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Reconstructing Ancestral Patterns of Colonization and Dispersal in the Hawaiian Understory Tree Genus *Psychotria* (Rubiaceae): A Comparison of Parsimony and Likelihood Approaches

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Abstract.— Systematic and biogeographical relationships within the Hawaiian clade of the pantropical understory shrub genus *Psychotria* (Rubiaceae) were investigated using phylogenetic analysis of 18S–26S ribosomal DNA internal (ITS) and external (ETS) transcribed spacers. Phylogenetic analyses strongly suggest that the Hawaiian *Psychotria* are monophyletic and the result of a single introduction to the Hawaiian Islands. The results of phylogenetic analyses of ITS and ETS partitions alone give slightly different topologies among basal lineages of the Hawaiian clade; however, such differences are not well supported. Relationships in the section *Straussia* clade in particular are not well resolved because of few nucleotide changes on internal branches, suggesting extremely rapid radiation in the lineage. Parsimony and likelihood reconstructions of ancestral geographical distributions using the topologies inferred from both parsimony and likelihood analysis of combined data and using different combinations of models and branch lengths gave highly congruent results. However, for one internal node (corresponding to the majority of the "*greenwelliae*" clade), parsimony reconstructions were unable to distinguish between three possible island states, whereas likelihood reconstructions resulted in clear ordering of possible states, with the island of O`ahu slightly more probable than other islands under all but one model and branch length combination considered (the Jukes–Cantor-like model with branch lengths inferred under parsimony, under which conditions Maui Nui is more probable). A pattern of colonization from oldest to youngest islands was inferred from the phylogeny, using maximum parsimony and maximum likelihood. Additionally, a much higher incidence of intraisland versus interisland speciation was inferred. [Ancestral character state reconstruction; biogeography; ETS; Hawai`i; island evolution; ITS; molecular systematics; *Psychotria*.]

The geological history of the Hawaiian Islands (Carson and Clague, 1995; Clague, 1996) has been correlated with historical patterns of dispersal and colonization in many plant and animal lineages and has been studied using an explicitly phylogenetic approach (e.g., Wagner and Funk, 1995; Givnish, 1998). Although many plant and animal groups studied conform to a west–east/older–younger island pattern of colonization, notable examples exist where initial colonization of the archipelago began on a younger island and progressed to older islands (e.g., *Tetramolopium* [Asteraceae]; Lowrey, 1995) or back-colonizations have occurred within a lineage (e.g., *Schiedea* [Caryophyllaceae]; Wagner et al., 1995). To explore various scenarios in the evolutionary and biogeographic history of closely related species and populations of the understory shrub genus *Psychotria* (Rubiaceae) on Hawai`i, we compare maximum parsimony and maximum likelihood models of ancestral character state reconstruction in estimating ancestral biogeographical states. In addition, we investigate whether biogeographical patterns inferred through these methods conform to a model of colonization and dispersal from older to younger islands, a pattern that has commonly been documented for many Hawaiian animal and plant groups (Wagner and Funk, 1995).

Modes of speciation in *Psychotria* are of interest because *Psychotria* is one of the most species-rich genera of flowering plants, with as many as 2000 species estimated worldwide (Hamilton, 1989a, 1989b, 1989c; Sanderson and Wojciechowski, 1996, Nepokroeff et al., 1999). *Psychotria* occurs pantropically and comprises mostly mesic to wet forest understory shrubs, with small, white, and generally inconspicuous flowers. The endemic Hawaiian *Psychotria* are an important and characteristic component of the native Hawaiian mesic to wet rain forests, along with *Acacia koa*, *Metrosideros polymorpha*, *Diospyros sandwichensis*, and other native species (Gagne and Cuddihy, 1990). The number of independent introductions of *Psychotria* to Hawai`i are in question, and adequate classification of the members of the Hawaiian section *Straussia* are thought to be one of the major problems in Hawaiian plant taxonomy (Sohmer, 1978). Members of section *Straussia* are thought to be currently undergoing very recent and rapid diversification both within and between islands, and hybridization is known to occur between sympatric species. Moreover, Hawaiian *Psychotria* are of special interest because they represent an example of 1 of 10 instances of autochthonous shift in breeding system from hermaphroditism to dicliny—the possession of two sexual floral morphs—that is frequent in the Hawaiian flora (Sakai et al., 1995).

A goal of this study was to investigate biogeographical and phylogeographical patterns among closely related species and populations of endemic Hawaiian *Psychotria* to address how colonization and dispersal may have occurred within a recent and actively evolving complex. Molecular phylogenetic analyses of Hawaiian *Psychotria* using both nuclear ribosomal DNA (rDNA) internal

transcribed spacer (ITS) and external transcribed spacer (ETS) regions were performed to address three issues: (1) to test competing hypotheses that the Hawaiian *Psychotria* are the result of either a single introduction (Fosberg, 1962) or two or three introductions with each of the two sections having separate origins (Sohmer, 1978), (2) to infer the direction of interisland colonization in the Hawaiian *Psychotria* and compare this pattern with those of other plant and animal groups having a well-established pattern of colonization from oldest to youngest islands (see Funk and Wagner, 1995), and (3) to compare the use of parsimony and likelihood reconstructions for estimating ancestral patterns of colonization.

Materials and Methods

Hawaiian Psychotria

Hawaiian *Psychotria* consists of 11 endemic species, which have been treated taxonomically by Fosberg (1962, 1964), Sohmer (1977, 1978) and Wagner et al. (1990). These authors have maintained two endemic sections of *Psychotria* in Hawai`i, separated by inflorescence architecture, flower size, anther attachment, and seed morphology. Recent phylogenetic studies (Nepokroeff et al., 1999) aligned the Hawaiian taxa with a group of western Pacific species of *Psychotria*. To address relationships among Hawaiian species of *Psychotria*, leaf material was collected in the field for 36 taxa, including populations of all 11 species of Hawaiian *Psychotria*, representing their respective ranges of distribution (Fig. 1; Table 1). In some cases, only one population of a given species was sampled when the species consisted of only one isolated population. In cases of widespread species, one to several populations were sampled per island, corresponding to geochronological formation of the major volcanoes that comprise the current high islands of the Hawaiian chain. Eight extra-Hawaiian species (*P. cadigiensis, P. hombroniana, P.* sp. Tinian Is., *P*. sp. Guam, *P. luzionensis, P. tahitiensis, P.pickeringii*, and *P*. sp. Fiji) were used as outgroups based on the results of several *Psychotria*-wide surveys (Andersson and Rova, 1999; Nepokroeff et al., 1999), with *P. luzoniensis* from the Philippines designated as the ultimate outgroup. The samples of Hawaiian species and populations of *Psychotria* included in this study were collected, shipped, processed, and frozen as outlined by Sytsma et al. (1993). Outgroup taxa were

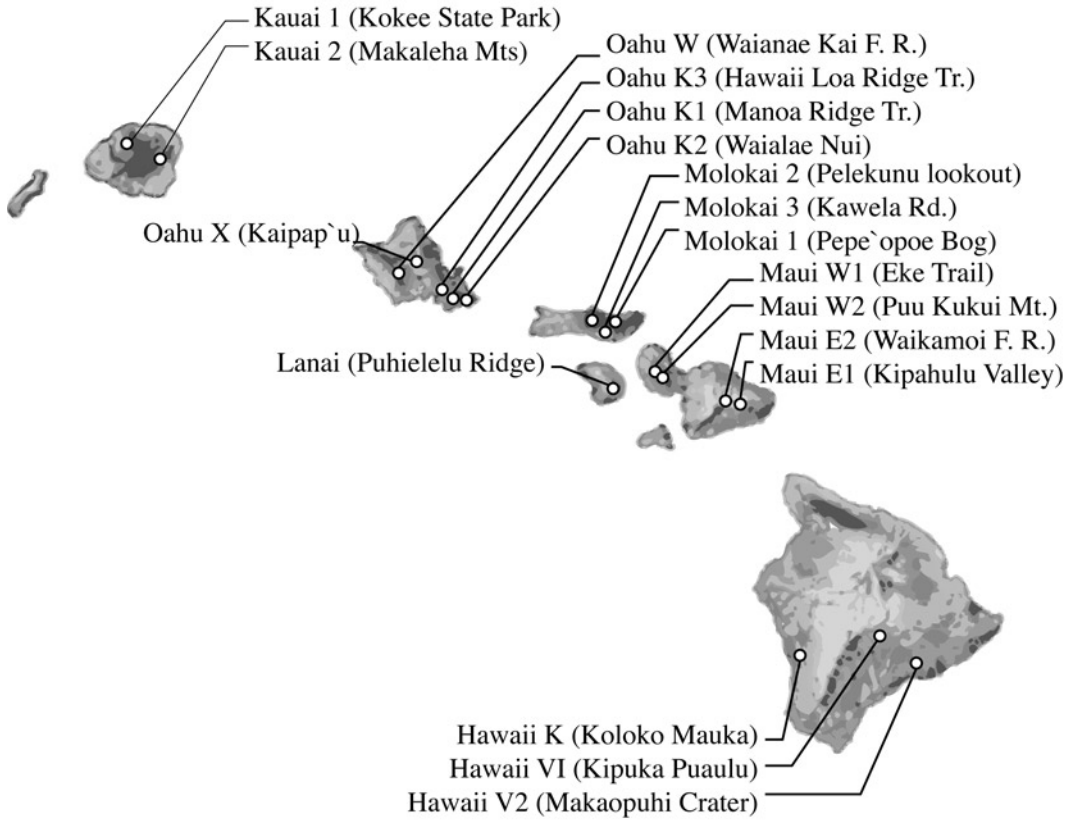

FIGURE 1. Collecting localities of Hawaiian *Psychotria* used in this study. Localities sampled represent geographical associations of major volcanoes that comprise the current high island chain. Codes for the localities: Kaua`i 1 = Kokee State Park; Kaua`i 2 = Makaleha Mts.; Kaua`i 3 = Hoary Head Range; O`ahu W = Waianae Mtns. (Waianae Kai Forest Reserve); O`ahu K = Koolau Mts., O`ahu K1 = Manoa Ridge Trail; O`ahu K2 = Waialae Nui; O`ahu K3 = Hawai`i Loa Ridge Trail; O`ahu X = Kaipapu; Maui W = West Maui; Maui W1 = Eke Trail; Maui W2 = Puu Kukui Mt.; Maui E = East Maui, Maui E1 = Kipahulu Valley; Maui E2 = Waikamoi Forest Resene; Moloka`i 1 = Pepeopoe Bog; Moloka`i 2 = Pelekunu Lookout; Moloka`i 3 = Kawela Rd.; Lanai`i = Puhielelu Ridge; Hawai`i V = Volcano; Hawai`i Volcano National Park, Hawai`i V1 = (Kipuka Puaulu; Hawai`i V2 = Makaopuhi Crater; Hawai`i K = Kona, Koloko Mauka.

TABLE 1. Collection data and GenBank accession numbers for Hawaiian *Psychotria* ITS and ETS sequences.

Taxon	Accession	Collection locality/Code	GenBank nos. ITS	GenBank nos. ETS
Psychotria mariniana	M. Nepokroeff 776	Awa`awapuhi Trail, Kokee State Park (Kaua`i 1)	AY350651	AY350680
Psychotria mariniana	M. Nepokroeff et al. 815	Wai`alae Nui Ridge, Honolulu Watershed, O`ahu (O`ahu K2)	AY350652	AY350681
Psychotria mariniana	M. Nepokroeff and C. Lutzow-Felling 793	Manoa Ridge Trail (O`ahu K1)	AY350653	AY350682
Psychotria mariniana	M. Nepokroeff, A. Medeiros and J. Y. Meyer 936	Kipahulu Valley, Haleakala Natl. Park, Maui (Maui E1)	AY350654	AY350683
Psychotria mariniana	M. Nepokroeff and S. Perlman 954	Puhielelu Ridge, Lana`i (Lana`i)	AY350656	AY350685
Psychotria hawaiiensis var. *hawaiiensis*	M. Nepokroeff 869 (pooled)	Makaopuhi Crater, Hawai`i Volcanoes National Park (Hawai`i V1)	AY350659	AY350688
Psychotria mariniana	M. Nepokroeff and J. Lau 922	Trail to Kawela, Kamakao Preserve, Moloka`i (Moloka`i 3)	AY350655	AY350684
Psychotria kaduana	M. Nepokroeff and H. Oppenheimer 860, 21 Oct., 1995	Pu'u Kukui Trail, Maui (Maui W2)	AY350657	AY350686
Psychotria kaduana	M. Nepokroeff et al. 901	Hawai`i Loa Ridge Trail, O`ahu (O`ahu K3)	AY350658	AY350687
Psychotria fauriei	M. Nepokroeff et al. 817	Wai`anae Kai F. R., O`ahu (O`ahu W)	AY350663	AY350692
Psychotria hathewayi	M. Nepokroeff et al. 802	Waialae Nui, O`ahu (O`ahu K2)	AY350664	AY350693
Psychotria hawaiiensis var. *hillebrandii*	M. Nepokroeff 930	Waikamoi F. R., Maui (Maui E2)	AY350660	AY350689
Psychotria mauiensis	M. Nepokroeff, H. Oppenheimer and S. Meidell 851, 19 Oct. 1995	Eke Trail, Maui (Maui W1)	AY350661	AY350690
Psychotria mauiensis	M. Nepokroeff and J. Lau 915	Pepe'opae Bog, Kamakao Preserve, Moloka`i (Moloka`i 1)	AY350662	AY350691
Psychotria greenwelliae	M. Nepokroeff 907	Awa`awapuhi Trail, Koke'e State Park, Kaua`i (Kaua`i 1)	AY350665	AY350695
Psychotria greenwelliae	M. Nepokroeff population sample 07	Awa`awapuhi Trail, Koke'e State Park, Kaua`i (Kaua`i 1)	AY350666	AY350694
Psychotria wawrae	D. Lorence 7428	Makaleha, Kaua`i (Kaua`i 2)	AY350672	AY350701
Psychotria hexandra	M. Nepokroeff s.n. June, 1993	Awa`awapuhi Trail, Koke'e State park, Kaua`i (Kaua`i 1)	AY350667	AY350696
Psychotria hexandra	D. Lorence 7405	Makaleha, Kaua`i (Kaua`i 2)	AY350668	AY350697
Psychotria hexandra subsp. *O`ahuensis*	K. R. Wood 7569	West rim of Kaipapa'u (O`ahu)	AY350669	AY350698
Psychotria grandiflora	M. Nepokroeff and K. R. Wood s.n.	Kalalau Lookout, Koke'e State Park, Kaua`i (Kaua`i 1)	AY350670	AY350699
Psychotria hobdyi	K. R. Wood 5010-C	Kuia NAR, Kaua`i (Kaua`i 1)	AY350671	AY350700
Psychotria cadigiensis	D. Heuschkel sn, Mt. Makiling, Philippines	cultivar, Ho'omaluhia Bot. Gardens, O`ahu; acc. 82.01	AY350673	AY350702
Psychotria hombroniana	D. Lorence 7844	Micronesia, Kosrae	AY350676	AY350705
P.sp. "Fiji A"	A. Whistler 24404	Fiji	AY350678	AY350707
Psychotria luzionensis	D. Heuschkel sn, Mt. Makiling, Laguna, Philippines	cultivar, Ho'omaluhia Bot. Gardens, O`ahu, 83.0537	AY350674	AY350703
Psychotria mariana	D. Lorence 7959 (ex. D. Hersbt s.n.,	Tinian Island	AY350677	AY350706
Psychotria pickeringii	A. Whistler 24414	Fiji	AY350679	AY350708
Psychotria tahitiensis	J. Y. Meyer 421	Tahiti, Tuauru Valley, trail to Mt. Orohena, Society Islands	AY350675	AY350704

obtained from botanical gardens and from field collected specimens. Voucher information is listed in Table 1.

Molecular Markers: ITS and ETS Sequences

ITS sequences have been used extensively for phylogenetic analysis in species level phylogenetic studies in plants (reviewed by Baldwin et al., 1995; Soltis and Soltis, 1998; Sytsma and Hahn, 2000). However, ITS sequences may not be evolving fast enough in some groups to permit phylogenetic inference among closely related species and populations within a species. To this end, we sequenced an additional region, the ETS region of the nuclear ribosomal array to provide more characters for phylogenetic analysis. The ETS region has been used recently (Baldwin and Markos, 1998; Bena-Gilles et al.,

1998a, 1998b; Clevinger and Panero, 2000; Linder et al., 2000; Andreasen and Baldwin, 2001) for inferring phylogenetic relationships at shallower levels of relationship, particularly among members of the Asteraceae, and has been useful as an additional source of sequence characters with respect to the ITS region.

Molecular Methods

DNA was extracted using a modified 6× CTAB (Smith et al., 1991) extraction procedure (Doyle and Doyle, 1987). Amplified double-stranded polymerase chain reaction (PCR) products were obtained for the entire ITS region, using primers LEU 1 (see Nepokroeff et al., 1999) and ITS 4 (White et al., 1990). The 50-μl reactions contained 33 μl sterile water, 2 μl DMSO, 1 μl of each 10 mM dNTP, 5 μl 10× buffer, 5 μl $MgCl_2$, 0.5 μl 5′ 20 μM primer, 0.5 μl 3′ 20 μM primer, 1.0 μl BSA (10 mg/ml), and 0.75 μl unquantified total genomic DNA. Amplification of some taxa required up to 5 μl DNA. PCR products were purified using differential centrifugation with either the QIAQuick columns (Qiagen Chatsworth, CA) or Ultrafree-MC tubes (Millipore Corp.) for ITS sequences at the University of Wisconsin or using PEG precipitation (20% PEG 8000/2.5 M NaCl) for ETS sequences at the Smithsonian Laboratory of Molecular Systematics.

ITS sequences were obtained for both strands using the primers LEU 1, ITS 3B, and ITS 4 (see Nepokroeff et al., 1999). Boundary regions of the coding regions for 18S, 5.8S, and 26S rDNA and spacer regions for ITS were determined by comparison to DNA sequences obtained from previous research on *Psychotria* (Nepokroeff et al., 1999). Sequences from ITS 1, the 5.8S region, and ITS 2 were included in the analyses.

The entire IGS region, including the ETS region, was amplified using long-distance PCR following the protocol outlined by Baldwin and Markos (1998) with primers 18S-IGS and 26S-IGS. The primer 18S-E, designed by Baldwin and Markos (1998) and situated at the 5′ border of 18S and ETS, was used to sequence upstream into the ETS region. A primer specific to *Psychotria* was designed for amplification of the 3′ end of the ETS region, ETS Psy1 (5′-GTG TGA GTG GTA AAT GGA TAG C-3′). The ETS Psy 1 primer was used in conjunction with the primer 18S-ETS designed by Baldwin and Markos (1998) for amplifying an approximately 460-bp fragment. The region was amplified using the protocol described by Baldwin and Markos (1998) with the exception that ProMega *Taq* polymerase was used in the amplifications, rather than AmpliTaq Gold. This 3′ ETS region was sequenced for both strands using the amplification primers.

Dideoxy sequencing was carried out directly from purified ITS and ETS PCR products using the dye terminator cycle sequencing protocol (Applied Biosystems) on a Perkin Elmer 2400 or 9700 thermocycler. Sequencing reactions were analyzed on an ABI 373 DNA automated DNA sequencer at the University of Wisconsin (ITS sequences) and an ABI 377 automated sequencer at the Smithsonian Laboratory of Molecular Systematics (ETS sequences). Contiguous alignments were edited using Sequencher 3.0 (Gene Codes Corp., Ann Arbor, MI).

Phylogenetic Analyses

Phylogenetic analyses were conducted using PAUP* 4.0b4a for UNIX for searching and PAUP* 4.0b4a-8 (PPC) for all other applications (Swofford, 2001). ITS and ETS sequences were manually aligned. Alignment of the sequences was unambiguous, due to low sequence divergence among taxa. In order to determine whether any sequences should be excluded from the data set for subsequent likelihood analyses because of low percentage sequence divergence/expected number of substitutions per site, the expected number of substitutions per site was calculated using the Kimura two-parameter model (K80+G) of sequence evolution (Kimura, 1980), the model determined to be the best fitting model for likelihood searches for both ITS + ETS combined (see below). Indels and missing data were ignored in the calculation of distance, and distances were corrected for rate heterogeneity across sites using the discrete approximation to the gamma distribution.

Phylogenetic analyses of ITS and ETS sequences, both separate and in combination, were conducted using maximum parsimony (MP) and maximum likelihood (ML) criteria to test the sensitivity of the data to the search algorithm. Base frequencies were examined for nucleotide bias among taxa using the chi-square test of homogeneity in PAUP*, and were not found to differ significantly ($P = 0.5831$). Heuristic searches were implemented under MP criteria using 5,000 random addition replicates of tree bisection–reconnection (TBR) branch swapping, saving 100 trees per replicate, mulpars on, and steepest descent activated. An unweighted "baseline" analysis was conducted for both separate ITS and ETS partitions and combined data (treating the gaps as missing data) on all 36 sequences. Subsequently, two taxa with pairwise sequence divergences of <1.0% (based on the K80+G model of sequence substitution, using PAUP*) were pruned from the analysis to speed likelihood analyses. One population of *P. hawaiiensis* var. *hillebrandii* (collected from Kipuka Puaulu, Hawai`i Volcanoes National Park, Hawai`i) and one individual of *P. greenwelliae* (from the Awa`awapuhi Trail population of Koke'e State Park, Kaua`i) were pruned from subsequent MP and ML analyses. The phylogenetic utility of indel characters has been widely discussed (Lloyd and Calder, 1991; Giribet and Wheeler, 1999; Lutzoni et al., 2000; Simmons and Ochoterena, 2000; Sanchis et al. 2001; Simmons et al., 2001). Potentially parsimony informative indels were coded as separate characters at the end of the data matrix and treated as missing data in the body of the nucleotide data matrix (GAPMODE=MISSING in PAUP*).

For parsimony searches, the effects of several weighting strategies of transversions relative to transitions (1.1:1 and 2:1) were explored using step matrices of user-defined character types on the substitution data alone (without indels). Support for internal branches was evaluated using 100 bootstrap replicates under the following

heuristic search parameters: 500 random addition replicates of TBR branch swapping, holding 10 trees per replicate, multrees on, and steepest descent on. Decay values (Bremer support) were calculated with the aid of AutoDecay 4.0 (Ericksson, 1999) used in conjunction with PAUP*.

Likelihood searches for separate ITS and ETS partitions and combined data employed an iterative approach (Sullivan et al., 1997) to evaluate models and then optimize model parameters for an initial set of trees resulting from parsimony analysis. Likelihood searches were then conducted under the fully defined model parameters. The program Modeltest 3.0 (Posada and Crandall, 1998) was used to evaluate models of DNA substitution that best fit the data. Likelihood scores for all models were evaluated using the likelihood ratio test statistic (Felsenstein, 1981; Goldman, 1993; Yang et al., 1995a) and the AIC criterion in Modeltest 3.0. In the event that the two evaluation criteria gave different models, the model with the fewest parameters needed to explain the data was chosen. The K80+G model was selected as the best-fit model of nucleotide substitution of the ITS partition alone; the HKY+G model (Hasegawa et al., 1985) was best for the ETS partition alone, and K80+G was best for the combined data. Heuristic ML analyses were implemented with a starting tree (tree 1 of respective MP searches, chosen arbitrarily as a reasonable starting estimate) obtained under the baseline MP searches described for each partition, separately and in combination. Searches were conducted under the fully defined model using 10 replicates of TBR branch swapping. Bootstrap analyses were performed under likelihood criteria using 100 replicates of FASTSTEP search in which searches in each replicate were performed using one random sequence addition with no branch swapping. The parameters of the most likely model were estimated in advance and then set to previous, with max trees = 1, and empirical base frequencies were used.

Biogeographical Analyses

Historical biogeographical events (e.g., dispersal and colonization events) may be reconstructed as ancestral character states (i.e., biogeographical states) at internal nodes of an independently constructed phylogenetic tree. Such approaches may make use of parsimony criteria, such as those methods commonly used in the program MacClade (Maddison and Maddison, 1992, 2001), although a likelihood method has been incorporated in Mesquite (Maddison and Maddison, 2002). Likelihood-based reconstructions use an explicit model of character evolution to estimate probabilities of all possible ancestral states at every node on a tree. Such probabilities are determined by a number of factors: the model of evolution used, the distribution of the character states in terminal taxa, the rate of evolution of the character, and the lengths of internal branches on the tree (Cunningham et al., 1998). It is possible for likelihood to prefer less parsimonious reconstructions, in part because branch length is explicitly considered. A strength of the likelihood method is that such approaches have revealed a considerable amount of uncertainty in ancestral character states, even when those states are unequivocally reconstructed under parsimony.

In this study, analysis of historical patterns of colonization were accomplished using two methods: (1) MP or Fitch reconstruction of ancestral geographical character states at internal nodes and branches of the trees and (2) ML reconstruction. Patterns of colonization and ancestral geographical localities of Hawaiian *Psychotria* were inferred using the single best tree topology obtained with an ML analysis of ITS and ETS sequences; this topology was identical to that of one of the best MP trees and to that of the strict consensus of the unweighted MP trees. (These trees were identical in topology because zero length branches in the single ML tree are represented as "hard" polytomies, i.e., multifurcations, and in the strict consensus of the MP trees these are represented as either "soft" or "hard" polytomies.) Minor differences in topology among external tips (on the (8) MP trees) do not change hypotheses of island dispersal order. The single best ML tree (and the MP trees) possessed "hard" polytomies, (interpreted as star-like speciation events, coded as zero-length branches in PAUP*). Additionally, we used one of the best MP trees (which was identical in topology to the single ML tree) for comparing inferred branch lengths under MP and ML criteria in reconstructing ancestral states. Although the Hawaiian island chain is comprised of eight high islands currently, these really comprise four island groups geologically (Carson and Clague, 1995): (1) Kaua`i/Ni`ihau; (2) O`ahu; (3) Moloka`i, Lana`i, Maui, and Kaho'olawe (together Maui Nui); and (4) Hawai`i. The islands of Maui, Moloka`i, Lana`i, and Kaho'olawe were formerly connected 300,000–400,000 years ago and represent a biogeographically meaningful unit (Carson and Clague, 1995). Populations of *Psychotria* are found only on Kaua`i, O`ahu, Moloka`i, Lana`i, Maui, and Hawai`i. For this reason, biogeographical character states were coded as one of four possible states, corresponding to the island or island groups of Kaua`i, O`ahu, Maui Nui, and Hawai`i. Only the geographical states of the ingroup (i.e., Hawaiian species and populations of *Psychotria*) were considered.

Both MP and ML reconstructions were conducted using a specially recoded version of PAUP* for four character states (written and kindly supplied by David Swofford, Smithsonian Institution). In PAUP*, the four island states were coded as one of four states A, C, G, and T (A = Kaua`i, C = O`ahu, G = Maui Nui, and T = Hawai`i) in a separate matrix. MP and ML reconstructions of biogeographical characters were subsequently conducted on the single best ML tree using the "gettrees" command in PAUP*. Under MP assumptions, transitions between character states were treated as unordered, and ancestral character states at internal nodes were reconstructed using the command "mprsets" in PAUP*. Under likelihood criteria, the command "allprobs=yes" was used to request marginal probabilities (="fossil likelihoods" in Discrete, Pagel,

1994) of each character state assignment for each internal node. We refer to these probabilities as relative probabilities for each node. Additional likelihood settings for both models investigated included "lset basefreq=equal userbrlens=yes." Rates of substitution of one geographical state to another were optimized by scaling all user-supplied branch lengths by a common factor estimated using maximum likelihood. We tested the sensitivity of the ML estimates to two factors: branch length and model of evolutionary change. We examined the effect of branch length on reconstructions by using (1) inferred branch lengths under likelihood criteria (branch lengths in expected number of substitutions per unit time, based on the optimal model used in searching), (2) inferred branch lengths under parsimony criteria (branch lengths equal to number of substitutions that have occurred in that branch), and (3) branch lengths equal to 1. Note that setting all branch lengths equal to 1 is often considered to be equivalent to assuming a punctuational model of evolutionary change, and has been advocated in clades that have undergone adaptive radiations (e.g. island radiations, Schluter and Nagel, 1995; Mooers et al., 1999). However, such a model may more appropriately be termed a model that assumes homogeneity of the amount of change across speciation events, because punctuational evolution implies change at speciation events, but the amount of change is not necessarily the same from one speciation event to another. PAUP* was modified to estimate a branch length scaler for ML reconstructions. The scaler estimates a value by ML under which the likelihood for the entire set of reconstructions (marginal probabilities) is maximized. This value can then be used to multiply with all branch lengths and obtain an optimal set of reconstructions that will have identical likelihoods, regardless of the branch lengths used. Such a scaler is necessary because branch lengths, here inferred from nucleotide substitution data and in units of expected number of substitutions per site, are arbitrary in the context of rates of island colonization, and also because branch length has a large effect on likelihood reconstructions (see Discussion). Pagel (1994, 1997, 1999b) described a continuous time Markov model of evolutionary change for reconstructing ancestral states of discrete characters, which was expanded upon by Schluter et al. (1997). The Markov process model assumes (1) that probability of change in a character depends only on the character's present state and not on any previous states; (2) changes along branches are independent of changes elsewhere on the tree; and (3) rates of change are constant throughout the tree. Here, we explored the use of two different models of evolutionary change. The Jukes–Cantor-like model (Jukes and Cantor, 1969) assumes that equilibrium frequencies of all states are the same and that character state changes occur at the same rate, i.e., dispersal from one island to any other is equally probable, and allows for a single type of substitution or character state change, equivalent to the Markov process model described above (employed using the likelihood setting "lset nst=1" in PAUP*). The general time reversible (GTR)-like model (Rodriguez et al., 1990) assumes that the overall rate of change from a given state i to j in a given time is the same as the rate of change from j to i but restricts some character state changes. The GTR-like model was used to limit dispersals between nearest neighboring islands only, i.e., from older to younger islands only (employed by using the likelihood setting "lset nst=6 rmat=(1 0.0001 0.0001 1 0.0001)" in PAUP*). The latter model incorporates a well-documented pattern of dispersal among islands of the Hawaiian chain in which colonization has been found to proceed successively in parallel to island age, i.e., Kaua`i to O`ahu, O`ahu to Maui Nui, and Maui Nui to Hawai`i (Wagner and Funk, 1995).

Estimating Rates of Dispersal

Branch lengths could be estimated by enforcing a molecular clock on a best ML tree to estimate the rates of dispersal for Hawaiian *Psychotria*. However, the presence of clocklike rates was tested first by comparing variation in rates across lineages using a tree-wide likelihood ratio test (Felsenstein, 1988; Huelsenbeck and Rannala, 1997) to compare rate-constant and rate-variable models. A likelihood search under the fully optimized model was repeated enforcing a molecular clock, and likelihoods for the best clock-constrained and unconstrained trees were compared using the likelihood ratio test statistic. Rate constancy was strongly rejected; thus, the unconstrained tree and saved branch lengths were subject to the nonparametric rate smoothing procedure (Sanderson, 1998) using the program TreeEdit 1.01 (Rambaut and Charleston, 2001). The rate-smoothed branch lengths were used to estimate rates of dispersal for Hawaiian *Psychotria*. A scaler was subsequently estimated in the recoded version of PAUP* via ML for this set of rate-smoothed, clocklike branch lengths. Multiplying the scaler by the branch length under molecular clock assumptions gives branch lengths that can be interpreted as equal to the number of dispersals per unit time (or relative rate of dispersal). Three internal calibration points within the *mariniana* clade (corresponding to branches labeled on Fig. 4) and one within the *greenwelliae* clade were used (with a "local" clock; Hillis et al., 1996) to convert relative time to absolute time. These points correspond to dispersal from (1) Kaua`i to O`ahu, (2) O`ahu to Maui Nui, and (3) Maui Nui to Hawai`i within the *mariniana* clade and (4) Kaua`i to O`ahu and (5) O`ahu to Maui in the *greenwelliae* clade (refer to Fig. 4). Calibrating these scaled branch lengths at the internal nodes by maximum age of the younger island gives an estimate of rate of dispersal per million years. Maximum age of the Hawaiian Islands has been estimated using K-Ar dating (Carson and Clague, 1989; Clague and Dalrymple, 1987). The maximum age used for O`ahu was 3.7 million years, for Maui Nui 1.9 million years, and for Hawai`i 0.5 million years.

Exploring scenarios for biogeographical patterns in a phylogenetic context can provide a meaningful method for interpreting colonization and dispersal despite the

inherent limitations involved in reconstructing ancestral character states under parsimony and other assumptions, which include dependence on the topology used (see Belshaw and Quicke, 2002) and sampling (Salisbury and Kim, 2001). While there is no *a priori* reason to believe that there are unequal (asymmetric) rates of gains and losses among distribution states in the Hawaiian *Psychotria* (i.e., dispersal vs. extinction of a population on an island), it is expected that rates of dispersal within the lineage may have been high (i.e., rapid radiation).

RESULTS

Sequence Variation

The aligned ITS region (702 bp) and the 3′ end of the ETS region (469 bp) comprise a combined matrix of 1,170 bp (GenBank accession numbers for the aligned sequences are given in Table 1). There were 168 phylogenetically informative characters in the entire matrix of combined sequences. Of these characters, 94 were provided by the ITS nucleotide data matrix and 74 were provided by the ETS partition. In addition, nine potentially informative gap characters were found, five in the ITS and four in the ETS region. The expected number of substitutions per site under the K80+G model of sequence evolution (ignoring missing data and indels and using the discrete approximation to the gamma) ranged from 0.00 between individuals of *P. greenwelliae* from Kaua`i to 11.160 between the outgroup *P. tahitiensis* and the ingroup Hawaiian species *P. fauriei*. Within the ingroup, expected number of substitutions per site ranged from 0 to 0.834 (the highest value was between *P. grandiflora* of sect. *Pelagomapouria* and *P. hathewayi* from sect. *Straussia*). GC content of the ITS region and the 5.8S subunit falls within ranges reported for other angiosperms (Baldwin et al., 1995); these areas are slightly GC rich (55.0% for the entire region) but not highly skewed. The ETS region showed similar (54%) mean GC content.

Phylogenetic Analysis

Parsimony searches.—Results of phylogenetic analyses are summarized in Table 2. Separate phylogenetic analyses resulted in 20 most-parsimonious trees for the ITS matrix, with a length of 223 steps (consistency index [CI] = 0.561, retention index [RI] = 0.673) and 11 most-parsimonious trees for the ETS matrix (length = 116, CI = 0.750, RI = 0.891). The combined data sets yielded eight MP trees, with a length of 341 (CI = 0.621, RI = 0.773) (Fig. 2). When transitions were downweighted 1 or 1.1 relative to transversions, 8 MP trees were found for the ITS matrix, these being a subset of the 20 trees obtained under Fitch assumptions, and 1 tree (one of those obtained under Fitch assumptions) was obtained for the ETS matrix. Downweighting transitions in the combined data set by 1 or 1.1 relative to transversions yielded four trees, which were a subset of those eight obtained under Fitch assumptions. Using a slightly higher weighting scheme of transversions weighted 2:1 over transitions resulted in 66 MP trees for the ITS matrix alone, which were one step longer than the shortest unweighted trees (not shown), and 1 tree for the ETS partition (one of those obtained under Fitch assumptions).

TABLE 2. Description of nuclear rDNA data sets, analyses, and resulting trees.

Region/analysis[a]	Aligned length (bp)	No. (%) potentially informative positions	No. informative indels	No. steps (MP, not including uninformative characters) or ML score	Consistency index	Retention index	No. trees, MP or ML	Model used (ML analyses)
ITS baseline	702	94 (7.5)		223	0.561	0.673	20 MP	N/A
ITS + indels	707	99 (7.1)	5	235	0.561	0.681	20 MP	N/A
ITS TI:TV 1:1.1	702	94 (7.5)		223 (under Fitch assumptions)	0.561	0.673	8 MP	N/A
ITS TI:TV 1:2	702	94 (7.5)		224 (under Fitch assumptions)	0.558 (under Fitch assumptions)	0.670 (under Fitch assumptions)	66 MP	N/A
ETS baseline	469	74 (6.3)		116	0.750	0.891	11 MP	N/A
ETS + indels	473	78 (6.1)	4	121	0.750	0.890	16 MP	N/A
ETS TI:TV 1:1.1	469	74 (6.3)		116 (under Fitch assumptions)	0.750	0.891	1 MP	N/A
ETS TI:TV 1:2	469	74 (6.3)		116 (under Fitch assumptions)	0.750	0.891	1 MP	N/A
Combined ITS + ETS baseline	1,170	168 (7.0)		341	0.621	0.773	8 MP	N/A
Combined ITS + ETS + indels	1,179	178 (15.1)	9	407	0.626	0.771	4 MP	N/A
Combined ITS + ETS TI:TV 1:1.1	1,170	168 (7.0)		341 (under Fitch assumptions)	0.622	0.773	4 MP	N/A
Combined ITS + ETS TI:TV 1:2	1,170	168 (7.0)		342 (under Fitch assumptions)	0.619	0.771	22 MP	N/A
Likelihood ITS baseline	702			−4022.56			1 ML	K80+G
Likelihood ETS baseline	469			−3470.39			1 ML	HKY+G
Likelihood ITS + ETS	1,170			−4743.98			1 ML	K80+G

[a]TI = transitions; TV = transversions.

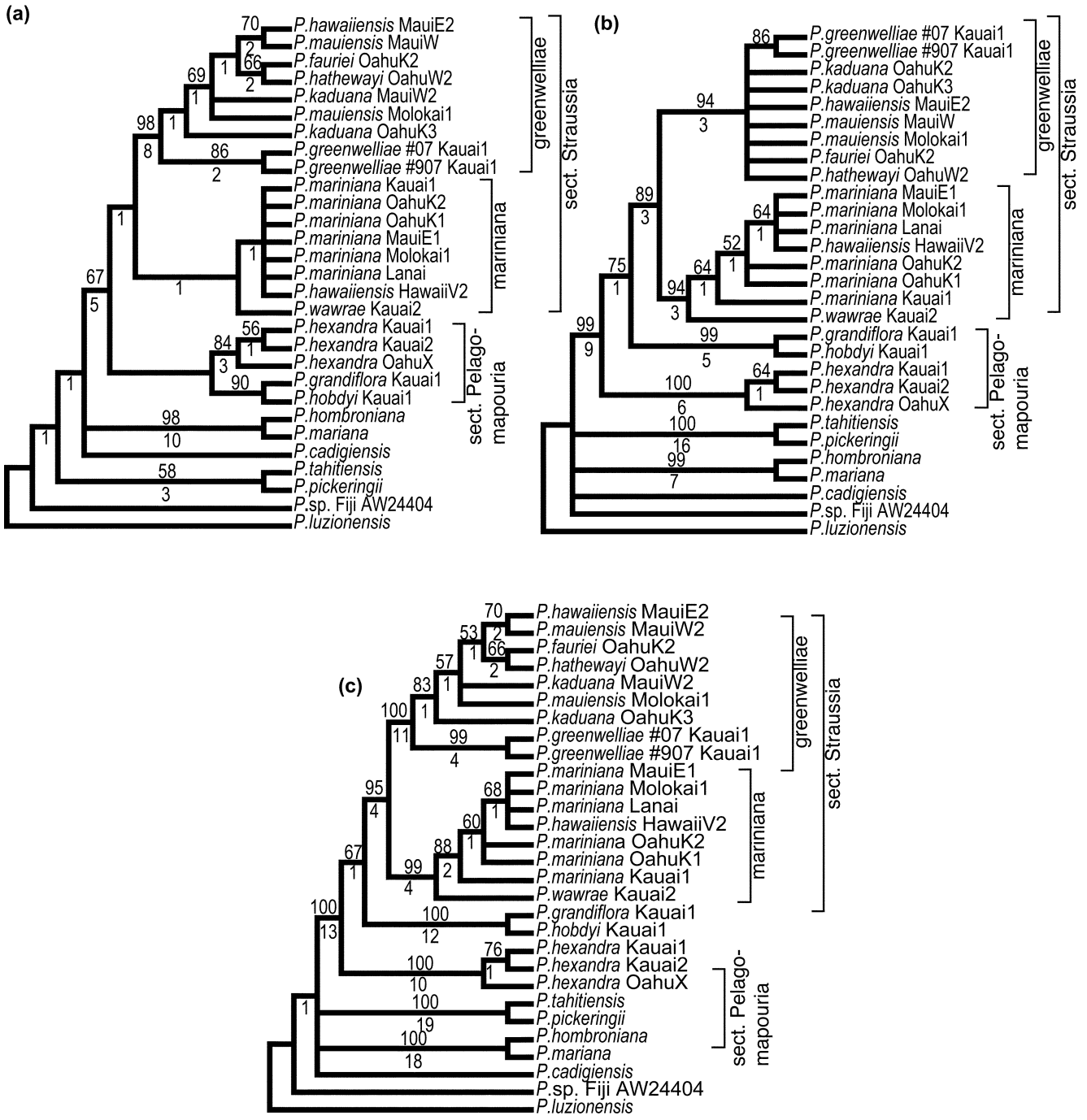

FIGURE 2. Comparison of trees resulting from phylogenetic analysis of ITS, ETS, and combined partitions. (a) Strict consensus of 20 MP trees resulting from phylogenetic analysis of ITS partition (baseline) only. (b) Strict consensus of 11 MP trees resulting from (baseline) phylogenetic analysis of ETS partition only. (c) Strict consensus of eight MP trees, combined ITS + ETS partitions (baseline analysis). Bootstrap values are given above branches, and decay values are below. Refer to Figure 1 for collecting locality codes.

Downweighting transitions 2:1 in the combined data set yielded 22 trees, and these trees were one step longer than the most-parsimonious trees under Fitch assumptions. Including five indel characters with the substitution data for the ITS sequences alone yielded 20 trees, with length of 235, CI = 0.561, and RI = 0.681, and inclusion of four indels together with the ETS sequences alone yielded 16 trees, length = 121, CI = 0.750, and RI = 0.890. The strict consensus trees from both ITS + indels and ETS + indels partitions are identical in topology to the strict consensus trees resulting from unweighted analyses. Bootstrap values were not improved by the addition

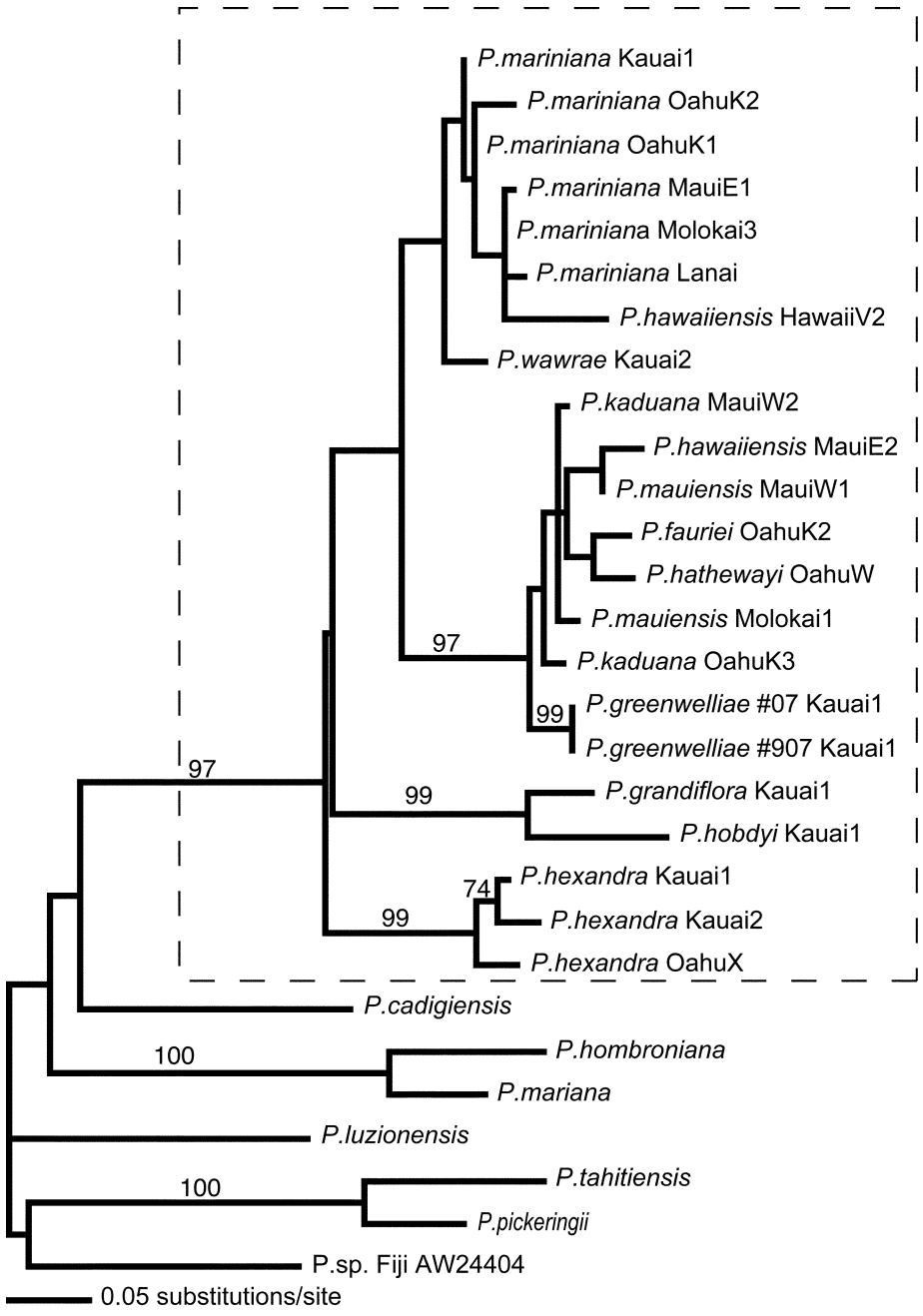

FIGURE 3. Single best ML tree resulting from analysis of combined ITS and ETS partitions under the K80+G model with fully optimized parameters. Likelihood bootstrap values are given above branches with >50% bootstrap values. Refer to Figure 1 for collecting locality codes.

of indels to either partition alone. The combined data + indels gave four MP trees, with a length of 407 (CI = 0.626, RI = 0.771). This tree is nearly identical to trees from the combined data set without indels, the only difference is that the strict consensus tree is more resolved among outgroups, although not supported by bootstrap values (not shown).

Likelihood searches.—The combined ITS and ETS partitions yielded a single ML tree with a negative log likelihood (−lnL) score of 4743.97506. The single best ML tree for the combined ITS and ETS data is shown in Figure 3 and possesses two "hard" polytomies within the terminal branches of the *mariniana* and *greenwelliae* clades, respectively. The topology of the single best ML tree is identical to that of the strict consensus of the eight baseline MP trees resulting from analysis of combined ITS and ETS partitions. Thus, this tree also shares the topological feature of the ETS partition trees in having the populations of *P. hexandra* sister to the rest of the clade, followed by *P. grandiflora* + *P. hobdyi* branching

off next and a monophyletic sect. *Straussia*. The single best ML tree resulting from analyses of the ITS partition alone differs in topology from the ML trees for the ETS alone and combined analyses in possessing a hard polytomy at the base of the Hawaiian clade (not shown), with three main lineages: (1) *P. grandiflora* + *P. hobdyi*, (2) *P. hexandra* accessions, and (3) a monophyletic sect. *Straussia*.

Phylogenetic Relationships

Phylogenetic analyses using MP and ML methods, with the ITS, ETS, and combined data matrices all support a monophyletic Hawaiian lineage (Figs. 2, 3). Monophyly of the Hawaiian lineage is supported with bootstrap values of 67% (decay value of 5) for the ITS alone, 99% (decay of 9) for ETS alone, and 100% (decay of 13) for the combined data. Analyses of the combined data and ETS partition alone (MP and ML analyses) support the populations of *P. hexandra* as sister to the rest of the Hawaiian clade (75% bootstrap and decay of 1 for ETS alone; 67% bootstrap and decay of 1 for combined data), with the clade containing *P. grandiflora* + *P. hobdyi* branching off next. Thus, these analyses support a paraphyletic relationship of sect. *Pelagomapouria* with respect to sect. *Straussia*. The ITS partition alone places accessions of *P. hexandra* in a clade with (and sister to) *P. grandiflora* and *P. hobdyi*; thus, sect. *Pelagomapouria* is either monophyletic (MP analyses) or is in a polytomy at the base of the Hawaiian clade with the *P. grandiflora* + *P. hobdyi* lineage and section *Straussia* (ML analyses). However, these relationships are not supported in the ITS analyses alone.

The sect. *Straussia* lineage is strongly supported with 89% and 95% bootstrap values in the ETS and combined analyses, respectively, and decay values of 3 and 4, respectively. The branch supporting the monophyly of sect. *Straussia* is only weakly supported in the ITS analysis alone, with a bootstrap value of 26% and decay of 1. However, all analyses (MP and ML) for all datasets recover two lineages within sect. *Straussia*: (1) populations of *P. mariniana* plus the Kaua`i endemic *P. wawrae* and *P. hawaiiensis* var. *hawaiiensis*, hereafter referred to as the *mariniana* clade (after a species that typifies the morphological characteristics) and (2) all the remaining species and populations of Hawaiian *Psychotria* except *P. grandiflora*, *P. hexandra* and *P. hobdyi*, hereafter referred to as the *greenwelliae* clade. The *greenwelliae* clade consists of all members of sect. *Straussia*, *except P. wawrae, P. mariniana*, and *P. hawaiiensis* subsp. *hawaiiensis* and is well supported (bootstrap value of 98%, decay of 8 for ITS; 94%, decay of 3 for ETS, 100%, decay of 11 for combined data).

In all analyses, accessions identified as members of a given "taxonomic" species form monophyletic groups with the following exceptions: *P. kaduana*, which has a paraphyletic relationship with respect to other members of the *greenwelliae* clade except *P. greenwelliae*; *P. mauiensis* which has a paraphyletic relationship to most other members of the *greenwelliae* clade (except *P. greenwelliae* and *P. kaduana* from O`ahu); and *P. mariniana*, which is paraphyletic with respect to the only population of *P. hawaiiensis* var. *hawaiiensis* sampled.

In general, the strict consensus of trees resulting from the combined data analysis is also more resolved and better supported than those resulting from analysis of either ITS or ETS data sets alone. In most cases, weighted parsimony analyses recovered either the same set of MP trees as the unweighted parsimony analyses or a smaller subset. Topologies resulting from weighted analyses did not differ from those resulting from unweighted analyses. Adding nine potentially informative indel characters to either partition separately or to the combined analysis did not change the topology of the trees and only resulted in fewer trees in the combined analysis.

Patterns of Colonization and Dispersal

Patterns of island colonization were compared using the topology of the single optimal ML tree resulting from analysis of combined ITS and ETS sequences under the K80+G model (Fig. 3), (see methods, above, for justification), which was identical to tree 6 of 8 MP trees. We explored the use of three types of branch length estimates: (1) those estimated under the fully defined likelihood model from the sequence data, (2) those estimated using parsimony from the sequence data, and (3) all branch lengths equal to 1 (punctuational model). On the MP trees, internal nodes range in bootstrap support from 53% to 100%, with six internal nodes supported at >90%, three nodes over 75%–90%, and seven nodes above 50%–75%. Topological differences between the shortest MP trees and the best ML tree do not result in major differences in the reconstructed pattern of island colonization.

MP reconstructions.—Ancestral state reconstructions using MP assumptions unequivocally reconstruct the root of the Hawaiian clade as Kaua`i (Fig. 4, comparison of MP and ML reconstructions). Populations of *P. hexandra*, which together are the sister to all other members of the Hawaiian clade in the best ML tree of combined ITS and ETS sequences (but form a monophyletic sect. *Pelagomapouria* together with *P. hobdyi* and *P. grandiflora* in the analysis of ITS sequences alone), are reconstructed to have had an ancestral distribution on Kaua`i and to have subsequently dispersed to the next younger island of O`ahu. The next clade to branch off, comprising the sister taxa *P. grandiflora* and *P. hobdyi*, is also reconstructed to have had an ancestral distribution on Kaua`i , although the clade never dispersed further. Additionally, there are no historical records of these taxa ever having been collected on any younger islands. The remaining two sister clades, the *greenwelliae* and the *mariniana* clades, also are reconstructed to have had ancestral distributions on Kaua`i. The *mariniana* clade follows a strict Kaua`i to O`ahu to Maui Nui to Hawai`i pattern of dispersal, although zero-length branches on the tree used to optimize the ancestral characters confound monophyly of the populations within a given island. All internal nodes of the *mariniana* clade are reconstructed unequivocally under MP. Dispersal patterns in the

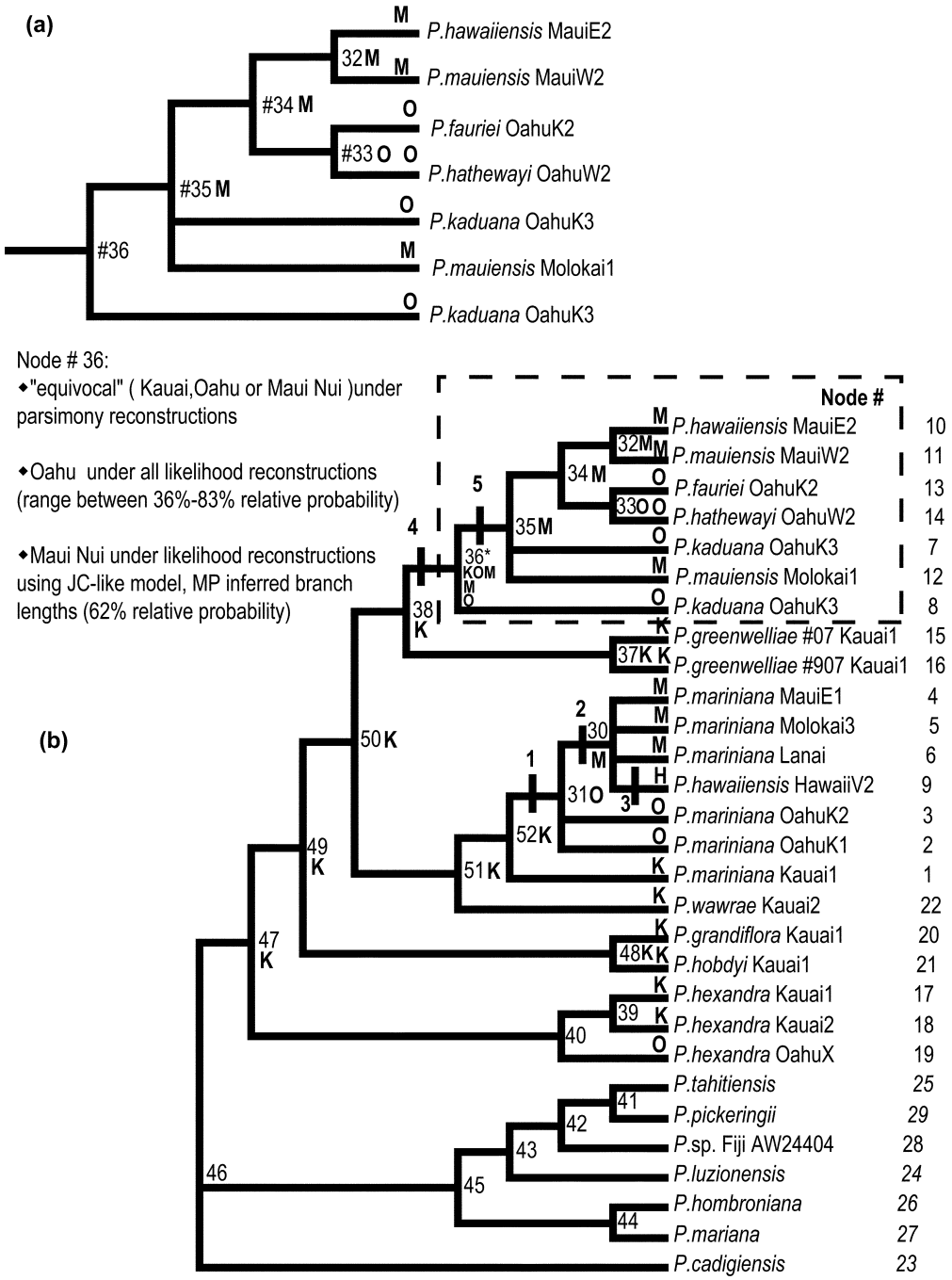

FIGURE 4. Comparison of MP and ML biogeographical reconstructions, using topology of single best ML tree for the combined ITS + ETS analysis. Branch lengths used are listed in Table 3 and include inferred branch lengths under the K80+G model, branch lengths = 1 (punctuational model), and branch lengths inferred under Fitch parsimony. (a) Reconstruction of ancestral states under parsimony and likelihood criteria is identical for all but one node 36, which defines the non-Kaua`i components of the *greenwelliae* clade. Refer to Figure 3 for branch lengths associated with this clade. (b) Node 36 is reconstructed equivocally under parsimony, with the states of Kaua`i, O`ahu, and Maui Nui equally likely. Under likelihood, however, node 36 is reconstructed as O`ahu, with a relative probability of only 36% under the JC-like punctuational model and as high 83% under the GTR-like punctuational model. However, when using branch lengths inferred under parsimony, with a JC-like model, node 36 is reconstructed as Maui Nui with 62% relative probability. Island state abbreviations: K = Kaua`i; O = O`ahu; M = Maui Nui complex; H = Hawai`i. Five internal calibration points are indicated within the *mariniana* and *greenwelliae* clades corresponding to dispersal from (1) Kaua`i to O`ahu, (2) O`ahu to Maui Nui, (3) Maui Nui to Hawai`i for the *mariniana* clade, (4) Kaua`i to O`ahu (and/or Maui Nui under parsimony reconstructions), and (5) O`ahu to Maui Nui for the *greenwelliae* clade.

TABLE 3. Comparison of ancestral bioegeographical reconstructions and relative probabilities (RP) of reconstructed states in Hawaiian *Psychotria* using MP and ML under different models of dispersal (JC or GTR-like) and branch lengths (punctuational evolution; inferred from ML; inferred from MP).

		Punctuatural evolution				Inferred from ML				Inferred from MP[a]			
		JC-like		GTR-like		JC-like		GTR-like		JC-like		GTR-like	
Node	MP (Reconstruction)	Island	RP	Island	RP	Island	RP	Island	RP	Island	RP	Island	RP
30	Maui	Maui	1.000	Maui	1.000	Maui	1.000	Maui	1.000	Maui	1.000	Maui	1.000
31	Oahu	Oahu	0.920	Oahu	0.983	Oahu	0.710	Oahu	0.965	Oahu	1.000	Oahu	1.000
32	Maui	Maui	0.982	Maui	0.946	Maui	0.963	Maui	0.954	Maui	1.000	Maui	1.000
33	Oahu	Oahu	0.907	Oahu	0.877	Oahu	0.497	Oahu	0.672	Oahu	0.695	Oahu	0.655
34	Maui	Maui	0.858	Maui	0.731	Maui	0.757	Maui	0.672	Maui	0.932	Maui	0.874
35	Maui	Maui	0.953	Maui	0.842	Maui	0.828	Maui	0.710	Maui	0.981	Maui	0.937
36	equivocal: K/O/M[b]	Oahu	0.356	Oahu	0.827	Oahu	0.469	Oahu	0.792	Maui	0.614	Oahu	0.617
37	Kauai	Kauai	0.987	Kauai	0.974	Kauai	1.000	Kauai	1.000	Kauai	1.000	Kauai	1.000
38	Kauai	Kauai	0.900	Kauai	0.830	Kauai	0.453	Kauai	0.606	Kauai	0.431	Kauai	0.490
39	Kauai	Kauai	0.973	Kauai	0.964	Kauai	0.902	Kauai	0.980	Kauai	0.980	Kauai	0.985
40	Kauai	Kauai	0.764	Kauai	0.749	Kauai	0.633	Kauai	0.874	Kauai	0.784	Kauai	0.860
41	Kauai	Kauai	0.346	Kauai	0.498	Kauai	0.251	Kauai	0.451	Kauai	0.273	Kauai	0.447
42	Kauai	Kauai	0.387	Kauai	0.537	Kauai	0.251	Kauai	0.443	Kauai	0.253	Kauai	0.347
43	Kauai	Kauai	0.447	Kauai	0.585	Kauai	0.252	Kauai	0.473	Kauai	0.253	Kauai	0.347
44	Kauai	Kauai	0.447	Kauai	0.585	Kauai	0.252	Kauai	0.473	Kauai	0.274	Kauai	0.447
45	Kauai	Kauai	0.534	Kauai	0.648	Kauai	0.250	Kauai	0.401	Kauai	0.277	Kauai	0.457
46	Kauai	Kauai	0.657	Kauai	0.730	Kauai	0.325	Kauai	0.773	Kauai	0.297	Kauai	0.503
47	Kauai	Kauai	0.835	Kauai	0.840	Kauai	0.328	Kauai	0.796	Kauai	0.482	Kauai	0.744
48	Kauai	Kauai	0.993	Kauai	0.989	Kauai	0.392	Kauai	0.906	Kauai	0.769	Kauai	0.900
49	Kauai	Kauai	0.967	Kauai	0.955	Kauai	0.416	Kauai	0.877	Kauai	0.471	Kauai	0.777
50	Kauai	Kauai	0.975	Kauai	0.953	Kauai	0.687	Kauai	0.936	Kauai	0.559	Kauai	0.791
51	Kauai	Kauai	1.000	Kauai	1.000	Kauai	0.995	Kauai	1.000	Kauai	0.740	Kauai	0.848
52	Kauai	Kauai	1.000	Kauai	1.000	Kauai	1.000	Kauai	1.000	Kauai	1.000	Kauai	1.000

[a]From tree 6 to 8 MP trees, identical in topology to single best ML tree.
[b]K/O/M = Kauai, Oahu, and Maui Nui, respectively.

greenwelliae clade, however, are confounded by equivocal reconstructions at one internal node (indicated in Fig. 4) corresponding to the *kaduana* clade (all members of the *greenwelliae* clade minus *P. greenwelliae*), which contains individuals representing *P. kaduana*, *P. mauiensis*, *P. hathewayi*, *P. faurei*, and *P. hawaiiensis* var. *hillebrandii*. This node is reconstructed equivocally under parsimony as Kaua`i, O`ahu, or Maui. Thus, several scenarios are equally likely under parsimony. One back dispersal is also inferred for the clade containing the sister taxa *P. fauriei* and *P. hathewayi* on O`ahu from ancestors distributed on Maui Nui.

ML reconstructions.—Under a simple JC-like model with branch lengths equal to 1, i.e., equivalent to a punctuational model of evolution (except for zero-length branches, which were coded at an arbitrarily low value of 0.00001), a branch length scaler of 0.271062 was estimated via ML. Applying this scaler value to all branches resulted in a −lnL of 24.84486 for the set of reconstructions. In general, under all likelihood models and branch length combinations used, including the JC-like model with branch lengths equal to 1, internal nodes were reconstructed identically, as under MP assumptions. However, under the JC-like model with all branch lengths equal to 1, the single node that is equivocally reconstructed under MP (node 36) is unequivocally reconstructed as O`ahu, although with a relative probability of only 35%. Nearly as likely for this node are reconstructions as Maui Nui (32%) and Kaua`i (29%), whereas Hawai`i receives only 3% probability of reconstruction. Table 3 lists relative probabilities of character state assignments at all internal nodes.

Under the GTR-like model with branch lengths equal to 1, a branch length scaler is estimated at 0.284631, and the −lnL for this set of reconstructions was 20.80196. Reconstructions under this model were again nearly identical to those reconstructed under MP assumptions. However, the *kaduana* clade node is reconstructed unequivocally as O`ahu with 83% probability, Kaua`i and O`ahu receive 10% and 7.7% probabilities, respectively, and Hawai`i receives <1% probability of reconstruction. The model used specifies higher probability of colonizing from nearest neighbor islands.

Using inferred branch lengths (those estimated from the ML search), under the JC-like model, the branch length scaler is estimated to be 137.801, a value several orders of magnitude higher than with branch lengths set equal to 1. With this scaler applied, the −lnL for the set of reconstructions is 28.34022. Again, the reconstructions are close to identical to those inferred under MP assumptions. The *kaduana* clade node is unequivocally reconstructed as O`ahu, with 47% probability (Maui Nui is nearly as likely with 44% probability, and Kaua`i and Hawai`i have 4.7% and 3.5% probability, respectively). Using inferred branch lengths under the GTR-like model, the estimated branch length scaler is 76.3651 and the −lnL for this set of reconstructions is 23.663. Under this model, the *kaduana* clade node is reconstructed

unequivocally as O`ahu with 78% probability, Maui Nui receives only 21%, and Kaua`i and Hawai`i each receive <1%.

Likelihood reconstructions based on inferred branch lengths obtained under parsimony (from tree 1, arbitrarily chosen from the 8 combined MP trees) are again nearly identical to those obtained reconstructed under MP assumptions with both JC-like and GTR-like models. The branch length scaler was estimated at 0.155602 for the MP tree under the JC-like model, and the −lnL of this set of reconstructions is 25.778. The single equivocal node under parsimony is reconstructed unequivocally as Maui Nui, however with 60% probability, rather than O`ahu, which receives 32% probability. Additionally, some nodes, while reconstructed unambiguously as a given state, received substantial probability for alternative states, a phenomenon not picked up with parsimony reconstructions alone. For example, although node 48, the root node, is reconstructed as Kaua`i with 31%, O`ahu receives 25% probability, and Maui Nui and Hawai`i each receive 22% probability. Node 39 (which defines the *greenwelliae* clade) is reconstructed with 48% probability as Kaua`i, but Maui Nui receives 28% probability and O`ahu receives 17%. Character states reconstructed under MP assumptions received between 83% and 100% probability under the JC-like model with branchlengths equal to 1.

Under rate-smoothed, clocklike branch lengths, we estimated that the rate of dispersal within the *mariniana* clade was 0.0163 dispersals per million years from Kaua`i to O`ahu, 0.0901 dispersals per million years from O`ahu to Maui Nui, and 0.9319 dispersals per million years from Maui Nui to Hawai`i. Within the *greenwelliae* clade, dispersals from Kaua`i to O`ahu and/or Maui Nui were confounded because those events correspond to node 36, which is equivocally reconstructed under parsimony. However, most likelihood reconstructions favor the ancestral state of O`ahu for node 36 (see Fig. 4), and if we assume O`ahu for this node, then a dispersal rate is estimated at 0.0213 dispersal per million years from Kaua`i to O`ahu and at 0.0217 dispersals per million years from O`ahu to Maui Nui.

DISCUSSION

Evolution of ITS and ETS Sequences in Hawaiian Psychotria

Sequence data from the nuclear ribosomal ITS and ETS regions provide useful information for inferring phylogenetic relationships at the broadest levels of taxonomic rank in the Hawaiian *Psychotria*. Expected numbers of substitutions per site among the Hawaiian species of *Psychotria* indicate low mean genetic divergence within each of three major clades recovered in the phylogenetic analysis: 0.155 within the *greenwelliae* clade, 0.087 within the *mariniana* clade, and 0.371 within members of sect. *Pelagomapouria*.

Low support for internal branches within the Hawaiian clade recovered in the ITS or ETS analyses alone is likely due to the low number of substitutions that occur along branches. The pattern of short branch lengths among lineages comprising the Hawaiian clade could result from either an artifact of taxonomic sampling or rapid radiation of the lineages. This pattern is commonly observed in molecular phylogenies of other Hawaiian plant groups, e.g., the Hawaiian silverswords (Baldwin, 1992) and the Hawaiian endemics *Scheidea* and *Alsinidendron* (Caryophyllaceae) (Soltis et al., 1996), and is consistent with the notion of rapid radiation following colonization by a single ancestor. Use of indel characters or weighting increases resolution in the strict consensus; however, complete resolution of terminal clades is not achieved through use of sequence data alone.

Origin and Monophyly of the Hawaiian Clade

Of the taxa sampled in the *Psychotria*-wide analysis (Nepokroeff et al., 1999), the Hawaiian *Psychotria* appear most closely related to a group of species in the subgenus *Psychotria* occurring in the Pacific Basin. The western Pacific members of subgenus *Psychotria* were hypothesized by both Fosberg (1964) and Sohmer (1977, 1978) to be closest relatives and to occur in the source area for Hawaiian *Psychotria*; our findings support that hypothesis. Although support for monophyly is low for ITS sequence data alone, the ETS sequence data alone and combined ITS + ETS sequence data strongly support the monophyly of the Hawaiian lineage. The most common source area for plant colonists to the Hawaiian Islands has been the Pacific islands to the south and west of Hawai`i, although several notable exceptions exist, including North America (Baldwin et al., 1991; Vargas et al., 1998; Alice and Campbell, 1999; Ballard and Sytsma, 2000; Lindqvist and Albert, 2002) and Africa (Kim et al., 1998).

Relationships Within the Hawaiian Psychotria

Although phylogenetic analysis of the ITS and ETS regions results in only somewhat different topologies with regard to sect. *Pelagomapouria* and sect. *Straussia*, such differences are not well supported, and the combined data weakly support a paraphyletic relationship, with sect. *Straussia* derived from section *Pelagomapouria* and *P. hexandra* as sister to all other species in the lineage (with 60% bootstrap support). Within the Hawaiian lineage, branch lengths are relatively short and support for branches among the three basal lineages (corresponding to *P. hexandra* populations, *P. grandiflora* + *P. hobdyi*; and sect. *Straussia*) is low. One possible explanation for lack of resolution among these basal lineages is that radiation upon initially colonizing the islands was extremely rapid. Alternatively, the current species of Hawaiian *Psychotria* may represent several separate lineages that had been in place before the formation of the modern high island of Kaua`i, either derived from separate and possibly now extinct colonists or from a single now-extinct colonist. However, low genetic divergence is less consistent with the idea of separate lineages. Further sampling among Pacific species of *Psychotria* would help to clarify these relationships. Fosberg (1964) suggested that sect.

Pelagomapouria gave rise to sect. *Straussia*. Our results based on combined data support that scenario (i.e., the paraphyletic relationship of sect. *Pelagomapouria* to sect. *Straussia*). Additionally, the results of all analyses suggest that the members of sect. *Pelagomapouria* (*P. grandiflora, P.hobdyi*, and *P. hexandra*) consistently occupy a basal (or at least sister) position to the rest of the Hawaiian clade.

Do the historical relationships inferred using the nuclear markers employed in this study accurately reflect the history of lineages of Hawaiian *Psychotria*? Several factors may influence the differing hypotheses resulting from phylogenetic analysis of nuclear markers (both together and separately) versus traditional—although not cladistic—hypotheses of relationship. Processes such as stochastic lineage sorting (Avise and Ball, 1990; Maddison, 1995), hybridization and introgression, and polyploidy may cause discordance between gene genealogies and species genealogies. As discussed by Shaw (1996), relationships influenced by lineage sorting (i.e. paralogs) would not be expected to be strongly correlated with biological or geographical criteria and would not result in meaningful monophyletic groups. Because the relationships inferred using ITS and ETS data are both highly correlated with geographical distribution (i.e., species and populations on neighboring islands and within the same island are each other's closest relatives) and biological criteria (e.g., leaf and floral morphology), lineage sorting of polymorphic forms of nuclear rDNA arrays are not likely to have occurred. Polyploidy has the potential to affect phylogeny reconstruction if the loci sampled represent paralogous rather than orthologous genes. However, polymorphic characters (i.e., with multiple peak bases at a position), which have been documented in some plant groups (Sang et al., 1995), were not encountered in this study. However, there is evidence that nuclear rDNA arrays may undergo differential lineage sorting following polyploidization in some plants (Wendel et al., 1995).

The Hawaiian species and populations of *Psychotria* present the opportunity to examine the reconstruction of historical relationships at the border between phylogenetic species (*sensu* Nixon and Wheeler, 1990) and phylogeographic or tokogenetic relationships, i.e., genealogical relationships between the individual organisms within a species and between populations. Factors such as small effective population size (Maddison, 1995), frequent colonization, and population extinction (Hudson, 1990) may contribute to recovery of monophyletic taxonomic species in many instances within the Hawaiian *Psychotria.* Furthermore, populations within a species, e.g., *P.mariniana*, appear to conform to expected (older to younger island) geographical patterns. However, relationships within members of the *kaduana* clade of sect. *Straussia* do not form well-supported monophyletic groups conforming to current species delimitations. Identification of species within this group is problematic, and characters used traditionally to circumscribe species may not accurately reflect species boundaries, particularly among more widespread species (*P. kaduana*, *P. mauiensis*, and *P. hawaiiensis*).

Parsimony Versus Likelihood Reconstructions of Ancestral Biogeographical States

In recent studies, the limitations of parsimony approaches to reconstructing character evolution have been discussed relative to reconstructing ancestral states under an ML framework (Omland, 1997; Schluter et al., 1997; Cunningham et al. 1998; Cunningham, 1999; Pagel, 1999a, 1999b). A likelihood framework has been adopted for reconstructing a variety of ancestral character states ranging from ancestral archosaur visual pigments (Chang et al., 2002), life history of ichneumonoid parasitoid wasps (Belshaw and Quicke, 2002), feeding behavior in Darwin's finches (Schluter et al., 1997), and floral symmetry in asterids (Ree and Donoghue, 1999). A likelihood model for estimating phylogenies from morphological data has also been presented (Lewis, 2001), underscoring the recent interest in developing and applying stochastic models for describing morphological change. Results of recent studies suggest that parsimony reconstructions can be misleading in instances when rates of evolution are rapid (Felsenstein, 1973) and when there are asymmetric probabilities of gains and losses (Schultz et al., 1996). Additionally, parsimony approaches reconstruct only a single ancestral state at a node (where unequivocal); the likelihood of that reconstructed state versus other states is unknown. Using a likelihood approach, we can assess the probability that a character is reconstructed as a given state, as well as the probability for reconstructing the character in alternative states.

In this study, colonization patterns inferred under both parsimony and likelihood criteria conform to a simple model of colonization from older to younger islands, with at least one instance of back colonization in the *greenwelliae* clade from the younger island group of Maui Nui to the older island of O`ahu for the clade containing the sister species *P. fauriei* and *P. hathewayi* on O`ahu and potentially for *P. kaduana* on O`ahu. All reconstructions (both MP and ML) suggest that the current radiation began on the oldest island of Kaua`i (3.8–5.6 million years old) or possibly an older island currently unable to sustain viable populations of *Psychotria*. Regardless of the topology used, all reconstructions point to an ancestral distribution on Kaua`i for all basal members of the Hawaiian clade: the sect. *Pelagomapouria* clade and the two radiations of sect. *Straussia*. The presence of at least two and possibly three well-defined lineages of *Psychotria* on Kaua`i suggests an additional hypothesis that these three lineages had already diverged at a time before the formation of Kaua`i and the other current high islands in the chain. Additionally, in general, patterns of dispersal and colonization in Hawaiian *Psychotria* follow a simple model of island hopping from oldest to youngest islands. Such a pattern, following Hennig's progression rule as outlined for Hawaiian biota by

Wagner and Funk (1995), has been previously suggested for other Hawaiian groups: lobelioids (Givnish et al., 1995), *Drosophila* (DeSalle, 1992), the heteropteran genus *Sarona* (Asquith, 1995), *Orsonwelles* spiders (Hormiga et al., 2003), and Hawaiian *Megalagrion* damselflies (Jordan et al., 2003). However, unlike the *Megalagrion* example, where closest relatives within clades represent radiations of ecological guilds, diversification in *Psychotria* does not appear to follow the pattern predicted for adaptive radiations (Schluter, 2000). In this respect, Hawaiian *Psychotria* exhibit a speciation pattern more similar to that inferred for the endemic Hawaiian Alsinoideae (Wagner et al., 1995; Sakai et al., 1997), the Hawaiian cricket genus *Laupala* (Shaw, 1995, 1996), and *Orsonwelles* spiders (Hormiga et al., 2003) in that most speciation events occur within islands rather than between islands. In most if not all cases, species of Hawaiian *Psychotria* have as their closest relatives other species occurring on the same island. In part, this is due to the fact that 7 of the 11 taxonomic species are single island endemics, 5 on Kaua`i and 2 on O`ahu. In these species, as well as all of the remaining species, our results are consistent with within-island cladogenesis, although alternative explanations could be postulated. The sect. *Pelagomapouria* clade has not radiated extensively and instead is almost exclusively present on only the oldest island of Kaua`i. One population of *P. hexandra* is reconstructed to have dispersed from Kaua`i to O`ahu. There is no historical record of either *P. grandiflora* or *P. hobdyi* occurring anywhere but in restricted areas on northwestern Kaua`i. *Psychotria hexandra* was formerly collected from several, rare localities on O`ahu and until recently was thought to be extinct on that island. The collection represented in this analysis is the only recent one of that species on O`ahu. In general, parsimony and likelihood methods gave similar results for reconstructing ancestral geographical states. One possible explanation for this similarity is that rates of dispersal may not be high within the Hawaiian *Psychotria*, and gains and losses (interpreted as dispersals and back-dispersals in the biogeographical context) may historically have been equally common.

At one node, (36) parsimony reconstruction gave equivocal results, whereas likelihood reconstructions gave a clear ordering of preferences to the island of O`ahu (in all but one case). Depending on the model used, the probability for this node being reconstructed as O`ahu was either the same as or only slightly higher than that for alternative states (JC-like, with scaled molecular branch lengths) or was much higher (GTR-like or all models with branch lengths equal to 1). Thus, likelihood reconstructions do help resolve ancestral biogeographical scenarios for the Hawaiian *Psychotria* in favor of an entirely older-to-younger island scenario of dispersal, and both MP and ML reconstructions suggest a pattern of colonization from older to younger islands.

A perceived advantage of likelihood-based reconstructions is the ability to incorporate information on branch lengths (and hence tempo of evolution) and thus to differentiate between possible ancestral states at a given node. However, even when parsimony and likelihood prefer the same reconstructions, the error associated with those reconstructions can be considerable. We find that depending on the model and branch lengths used, the degree to which one state is favored over other possible states at nodes that are equivocally reconstructed under parsimony varies widely (Table 3). Under simple JC-like models with branch lengths inferred from nucleotide data (either under ML or MP criteria), the state with the greatest relative probability is in general only slightly favored over other possible states, but under more complex GTR-like models, the state with the greatest relative probability is much more highly favored, regardless of the branch lengths used. The same is also true even for those nodes that are unequivocally reconstructed under the parsimony criterion; for example, node 38, which corresponds to the origin of the *greenwelliae* clade (Fig. 4), is reconstructed unequivocally as Kaua`i under the parsimony criterion but receives a relative probability of only 0.453 under the JC-like model with branch lengths inferred under likelihood and 0.431 with branch lengths inferred under parsimony. Note that nodes 41–46 represent ancestral nodes of the outgroup, which were coded as unknown (not from Hawaiian chain); thus, their ancestral states are all reconstructed (erroneously) as Kaua`i as an artifact of this scoring. These nodes also receive very low relative probabilities but should be disregarded. A special case of reconstruction is the root node, node 47 in Figure 4 (the Hawaiian lineage). This node, as well as #48, also near the base of the lineage, receive very low relative probabilities (0.328 and 0.482 for node 47 under a JC-like model with likelihood inferred and parsimony inferred branch lengths, respectively). Such low relative probabilities likely reflect the fact that there is a higher degree of uncertainty in ancestral reconstruction of nodes closer to the root of a phylogeny. Thus, depending on the model and branch length used, the degree to which likelihood reconstructions favor one ancestral state over an alternative state can vary greatly. In general, simpler JC-like models and inferred branch lengths (especially parsimony inferred branch lengths) tend to have lower relative probabilities than do alternative states; thus those conditions tend toward nearly equal relative probabilities for alternative states and hence toward the parsimony solution of equivocal reconstructions at those nodes.

Rates of Dispersal

Rates of dispersal estimated using rate-smoothed, clocklike branch lengths suggest that within the *mariniana* clade, although the model used assumed that the rates of dispersal between neighboring islands was equal, the numbers of reconstructed dispersals between O'ahu and Maui Nui and between Maui Nui and Hawai`i were higher than that between the islands of Kaua'i and O'ahu. Within the *greenwelliae* clade, dispersal rates between Kaua`i and O`ahu and between O`ahu and Maui Nui were roughly the same. The *mariniana* clade mostly comprises populations of a single species

(*P. mariniana*), which we suspect to be actively speciating on the youngest island, Hawai`i. The distance between Maui and Hawai`i is currently 28 mil., compared with 72 mil. between Kaua`i and O`ahu and 25 mil. between O`ahu and Maui Nui. If we accept that the rates of dispersal estimated here are accurate, then the high rate of dispersal between O`ahu and the islands of the Maui Nui complex and between Maui Nui and Hawai`i for the *mariniana* clade in particular may reflect the short distance between these island groups in recent history, as compared with the other island groups. An additional fact that may influence these estimates of dispersal rates is that O`ahu and Maui Nui were once united by low elevation terrestrial terrain (the Penguin bank) which lasted for >100,000 years (Carson and Clague, 1995). Several taxonomic species are found only on a single island; *P. grandiflora*, *P. hobdyi*, *P. greenwelliae*, and *P. wawrae* are known only from Kaua`i, and *P. fauriei* and *P. hathewayi* are known only from O`ahu. It is not clear why some taxa have not dispersed to other younger islands, but perhaps local adaptations to pollinators or avian dispersers with reduced ranges have played a role in these dispersal patterns.

In this study, we explored the use of both parsimony and likelihood frameworks in reconstructing ancestral geographical distributions and historical dispersal events. Reconstructing ancestral biogeographical states for the Hawaiian *Psychotria* using these alternative methods did not result in strong differences for most nodes. In our example, it may be that conditions in which parsimony may perform poorly, i.e., when rate of character change is moderate to high (Maddison, 1994; Yang et al., 1995b; Omland, 1997), were not violated. However, likelihood methods were able to distinguish between alternative states in the case of one node that was reconstructed equivocally under parsimony. The degree to which alternative states are favored over other states varies depending on the combination of model and branch lengths used. Additionally, a high degree of uncertainty about ancestral states reconstructed unambiguously under parsimony criteria was detected. One of the strengths of the likelihood approach is the ability to consider branch length when inferring possible ancestral states at nodes (Swofford et al., 1996). However, the issue of arbitrariness of branch lengths in reconstructing discrete ancestral characters under likelihood is raised by this study, and practitioners of a likelihood framework should be cautious to explore all available options. Branch lengths inferred under likelihood criteria from nucleotide data in particular may be inappropriate in the context of inferring past rates of dispersal between islands (Lewis, 2001). Branch lengths may be long because they represent long periods of time or because the rate of substitution has been high, and it may be impossible to tease these two issues apart (Swofford et al., 1996). More empirical information on historical rates of dispersal in different groups of Hawaiian organisms, perhaps using information from fossil records, would provide a meaningful and more realistic model for reconstructing historical patterns of dispersal and colonization. Despite these limitations, however, it should be emphasized that a real strength of the likelihood approach is the valuable perspective provided by relative probabilities of ancestral states, in contrast to the absence of such perspective obtained with parsimony reconstructions. The high degree of uncertainty about ancestral states reconstructed even unambiguously under parsimony criteria is surprising and justifies the use of model-based methods for ancestral character state reconstructions.

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REFERENCES

ALICE, L. A., AND C. S. CAMPBELL. 1999. Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot. 86:81–97.

ANDERSSON, L., AND J. H. E. ROVA. 1999. The rps16 intron and the phylogeny of the Rubioideae (Rubiaceae). Plant Syst. Evol. 214:161–186.

ANDREASEN, K., AND B. G. BALDWIN. 2001. Unequal evolutionary rates between annual and perennial lineages of checker mallows (*Sidalcea*, Malvaceae): Evidence from 18S–26S rDNA internal and external transcribed spacers. Mol. Biol. Evol. 18:936–944.

ASQUITH, A. 1995. Evolution of *Sarona* (Heteroptera, Miridae): Speciation on geographic and ecological islands. Pages 90–120 in Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Fun, eds.). Smithsonian Institution Press, Washington, D.C.

AVISE, J. C., AND J. R. M. BALL. 1990. Gene genealogies and the coalescent process. Oxf. Surv. Evol. Biol. 7:43–67.

BALDWIN, B. G. 1992. Phylogenetic utility of the transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. Mol. Phylogenet. Evol. 1:3–16.

BALDWIN, B. G., D. W. KYHOS, J. DVORAK, AND G. D. CARR. 1991. Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae). Proc. Natl. Acad. Sci. USA 88:1840–1843.

BALDWIN, B. G., AND S. MARKOS. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). Mol. Phylogenet. Evol. 10:449–463.

BALDWIN, B. G., M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The

ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Ann. Mo. Bot. Gard. 82:247–277.
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.
BELSHAW, R., AND D. L. J. QUICKE. 2002. Robustness of ancestral state estimates: Evolution of life history strategy in ichneumonoid parasitoids. Syst. Biol. 51:450–477.
BENA, G., M. JUBIER, I. OLIVIERI, AND B. LEJEUNE. 1998A. Ribosomal external and internal transcribed spacers: Combined use in the phylogenetic analysis of *Medicago* (Leguminosae). J. Mol. Evol. 46:299–306.
BENA, G., B. LEJEUNE, J. PROSPERI, AND I. OLIVIERI. 1998B. Evolution of annual species of the genus *Medicago*: A molecular phylogenetic approach. Mol. Phylogenet. Evol. 9:552–559.
CARSON, H. L., AND D. A. CLAGUE. 1995. Geology and biogeography of the Hawaiian islands. Pages 12–29 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution Press, Washington, D.C.
CHANG, B. S. W., K. JONSSON, M. A. KAZMI, M. J. DONOGHUE, AND T. P. SAKMAR. 2002. Recreating a functional ancestral archosaur visual pigment. Mol. Biol. Evol. 19:1483–1489.
CLAGUE, D. A. 1996. The growth and subsidence of the Hawaiian–Emperor volcanic chain. Pages 35–50 in The origin and evolution of Pacific island biotas, New Guinea to eastern Polynesia: Patterns and processes (A. Keast and S. E. Miller eds.). SPB Academic, Amsterdam.
CLAGUE, D. A., AND G. B. DALRYMPLE. 1987. Tectonics, geochronology and the origin of the Hawaiian–Emperor volcanic chain. Pages 1–54 *in* Volcanism in Hawai`i (R. W. Decker, T. L. Wright, and P. H. Stauffer, eds.). U.S. Geological Survey Professional Paper 1350. U.S. Government Printing Office, Washington, D.C.
CLEVINGER, J. A., AND J. L. PANERO. 2000. Phylogenetic analysis of *Silphium* and subtribe Engelmanniinae (Asteraceae: Heliantheae) based on ITS and ETS sequence data. Am. J. Bot. 87:565–572.
CUNNINGHAM, C. W. 1999. Some limitations of ancestral character-state reconstruction when testing evolutionary hypotheses. Syst. Biol. 48:665–674.
CUNNINGHAM, C. W., K. E. OMLAND, AND T. H. OAKLEY. 1998. Reconstructing ancestral character states: A critical reappraisal. Trends Ecol. Evol. 13:361–366.
DESALLE, R. 1992. The origin and possible time of divergence of the Hawaiian Drosophilidae: Evidence from DNA sequences. Mol. Biol. Evol. 9:905–916.
DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.
ERICKSSON, T. 1999. AutoDecay, version 4.0 (program distributed by the author). Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm.
FELSENSTEIN, J. 1973. Maximum likelihood and minimum-steps methods for estimating evolutionary trees from discrete characters. Syst. Zool. 22:240–249.
FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. J. Mol. Evol. 37:368–376.
FELSENSTEIN, J. 1988. Phylogenies from molecular sequences: Inference and reliability. Annu. Rev. Genet. 22:521–566.
FOSBERG, F. R. 1962. Miscellaneous notes on Hawaiian plants—3. Occas. Pap. Bernice P. Bishop Mus. 23:42–44.
FOSBERG, F. R. 1964. Studies in Pacific Rubiaceae: V. Brittonia 16:255–271.
FUNK, V. A., AND W. L. WAGNER. 1995. Biogeographic patterns in the Hawaiian Islands. Pages 379–419 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution Press, Washington, D.C.
GAGNE, W. C., AND L. W. CUDDIHY. 1990. Vegetation. Pages 45–114 *in* Manual of the flowering plants of Hawai`i, Volume 1 (W. L. Wagner, D. R. Herbst, and S. H. Sohmer, eds.). Bishop Museum, Honolulu, Hawaii.
GIRIBET, G., AND W. C. WHEELER. 1999. On gaps. Mol. Phylogenet. Evol. 13:132–143.
GIVNISH, T. J. 1998. Adaptive plant evolution on islands: Classical patterns, molecular data, new insights. Pages 281–304 *in* Evolution on islands (P. R. Grant, ed.). Oxford Univ. Press, New York.
GIVNISH, T. J., K. J. SYSTMA, J. F. SMITH, AND W. J. HAHN. 1995. Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae, Lobelioideae). Pages 288–337 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution Press, Washington, D.C.
GOLDMAN, N. 1993. Statistical tests of models of DNA substitution. J. Mol. Evol. 36:182–198.
HAMILTON, C. W. 1989a. A revision of Mesoamerican *Psychotria* subgenus *Psychotria* (Rubiaceae). Part I: Introduction and species 1–16. Ann. Mo. Bot. Gard. 76:67–111.
HAMILTON, C. W. 1989b. A revision of Mesoamerican *Psychotria* subgenus *Psychotria* (Rubiaceae). Part II: Species 17–47. Ann. Mo. Bot. Gard. 76:386–429.
HAMILTON, C. W. 1989c. A revision of Mesoamerican *Psychotria* subgenus *Psychotria* (Rubiaceae). Part III: Species 48–61. Ann. Mo. Bot. Gard. 76:886–916.
HASEGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 21:160–174.
HILLIS, D. M., B. K. MABLE, AND C. MORITZ. 1996. Applications of molecular systematics: The state of the field and a look to the future. Pages 515–543 *in* Molecular systematics, 2nd edition (D. M. Hillis, C. Moritz and B. K. Mable, eds.). Sinauer, Sunderland, Massachusetts.
HORMIGA, G., M. ARNEDO, AND R. G. GILLESPIE. 2003. Speciation on a conveyor belt: Sequential colonization of the Hawaiian islands by *Orsonwelles* spiders (Aranae, Linyphiidae). Syst. Biol. 52: 70–88.
HUDSON, R. R. 1990. Gene genealogies and the coalescent process. Oxf. Surv. Evol. Biol. 7:1–44.
HUELSENBECK, J. P., AND B. RANNALA. 1997. Phylogenetic methods come of age: Testing hypotheses in an evolutionary context. Science 276:227–232.
JORDAN, S., C. SIMON, AND D. POLHEMIS. 2003. Molecular systematics and adaptive radiation of Hawaii's endemic damselfly genus Megalagrion (Odonata: Coenagrionidae). Syst. Biol. 52:89–109.
JUKES, T. H., AND C. R. CANTOR. 1969. Evolution of protein molecules. Pages 21–132 *in* Mammalian protein metabolism (H. M. Munro, ed.). Academic Press, New York.
KIM, H. G., S. C. KEELEY, P. S. VROOM, AND R. K. JANSEN. 1998. Molecular evidence for an African origin of the Hawaiian endemic *Hesperomannia* (Asteraceae). Proc. Natl. Acad. Sci. USA. 95:15440–15445.
KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.
LEWIS, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50:913–925.
LINDER, C. R., L. R. GOERTZEN-LESLIE, B. VANDEN HEUVEL, J. FRANCISCO-ORTEGA, AND R. K. JANSEN. 2000. The complete external transcribed spacer of 18S–26S rDNA: Amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely allied families. Mol. Phylogenet. Evol. 14:285–303.
LINDQVIST, C., AND V. A. ALBERT. 2002. Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). Am. J. Bot. 89:1709–1724.
LLOYD, D. G., AND V. L. CALDER. 1991. Multi-residue gaps, a class of molecular characters with exceptional reliability for phylogenetic analyses. J. Evol. Biol. 4:9–21.
LOWREY, T. K. 1995. Phylogeny, adaptive radiation, and biogeography of Hawaiian *Tetramolopium* (Asteraceae, Astereae). Pages 195–220 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner, and V. A. Funk, eds.). Smithsonian Institution Press, Washington, D.C.
LUTZONI, F., P. WAGNER, V. REEB, AND S. ZOLLER. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst. Biol. 49:628–651.

MADDISON, D. R. 1994. Phylogenetic methods for inferring the evolutionary history and processes of change in discretely valued characters. Annu. Rev. Entomol. 39:267–292.

MADDISON, D. R., AND W. P. MADDISON. 2001. MacClade: Analysis of phylogeny and character evolution, version 4.03. Sinauer, Sunderland, Massachusetts.

MADDISON, W. P. 1995. Phylogenetic histories within and among species. Monogr. Syst. M. Bot. Gard. 53:273–287.

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

MADDISON, W. P., AND D. R. MADDISON. 2002. Mesquite: A modular system for evolutionary analysis, version 0.99. Available at http://mesquiteproject.org.

MOOERS, A., S. M. VAMOSI, AND D. SCHLUTER. 1999. Using phylogenies to test macroevolutionary hypotheses of trait evolution in cranes (Gruinae). Am. Nat. 154:249–259.

NEPOKROEFF, M., B. BREMER, AND K. J. SYTSMA. 1999. Reorganization of the genus *Psychotria* and tribe Psychotrieae (Rubiaceae) inferred from ITS and *rbc*L sequence data. Syst. Bot. 24:5–27.

NIXON, K. C., AND Q. D. WHEELER. 1990. An amplification of the phylogenetic species concept. Cladistics 6:211–223.

OMLAND, K. E. 1997. Examining two standard assumptions of ancestral reconstructions: Repeated loss of dimorphism in dabbling ducks (Anatini). Evolution 51:1636–1646.

PAGEL, M. 1994. Detecting correlated evolution on phylogenies: A general method for the comparative analysis of discrete characters. Proc. R. Soc. Lond. B 255:37–45.

PAGEL, M. 1997. Inferring evolutionary processes from phylogenies. Zool. Scr. 26:331–348.

PAGEL, M. 1999a. Inferring the historical patterns of biological evolution. Nature 401:877–884.

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

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

RAMBAUT, A., AND M. CHARLESTON. 2001. TreeEdit: Phylogenetic tree editor, version 1.0, alpha 8. Available at http://evolve.zoo.ox.ac.uk/.

REE, R. H., AND M. J. DONOGHUE. 1999. Inferring rates of change in flower symmetry in asterid angiosperms. Syst. Biol. 48:633–641.

RODRIGUEZ, F., J. F. OLIVER, A. MARIN, AND J. R. MEDINA. 1990. The general stochastic model of nucleotide substitutions. J. Theor. Biol. 142:485–501.

SAKAI, A. K., W. L. WAGNER, D. M. FERGUSON, AND D. R. HERBST. 1995. Biogeographical and ecological correlates of dioecy in the Hawaiian flora. Ecology 76:2530–2543

SAKAI, A. K., S. G. WELLER, W. L. WAGNER, P. S. SOLTIS, AND D. E. SOLTIS. 1997. Phylogenetic perspectives on the evolution of dioecy: Adaptive radiation in the endemic Hawaiian genera *Schiedea* and *Alsinidendron* (Caryophyllaceae: Alsinoideae). Pages 455–473 *in* Molecular evolution and adaptive radiation (T. J. Givnish and K. J. Sytsma, eds.). Cambridge Univ. Press, New York.

SALISBURY, B. A., AND J. H. KIM. 2001. Ancestral state estimation and taxon sampling density. Syst. Biol. 50:557–564.

SANCHIS, A., J. M. MICHELENA, A. LATORRE, D. L. J. QUICKE, U. GARDENFORS, AND R. BELSHAW. 2001. The phylogenetic analysis of variable-length sequence data: Elongation factor-1 alpha introns in European populations of the parasitoid wasp genus *Pauesia* (Hymenoptera: Braconidae: Aphidiinae). Mol. Biol. Evol. 18:1117–1131.

SANDERSON, M. J. 1998. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14:1218–1231.

SANDERSON, M. J., AND M. F. WOJCIECHOWSKI. 1996. Diversification in a temperate legume clade: Are there "so many species" of *Astragalus* (Fabaceae)? Am. J. Bot. 83:1488–1502.

SANG, T., D. J. CRAWFORD, AND T. F. STUESSY. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: Implications for biogeography and concerted evolution. Proc. Natl. Acad. Sci. USA 92:6813–6817.

SCHLUTER, D. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford, U.K.

SCHLUTER, D., AND L. NAGEL. 1995. Parallel speciation by natural selection. Am. Nat. 146:292–301.

SCHLUTER, D., T. PRICE, A. Ø. MOOERS, AND D. LUDWIG. 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51:1699–1711.

SCHULTZ, T. R., R. B. CROCROFT, AND G. A. CHURCHILL. 1996. The reconstruction of ancestral character states. Evolution 50:504–511.

SHAW, K. L. 1995. Biogeographic patterns of two independent Hawaiian cricket radiations (*Laupala* and *Prognathogryllus*). Pages 39–56 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution Press, Washington, D.C.

SHAW, K. L. 1996. Sequential radiations and patterns of speciation in the Hawaiian cricket genus *Laupala* inferred from DNA sequences. Evolution 50:237–266.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49:369–381.

SIMMONS, M. P., H. OCHOTERENA, AND T. G. CARR. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analysis. Syst. Biol. 50:454–462.

SMITH, J. F., K. J. SYTSMA, J. S. SHOEMAKER, AND R. L. SMITH. 1991. A qualitative comparison of total cellular DNA extraction protocols. Phytochem. Bull. 23:2–9.

SOHMER, S. 1977. *Psychotria* L. (Rubiaceae) in the Hawaiian islands. Lyonia 1:103–186.

SOHMER, S. 1978. Morphological variation and its taxonomic and evolutionary significance in the Hawaiian *Psychotria* (Rubiaceae). Brittonia 30:256–264.

SOLTIS, D. E., AND P. S. SOLTIS. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. Pages 1–42 *in* Molecular systematics of plants, Vol. II. DNA sequencing (D. E. Soltis, P.S. Soltis, and J. J. Doyle, eds.). Kluwer Academic, Norwell, Massachusetts.

SOLTIS, P. S., D. E. SOLTIS, S. G. WELLER, A. K. SAKAI, AND W. L. WAGNER. 1996. Molecular phylogenetic analysis of the Hawaiian endemics *Schiedea* and *Alsinidendron* (Caryophyllaceae). Syst. Bot. 21:365–379.

SULLIVAN, J., J. A. MARKERT, AND C. W. KILPATRICK. 1997. Phylogeography and molecular systematics of the *Peromyscus aztecus* group (Rodentia: Muridae) inferred using parsimony and likelihood. Syst. Biol. 46:426–440.

SWOFFORD, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b6. Sinauer, Sunderland, Massachusetts.

SWOFFORD, D. L ., G. J. OLSEN, P. J. WADDELL, AND D. M. HILLIS. 1996. Phylogenetic inference. Pages 407–509 *in* Molecular systematics, 2nd edition (D. M. Hillis, C. Moritz, and B. Mable, eds.). Sinauer, Sunderland, Massachusetts.

SYTSMA, K. J., T. J. GIVNISH, J. F. SMITH, AND W. J. HAHN. 1993. Obtaining and storing land plant samples for macromolecular comparisons. Methods Enzymol. 224:23–37.

SYTSMA, K. J., AND W. H. HAHN. 2000. Molecular systematics: 1997–1999. Prog. Bot. 62: 307–339.

VARGAS, P., B. G. BALDWIN, AND L. CONSTANCE. 1998. Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of *Sanicula* (Apiaceae). Proc. Natl. Acad. Sci. USA 95:235–240.

WAGNER, W. L., AND V. A. FUNK. (eds.). 1995. Hawaiian biogeography: Evolution on a hot spot archipelago. Smithsonian Institution Press, Washington, D.C.

WAGNER, W. L., D. R. HERBST, AND S. H. SOHMER. 1990. Manual of the flowering plants of Hawai`i, Volume 2. Bishop Museum, Honolulu, Hawai`i.

WAGNER, W. L., S. G. WELLER, AND A. K. SAKAI. 1995. Phylogeny and biogeography in *Scheidea* and *Alsinidendron* (Caryophyllaceae). Pages 251–258 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution Press, Washington, D.C.

WENDEL, J. F., A. SCHANBEL, AND T. SEELANAN. 1995. Bidirectional interlocus concerted evolution following allopolyploid

speciation in cotton (*Gossypium*). Proc. Natl. Acad. Sci. USA 92:280–284.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genews for phylogenetics. Pages 315–322 *in* PCR protocols: A guide to methods and applications (M. Innis, D. Gelfand, J. Sninsky, and T. White, eds.). Academic Press, San Diego, California.

YANG, Z., N. GOLDMAN, AND A. FRIDAY. 1995a. Maximum likelihood trees from DNA sequences. J. Mol. Evol. 39:315–329.

YANG, Z., S. KUMAR, AND M. NEI. 1995b. A new method of inference of ancestral nucleotide and amino acid sequences. Genetics 141:1641–1650.

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