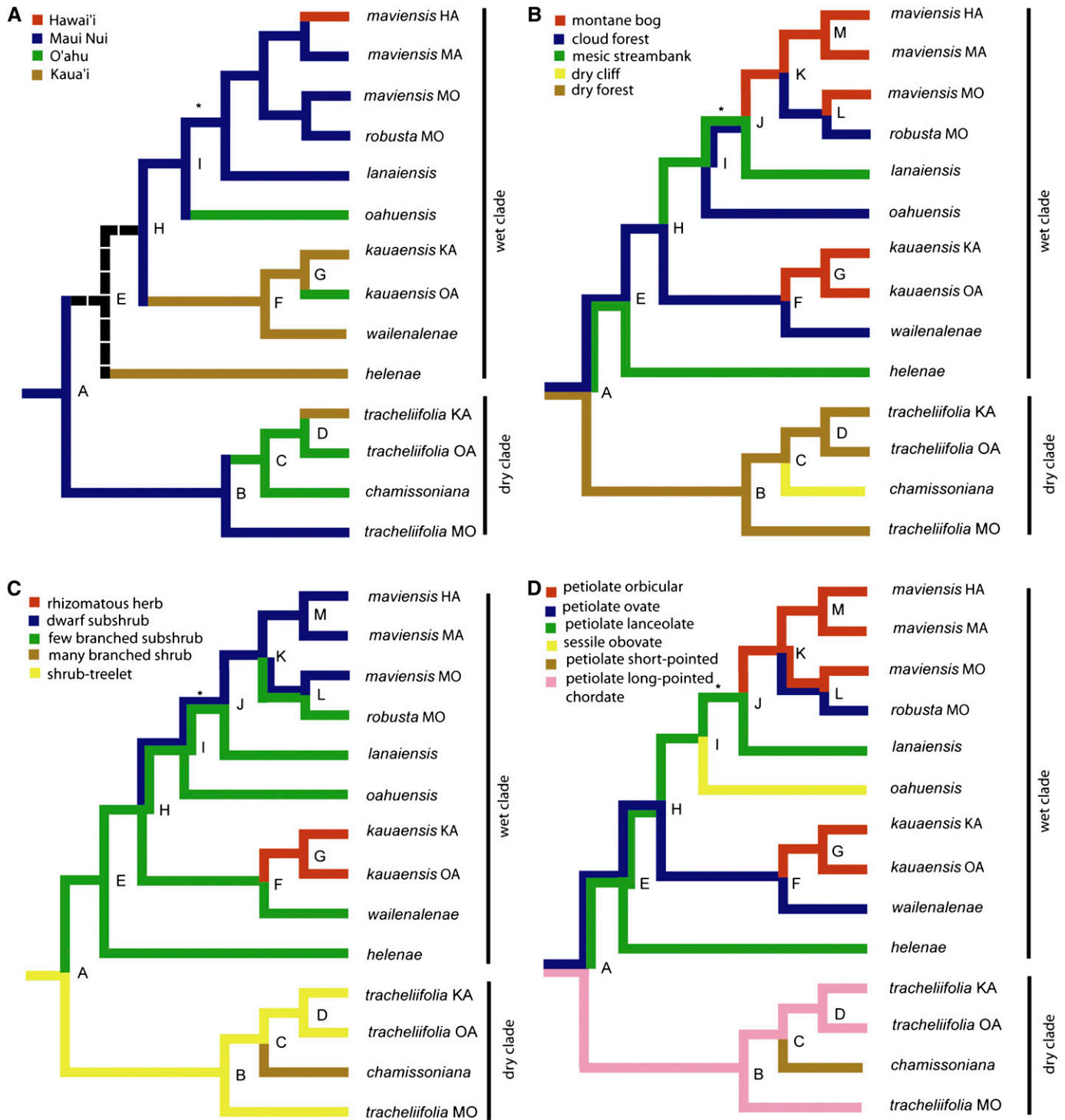
Fig. 4. Biogeographic and trait evolution hypotheses in Hawaiian violets from BayesMultistate analyses. Significant posterior probability values mapped onto ancestral nodes of the maximum-likelihood tree (Fig. 2A). Outgroups are not depicted on trees. (A) Interisland biogeography. Maximum likelihood and posterior probabilities are in Appendix 1. (B) Habitat associations; the ancestral condition is a bog habitat. Maximum likelihood and posterior probabilities are in Appendix 2. (C) Growth form; the ancestral condition is rhizomatous herb. Maximum likelihood and posterior probabilities are in Appendix 3. (D) Leaf morphology; the ancestral condition is petiolate orbicular. Maximum likelihood and posterior probabilities are in Appendix 4. Equivocal regions are marked with dashes. Branches are marked with two states when ML and BA give conflicting signals, or when two states are approximately equally supported. (Asterisk marks the collapse of the *V. lanaiensis*, *V. oahuensis*, and *V. maviensis*/*V. robusta* nodes.)

A

B

$q_{21} = 123$

$q_{12} = 131$

$q_{03} = 29$

$q_{32} = 3$

$q_{34} = 107$

$q_{31} = 41$

Beringia

Fig. 5. Biogeography of the Hawaiian violets inferred from BayesMultistate interisland analysis. (A) Inferred biogeographic pattern of colonization, dispersal, and speciation. Uncertainty in reconstruction of ancestral islands states around the lineage leading to *V. helenae* is indicated by a dashed line. Scale bar for plant habit diagrams represents 10 cm. (B) Rates of interisland transition as estimated by maximum likelihood (ML). Of the 20 possible rates, only nonzero rates are shown.
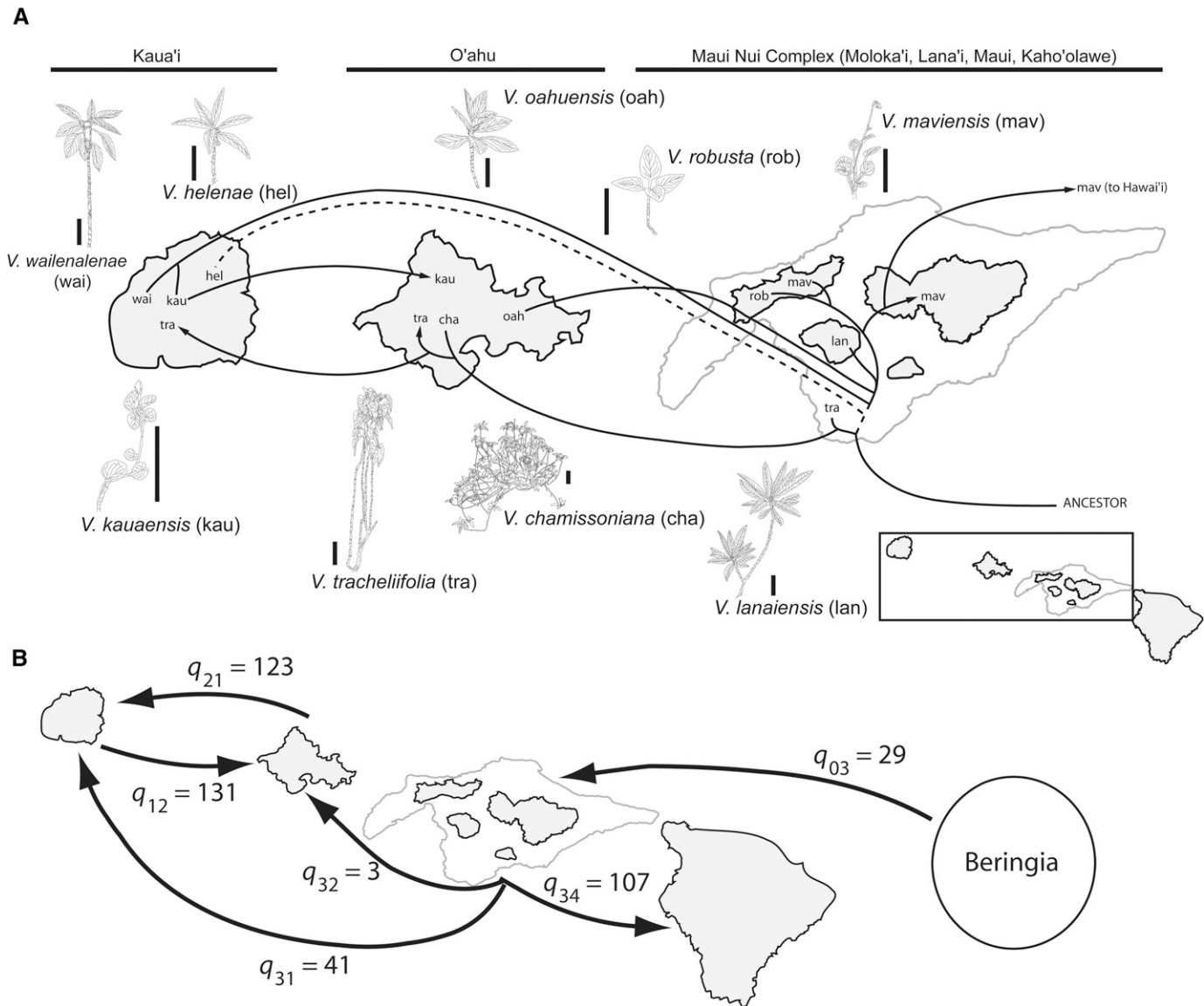
V. kauaensis subclades. As seen with both habitat (bog conditions in *V. maviensis* and *V. kauaensis*) and habit (rhizomatous herb growth form in *V. kauaensis*), these parallel shifts to petiolate-orbicular leaves are reversals back to the condition of the sister species of the lineage, *V. langsdorffii*.

Molecular evolution rates—Between the two lineages of violets within the wet clade that were investigated, differences in relative rates of molecular evolution between interisland populations of conspecific species are only seen on the younger islands/island systems (Table 2). Disparate populations of *V. kauaensis* on Kaua'i and O'ahu show equivalent rates of molecular evolution ($P = 0.76$) (Table 2). There is no significant difference between the rates of molecular evolution between

herbaceous (*V. kauaensis*) and woody (*V. wailenalenae*) species of Hawaiian violet ($P = 0.73$).

On the younger island systems of Maui Nui and Hawai'i, interisland populations of *V. maviensis* show nonequivalent rates of molecular evolution (Table 2). No statistical difference is seen between the relative rates of molecular evolution of herbaceous (*V. kauaensis*) and woody (*V. wailenalenae*) sister species in the lineage ($P = 0.7388$). Relative rates of molecular evolution among conspecific populations on Kaua'i and O'ahu do not differ (Table 2). Violet species on the relatively younger islands of Maui Nui and Hawai'i do show significant differences in their relative rates of molecular evolution. In these groups, differences in relative rates of molecular evolution are not seen between herbaceous and woody species, but between

montane bog and cloud forest violet species: *V. maviensis* and *V. robusta* on Moloka'i (*P* = 0.01431) and between interisland populations of *V. maviensis*: *V. maviensis* on Maui and Moloka'i (*P* = 0.02535); and Maui and Hawai'i (*P* = 0.01431) (Table 2).

DISCUSSION

The Hawaiian violet lineage consists of two clades differentiated by habitat: a wet clade (distributed in montane bogs, cloud forests, and mesic streambanks), and a dry clade (distributed in dry forests, mesic forests, and dry cliffs). Within the wet clade, two parallel lineages are evident: one on Kaua'i and one on Maui Nui. Species distributed in similar habitat types across the two islands have remarkable similarities in growth form and leaf morphology.

The Hawaiian violet lineage consists of a dry and a wet clade—The enlarged taxonomic sampling in this study confirms the tentative conclusion of Ballard and Sytsma (2000) that the Hawaiian violet lineage has a basal split into dry and wet clades (Figs. 2, 3B). *Viola tracheliifolia* and *V. chamissoniana*, representatives of the dry forest and dry cliff, respectively, comprise the dry clade. Habitats of these species are located in the leeward region of the islands, and experience 1000 mm or less annual rainfall (Giambelluca et al., 1986). The remaining species of Hawaiian violets comprise the more weakly supported wet clade and are restricted to high-elevation mesic forests, cloud forests, and montane bogs. These regions may receive from 2000 to 4000 mm annual rainfall (Giambelluca et al., 1986).

Paraphyletic groups are present in both the wet and dry clades (Fig. 2). *Viola maviensis* is paraphyletic with regards to *V. robusta*, while *V. tracheliifolia* is paraphyletic with respect to *V. chamissoniana* (Fig. 2). Additional population genetic research is warranted to elucidate the relationships between these paraphyletic groups. Due to their morphological differences (Fig. 1), we are maintaining *V. robusta* and *V. chamissoniana* at the species rank.

Patterns of growth form and leaf evolution mirror habitat shifts in parallel—Adaptive radiations of plants in island settings often are accompanied both by striking changes among close relatives and by similarities among distant relatives. These patterns are often only fully recognized when molecular phylogenetic tools are applied (Baldwin et al., 1990; Baldwin and Robichaux 1995; Givnish et al., 1995, 2009; Givnish 1997, 1998; Givnish and Sytsma, 1997). In the Hawaiian violets, the evolution in habit and leaf shape is clearly tied to shifts in habitat (Fig. 4B–D).

Parallelisms in growth form and leaf morphology accompanying independent habitat shifts are dramatically apparent in the wet clade of Hawaiian violets. Changes in growth form and leaf shape are accompanied by parallel shifts into the montane bog and cloud forest habitats by the two pairs of sister species of montane bog (*V. kauaensis* and *V. maviensis*) and cloud forest (*V. wailenalenae* and *V. robusta*) violets as well as the two representatives of the mesic forest (*V. helenae* and *V. lanaiensis*) (Fig. 4B–D). The montane bog violets have a small, compact growth form and small, ovate to reniform leaves. This growth form is common in bog plants and may facilitate retention of warmth in a wetter, high elevation environment (Givnish, 1987). The sorting of sister species between the bog and cloud

forest is seen in many plant radiations including, but not limited to *Cyanea* (Givnish et al., 1995, 2004, 2009), and *Dubautia* (Baldwin and Robichaux, 1995; Baldwin, 1997). The dramatic parallelisms in leaf morphology and growth form among members of the wet clade of Hawaiian violets strongly suggest that the radiation of the lineage across the archipelago is adaptive. Comparative physiological investigations of the violets, similar to those investigations of the Hawaiian silverswords (Robichaux et al., 1990), lobeliads (Givnish et al., 2004), and *Schiedea* (Culley et al., 2006), are warranted to reveal mechanisms underlying morphological parallelisms.

The Hawaiian violet lineage was most likely established on Maui Nui—At approximately 5 mya, Kaua'i was the only extant Hawaiian Island capable of receiving propagules through long-distance dispersal (Price and Clague 2002). For several million years before Kaua'i's establishment, there were no other large islands capable of receiving propagules (Price and Clague, 2002). The majority of extant plant and animal taxa were established following this pre-Kaua'i gap (ca. 5 mya) (Price and Clague, 2002). Among the Hawaiian lineages whose origins have been dated, only the diverse Hawaiian *Drosophila* (ca. 30 mya, Tamura et al., 2004), *Megalagrion* damselflies (9.6 mya, Jordan et al., 2003), the Hawaiian sumac *Rhus sandwicensis* (13.5 mya, Yi et al., 2004), the Hawaiian Honeyeaters (14–17 mya, Fleischer et al., 2008), the small fern genus *Diellia* (23 mya, Schneider et al., 2005), and the Hawaiian lobeliads (13 mya, Givnish et al., 2009) date to islands prior to Kaua'i. Many endemic Hawaiian plant and animal lineages show evidence of a west-to-east progression across the islands with early diverging species on Kaua'i and more derived species on the younger islands (Funk and Wagner, 1995; Givnish et al., 2009). Radiation from a central location in the archipelago (indicative of a younger lineage) is seen in *Tetramolopium* (Lowrey, 1995).

Ballard and Sytsma (2000) had suggested that the origin of the Hawaiian violets involved an initial colonization of Kaua'i around 3.7 mya followed by an early dispersal to O'ahu. The data presented in this paper disagree with this previous hypothesis and suggest that the ancestor to the Hawaiian violets was most likely established on Maui Nui, thus no earlier than 1.2–2 mya (Price and Elliott-Fisk, 2004), with subsequent radiations to the older islands of Kaua'i and O'ahu, and later to the younger island of Hawai'i as it formed beginning 600 000 yr ago. ML and Bayesian inference reconstructions strongly support (0.9222, 0.7565, respectively) an origin of the entire clade on Maui Nui. A Kaua'i (or any other island) origin for the entire Hawaiian violet lineage is strongly rejected by ML and Bayesian inference (0.0000, 0.1161, respectively).

The pattern of Hawaiian violet colonization, subsequent radiation, and dispersal as represented by an overlay of the phylogenetic tree onto the islands (Fig. 5A) and by transition rates between islands (Fig. 5B) differs from most other Hawaiian groups. Once arriving in Maui Nui, the lineage diverged into two clades with subsequent radiation into diverse habitats within each clade. Presumably early dispersal events involving both clades from Maui Nui occurred back to habitat-rich, older islands of Kaua'i and O'ahu (one or two dispersals each). Subsequently, *V. tracheliifolia* from O'ahu and *V. kauaensis* from Kaua'i dispersed to form new isolated populations on Kaua'i and O'ahu, respectively. A far more recent dispersal within the last 600 000 yr by *V. maviensis* occurred from Maui Nui to Hawai'i.

TABLE 2. Relative rate tests of molecular evolution between the Hawaiian violets. Results for the Tajima test for seven independent comparisons of three sequences each.

			Unique differences in sequence					
m_{ijj}	m_{iji}	m_{iij}	m_{ijj}	m_{iji}	m_{iij}	χ^2	P	df
V. kauaensis - OH	*V. kauaensis* - KA	*V. wailenalenae*	5	6	4	0.09	0.76302	1
V. kauaensis - KA	*V. wailenalenae* - KA	*V. helenae*	4	5	13	0.11	0.73880	1
V. kauaensis - OH	*V. wailenalenae* - KA	*V. helenae*	5	5	13	0.00	1.00000	1
V. maviensis - MO	*V. robusta* - MO	*V. lanaiensis*	0	6	1	6.00	0.01431*	1
V. maviensis - MO	*V. maviensis* - HA	*V. lanaiensis*	1	0	0	1.00	0.31731	1
V. maviensis - MO	*V. maviensis* - MA	*V. lanaiensis*	0	5	1	5.00	0.02535*	1
V. maviensis - MA	*V. maviensis* - HA	*V. lanaiensis*	6	0	0	6.00	0.01431*	1

Notes: OH = O'ahu, KA = Kaua'i, MA = Maui, MO = Moloka'i. * $P < 0.05$ was used to reject the null hypothesis of equal rates between lineages.

The montane bog and dry forest violets show the greatest incidence of interisland dispersal and establishment within the Hawaiian violet lineage. Both bog violet representatives have undergone dispersal events to their nearest eastern neighbor island: *V. kauaensis* to O'ahu and *V. maviensis* to Hawai'i (Fig. 4A). The apparent lack of interisland dispersal and establishment in the closed canopy cloud forest species and more frequent dispersibility in the open (bog and dry forest) habitats is similar to the pattern seen in Hawaiian *Cyanea* (Givnish et al., 1995, 2009). The abundance of avian taxa that migrate across the high-elevation bogs and dry forests of the Hawaiian archipelago (Scott et al., 1986) may facilitate the dispersal of violets via endo- or exozoochory. Seeds of Hawaiian violets have been shown to be capable of bird-mediated endozoochorous dispersal (Ballard and Sytsma, 2000). It is unlikely that seeds could have been dispersed via oceanic rafting or wind dispersal. Despite the similarities in the dispersal between *Viola* and *Cyanea*, seeds of Hawaiian violets lack the fleshy fruits seen in *Cyanea*, which would potentially limit the frequency of violet dispersal events. The perceived lack of dispersibility in the cloud forest violets may also be due to the apparent sedentary nature of cloud forest bird species (Diamond et al., 1976; Givnish et al., 1995).

Dispersals from both older and younger islands to the ancestral island complex of Maui Nui are not seen (Fig. 5A, B). The early colonization of Maui Nui and within-island diversification into many different habitat types involving two main clades might have saturated available niches for the violets. Species dispersing back to Maui Nui would face strong competition from violets already occupying dry to wet niches.

The current biogeographic hypothesis of the Hawaiian violets does not account for extinct populations of members of the dry or wet clade on younger islands. For example, a population of dry habitat violets may have colonized Maui Nui from a younger island prior to going extinct. This extinction pattern (Funk and Wagner, 1995) is not currently possible to test. Despite the strong support for the origin of the Hawaiian violet lineage on Maui Nui (provided by ML and Bayesian inference), the parsimony analysis (Fig. 3) suggests an origin of the lineage on Kaua'i (as Ballard and Sytsma [2000] initially hypothesized). Additional population-level investigations that incorporate data from multiple populations of each species on each island are warranted to further elucidate and increase the robustness of these biogeographic patterns.

Woody and herbaceous violets exhibit equal relative rates of molecular evolution—The statistically similar relative rates of molecular evolution seen in *V. wailenalenae* and *V. kauaensis* on Kaua'i disagree with the findings of Dodd et al. (1999) and Eriksson and Bremer (1992). Those authors showed that the herbaceous growth form in other groups is associated with a higher speciation rate than that in woody species. The lack of seasonality in the Hawaiian archipelago (Carlquist, 1970) may facilitate the year-round production of seeds in both *V. kauaensis* and *V. wailenalenae*. Herbarium and field surveys of *V. kauaensis* and *V. wailenalenae* have shown that the two species do flower throughout the entire year (Havran and Ballard, 2007). The congruent rates of molecular evolution in these species suggest that they may have equal generation times, despite their different growth forms.

Violets on Maui Nui and Hawai'i are undergoing different relative rates of molecular evolution—Because of the relatively young age of the Hawaiian Islands, the endemic Hawaiian flora has undergone a relatively rapid evolution (Price and Clague, 2002). Within the Hawaiian silversword alliance, Baldwin (1997) has shown that there are equal rates of molecular evolution between morphologically and ecologically distinct species. Differences in molecular evolution rates between congeneric sublineages of Hawaiian angiosperms have not been previously investigated. Cloud forest and bog violets of Moloka'i have unequal relative rates of molecular evolution, while their ecological analogues on Kaua'i possess statistically similar relative rates (Table 2). These results suggest that species on the geologically younger islands of Maui Nui and Hawai'i are diverging at a greater rate than their analogues on Kaua'i.

The incongruent rates of molecular evolution between interisland populations of *V. maviensis* (Table 2) provide strong evidence for incipient speciation on the younger Hawaiian Islands. If interisland dispersal events result in speciation in the Hawaiian violet lineage (as is common in the majority of Hawaiian angiosperm lineages), then *V. maviensis* may represent a relatively recently dispersed species currently undergoing allopatric speciation across Maui, Moloka'i, and Hawai'i. St. John (1989) described populations of *V. maviensis* on Hawai'i as three distinct species. His determinations were chiefly based on vegetative and reproductive features. Since his publication, Wagner et al. (1999) have recognized St. John's species as interisland populations of *V. maviensis*. The molecular evolution rates presented in this paper, along with the taxonomic history of *V. maviensis*, suggest that this species warrants additional attention. Careful comparisons of morphology, ecophysiology, and reproductive biology among interisland populations of *V. maviensis* are required to further characterize its incipient speciation.

In this study, we have presented new data that suggest a revised origin of the Hawaiian violets on the Hawaiian archipel-

ago. The putative establishment of the Hawaiian violets on the Maui Nui conflicts with the west-to-east progression pattern seen in many, but not all, endemic Hawaiian lineages. The evolution of similar morphologies in similar habitats in isolation strongly suggests that the radiation is adaptive. In addition, the identification of unequal molecular evolution rates of *V. maviensis* on the younger Hawaiian Islands provides future opportunities to elucidate the patterns and processes of morphological and molecular evolution within incipient species.

LITERATURE CITED

ACKERLY, D. D., S. A. DUDLEY, S. E. SULTAN, J. SCHMITT, J. S. COLEMAN, C. R. LINDER, D. R. SANDQUIST, ET AL. 2000. The evolution of plant ecophysiological traits: Recent advances and future directions. *Bioscience* 50: 979–996.

ACKERLY, D. D., AND R. K. MONSON. 2003. Waking the sleeping giant: The evolutionary foundations of plant function. *International Journal of Plant Sciences* 164 (supplement 3): S1–S6.

ARCHIE, J. W. 1989. Homoplasy excess ratios: New indices for measuring levels of homoplasy in phylogenetic systematics and a critique of the consistency index. *Systematic Zoology* 38: 253–269.

BALDWIN, B. G. 1997. Adaptive radiation of the Hawaiian silversword alliance: Congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. *In* T. J. Givnish and K. J. Sytsma [eds.], Molecular evolution and adaptive radiation, 103–128. Cambridge University Press, New York, New York, USA.

BALDWIN, B. G., C. G. CAMPBELL, J. M. PORTER, M. J. SANDERSON, M. F. WOJCIECHOWSKI, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

BALDWIN, B. G., D. W. KYHOS, AND J. DVORAK. 1990. Chloroplast DNA evolution and adaptive radiation in the Hawaiian silversword alliance (Asteraceae-Madiinae). *Annals of the Missouri Botanical Garden* 77: 96–109.

BALDWIN, B. G., AND R. H. ROBICHAUX. 1995. Historical biogeography and ecology of the Hawaiian silversword alliance (Asteraceae). New molecular phylogenetic perspectives. *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: Evolution on a hotspot archipelago, 259–287. Smithsonian Institution Press, Washington, D.C., USA.

BALLARD, H., K. J. SYTSMA, AND R. R. KOWAL. 1998. Shrinking the violets: Phylogenetic relationships of infrageneric groups in *Viola* (Violaceae) based on internal transcribed spacer DNA sequences. *Systematic Botany* 23: 439–458.

BALLARD, H. E., AND K. J. SYTSMA. 2000. Evolution and biogeography of the woody Hawaiian violets (*Viola*, Violaceae): Arctic origins, herbaceous ancestry and bird dispersal. *Evolution* 54: 1521–1532.

BAUM, D. A., K. J. SYSTMA, AND H. C. HOCH. 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. *Systematic Botany* 19: 363–388.

CARLQUIST, S. 1970. Hawaii: A natural history. Natural History Press, New York, New York, USA.

CARLQUIST, S., B. G. BALDWIN, AND G. D. CARR. 2003. Tarweeds and silverswords: Evolution of the Madiinae (Asteraceae). Missouri Botanical Garden Press, St. Louis, Missouri, USA.

CARSON, H. L., AND D. A. CLAGUE. 1995. Geology and biogeography of the Hawaiian Islands. *In* W. L. Wagner and V.A. Funk [eds.], Hawaiian biogeography: Evolution on a hot spot archipelago, 14–29. Smithsonian Institution Press, Washington, D.C., USA.

CULLEY, T. M., A. K. DUNBAR-WALLIS, A. K. SAKAI, S. G. WELLER, M. MISHIO, D. R. CAMPBELL, AND M. HERZENACH. 2006. Genetic variation of ecophysiological traits in two gynodioecious species of *Schiedea* (Caryophyllaceae). *New Phytologist* 169: 589–601.

DIAMOND, J. M., M. E. GILPIN, AND E. MAYR. 1976. Species-distance relation for birds of the Solomon archipelago, and the paradox of the great speciators. *Proceedings of the National Academy of Sciences, USA* 73: 2160–2164.

DODD, M. E., J. SILVERTOWN, AND M. W. CHASE. 1999. Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. *Evolution* 53: 732–744.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

ERIKSSON, O., AND B. BREMER. 1992. Pollination systems, dispersal modes, life forms, and diversification rates in angiosperm families. *Evolution* 46: 258–266.

FARRIS, J. S. 1989. The retention index and homoplasy excess. *Systematic Zoology* 38: 406–407.

FARRIS, J. S., V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.

FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* 17: 368–376.

FELSENSTEIN, J. 1985. Confidence limits of phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.

FITCH, W. M. 1971. Toward defining the course of evolution: Minimum change for a specific tree topology. *Systematic Zoology* 20: 406–416.

FLEISCHER, R. C., H. F. JAMES, AND S. L. OLSON. 2008. Convergent evolution of Hawaiian and Australo-Pacific honeyeaters from distant songbird ancestors. *Current Biology* 18: 1927–1931.

FUNK, V. A., AND W. L. WAGNER. 1995. Biogeographic patterns in the Hawaiian Islands. *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: Evolution on a hot spot archipelago, 379–419. Smithsonian Institution Press, Washington, D.C., USA.

GIAMBELLUCA, T. W., M. A. NULLETT, AND T. A. SCHROEDER. 1986. Hawaii rainfall atlas. Hawaii Division of Water and Land Development, Department of Land and Natural Resources, Honolulu, Hawaii, USA.

GIVNISH, T. J. 1987. Comparative studies of leaf form: Assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106 (supplement): 131–160.

GIVNISH, T. J. 1997. Adaptive radiation and molecular systematics: Issues and approaches. *In* T. J. Givnish and K. J. Sytsma [eds.], Molecular evolution and adaptive radiation, 1–54. Cambridge University Press, New York, New York, USA.

GIVNISH, T. J. 1998. Adaptive plant evolution on islands: Classical patterns, molecular data, new insights. *In* P. R. Grant [ed.], Evolution on islands, 281–304. Oxford University Press, Oxford, UK.

GIVNISH, T. J., K. C. MILLAM, A. R. MAST, T. B. PATERSON, T. J. THEIM, A. L. HIPP, J. M. HENSS, ET AL. 2009. Origin, adaptive radiation, and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society of London, B, Biological Sciences* 276: 407–417.

GIVNISH, T. J., R. A. MONTGOMERY, AND G. GOLDSTEIN. 2004. Adaptive radiation of photosynthetic physiology in the Hawaiian lobeliads: Light regimes, static light responses, and whole-plant compensation points. *American Journal of Botany* 91: 228–246.

GIVNISH, T. J., AND K. J. SYTSMA. 1997. Homoplasy in molecular vs. morphological data: The likelihood of correct phylogenetic inference. *In* T. J. Givnish and K. J. Sytsma [eds.], Molecular evolution and adaptive radiation, 55–103. Cambridge University Press, New York, New York, USA.

GIVNISH, T. J., K. J. SYTSMA, J. F. SMITH, AND W. J. HAHN. 1995. Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae, Lobelioideae). *In* W. L. Wagner and V.A. Funk [eds.], Hawaiian biogeography: Evolution on a hot spot archipelago, 228–361. Smithsonian Institution Press, Washington, D.C., USA.

HAVRAN, J. C., AND H. E. BALLARD. 2007. Evolutionary mechanisms of pre-zygotic isolation in the Hawaiian violets (*Viola*: Violaceae). Plant Biology and Botany 2007: Chicago, Illinois, USA. [online abstract: http://www.2007.botanyconference.org/engine/search/index.php?func=detail&aid=1173].

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

JORDAN, S. D., C. SIMON, AND D. A. POLHEMUS. 2003. Molecular systematics and adaptive radiation of Hawaii's endemic damselfly genus *Megalagrion*. *Systematic Biology* 52: 89–109.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18: 1–32.

LOSOS, J. B., R. E. GLOR, J. J. KOLBE, AND K. NICHOLSON. 2006. Adaptation, speciation, and convergence: A hierarchical analysis of adaptive radiation in Caribbean *Anolis* lizards. *Annals of the Missouri Botanical Garden* 93: 24–33.

LOSOS, J. B., T. R. JACKMAN, A. LARSON, K. DEQUEIROZ, AND L. RODRIGUEZ-SCHETTINO. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. *Science* 279: 2115–2118.

LOWREY, T. K. 1995. Phylogeny, adaptive radiation, and biogeography of Hawaiian *Tetramolopium* (Asteraceae, Astereae). *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: Evolution on a hotspot archipelago, 195–220. Smithsonian Institution Press, Washington, D.C., USA.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade 3.0: Analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.

MUELLER-DOMBOIS, D., AND F. R. FOSBERG. 1998. Vegetation of the tropical Pacific islands. Springer-Verlag, New York, New York, USA.

MULLIS, K. B., AND F. A. FALOONA. 1987. Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction. *Methods in Enzymology* 155: 335–350.

PAGEL, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48: 612–622.

PAGEL, M., AND A. MEADE. 2007. BayesTraits v.1.0 [computer program]. Website http://www.evolution.rdg.ac.uk/BayesTraits.html

PAGEL, M., A. MEADE, AND D. BARKER. 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* 53: 673–684.

POSADA, D., AND T. R. BUCKLEY. 2004. Model selection and model averaging in phylogenetics: Advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: Testing the model of DNA substitution. 14: 817–818.

PRICE, J. P., AND D. A. CLAGUE. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society of London, B, Biological Sciences* 269: 2429–2435.

PRICE, J. P., AND D. ELLIOTT-FISK. 2004. Tophographic history of the Maui Nui Complex, Hawai'i, and its implications for biogeography. *Pacific Science* 58: 27–45.

PRICE, J. P., AND W. L. WAGNER. 2004. Speciation in Hawaiian angiosperm lineages: Cause, consequence, and mode. *Evolution* 58: 2185–2200.

ROBICHAUX, R. H., G. D. CARR, M. LEIBMAN, AND R. W. PEARCY. 1990. Adaptive radiation of the Hawaiian silversword alliance (Compositae-Madiinae): Ecological, morphological, and physiological diversity. *Annals of the Missouri Botanical Garden* 77: 64–72.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

SANDERSON, M. J. 1998. Estimating rate and time in molecular phylogenies: Beyond the molecular clock? *In* P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants II: DNA sequencing, 242–264. Kluwer, New York, New York, USA.

SCHLUTER, D. 2000. The ecology of adaptive radiation. Oxford University Press, New York, New York, USA.

SCHNEIDER, H., T. A. RANKER, S. J. RUSSELL, R. CRANFILL, J. M. O. GEIGER, R. AGURAIUJA, K. R. WOOD, ET AL. 2005. Origin of the endemic fern genus *Diellia* coincides with the renewal of Hawaiian terrestrial life in the Miocene. *Proceedings of the Royal Society of London, B, Biological Sciences* 272: 455–460.

SCOTT, J. M., S. MOUNTAINSPRING, F. L. RAMSEY, AND C. B. KEPLER. 1986. Forest bird communities of the Hawaiian Islands: Their dynamics, ecology, and conservation. Studies in Avian Biology No. 9, Cooper Ornithological Society. Allen Press, Lawrence, Kansas, USA.

SKOTTSBERG, C. 1940. Observations on Hawaiian violets. *Meddelanden Fran Goteborgs Botaniska Tradgard* 13: 451–528.

ST. JOHN, H. 1989. Hawaiian plant studies 135: Revision of the Hawaiian species of *Viola* (Violaceae). *Botanische Jahrbücher für Systematik* 111: 165–204.

SWOFFORD, D. 1998. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, Massachusetts, USA.

SWOFFORD, D. L., AND W. P. MADDISON. 1987. Reconstructing ancestral character states under Wagner parsimony. *Mathematical Biosciences* 87: 199–229.

TAJIMA, F. 1993. Simple methods for testing molecular clock hypothesis. *Genetics* 135: 599–607.

TAMURA, K., J. L. DUDLEY, M. NEI, AND S. S. KUMAR. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software v. 4.0. *Molecular Biology and Evolution* .

TAMURA, K., S. SUBRAMANIAN, AND S. KUMAR. 2004. Temporal patterns of fruit fly (*Drosophila*) evolution revealed by mutation clocks. *Molecular Biology and Evolution* 21: 36–44.

VITOUSEK, P. 2004. Nutrient cycling and limitation: Hawai'i as a model system. Princeton University Press, Princeton, New Jersey, USA.

WAGNER, W. L., D. R. HERBST, AND S. H. SOHMER. 1999. Manual of the flowering plants of Hawai'i, revised ed. University of Hawai'i Press and Bishop Museum Press, Honolulu, Hawai'i, USA.

WEBB, C. O., D. D. ACKERLY, M. A. MCPEEK, AND M. J. DONOGHUE. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33: 475–505.

WEBB, C. O., J. B. LOSOS, AND A. A. AGRAWAL. 2006. Integrating phylogenies into community ecology. *Ecology* 87 (sp7): 1–2.

WHITE, T. J., T. BRUNS, S. LEE, AND J. W. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: A guide to methods and applications, 315–322. Academic Press, San Diego, California, USA.

YI, T., A. J. MILLER, AND J. YEN. 2004. Phylogenetic and biogeographic diversification of *Rhus* (Anacardiaceae) in the Northern Hemisphere. *Molecular Phylogenetics and Evolution* 33: 861–879.

APPENDIX 1. Maximum likelihood (ML) and posterior probabilities (PP) for island biogeographic hypotheses.

Island	A (ML | PP)	B (ML | PP)	C (ML | PP)	D (ML | PP)	E (ML | PP)	F (ML | PP)	G (ML | PP)	H (ML | PP)	I (ML | PP)
K	0.0000 | 0.1161	0.0000 | 0.0005	0.3133 | 0.1682	0.2883 | 0.1972	0.0000 | **0.4195**	**0.6029 | 0.7390**	**0.9975 | 0.9981**	0.0000 | 0.3147	0.0000 | 0.0415
O	0.0000 | 0.0415	0.0000 | 0.0011	**0.3738 | 0.4427**	**0.4529 | 0.4761**	0.0000 | 0.0871	0.1459 | 0.0650	0.0010 | 0.0006	0.0000 | 0.0690	0.0000 | 0.0565
M	**0.9222 | 0.7565**	**0.9973 | 0.9977**	0.0844 | 0.1421	0.0520 | 0.1225	**0.6617** | 0.3195	0.0769 | 0.0696	0.0003 | 0.0005	**0.7047 | 0.4826**	**0.9042 | 0.8253**
H	0.0732 | 0.0489	0.0027 | 0.0004	0.2234 | 0.1262	0.2064 | 0.1063	0.3322 | 0.0972	0.1735 | 0.0706	0.0012 | 0.0004	0.2930 | 0.0781	0.0947 | 0.0412

APPENDIX 2. Maximum likelihood (ML) and posterior probabilities (PP) for habitat biogeographic hypotheses.

Habitat	A (ML | PP)	B (ML | PP)	C (ML | PP)	D (ML | PP)	E (ML | PP)	F (ML | PP)	G (ML | PP)	H (ML | PP)	I (ML | PP)
MB	0.0013 | 0.0346	0.0000 | 0.0000	0.0000 | 0.0622	0.0001 | 0.0399	0.0065 | 0.0977	0.0527 | 0.0660	**0.9991 | 0.9991**	0.0083 | 0.1194	0.0212 | 0.2119
CF	**0.8920** | 0.1541	0.0002 | 0.0001	0.0085 | 0.1114	0.0128 | 0.0659	**0.5839 | 0.4018**	**0.6886 | 0.7451**	0.0001 | 0.0003	**0.5707 | 0.5855**	**0.3203 | 0.2854**
MS	0.1067 | 0.1419	0.0000 | 0.0002	0.0011 | 0.1185	0.0018 | 0.0694	**0.4096 | 0.3365**	0.2587 | 0.0689	0.0008 | 0.0002	**0.4210** | 0.1791	**0.6585 | 0.3683**
DC	0.0000 | 0.0604	0.0000 | 0.0002	0.0000 | 0.2149	0.0000 | 0.0702	0.0000 | 0.0803	0.0000 | 0.0613	0.0000 | 0.0002	0.0000 | 0.0604	0.0000 | 0.0704
DF	0.0000 | **0.6090**	**1.0000 | 0.9994**	**0.9903** | 0.4931	**0.9853 | 0.7546**	0.0000 | 0.0809	0.0000 | 0.0587	0.0000 | 0.0002	0.0000 | 0.0557	0.0000 | 0.0639

Habitat	J (ML | PP)	K (ML | PP)	L (ML | PP)	M (ML | PP)
MB	0.0647 | 0.2989	**0.6606 | 0.6702**	**0.4021** | 0.2360	**0.9998 | 0.9998**
CF	**0.2375** | 0.1386	0.1204 | 0.1600	**0.4042 | 0.4293**	0.0000 | 0.0000
MS	**0.6878 | 0.4266**	0.2190 | 0.0626	0.1939 | 0.1228	0.0002 | 0.0000
DC	0.0000 | 0.0716	0.0000 | 0.0563	0.0000 | 0.1089	0.0000 | 0.0000
DF	0.0000 | 0.0654	0.0000 | 0.0490	0.0000 | 0.1031	0.0000 | 0.0000

APPENDIX 3. Maximum likelihood (ML) and posterior probabilities (PP) for leaf shape biogeographic hypotheses.

Leaf shape	A (ML | PP)	B (ML | PP)	C (ML | PP)	D (ML | PP)	E (ML | PP)	F (ML | PP)	G (ML | PP)	H (ML | PP)	I (ML | PP)
OR	0.0010 | 0.0421	0.0000 | 0.0001	0.0001 | 0.0765	0.0001 | 0.0490	0.0040 | 0.1075	0.0505 | 0.0615	**0.9988 | 0.9986**	0.0049 | 0.1459	0.0070 | 0.2189
OV	**0.7489** | 0.1144	0.0000 | 0.0002	0.0108 | 0.1164	0.0158 | 0.0065	0.4093 | 0.2712	**0.6302 | 0.6820**	0.0001 | 0.0003	**0.4361 | 0.4051**	0.1893 | 0.1114
LA	0.1980 | 0.1344	0.0000 | 0.0002	0.0021 | 0.1173	0.0035 | 0.0684	**0.3614 | 0.3274**	0.1767 | 0.0675	0.0004 | 0.0003	**0.3284 | 0.2015**	**0.4536 | 0.3595**
OB	0.0521 | 0.0707	0.0000 | 0.0002	0.0008 | 0.1194	0.0013 | 0.0651	**0.2253 | 0.1160**	0.1425 | 0.0659	0.0006 | 0.0003	0.2306 | 0.1156	**0.3501** | 0.1649
SP	0.0000 | 0.0631	0.0000 | 0.0003	0.0000 | 0.1838	0.0000 | 0.0774	0.0000 | 0.0928	0.0000 | 0.0641	0.0000 | 0.0003	0.0000 | 0.0775	0.0000 | 0.0783
LP	0.0000 | **0.5752**	**1.0000 | 0.9991**	**0.9827** | 0.4071	**0.9793 | 0.6735**	0.0000 | 0.0852	0.0000 | 0.0590	0.0000 | 0.0002	0.0000 | 0.0678	0.0000 | 0.0670

Leaf shape	J (ML | PP)	K (ML | PP)	L (ML | PP)	M (ML | PP)
OR	0.0527 | 0.2872	**0.5784 | 0.6776**	0.1495 | **0.2173**	**0.9996 | 0.9997**
OV	0.1930 | 0.1128	0.0713 | 0.1122	0.2826 | **0.3463**	0.0000 | 0.0001
LA	**0.4995 | 0.3686**	0.1589 | 0.0567	0.2953 | 0.1157	0.0001 | 0.0001
OB	0.2548 | 0.0823	0.1914 | 0.0543	0.2727 | 0.1117	0.0002 | 0.0001
SP	0.0000 | 0.0795	0.0000 | 0.0527	0.0000 | 0.1083	0.0000 | 0.0001
LP	0.0000 | 0.0696	0.0000 | 0.0465	0.0000 | 0.1001	0.0000 | 0.0000

APPENDIX 4. Maximum likelihood (ML) and posterior probabilities (PP) for growth form biogeographic hypotheses.

Growth form	A (ML | PP)	B (ML | PP)	C (ML | PP)	D (ML | PP)	E (ML | PP)	F (ML | PP)	G (ML | PP)	H (ML | PP)	I (ML | PP)
RH	0.0001 | 0.0167	0.0000 | 0.0000	0.0010 | 0.0406	0.0018 | 0.1090	0.0000 | 0.0118	0.0009 | 0.0541	**0.9996 | 0.9997**	0.0000 | 0.0090	0.0000 | 0.0089
DS	0.0000 | 0.0248	0.0000 | 0.0000	0.0000 | 0.0475	0.0000 | 0.0209	0.0000 | 0.0307	0.0000 | 0.0297	0.0000 | 0.0000	0.0000 | 0.0497	0.0000 | 0.2070
FB	0.0434 | **0.3913**	0.0000 | 0.0000	0.0002 | 0.0614	0.0003 | 0.0262	**0.9752 | 0.9226**	**0.9367 | 0.8441**	0.0001 | 0.0000	**0.9900 | 0.9173**	**0.9983 | 0.7504**
ST	**0.9445 | 0.5393**	**0.9997 | 0.9998**	**0.6673 | 0.6412**	**0.8734 | 0.9069**	0.0244 | 0.0186	0.0559 | 0.0395	0.0000 | 0.0000	0.0099 | 0.0126	0.0017 | 0.0173
BS	0.0120 | 0.0272	0.0003 | 0.0001	0.3316 | 0.2093	0.1244 | 0.0270	0.0005 | 0.0164	0.0065 | 0.0326	0.0003 | 0.0000	0.0001 | 0.0115	0.0000 | 0.0165

Growth form	J (ML | PP)	K (ML | PP)	L (ML | PP)	M (ML | PP)
RH	0.0000 | 0.0184	0.0000 | 0.0038	0.0000 | 0.0185	0.0000 | 0.0000
DS	0.0000 | **0.4731**	**0.9953 | 0.9777**	**0.9702 | 0.8919**	**1.0000 | 1.0000**
FB	**0.9923** | 0.4497	0.0047 | 0.0076	0.0292 | 0.0369	0.0000 | 0.0000
ST	0.0077 | 0.0302	0.0000 | 0.0052	0.0005 | 0.0253	0.0000 | 0.0000
BS	0.0000 | 0.0287	0.0000 | 0.0058	0.0000 | 0.0275	0.0000 | 0.0000