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# Molecular phylogenetics and historical biogeography of Hawaiian *Dryopteris* (Dryopteridaceae)

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**Abstract**

The fern genus *Dryopteris* (Dryopteridaceae) is represented in the Hawaiian Islands by 18 endemic taxa and one non-endemic, native species. The goals of this study were to determine whether *Dryopteris* in Hawai'i is monophyletic and to infer the biogeographical origins of Hawaiian *Dryopteris* by determining the geographical distributions of their closest living relatives. We sequenced two chloroplast DNA fragments, *rbcL* and the *trnL-F* intergenic spacer (IGS), for 18 Hawaiian taxa, 45 non-Hawaiian taxa, and two outgroup species. For individual fragments, we estimated phylogenetic relationships using Bayesian inference and maximum parsimony. We performed a combined analysis of both cpDNA fragments employing Bayesian inference, maximum parsimony, and maximum likelihood. These analyses indicate that Hawaiian *Dryopteris* is not monophyletic, and that there were at least five separate colonizations of the Hawaiian Islands by different species of dryopteroid ferns, with most of the five groups having closest relatives in SE Asia. The results suggest that one colonizing ancestor, perhaps from SE Asia, gave rise to eight endemic taxa (the glabra group). Another colonizing ancestor, also possibly from SE Asia, gave rise to a group of five endemic taxa (the exindusiate group). *Dryopteris fusco-atra* and its two varieties, which are endemic to Hawai'i, most likely diversified from a SE Asian ancestor. The Hawaiian endemic *Nothoperanema rubiginosum* has its closest relatives in SE Asia, and while the remaining two species, *D. wallichiana* and *D. subbipinnata*, are sister species, their biogeographical origins could not be determined from these analyses due to the widespread distributions of *D. wallichiana* and its closest non-Hawaiian relative.


*Keywords:* *Dryopteris*; Hawaiian Islands; Molecular phylogenetics; *trnL-F* IGS; *rbcL*; Biogeography

## 1. Introduction

The Hawaiian Island chain is approximately 80 million years old, however, most islands eroded to below sea level long ago. The oldest of the current high islands is Kaua'i, which is about 5.0 million years old and the youngest is the Big Island of Hawai'i, which is about 0.5 million years old (Carson and Clague, 1995). On the current high islands, most of the mid to high elevation Hawaiian flora and fauna evolved in isolation for up to 5 million years (Clague, 1996; Price and Clague, 2002) before the arrival of the Polynesians about 1600 years ago (Kirch, 1982). The result of this isolation was the production of a unique and distinctive flora. Among Hawaiian flowering plant species, approximately 89% are endemic to the archipelago (Wagner et al., 1999b) and among Hawaiian pteridophytes, approximately 71% are endemic (Palmer, 2003). These are among the highest rates of endemism for any known flora (Sohmer and Gustafson, 1987). The characteristically insular nature of the flora is due to a limited number of original colonizers and the diversity of their origin (Fosberg, 1948; Wagner et al., 1999b). For angiosperms, it appears to have been a rare occurrence that more than one species of a

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recognized genus reached the islands due to dispersal limitations (Carlquist, 1980) and it is commonly thought that dispersal events to the islands are not a large source of the observed diversity. It has been estimated that the ca. 1000 native angiosperm species were derived from only 272–282 natural introductions (Fosberg, 1948; Wagner et al., 1999b). Of the 161 native species of Hawaiian pteridophytes (Palmer, 2003), however, there were an estimated 115 colonizing ancestral species (Wagner, 1988). A likely explanation for this difference is that pteridophytes are generally more easily dispersed long distances due to their small, windblown spores, whereas the seeds or fruits of most angiosperms are much larger and less easily dispersed (Carlquist, 1980; Ranker et al., 1994, 2000; Smith, 1972; Tryon, 1970). Hence, within the archipelago (and elsewhere), speciation rates of pteridophytes could be depressed relative to those of angiosperms due to continued gene flow between newly founded and source populations (Ranker et al., 2000).

Biogeographical histories of endemic Hawaiian plants may be more complicated and obscure than those of plants of other volcanic oceanic archipelagos, mainly due to the large distances from source populations and the long existence of the chain of the Hawaiian Islands (Kim et al., 1998). Recent studies of specific groups of Hawaiian angiosperms have indicated that the diversity of Hawaiian species for many genera or groups of confamilial genera is usually the result of a single dispersal event to the Islands with subsequent phylogenetic radiations (e.g., the silversword alliance, Baldwin et al., 1991; *Hesperomannia*, Kim et al., 1998; Hawaiian geraniums, Pax et al., 1997). However, exceptions in which more than one dispersal event to Hawai'i of congeneric species has been discovered include *Rubus* (Howarth et al., 1997) and *Scaevola* (Howarth et al., 1999).

In contrast to flowering plants, of the two studies performed specifically on Hawaiian pteridophytes, one provides evidence of multiple dispersal events to the islands by a single species, *Asplenium adiantum-nigrum* (Ranker et al., 1994), while the other supports a single colonization hypothesis for the endemic genus *Adenophorus* plus its sister taxon *Grammitis tenella* (Ranker et al., 2003). Various groups of angiosperms in Hawai'i continue to be studied from a phylogenetic perspective (e.g., see Powell and Kron, 2002; Wagner and Funk, 1995), but to date there are only five published phylogenetic studies that include Hawaiian pteridophytes (Haufler and Ranker, 1995; Hennequin et al., 2003; Ranker et al., 2003, 2004; Schneider et al., 2004a) and few population genetic studies on Hawaiian fern groups (Ranker et al., 1994, 1996, 2000; Russell et al., 1999). Pteridophytes comprise about one-sixth of the native vascular plant species in Hawai'i and they physically dominate some communities, thus the paucity of phylogenetic information about pteridophytes represents a gap in our understanding of the evolution of the Hawaiian flora.

### 1.1. Background on Hawaiian Dryopteris

*Dryopteris* Adans. (Dryopteridaceae) is a cosmopolitan genus comprising approximately 225 species, mostly occurring in temperate forests and montane areas of the tropics (Hoshizaki and Wilson, 1999). An estimated eight to 17 species occur in the Hawaiian Islands, all of which are endemic to the Islands except one, which is indigenous (*D. wallichiana*). Individual Hawaiian *Dryopteris* species generally occur on all or most of the islands, however, depending on the taxonomic classification followed, there may be several single-island endemics. All Hawaiian taxa are found in mesic to very wet, montane forests.

*Dryopteris* has been a difficult group to understand taxonomically in Hawai'i (Wagner, 1995; Wagner et al., 1999a) and throughout the world (Hoshizaki and Wilson, 1999). There has been much debate and confusion regarding specific and subspecific classifications and in the understanding of the evolutionary relationships among the taxa, especially for the Hawaiian species (Fraser-Jenkins, 1986, 1994; Herat, 1979; Palmer, 2003; Wagner, 1993, 1995; Wagner et al., 1999a). There have also been conflicting views regarding the historical biogeography of these taxa and their origins in the Islands. Fosberg (1948) estimated that there were 25 Hawaiian *Dryopteris* species, each a result of a separate dispersal and colonization event to the Islands. Herat (1979) proposed that there were only eight species of *Dryopteris* in Hawai'i that resulted from five separate introductions. Fraser-Jenkins (1994) recognized nine species, with 10 varieties, and suggested at least three separate colonization events. In various publications, Wagner (1993, 1995) and Wagner et al. (1995b, 1999b) recognized as many as 17 species. Most recently, Palmer (2003) revised the classification of these species and recognized 10 species and 11 varieties. He placed five species and nine varieties into two morphologically distinct groups. Palmer recognized an "exindusiate" group (as it will be referred to below), with 3- to 5-pinnate leaves, as likely being monophyletic. Included in this group are *D. sandwicensis*, *D. tetrapinnata*, and *D. unidentata* (and varieties). However, two species, *D. mauiensis* and *D. crinalis* (and varieties), which also lack indusia, were not recognized in this group. Palmer (2003) also recognized a second likely monophyletic group, which includes *D. glabra* plus varieties and *D. hawaiiensis*. However, Palmer suggested that *D. hawaiiensis* is probably unrelated to *D. glabra*. Species in this group, which will be referred to as the "glabra" group, have indusia and the leaves are characteristically 2-, 3-, to 5-pinnate. Palmer did not propose potential relationships for the remaining species (*D. mauiensis*, *D.*

*crinalis* and varieties, *D. wallichiana*, *D. subbipinnata*, and *D. fusco-atra* and varieties) but suggested seven colonization events that gave rise to this species diversity of *Dryopteris* in Hawai'i.

The goals of the present study were 3-fold. The first goal was to elucidate the phylogenetic relationships among species of Hawaiian *Dryopteris* and their non-Hawaiian relatives using chloroplast DNA (cpDNA) sequence variation. Our second goal was to test alternative hypotheses concerning the monophyly of Hawaiian *Dryopteris*. Specifically we tested whether (a) Hawaiian *Dryopteris* is monophyletic or (b) Hawaiian *Dryopteris* is polyphyletic, consisting of monophyletic groups of species, each of which are descendants of distinct colonizing ancestors to Hawai'i. Our third goal was to infer the geographic origin(s) of Hawaiian *Dryopteris*.

## 2. Materials and methods

### 2.1. Taxon sampling and DNA extraction

We used *Arachniodes aristata* as one outgroup taxon based on the family level *rbcL* phylogeny of Hasebe et al. (1995) where *Arachniodes* was consistently supported as the sister taxon to *Dryopteris*, when it was included in analyses. A second species, *Nothoperanema rubiginosum*, which is a Hawaiian endemic thought to be closely related to *Dryopteris* (Smith and Palmer, 1995), was also used as an outgroup taxon.

Samples of all species of Hawaiian *Dryopteris* were collected in Hawai'i. Leaf material was stored in silica gel until DNA was extracted. Samples of non-Hawaiian taxa were supplied by colleagues, extracted from fragments of COLO or PTBG herbarium specimens, or spores were provided from the British Pteridological Society Spore Exchange and the American Fern Society Spore Exchange (Table 1). We sampled *Dryopteris* species broadly from around the Pacific Rim and elsewhere. We also sampled common species and species that have been described as being morphologically similar to some of the Hawaiian species. When spores were provided, the spores were germinated and resulting gametophytes were cultured on a nutrient-enriched agar following the methods of Ranker et al. (1996). DNA was then extracted from the gametophytes (method cited below). Table 1 lists (when available) locality, collector, collection number, herbarium of deposit for voucher specimens, and GenBank accession numbers for all taxa and DNA sequences studied, including the outgroup species. We extracted total cellular DNA using the CTAB method of Doyle and Doyle (1987) modified by adding 3% PVP-40 and 5 mM ascorbic acid. Sample DNA concentrations were quantified using a minifluorometer and standardized to 10 ng/μl.

### 2.2. PCR amplification and sequencing

We PCR-amplified and sequenced two segments of the cpDNA genome: a 1311 basepair (bp) fragment of the *rbcL* gene and the *trnL* (UAA) 3′ exon-*trnF* (GAA) intergenic spacer (IGS). PCR amplification of *rbcL* was accomplished as in Ranker et al. (2003). Primer sequences are listed in Wolf et al. (1994) and Ranker et al. (2003) with the exception of D876R, which was designed specifically for this study. The sequence of D876R is: 5′-ATGAAGAAGCAGCCCYTTGTC-3′. PCR conditions were described in Haufler and Ranker (1995). Amplification of the *trnL-F* IGS was achieved with primers "e" and "f" of Taberlet et al. (1991). PCR conditions were 94 °C (180 s), followed by 5 cycles of 94 °C (30 s), 45 °C (30 s), and 72 °C (30 s), followed by 37 cycles of 94 °C (30 s), 60 °C (30 s), and 72 °C (30 s), ending with 10 min at 72 °C after cycling was completed. The same primers were used individually for sequencing each strand of the spacer. PCR products were purified with the Promega Wizard PCR Preps Purification System. Sequencing reactions were performed with the ABI Prism BigDye Terminator Cycle Sequencing Kit, employing 1/4 reactions. Sequencing products were purified with AutoSeq G-50 Sephadex columns from Amersham–Pharmacia Biotech. Sequences were detected on ABI automated sequencers at the Iowa State University DNA Sequencing and Synthesis Facility.

### 2.3. Phylogenetic analyses

Sequence fragments were edited by visual inspection of electropherograms in Sequencher (Gene Codes) and aligned manually (*rbcL*) or with ClustalX (Thompson et al., 1997) and then manually adjusted to achieve more parsimonious alignments (*trnL-F* IGS).

We conducted phylogenetic analyses on three different data sets: (1) *rbcL* alone, (2) *trnL-F* alone, and (3) a combined data set of *rbcL* and *trnL-F*. For each data set we first performed maximum parsimony (MP) analysis as implemented in PAUP* 4.0b10 (Swofford, 2002). We conducted two MP analyses per data set, following two criteria: (1) with all characters unordered and equally weighted and (2) unordered and with a transition-to-transversion bias. We estimated transition-to-transversion bias by comparing the length of the MP trees with transversions omitted to the length of MP trees with all variable sites included (see Martin and Naylor, 1997). The transversion bias was six for the *rbcL* data set and three for the *trnL-F* data set. For the combined data set, each data partition was defined with its determined transversion bias using a step-matrix as described above. For the separate MP analyses we excluded all uninformative characters and employed the heuristic search algorithm with 1000 random addition sequence replicates with MulTrees activated, and with TBR branch

Table 1
Species list, collection, and voucher information (when available), and GenBank accession numbers

| Species | Collection locality | Collector, number, and herbarium | Native distribution | GenBank accession number | |
|---|---|---|---|---|---|
| | | | | *trnL-F* | *rbcL* |
| *Arachnoides aristata* (Forst.) Tindale | American Samoa | Lorence 9762; PTBG | Marquesas | AY268782 | AY268851 |
| *Dryopteris aemula* (Aiton) Kuntze | BPSSE | Unknown | W. Europe | AY268816 | AY268881 |
| *Dryopteris affinis* subsp. *affinis* (Lowe) Fraser-Jenk. | Stowey Parish | Crabbe 11824; COLO | China, Japan | AY268780 | AY268849 |
| *Dryopteris affinis* subsp. *borreri* (Newman) Fraser-Jenk. | Caucasus | V. Vasak; COLO | NZ, Europe; SW Asia | AY268778 | AY268847 |
| *Dryopteris amurensis* Christ | Label not legible | Unknown; COLO | E. Asia | AY268802 | AY268867 |
| *Dryopteris aquilinoides* (Desv.) C. Chr. | La Réunion | Ranker 1536; COLO | Réunion; Mauritius | AY268803 | AY268868 |
| *Dryopteris ardechensis* Fraser-Jenk. | BPSSE | Unknown | W. Europe, France | AY268817 | AY268882 |
| *Dryopteris bissetiana* (Baker) C. Chr. | Cultivated, NY | R. Moran; COLO | China | AY268796 | AY268862 |
| *Dryopteris campyloptera* (Kunze) Clarkson | Partridge Island | Cody 23484; COLO | NE USA | AY268801 | AY268866 |
| *Dryopteris carthusiana* (Villars) H. P. Fuchs | BPSSE | Unknown | NA/Euraisa | AY268818 | AY268883 |
| *Dryopteris carthusiana* | Quebec, Canada | Argus 9327; COLO | NA/Eurasia | AY268777 | AY268846 |
| *Dryopteris championii* (Benth.) C. Chr. | Cultivated, NY | R. Moran; COLO | China | AY268797 | AY268863 |
| *Dryopteris corleyi* Fraser-Jenk. | AFSSE | Unknown | W. Europe | AY268808 | AY268873 |
| *Dryopteris crassirhizoma* Nakai | AFSSE | Unknown | China, Japan | AY268805 | AY268870 |
| *Dryopteris crinalis* (Hook. et Arn.) C. Chr. | Mau'i, Hawai'i | Oppenheimer H50044; COLO | Hawai'i | AY268774 | AY268835 |
| *Dryopteris crispifolia* Rasbach, Reichst. & Vida | BPSSE | Unknown | W. Europe, Portugal | AY268819 | AY268884 |
| *Dryopteris cystolepidota* (Miq.) Makino | AFSSE | Unknown | Japan, Korea | AY268813 | AY268878 |
| *Dryopteris dickinsii* (Franch. & Sav.) C. Chr. | BPSSE | Unknown | China, Japan | AY268820 | AY268885 |
| *Dryopteris dilatata* (Hoffm.) A. Gray | Siberia, Russia | Krasnobovov 679; COLO | China, NZ | AY268779 | AY268848 |
| *Dryopteris erythrosora* (D. Eaton) Kuntze | Cultivated, DBG | Geiger 94; COLO | China, Japan | AY268787 | AY268852 |
| *Dryopteris expansa* (C. Presl) Fraser-Jenk. & Jermy | Unknown | Nelson 7921; COLO | widespread | AY268775 | AY268844 |
| *Dryopteris filix-mas* (L.) Schott | Boulder, Colorado | Hogan 1421; COLO | widespread | AY268776 | AY268845 |
| *Dryopteris formosana* (Christ) C. Chr. | Cultivated, NY | R. Moran; COLO | China, Japan | AY268793 | AY268857 |
| *Dryopteris fragrans* (L.) Schott | Alaska, USA | Kelso 83-221; COLO | China, Japan | AY268800 | AY268865 |
| *Dryopteris fusco-atra* var. *fusco-atra* (Hillebr.) W. J. Rob. | Mau'i, Hawai'i | Geiger 4; COLO | Hawai'i | AY268760 | AY268837 |
| *Dryopteris fusco-atra* var. *lamoureuxii* Fraser-Jenk. | Mau'i, Hawai'i | Geiger 77; COLO | Hawai'i | AY268783 | AY268841 |
| *Dryopteris glabra* (Brack.) Kuntze var. *alboviridis* (W. H. Wagner) D. D. Palmer | Kaua'i, Hawai'i | Geiger 25; COLO | Hawai'i | AY268768 | AY268831 |
| *Dryopteris glabra* var. *flynnii* D. D. Palmer | Kaua'i, Hawai'i | Geiger 23; COLO | Hawai'i | AY268764 | AY268829 |
| *Dryopteris glabra* var. *glabra* | Kaua'i, Hawai'i | Geiger 24; COLO | Hawai'i | AY268767 | AY268830 |
| *Dryopteris glabra* var. *hobdyana* (W. H. Wagner) D. D. Palmer | Mau'i, Hawai'i | Palmer; COLO | Hawai'i | AY268773 | AY268839 |
| *Dryopteris glabra* var. *nuda* (Underw.) Fraser-Jenk. | O'ahu, Hawai'i | Geiger 90; COLO | Hawai'i | AY268785 | AY268843 |
| *Dryopteris glabra* var. *pusilla* (Hillebr.) Fras-Jenk. | Kaua'i, Hawai'i | Geiger 21; COLO | Hawai'i | AY268763 | AY268828 |
| *Dryopteris glabra* var. *soripes* (Hillebr.) Herat ex Fraser-Jenk. | Moloka'i, Hawai'i | Geiger 41; COLO | Hawai'i | AY268786 | AY268842 |
| *Dryopteris goeringiana* (Kunze) Koidz. | Cultivated, NY | R. Moran; COLO | China | AY268790 | AY268855 |
| *Dryopteris hawaiiensis* (Hillebr.) W. J. Rob. | Mau'i, Hawai'i | Geiger 74; COLO | Hawai'i | AY268784 | AY268840 |
| *Dryopteris hondoensis* Koidz. | Cultivated, NY | R. Moran; COLO | Japan | AY268791 | AY268856 |
| *Dryopteris intermedia* (Muhlenb. ex Willd.) A. Gray subsp. *maderensis* Fraser-Jenk. | BPSSE | Unknown | NE USA | AY268821 | AY268886 |
| *Dryopteris juxtaposita* Christ | AFSSE | Unknown | China | AY268810 | AY268875 |
| *Dryopteris lacera* (Thunb.) Kuntze | Cultivated, NY | R. Moran; COLO | China, Japan | AY268794 | AY268860 |
| *Dryopteris lepidopoda* Hayata | Cultivated, DBG | Geiger 96; COLO | China | AY268789 | AY268854 |
| *Dryopteris mauiensis* C. Chr. | Kaua'i, Hawai'i | Geiger 29; COLO | Hawai'i | AY268770 | AY268833 |
| *Dryopteris munchii* A. Reid Smith | BPSSE | Unknown | Chiapas, Mesoamericana | AY268822 | AY268887 |
| *Dryopteris odontoloma* (Beddome) C. Chr. | AFSSE | Unknown | China | AY268807 | AY268872 |
| *Dryopteris oreades* Fomin. | Caucasus centralis | V. Vasak; COLO | Europe | AY268781 | AY268850 |

(*continued on next page*)

Table 1 (*continued*)

| Species | Collection locality | Collector, number, and herbarium | Native distribution | GenBank accession number | |
|---|---|---|---|---|---|
| *Dryopteris pacifica* (Nakai) Tag. | AFSSE | Unknown | China, Japan | AY268814 | AY268879 |
| *Dryopteris pallida* (Bory) C. Chr. ex Maire & Petitm. | AFSSE | Unknown | W. Europe, Balearic Islands | AY268809 | AY268874 |
| *Dryopteris patula* (Sw.) L. Underw. | BPSSE | Unknown | Guatemala, Chiapas, Mexico | AY268823 | AY268888 |
| *Dryopteris polylepis* (Franch. & Sav.) C. Chr. | Cultivated, NY | R. Moran; COLO | China, Japan | AY268798 | AY268864 |
| *Dryopteris pulcherrima* Ching | AFSSE | Unknown | China | AY268811 | AY268876 |
| *Dryopteris pycnopteroides* (Christ) C. Chr | Cultivated, NY | R. Moran; COLO | China | AY268799 | AY268859 |
| *Dryopteris remota* (A. Braun ex Doell) Druce, non Hayata | Cultivated, NY | R. Moran; COLO | Asia/Europe | AY268792 | AY268858 |
| *Dryopteris sacrosancta* Koidz. | AFSSE | Unknown | E. Asia | AY268812 | AY268877 |
| *Dryopteris sandwicensis* (Hook. & Arn.) C. Chr. | Kaua'i, Hawai'i | Flynn 6675; PTBG | Hawai'i | AY268762 | AY268827 |
| *Dryopteris sichotensis* V. Komarov | Label not legible | Unknown; COLO | NE Asia | AY268804 | AY268869 |
| *Dryopteris sieboldii* (Van Houtte ex Mett.) Kuntze | AFSSE | Unknown | Japan | AY268815 | AY268880 |
| *Dryopteris stenolepis* (Baker) C. Chr. | BPSSE | Unknown | China | AY268824 | AY268889 |
| *Dryopteris subbipinnata* W. H. Wagner & Hobdy | Mau'i, Hawai'i | Oppenheimer H50074; COLO | Hawai'i | AY268765 | AY268834 |
| *Dryopteris sublacera* Christ | Cultivated, DBG | Geiger 95; COLO | China | AY268788 | AY268853 |
| *Dryopteris tetrapinnata* W. H. Wagner & Hobdy | Mau'i, Hawai'i | Geiger 17; COLO | Hawai'i | AY268772 | AY268853 |
| *Dryopteris tokyoensis* (Matsum. & Makino) C. Chr. | Cultivated, NY | R. Moran; COLO | Japan | AY268795 | AY268861 |
| *Dryopteris unidentata* var. *paleacea* (Hillebr.) Herat ex Fraser-Jenk | Kaua'i, Hawai'i | Geiger 28; COLO | Hawai'i | AY268769 | AY268832 |
| *Dryopteris unidentata* var. *unidentata* (Hook. & Arn.) C. Chr. | Kaua'i, Hawai'i | Flynn 6666; PTBG | Hawai'i | AY268766 | AY268825 |
| *Dryopteris uniformis* (Makino) Makino | AFSSE | Unknown | E. Asia | AY268806 | AY268871 |
| *Dryopteris wallichiana* (Spreng.) Hyl. | Kaua'i, Hawai'i | Flynn 6671; COLO | Hawai'i | AY268761 | AY268826 |
| *Nothoperanema rubiginosum* Smith & Palmer | Mau'i, Hawai'i | Geiger 2; COLO | Hawai'i | AY268771 | AY268836 |

*Abbreviations:* AFSSE, American Fern Society Spore Exchange; BPSSE, British Pteridological Society Spore Exchange; DBG, Denver Botanic Garden, Denver, CO, USA; NY, New York, USA.

swapping. For the combined data set, the MP settings were the same as above, except 5000 random addition sequence replicates were performed. Also for the combined data set, we performed bootstrap (BS) analysis with 1303 repetitions and 10 random stepwise addition replicates each with the transversion bias excluded. We conducted a decay analysis of branch support (Bremer, 1988; Donoghue et al., 1992) on the combined data set with AutoDecay 4.0.1 (Eriksson, 1998).

For each data set we used ModelTest (Posada and Crandall, 1998) to determine the model of evolution that best explained the data for use in maximum likelihood (ML) and Bayesian inference analyses. The TrN + I + G (Tamura and Nei, 1993) model best explained the *rbcL* data set, the HKY + G (Hasegawa et al., 1985) evolutionary model best fit the *trnL-F* data set, and the TrNef + I + G (TrN equal base frequencies; Tamura and Nei, 1993) model best explained the combined data set. For the individual data sets, it was computationally difficult to perform ML analyses due to the paucity of phylogenetically informative characters and the large number of taxa, even with the selected models enforced. We conducted a ML bootstrap analysis (ML BS), therefore, on the combined data set only, running 1000 repetitions and 10 random stepwise addition replicates each.

We performed Bayesian analysis as implemented in MrBayes 2.01 (Huelsenbeck and Ronquist, 2001) on each data set, enforcing the models listed above. For each analysis, we ran 1,000,000 generations of which one tree was sampled per 100 trees generated. We obtained posterior probability values for each node in PAUP* by computing the majority-rule consensus tree of the last 8000 sampled trees, excluding the first 2000 trees sampled during the "burn-in period."

We attempted to perform 1,000 random addition sequence replicates of the incongruence length difference test (ILD; Farris et al., 1994, 1995) with constant characters excluded as implemented in PAUP* to determine whether the *rbcL* and *trnL-F* data sets were combinable. However, after approximately 6 days of running time, the analysis had only reached replicate 25b, thus the results of this test were based on 25 replicates. As an additional means of assessing combinability, we compared the topologies of the strict consensus trees from the separate data sets with the Shimodaira–Hasegawa (SH) test as implemented in PAUP* (Goldman et al., 2000; Shimodaira and Hasegawa, 1999; Schneider et al., 2004b). The individual topologies did not differ significantly ($P = 0.174$).

Because it is an apparently rare event that two or more congeneric species independently colonized the Hawaiian Islands, as our results suggest occurred with *Dryopteris* (see Section 3), we performed the topological tests discussed below to add confidence to our results.

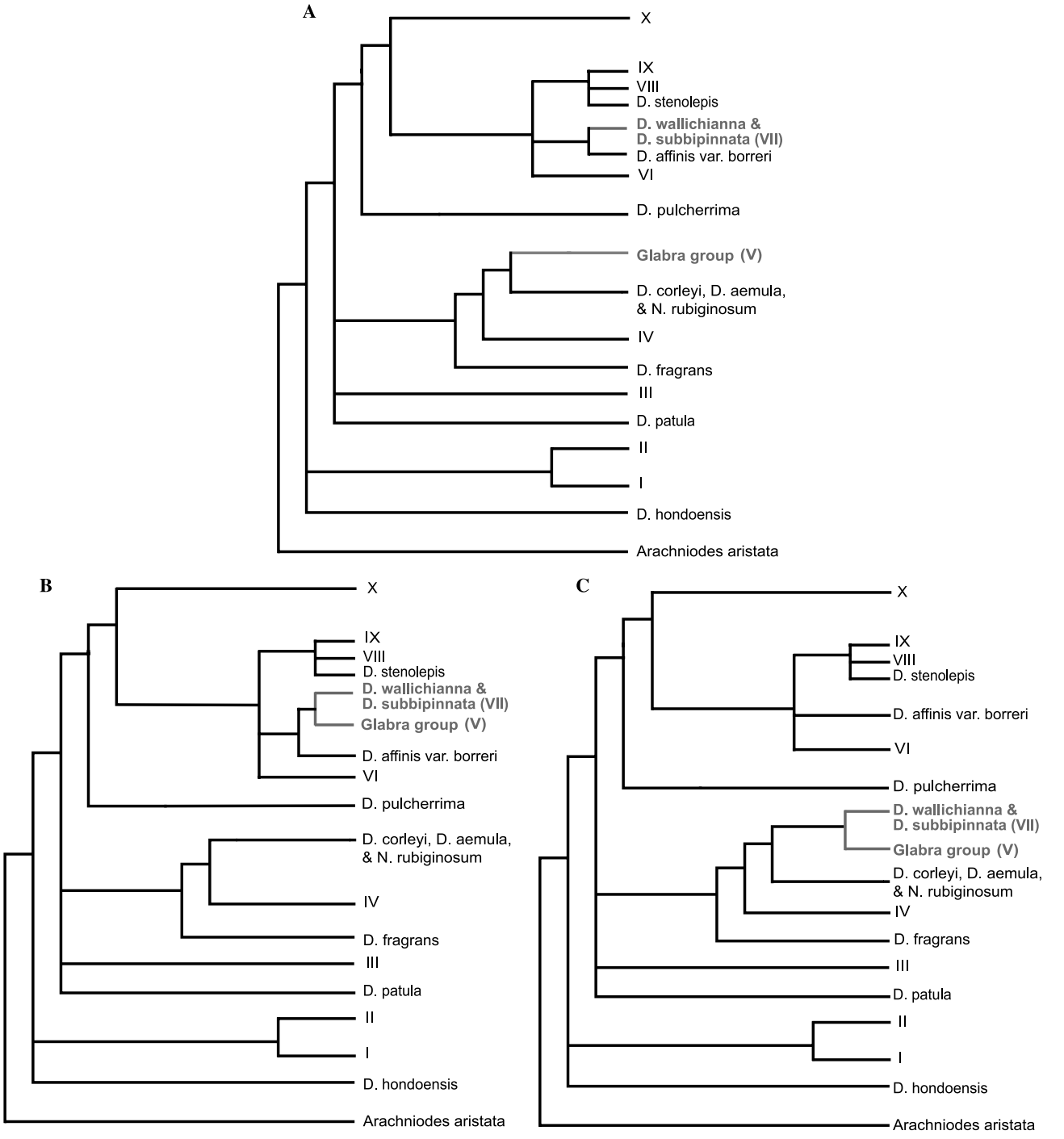


Fig. 1. Example of topologies used for testing alternative phylogenetic hypotheses. (A) Topology found by combined Bayesian analysis; (B) Hawaiian Clade VII and Hawaiian group of Clade V are monophyletic and diverge at the place in the topology where Clade VII diverged in the combined Bayesian analysis; (C) Hawaiian Clade VII and Hawaiian group of Clade V are monophyletic and diverge at the position in the topology where Clade V diverged in the combined Bayesian analysis.

With the topology from the Bayesian analysis (which did not differ significantly topologically from either the ML tree or MP consensus tree) as our phylogenetic hypothesis, we performed likelihood-ratio tests employing the Shimodaira–Hasegawa (SH) test in PAUP* to compare different hypothetical topologies (Shimodaira and Hasegawa, 1999). Using MacClade (Maddison and Maddison, 2000) we created topologies to test the support of the polyphyly of Hawaiian *Dryopteris* (see Section 3). We used roman numerals to label clades for explanatory simplicity (see Fig. 1). By constraining relationships, we tested the following hypotheses: (1) Hawaiian pairs of monophyletic groups (found in phylogenetic analyses; see Section 3) share common ancestry and thus evolved

from a single colonizing ancestor (i.e., that the Hawaiian taxa of Clade V (glabra group) are sister to the Hawaiian taxa of Clade VII (*D. subbipinnata* + *D. wallichiana*) or that the Hawaiian Clade II (exindusiate group) is sister to the Hawaiian taxa of Clade V (glabra group); see Fig. 1 for a graphical explanation of topological constraints); and (2) Hawaiian *Dryopteris* is monophyletic. For the pairwise hypotheses, each sister relationship was tested in two ways: (1) in the position on the tree where one of the two Hawaiian clades was resolved in the Bayesian phylogeny and (2) in the position on the tree where the second of the two clades was resolved in the Bayesian phylogeny. For example, when hypothesized that the glabra group (Clade V) is sister to *D. wallichiana* and *D. subbipinnata* (Clade VII), we first constrained the glabra group of Clade V to be sister to the Hawaiian taxa of Clade VII to the exclusion of *D. affinis* var. *borreri*, which is the sister taxon to the Hawaiian members of Clade VII (see Fig. 1). Second, we placed the Hawaiian taxa of Clade VII as sister to the Hawaiian taxa of Clade V to the exclusion of *D. aemula* + *D. corleyi* + *Nothoperanema rubiginosum* and Clade IV. To test whether Hawaiian *Dryopteris* is monophyletic, we constrained all Hawaiian species together and tested the likelihood of each of the four hypotheses against the likelihood of the Bayesian phylogeny. In four different tests, we placed monophyletic Hawaiian *Dryopteris* in the position on the Bayesian tree where the Hawaiian Clades II, V, VII, and VIII diverged, respectively. We did not make any assumptions about the relationships among the Hawaiian *Dryopteris* species, thus the relationships within monophyletic Hawaiian *Dryopteris* were always unresolved in our tests.

## 3. Results

### 3.1. Phylogenetic analyses

Sequences of *rbcL* and *trnL-F* were obtained for all 63 samples of *Dryopteris* and the outgroups, *Arachniodes aristata* and *Nothoperanema rubiginosum*. Across the 63 *Dryopteris rbcL* sequences obtained, 1090 bp were invariant, 221 bp were variable, and 136 bp were parsimony informative. Across the 63 ingroup sequences of *trnL-F* obtained, 255 bp were invariant, 124 bp were variable, and 62 bp were parsimony informative.

### 3.2. rbcL only analyses

The heuristic MP analysis of the *rbcL* data set with no transversion bias found 30,622 equally parsimonious trees. Each tree was characterized by a length ($L$) of 420 steps, a consistency index (CI) of 0.59, and a retention index (RI) of 0.84. The heuristic MP analysis of the *rbcL* data set run with a transversion-to-transition ratio of 6:1 resulted in 18,283 most parsimonious trees. Each tree had an $L$ of 542 steps, a CI of 0.55, and a RI of 0.87. The strict consensus trees from both of these analyses (not shown) were not completely dichotomously resolved but did have the same topologies.

The 50% majority-rule consensus tree obtained from 8000 equally likely trees from the Bayesian analysis was nearly completely dichotomously resolved (Fig. 2). Clade credibility (= posterior probability, PP) values were often above 85% and were frequently above 95%. There were no major conflicts among the topologies resulting from the MP (not shown) and Bayesian analyses.

From these analyses, Hawaiian *Dryopteris* was not supported as monophyletic (see Fig. 2). *Dryopteris wallichiana* and *D. subbipinnata* comprised a clade sister to non-Hawaiian *D. affinis* var. *borreri*, but were in the same larger clade with the *D. fusco-atra* varieties. There was poor resolution among the groups in this clade, and the sister-taxon relationships of the *D. fusco-atra* varieties were not resolved. The exindusiate group was supported as monophyletic, however, with the inclusion of *D. mauiensis* and *D. crinalis* and this clade was sister to a group, which included the non-Hawaiian *D. odontoloma*, *D. pallida*, and *D. tokyoensis*. The glabra group was supported as paraphyletic, as this unresolved clade also included *D. corleyi* and *D. aemula*, which do not occur in Hawai'i. This group was sister to the Hawaiian species *Nothoperanema rubiginosum*, which had been included in the analysis as an outgroup. Additionally, *D. hawaiiensis* was in this unresolved clade.

The glabra clade (including *Nothoperanema rubiginosum*) was strongly supported as sister to a clade that included the non-Hawaiian species *D. erythrosora*, *D. cystolepidota*, *D. formosana*, *D. pacifica*, *D. championii*, *D. bissetiana*, and *D. sacrosancta*. In neither of the analyses, MP or Bayesian, were any of the four Hawaiian groups supported as sister clades.

### 3.3. TrnL-F only analyses

The total aligned length of *trnL-F* IGS was 379 bp. The MP heuristic analysis of the *trnL-F* data set without a transversion bias found 16 most parsimonious trees. Each tree had $L = 233$, CI = 0.743, and RI = 0.88. When a transition-to-transversion bias of 3:1 was enforced in an MP heuristic search, 10 most parsimonious trees were found. These 10 trees each had a total $L = 345$, CI = 0.82, and RI = 0.90. The strict consensus trees (not shown) produced by both of these analyses did not differ in topology and had well-resolved terminal relationships, although deep relationships were not resolved.

Bayesian analysis resulted in an almost fully resolved 50% majority-rule consensus tree of the 8000 trees included (Fig. 3). Many of the clades had PP values above 90% and the topology was identical to the MP strict consensus trees.

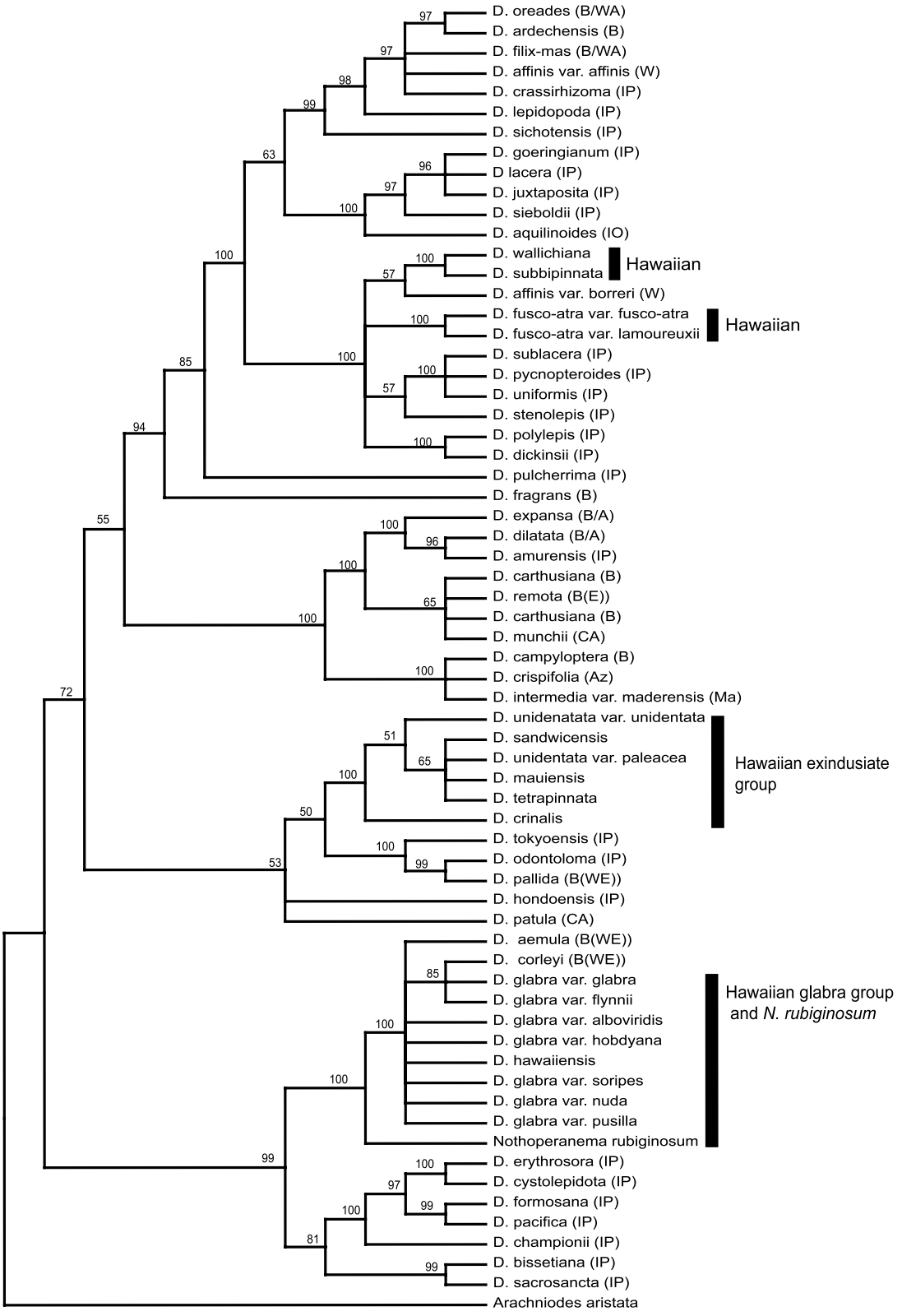


Fig. 2. Topology from Bayesian analysis of the individual *rbcL* data set. Numbers above the branches represent posterior probabilities (PP). Abbreviations in parentheses following each taxon name represent the geographic distribution of that taxon. An abbreviation in an internal second set of parentheses indicates a restricted distribution of the taxon within the broader region. A slash between two regions with parentheses indicates it is distributed in both regions. A, Asia; Az, Azores; B, Boreal; CA, Central America; E, Europe; IO, Indian Ocean; IP, Indo-Pacific; Ma, Madeira; W, Widespread; WA, Western Asia; and WE, Western Europe.

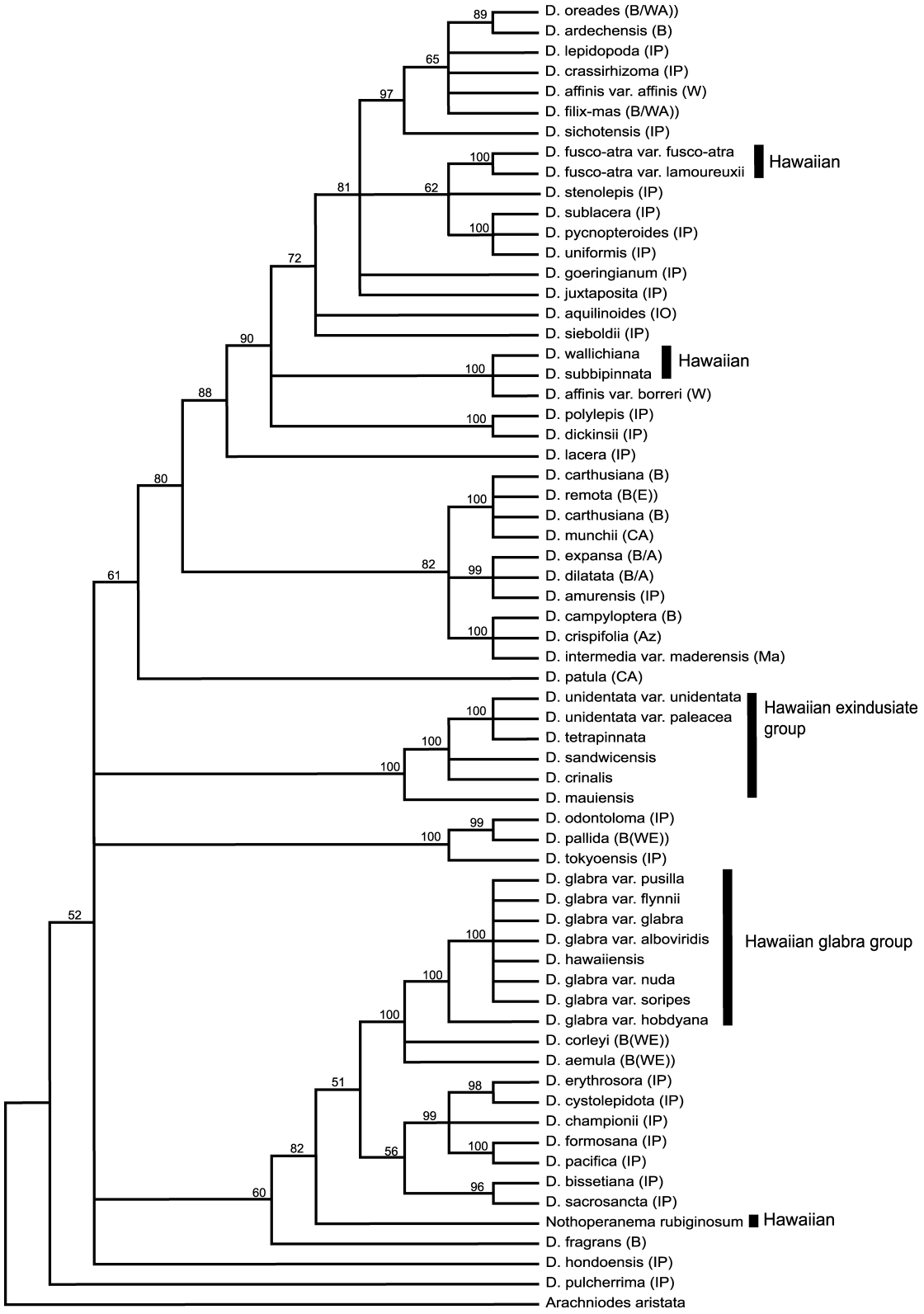


Fig. 3. Topology from Bayesian analysis of the individual *trnL-F* IGS data set. Numbers above the branches represent posterior probabilities (PP). Abbreviations in parentheses following each taxon name represent the geographic distribution of that taxon. A distribution in a second set of parentheses indicates the restricted distribution of the taxon within a broader region. A slash between two regions with parentheses indicates it is distributed in both regions. A, Asia; Az, Azores; B, Boreal; CA, Central America; E, Europe; IO, Indian Ocean; IP, Indo-Pacific; Ma, Madeira; W, Widespread; WA, Western Asia; and WE, Western Europe.

Analyses based on *trnL-F* did not support a monophyletic Hawaiian *Dryopteris*. *Dryopteris wallichiana* and *D. subbipinnata* were grouped with non-Hawaiian *D. affinis* var. *borreri*. The *D. fusco-atra* varieties were grouped together and closely associated with the non-Hawaiian *D. stenolepis*, *D. sublacera*, *D. pycnopteroides*, and *D. uniformis*. The exindusiate group was supported as monophyletic, including *D. crinalis* and *D. mauiensis*. This group's relationship to any other clade was unresolved in this analysis. The glabra group was supported as monophyletic with its closest relatives being European *D. corleyi* and *D. aemula*. This clade was supported as sister to the same group of species indicated from the *rbcL* analysis and *N. rubiginosum* was sister to those two sister clades. This result is different from the *rbcL* analysis in which *N. rubiginosum* was sister only to the glabra group + *D. corleyi* and *D. aemula*. Similar to the *rbcL* analysis, none of the four Hawaiian clades was supported as sister to another Hawaiian clade.

### 3.4. Combinability

The ILD test was not significant ($P > 0.40$) indicating that we could not reject the null hypothesis of data set homogeneity. Other than the placements of *D. hondoensis*, *D. patula*, *D. pulcherrima*, *D. fragrans*, and *N. rubiginosum*, there were not strong incongruencies in clade composition among single-sequence topologies (see Figs. 2 and 3). The SH test also indicated no significant difference between the topologies ($P = 0.174$). Given the lack of topological incongruence, the result of the ILD test, the result of the SH test, and that the two sequenced regions are chloroplastic, we felt justified in combining the data sets for a more robust analysis.

### 3.5. rbcL + trnL-F combined analyses

The MP heuristic search, in which no transversion bias was enforced, found 80 most parsimonious trees, each tree with $L = 662$, CI = 0.64, and RI = 0.85. When each partition had a transversion bias applied (6:1 for *rbcL* and 3:1 for *trnL-F*), 48 most parsimonious trees were found with $L = 1104$, CI = 0.69, and RI = 0.87. The transversion-biased topology was better resolved, especially at deep nodes. The main differences between the two topologies were the relative resolutions at deeper nodes and the differential placement of *D. fragrans*. A SH test under the likelihood settings indicated no significant difference between the topologies ($P = 0.174$).

The ML bootstrap analysis, based on 1000 replicates of 10 random addition sequence replicates each, was nearly completely resolved except deep within the phylogeny. The resulting topology is not shown here, however, it was mostly congruent with the Bayesian and MP topologies. The only differences were the placements of *D. fragrans*, *D. patula*, *D. pulcherrima*, and *D. sandwicensis*. The −log likelihood value of this topology was 6237.46.

The 50% majority-rule consensus tree found from the Bayesian analysis is shown in Fig. 4 and resulted in nearly the same topology as the ML BS and the MP BS analyses (not shown), although based on a SH test it had a significantly higher ($P = 0.028$) −log likelihood value (6205.28) than the ML topology. The major clades in Fig. 4 are identified with Roman numerals. Posterior probability values (PP) from Bayesian analysis, MP BS values, and decay values are indicated in Fig. 4, however, because the ML BS values were nearly identical to MP BS values and did not affect our interpretation of the results, they are not reported here. Posterior probability values were, without exception, equal to or higher than MP BS and ML BS (not shown) values. It has been suggested that Bayesian analysis overestimates node support (Suzuki et al., 2002), however, others have suggested that the posterior probability estimation methods are more sensitive to phylogenetic signal in data sets than are bootstrapping methods (Alfaro et al., 2003).

Consistent with the topologies of the individual data sets, the combined analyses strongly suggest that Hawaiian *Dryopteris* is not monophyletic. Again, *D. wallichiana* and *D. subbipinnata* were sisters to each other and non-Hawaiian *D. affinis* var. *borreri* was strongly supported (PP 100, MP BS 95, d4) as sister to them. The *D. fusco-atra* varieties were sister to each other, falling within a clade that was supported with 99% PP support, a MP BS value of 70, and included the same species as listed in the *trnL-F* analysis. The glabra group was monophyletic with 100% PP support and a MP BS value of 72. European *D. corley*i and *D. aemula* were strongly supported as sharing a common ancestor with the glabra group (PP 100, MP BS 100, d11), and *N. rubiginosum* was sister to the glabra-group + *D. corleyi* + *D. aemula* clade (PP 94, MP BS 63, d6). As in the *rbcL* analysis, the exindusiate group, including *D. crinalis* and *D. mauiensis*, was strongly supported as monophyletic (PP 100, MP BS 100, d13) and was weakly supported as sister to a clade including *D. tokyoensis*, *D. odontoloma*, and *D. pallida*.

Similar to patterns shown in the single-sequence analyses, no Hawaiian clade was ever most closely related to another Hawaiian clade. When we tested the likelihood of alternative phylogenetic hypotheses using the topological incongruency SH tests, no hypothesis from the pairwise comparisons was as likely as the Bayesian topology (*P* values were not larger than 0.009 for any comparison and were <0.001 for eight of the 12 pairwise comparisons). Similarly, no hypothetical topology in which Hawaiian *Dryopteris* was monophyletic was as likely as the supported topology ($P < 0.001$ for each comparison). In every case, the constrained hypotheses were significantly less likely than the Bayesian topology. Although not all plausible trees with each specific group

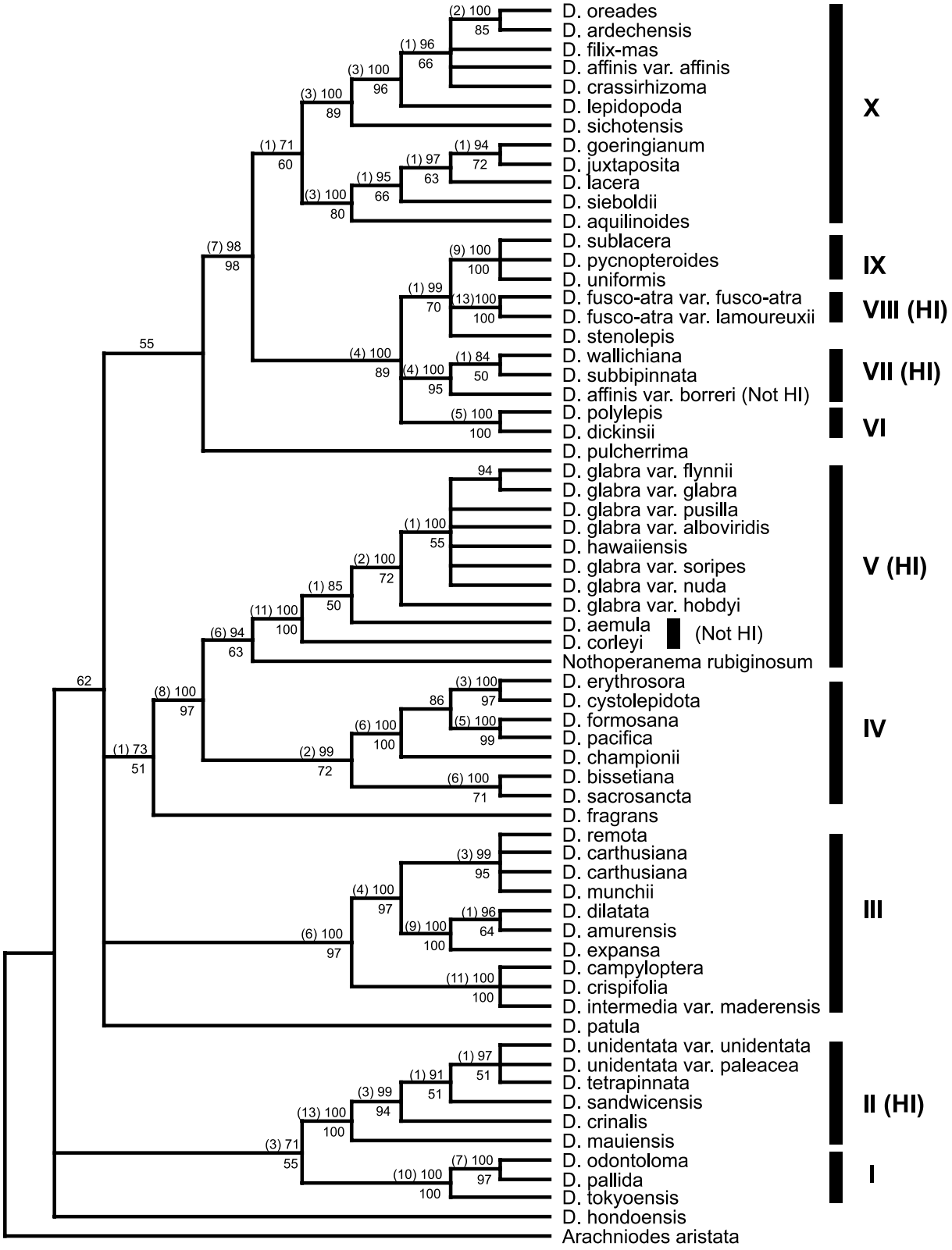


Fig. 4. Topology from the combined Bayesian analysis. The numbers above branches, not in parentheses, are posterior probability values (PP) from Bayesian analysis, the numbers, in parentheses, above branches are decay values (*d*) from decay analysis, and the numbers below are bootstrap (BS) values from maximum parsimony (MP) analysis. For nodes not present in the MP BS analysis, no *d* or BS values are listed.

monophyletic were included in these analyses, due to the very low *P* values we believe the results would hold even if all plausible trees were tested. Additionally, forcing monophyly on the Hawaiian groups would require breaking up strongly supported clades in every case.

## 4. Discussion

### 4.1. Phylogenetic relationships

Although our analyses were based only on cpDNA sequences, we will discuss putative relationships among *Dryopteris* taxa. As mentioned above, clades in Fig. 4 have been numbered with Roman numerals for simplicity in discussing the results.

Each of the three data sets, *rbcL*, *trnL-F*, and combined, yielded similar results regarding relationships among Hawaiian taxa. Each type of analysis, MP, ML (combined only), and Bayesian, supports the hypothesis that Hawaiian *Dryopteris* is polyphyletic, and suggests that there are four or five independently derived groups (including *N. rubiginosum*) in Hawai'i, which do not share a unique common ancestor. Our results indicate that the Hawaiian clades have closer relatives in other parts of the world. We hypothesize then that there have been five successful colonizations of different *Dryopteris* species in the Hawaiian Islands. This hypothesis assumes that mainland *Dryopteris* species did not arise from the Hawaiian taxa (but, see below).

*Dryopteris wallichiana* and *D. subbipinnata*, part of Clade VII, consistently grouped together (Figs. 2–4; PP 84, MP BS 50, d1) and without exception were strongly supported as sister to *D. affinis* var. *borreri* (PP 100, MP BS 95, d4). *Dryopteris wallichiana* is a pantropical species and is the only Hawaiian *Dryopteris* that is not endemic. It has been reported as a tetraploid in Hawai'i ($n=82$; F.S. Wagner, pers. comm.; the base chromosome number in *Dryopteris* in $x=41$), but Fraser-Jenkins (1994) rather suggests that it is an apomictic diploid that has been mistaken for a tetraploid. Plants of *D. wallichiana* from Asia have been counted three separate times as diploids ($n=41$; Gibby, 1985; Khullar et al., 1988; Punetha, 1989) and Fraser-Jenkins (1994) suggests that it is normally an apomictic diploid. *Dryopteris subbipinnata* is morphologically similar to *D. wallichiana*, but differs in that it has a longer petiole, is wider at the laminar base, and the pinnules are longer and more shallowly lobed (Fraser-Jenkins, 1994, and personal observations). It has been reported as a hexaploid ($n=123$; Wagner (1993)), however, Fraser-Jenkins (1994) suggests it is likely an apomictic triploid rather than a hexaploid.

Further cytological and experimental investigations are needed to clarify the ploidy levels and mating systems of *D. wallichiana* and *D. subbipinnata*. Nevertheless, it is not surprising that these analyses indicate that these two species are closely related. It has been hypothesized that *D. subbipinnata* is of allopolyploid hybrid origin and that *D. wallichiana* is one of its parental species (Fraser-Jenkins, 1994). There is low molecular divergence between these two species (*rbcL* = 0.15% divergence; *trnL-F* = 0% divergence), suggesting that the separation of *D. subbipinnata* from *D. wallichiana* (and/or its other parent) is a relatively recent event. A phylogeny based on a nuclear gene or other biparentally inherited markers might assist in elucidating whether *D. subbipinnata* is of alloploid hybrid origin or is an *in situ* autoploid derivative of *D. wallichiana* or another closely related unknown species.

Clade VII was part of a larger clade (not numbered) that included two other Hawaiian taxa, *D. fusco-atra* var. *fusco-atra* and *D. fusco-atra* var. *lamoureuxii*, Clade VIII. *Dryopteris fusco-atra* var. *fusco-atra* has been reported to be a hexaploid with $n=$ c. 123 (F.S. Wagner, pers. comm.). The two taxa were strongly supported as sister (Fig. 4; PP 100, MP BS 100, d13), and belonged to an unresolved clade including *D. stenolepis*, *D. sublacera*, *D. pycnopteroides*, and *D. uniformis* (PP 99; MP BS 70, d1). Additional data will need to be collected to identify the closest living relatives of *D. fusco-atra*. The two *D. fusco-atr* a varieties had identical sequences for *trnL-F* and for *rbcL* they were only 0.07% different. *Dryopteris fusco-atra* var. *lamoureuxii* is only found on E. Maui and these results suggest that it may be only an ecotype of the more widespread taxon, *D. fusco-atra* var. *fusco-atra*. Additional study is needed to determine if *D. fusco-atra* is of allo- or autopolyploid origin and what other taxa may have contributed to its genome.

Analyses based on *trnL-F* and the combined data set each supported a monophyletic glabra group as being closely related to *D. corleyi*, *D. aemula*, and *N. rubiginosum* (Clade V, Figs. 3 and 4, PP 100, MP BS 72, d2). In contrast, the *rbcL* analyses included *D. corleyi* and *D. aemula* within the glabra group (Fig. 2). The glabra group, as described by Palmer (2003), includes *D. glabra*, as well as *D. hawaiiensis*, although Palmer (2003) described *D. hawaiiensis* as "unrelated" to *D. glabra*. Our data suggest, however, that the hexaploid *D. hawaiiensis* ($n=123$; F.S. Wagner, pers. comm.) is closely related to *D. glabra*, which has been counted as a diploid ($n=41$; F.S. Wagner, pers. comm.). Sequences from *rbcL* showed only 0.3% base differences between *D. hawaiiensis* and six other taxa in the glabra group and it had identical *trnL-F* sequences as five other glabra group taxa. It is unknown whether *D. hawaiiensis* is an autopolyploid or an allopolyploid. Regardless of its polyploid origins, it appears either that *D. hawaiiensis* has inherited its chloroplast genome from *D. glabra* or that the two species share a very recent common ancestor.

Circumscription of and relationships among the members of the glabra group remain unresolved. This is perhaps not surprising as this group has recently been

the source of much taxonomic disagreement (Fraser-Jenkins, 1994; Herat, 1979; Palmer, 2003; Wagner, 1993, 1988; Wagner et al., 1995a, 1999a). These authors have disagreed about whether the varieties of *D. glabra* are in fact morphologically distinct enough to be considered separate species. Our analyses indicate that there is little chloroplastic molecular divergence among the *D. glabra* varieties. Pairwise divergences varied from 0 to 0.32% for *rbcL* and from 0 to 0.15% for *trnL-F*. These molecular results alone do not provide evidence warranting the recognition of varieties as species or vice-versa. However, as more data are added, this may change. We have completed studies using more variable molecular markers and morphological data in an attempt to objectively resolve this issue, which will be reported elsewhere (Geiger and Ranker, unpublished).

Analyses of the three data sets consistently placed two European species, *D. corleyi* and *D. aemula*, as closely related to, or within, the glabra group. Analyses based on *rbcL* placed both species within the glabra group, making the glabra group paraphyletic. However, analyses from both the *trnL-F* and combined data sets suggest that the two European species are basal to a monophyletic glabra group. Pairwise distances for *rbcL* between *D. corleyi* and members of the glabra group (range = 0.0030–0.0053) were greater than the pairwise distances between *D. aemula* and members of the glabra group (range = 0.0010–0.0038). However, for *trnL-F*, *D. corleyi* and *D. aemula* shared identical sequences, thus there was no difference in the pairwise distance ranges between the two species when compared with the glabra group (range = 0.0030–0.0064). That *D. corleyi* and *D. aemula* were supported as close relatives is not surprising as *D. corleyi* is thought to be an allopolyploid species that arose from chromosome doubling after a hybridization event between *D. oreades* and *D. aemula* (Fraser-Jenkins and Widen, 1993). From these analyses it would appear that *D. corleyi* inherited its chloroplast genome from *D. aemula*, as the two putative parental species, *D. oreades* and *D. aemula*, are resolved in very different parts of the tree.

Another interesting result regarding this group is that the Hawaiian endemic, *Nothoperanema rubiginosum*, was supported by both *rbcL* and the combined dataset as sister to the glabra + *D. corleyi* + *D. aemula* clade (Figs. 2, 4 PP 94, MP BS 63, d1), but the *trnL-F* analysis places *N. rubiginosum* as sister to the aforementioned clade and the non-Hawaiian clade (Fig. 3; Clade IV of Fig. 4). *Nothoperanema rubiginosum* has been variously treated in the genera *Lastrea*, *Ctenitis*, and *Dryopteris* (see Smith and Palmer, 1995). Smith and Palmer (1995) suggest that *Nothoperanema* is closely related to *Dryopteris* but is separated from it based on laminar scale differences. Our results suggest that *N. rubiginosum* should be placed in the genus *Dryopteris* to preserve the monophyly of the latter. As suggested by Fraser-Jenkins (1994) and by our results, further studies of the genus *Nothoperanema* in conjunction with the genus *Dryopteris* are needed to resolve whether *Nothoperanema* species should be treated in the genus *Dryopteris*.

Our analyses suggest close relationships among members of the so-called exindusiate group, Clade II (Figs. 2–4; PP 100, MP BS 100, d13). The leaves of these species range in length from 0.30–0.61 to 2.44 m (Palmer, 2003), and all species lack indusia. Our results support Palmer's (2003) hypothesis that *D. unidentata*, *D. sandwicensis*, and *D. tetrapinnata* are closely related. Although not as closely related as the above mentioned three species, our data indicate that *D. crinalis* and *D. mauiensis* also belong in this clade, with all five species sharing a putative unique common ancestor. Pairwise distances among species in this group ranged from 0.0008 to 0.0076 for *rbcL* and from 0 to 0.0096 for *trnL-F*. The exindusiate group was supported as sister to the non-Hawaiian Clade I in the *rbcL* and combined analyses, although weakly, and its relationship to the rest of the ingroup was unresolved in the *trnL-F* analysis. None of the species in Clade I lack indusia. There are, however, non-Hawaiian exindusiate species of *Dryopteris* of various geographical origins, thus it is possible that we have not yet sampled the closest relatives of the exindusiate group. Another possible explanation is that we have sampled this group's closest extant relatives, but the ancestor of the exindusiate Hawaiian species lost the indusium subsequent to colonization, and that the extant species inherited this trait.

Sequence divergences among species within each of the monophyletic groups in Hawai'i were very low. This result is not unusual for closely related species in Hawai'i. Many studies have shown that although there is considerable morphological divergence between related Hawaiian species, there is often little molecular divergence (Helenurum and Ganders, 1985; Lowrey and Crawford, 1985; Lowrey, 1995; Witter and Carr, 1988; Witter, 1990). Our study provides another example of this phenomenon and may reflect recent diversification of these clades.

### 4.2. Biogeographical implications

In each analysis, the four Hawaiian clades of *Dryopteris* were more closely related to non-Hawaiian species than they were to each other (Figs. 2–4). The Hawaiian endemic, *Nothoperanema rubiginosum*, was strongly supported as closely related to, but not sister to, the Hawaiian glabra group. These results suggest that there were five separate introductions of dryopteroid ferns to the Hawaiian archipelago. This finding, although not entirely surprising when considering the dispersal ability of fern spores via wind, is contrary to what is believed to have occurred for several other endemic plant groups occurring in Hawai'i (Baldwin et al., 1991; Baldwin and Robichaux, 1995; Pax et al., 1997; Wagner et al., 1995b). In each of those groups, the evidence suggests

that there was a single colonizing ancestor and subsequent evolutionary radiation. Only three studies of flowering plants have suggested multiple, independent colonizations of congeneric species to the Hawaiian Islands (*Rubus*, Howarth et al., 1997; *Chamaesyce*, Raz et al., 1998; and *Scaevola*, Howarth et al., 1999), and only one study has provided evidence for multiple colonizations of a single fern species (Ranker et al., 1994) in Hawai'i. However, Motley and Raz (2004) reported new evidence indicating that the endemic Hawaiian *Chamaesyce* taxa are monophyletic resulting from only one colonization event. Outside of Hawai'i, multiple colonizations of oceanic islands have been hypothesized for *Lavatera* (Fuertes-Aguilar et al., 2002) and *Ilex* (Cuenoud et al., 2000) in the Canary Islands, and *Gossypium* in the Galápagos (Wendel et al., 1995).

Our results do not allow for a strongly supported hypothesis on the geographical origins of *D. wallichiana* and *D. subbipinnata* (Clade VII) in Hawai'i. These two species are clearly supported as having a different phylogenetic history from the other Hawaiian species, but their geographical origin is unclear as *D. wallichiana* has a pantropical distribution. The close relationship with *D. affinis* subsp. *borreri* does not help elucidate the matter, as it, too, is geographically widespread throughout Europe to W. Asia. Phylogeographic studies of populations of *D. wallichiana* may assist in clarifying the origins of the Hawaiian populations of this species.

*Dryopteris fusco-atra* is related to Clades VI, VII, IX, and to *D. stenolepis*. Our data support the hypothesis that the presence of *D. fusco-atra* in Hawai'i is the result of an independent colonization event. The three species in Clade IX and *D. stenolepis* are all native to SE Asia. The two species in Clade VI are also native to SE and eastern Asia, specifically China and Japan. Although the closest relative of *D. fusco-atra* has yet to be identified, this study provides support for this species being of East Asian origin.

The exindusiate group, Clade II, is supported as a third monophyletic *Dryopteris* clade in Hawai'i. *Dryopteris tokyoensis* and *D. odontoloma* of Clade I, which is the sister group to Clade II in the *rbcL* and combined analyses (Figs. 2 and 4), are geographically distributed throughout E. Asia. *Dryopteris pallida* of Clade I is a W. European taxon. It is possible that the Hawaiian Clade II arose from a SE Asian species that dispersed to Hawai'i and a related species also dispersed to W. Europe. However, the relationship between Clades I and II is weakly supported by the combined analysis (Fig. 4; PP 71, MP BS 55, d3). It is probable that we have not yet sampled the closest relatives of the exindusiate group, and thus any specific biogeographical hypotheses are likely to be misguided at this time.

Our data indicate that the Hawaiian *Dryopteris* taxa of Clade V, the glabra group, are closely related to the W. European species *D. aemula* and *D. corleyi* (Fig. 4, PP 100, MP BS 100, d11). The relationship between *D. aemula* and *D. corleyi* is discussed above. Hillebrand (1888) recognized the close relationship between *D. aemula* and Hawaiian *D. glabra* as did Fraser-Jenkins (1994). Fraser-Jenkins (1994) comments that "it is unlikely to have been mere chance long-distance dispersal with such a restricted species [*D. aemula*]." Indeed, it is difficult to explain this type of biogeographical disjunction. However, from our data, and from previous morphological observations by Hillebrand (1888) and Fraser-Jenkins (1994), it would seem that there must have been a long distance dispersal event of *D. aemula* to the Hawaiian Islands, and the lack of gene flow between the Hawaiian populations and W. European populations allowed the genetic separation of *D. glabra*. There are, however, at least two alternative hypotheses. The sister clade to the Hawaiian + W. European Clade V + Hawaiian *N. rubiginosum*, Clade IV, is comprised of seven species, all of which occur in SE Asia. It is possible that *D. aemula* and *D. glabra* share an unsampled common ancestor (or an extinct common ancestor) that was from SE Asia. This ancestor could have recently dispersed to both W. Europe and Hawai'i separately. Alternatively, *D. aemula* could have arisen in the Hawaiian Islands and dispersed to W. Europe, since in our analyses it is embedded in a clade of endemic Hawaiian species. We cannot reject any of these scenarios with our phylogenetic hypotheses.

*Nothoperanema rubiginosum* may represent a fifth dispersal event to the Hawaiian Islands. This species was sister to the Hawaiian glabra group + *D. corleyi* + *D. aemula*. Given the close relationship of the entire clade to the SE Asian Clade IV, a SE Asian origin for this species is the best supported hypothesis. However, there are five other described species of Asiatic *Nothoperanema* that should be sampled and included in subsequent phylogenetic analyses to best infer the geographical origins of this Hawaiian endemic.

Our analyses suggest that three, perhaps four, of the five Hawaiian clades have Asian geographical associations. We may have expected to find this pattern for several reasons. First, Fraser-Jenkins (1994) commented that the Hawaiian fern flora appears to have a considerable resemblance to the Sino-Japanese and Sino-Himalayan floras and Fosberg (1948) hypothesized that 14 of the 25 *Dryopteris* species he recognized were of Indo-Pacific origin. Second, the jetstream occurs as a latitudinally undulating band of fast-moving air, ~5500–17,000 meters in altitude, flowing from west to east. The jetstream accelerates as it moves eastward from SE Asia (up to 195 kph) and decelerates as it moves over the Hawaiian Islands (down to ~115 kph; Carlquist, 1980). Spores could be moved up into the jetstream by storms and transported from SE Asia/Malaysia to Hawai'i in 2–4 days. Third, the taxa included in our analyses are mostly native to Asia, thus our analyses could be biased. Until we have sampled all *Dryopteris* and *Nothopera-*

*nema* species and obtained sequence data from both chloroplast and nuclear markers, we will not have a complete picture of the origins of each Hawaiian clade, at least from a molecular perspective.

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## References

Alfaro, M.E., Zoller, S., Lutzoni, F., 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol. Biol. Evol. 20, 255–266.

Baldwin, B.G., Robichaux, R.H., 1995. Historical biogeography and ecology of the Hawaiian Silversword alliance (Asteraceae). In: Wagner, W.L., Funk, V.A. (Eds.), Hawaiian Biogeography: Evolution on a Hot Spot Archipelago. Smithsonian Institution Press, Washington, DC, pp. 259–285.

Baldwin, B.G., Kyhos, D.W., Dvorák, J., Carr, G.D., 1991. Chloroplast DNA evidence for a North American origin of the Hawaiian Silversword Alliance (Asteraceae). Proc. Natl. Acad. Sci. USA 88, 1840–1843.

Bremer, K., 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42, 795–803.

Carlquist, S., 1980. Hawai'i: A Natural History, 2nd ed. Pacific Tropical Botanical Garden, Lawai.

Carson, H.L., Clague, D.A., 1995. Geology and biogeography of the Hawaiian Islands. In: Wagner, W.L., Funk, V.A. (Eds.), Hawaiian Biogeography: Evolution on a Hot Spot Archipelago. Smithsonian Institution Press, Washington, DC, pp. 14–29.

Clague, D.A., 1996. The growth and subsidence of the Hawaiian-Emperor volcanic chain. In: Miller, S.E. (Ed.), The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes. SPB Academic Publishing, Amsterdam, pp. 35–50.

Cuenoud, P., Martinez, M.A.D., Loizeau, P.A., Spichiger, R., Andrews, S., Manen, J.F., 2000. Molecular phylogeny and biogeography of the genus *Ilex* L. (Aquifoliaceae). Ann. Bot. 85, 111–122.

Donoghue, M.J., Olmstead, R.G., Smith, J.F., Palmer, J.D., 1992. Phylogenetic relationships of Dipsacales based on *rbcL* sequences. Ann. Missouri Bot. Gard. 79, 333–345.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Eriksson, T., 1998. AutoDecay ver. 4.0.1 (program distributed by the author). Department of Botany, Stockholm University, Stockholm.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.

Farris, J.S., Källersjö, J., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.

Fosberg, F.R., 1948. Derivation of the flora of the Hawaiian Islands. In: Zimmerman, E.C. (Ed.), Insects of Hawai'i. University Press, Honolulu, pp. 107–119.

Fraser-Jenkins, C.R., 1986. A classification of the genus *Dryopteris* (Pteridophyta: Dryopteridaceae). Bull. Brit. Mus. Nat. Hist. 14, 183–218.

Fraser-Jenkins, C.R., 1994. *Dryopteris* (Pteridophyta) of Hawai'i—a monographic study. Thaiszia-J. Bot., Kosice 4, 15–47.

Fraser-Jenkins, C.R., Widen, C.J., 1993. Phloroglucinol derivatives in *Dryopteris ardechensis* and *D. corleyi* (Pteridophyta, Dryopteridaceae) and their putative ancestors. Annales Botanici Fennici 30, 43–51.

Fuertes-Aguilar, J., Ray, M.E., Francisco-Ortega, J., Santos-Guerra, A., Jansen, R.K., 2002. Molecular evidence from chloroplast and nuclear markers for multiple colonizations of *Lavatera* (Malvaceae) in the Canary Islands. Syst. Bot. 27, 74–83.

Gibby, M., 1985. Cytological observations on Indian subcontinent and Chinese *Dryopteris* and *Polystichum* (Pteridophyta: Dryopteridaceae). Bull. Brit. Mus. Nat. Hist. (Bot.) 14, 1–42.

Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–670.

Hasebe, M., Wolf, P.G., Pryer, K.M., Ueda, K., Ito, M., Sano, R., Gastony, G.J., Yokayama, J., Manhart, J.R., Murakami, N., Crane, E.H., Haufler, C.H., Hauk, W.D., 1995. Fern phylogeny based on *rbcL* nucleotide sequences. Am. Fern. J. 85, 134–181.

Hasegawa, M., Kishino, K., Yano, T., 1985. Dating the human–ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.

Haufler, C.H., Ranker, T.A., 1995. *RbcL* sequences provide phylogenetic insights among sister species of the fern genus *Polypodium*. Am. Fern. J. 85, 361–374.

Helenurum, K., Ganders, F.R., 1985. Adaptive radiation and genetic differentiation in Hawaiian *Bidens*. Evolution 39, 753–765.

Hennequin, S., Ebihara, A., Ito, M., Iwatsuki, K., Dubuisson, J-Y., 2003. Molecular systematics of the fern genus *Hymenophyllum* s. l. (Hymenophyllaceae) based on chloroplastic coding and noncoding regions. Mol. Phylogenet. Evol. 23, 283–301.

Herat, T.R., 1979. A Systematic Study of the Genus *Dryopteris* in Hawai'i. University of Hawai'i, Honolulu.

Hillebrand, W., 1888. Flora of the Hawaiian Islands: A Description of their Phanerogams and Vascular Cryptogams. Lubrecht and Cramer, Monticello, NY.

Hoshizaki, B.J., Wilson, K.A., 1999. The cultivated species of the fern genus *Dryopteris* in the United States. Am. Fern J. 89, 1–98.

Howarth, D.G., Gardner, D.E., Morden, C.W., 1997. Phylogeny of *Rubus* subgenus *Idaeobatus* (Rosaceae) and its implications toward colonization of the Hawaiian Islands. Syst. Bot. 22, 433–441.

Howarth, D.G., Gustafsson, M.H.G., Motley, T.J., 1999. Phylogenetics and biogeography of *Scaevola* in the Pacific basin. XVI International Botanical Congress Abstracts, 4539, St. Louis, Missouri, USA.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Kim, H., Keeley, S.C., Vroom, P.S., Jansen, R.K., 1998. Molecular evidence for an African origin of the Hawaiian endemic *Hesperomannia* (Asteraceae). Proc. Natl. Acad. Sci. USA 95, 15440–15445.

Kirch, P., 1982. The impact of the prehistoric Polynesians on the Hawaiian ecosystem. Pacific Sci. 36, 1–14.

Khullar, S.P., Sharma, S.S., Verma, S.C., 1988. SOCGI plant chromosome number reports VI. J. Cytol. Genet. 23, 38–52.

Lowrey, T.K., 1995. Phylogeny, adaptive radiation, and biogeography of Hawaiian *Tetramolopium* (Asteraceae, Astereae). In: Wagner, W.L., Funk, V.A. (Eds.), Hawaiian Biogeography: Evolution on a

Hot Spot Archipelago. Smithsonian Institution Press, Washington, DC, pp. 195–220.
Lowrey, T.K., Crawford, D.J., 1985. Allozyme divergence and evolution in *Tetramolopium* (Compositae: Astereae) on the Hawaiian Islands. Syst. Bot. 10, 64–72.
Maddison, D.R., Maddison, W.P., 2000. MacClade 4. Sinauer Associates, Massachusetts.
Martin, A.P., Naylor, G.J.P., 1997. Independent origins of filter-feeding in megamouth and basking sharks (Order Lamniformes) inferred from phylogenetic analysis of cytochrome *b* gene sequences. In: Yano, K., Morrissey, J.F., Yabumoto, Y., Nakaya, K. (Eds.), Biology of Megamouth Shark. Tokai University Press, Tokyo, pp. 39–50.
Motley, T.J., Raz, L., 2004. Origins, evolution, and systematics of Hawaiian and Pacific *Chamaesyce* (Euphorbiaceae). Botany 2004 Abstracts, 522, Snowbird, Utah, USA.
Palmer, D.D., 2003. Hawai'i's Ferns and Fern Allies. University of Hawai'i Press, Honolulu.
Pax, D.L., Price, R.A., Michaels, H.J., 1997. Phylogenetic position of the Hawaiian geraniums based on *rbcL* sequences. Am. J. Bot. 84, 72–78.
Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Powell, E.A., Kron, K.A., 2002. Hawaiian blueberries and their relatives—a phylogenetic analysis of *Vaccinium* sections *Macropelma*, *Myrtillus*, and *Hemimyrtillus* (Ericaceae). Syst. Bot. 27, 768–779.
Price, J.P., Clague, D.A., 2002. How old is the Hawaiian biota. Geology and phylogeny suggest recent divergence. Proc. R. Soc. Lond. B 269, 2429–2435.
Punetha, N., 1989. Cytological observations on ferns of Kumoan (N.W. Himalaya). Aspects Plant Sci. 11, 459–465.
Ranker, T.A., Floyd, S.K., Trapp, P.G., 1994. Multiple colonizations of *Asplenium adiantum-nigrum* onto the Hawaiian archipelago. Evolution 48, 1364–1370.
Ranker, T.A., Gemmill, C.E.C., Trapp, P.G., 2000. Microevolutionary patterns and processes of the native Hawaiian colonizing fern *Odontosoria chinensis* (Lindsaeaceae). Evolution 54, 828–839.
Ranker, T.A., Gemmill, C.E.C., Trapp, P.G., Hambleton, A., Ha, K., 1996. Population genetics and reproductive biology of lava-flow colonising species of Hawaiian *Sadleria* (Blechnaceae). In: Johns, R.J. (Ed.), Pteridology in Perspective. Royal Botanical Gardens, Kew, pp. 581–598.
Ranker, T.A., Geiger, J.M.O., Kennedy, S.C., Smith, A.R., Haufler, C.H., Parris, B.S., 2003. Molecular phylogenetics and evolution of the endemic Hawaiian genus *Adenophorus*. Mol. Phylogenet. Evol. 26, 337–347.
Ranker, T.A., Smith, A.R., Parris, B.S., Geiger, J.M.O., Haufler, C.H., Straub, S.C.K., Schneider, H., 2004. Phylogeny and evolution of grammitid ferns (Grammitidaceae): a case of rampant morphological homoplasy. Taxon 53, 415–428.
Raz, L., Albert, V.A., Motley, T.J., 1998. Molecular systematics of Hawaiian *Chamaesyce* (Euphorbiaceae). Am. J. Bot. 85 (supplement), 153–154.
Russell, A.E., Ranker, T.A., Gemmill, C.E.C., Farrar, D.R., 1999. Patterns of clonal diversity in *Dicranopteris linearis* on Mauna Loa, Hawai'i. Biotropica 31, 449–459.
Schneider, H., Russell, S.J., Cox, C.J., Bakker, F., Henderson, S., Rumsey, F., Barrett, J., Gibby, M., Vogel, J.C., 2004a. Chloroplast phylogeny of Asplenioid ferns based on *rbcL* and *trnL-F* spacer sequences (Polypodiidae, Aspleniaceae) and its implications for biogeography. Syst. Bot. 29, 260–274.
Schneider, H., Smith, A.R., Cranfill, R., Hildebrand, T., Haufler, C.H., Ranker, T.A., 2004b. Unraveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): exploring aspects of the diversification of epiphytic plants. Mol. Phylogenet. Evol. 31, 1041–1063.
Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.
Smith, A.R., 1972. Comparison of fern and flowering plant distributions with some evolutionary interpretations for ferns. Biotropica 4, 4–9.
Smith, A.R., Palmer, D.D., 1995. *Ctenitis rubiginosa*, from Hawai'i, belongs in *Nothoperanema*. Am. Fern J. 85, 63.
Sohmer, S.H., Gustafson, R., 1987. Plants and Flowers of Hawai'i. University of Hawai'i Press, Honolulu.
Suzuki, Y., Glazko, G.V., Nei, M., 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proc. Natl. Acad. Sci. USA 99, 16138–16143.
Swofford, D.L., 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Sinauer Associates, Sunderland, MA.
Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.
Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 16, 1292–1299.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequences alignment aided by quality analysis tools. Nucleic Acid Res. 24, 4876–4882.
Tryon, R., 1970. Development and evolution of fern floras of oceanic islands. Biotropica 2, 76–84.
Wagner Jr., W.H., 1988. Status of the Hawaiian fern flora. Fiddlehead Forum 15, 11–14.
Wagner Jr., W.H., 1993. New species of Hawaiian pteridophytes. Contr. Univ. Michigan Herb. 19, 63–82.
Wagner Jr., W.H., 1995. Evolution of Hawaiian ferns and fern allies in relation to their conservation status. Pacific Sci. 49, 31–41.
Wagner Jr., W.H., Wagner, F.S., Flynn, T., 1995a. Taxonomic notes on the pteridophytes of Hawai'i. Contr. Univ. Michigan Herb. 20, 241–260.
Wagner Jr., W.H., Wagner, F.S., Palmer, D.D., Hobdy, R.W., 1999a. Taxonomic notes on the pteridophytes of Hawai'i-II. Contr. Univ. Michigan Herb. 22, 135–187.
Wagner, W.L., Funk, V.A., 1995. Hawaiian Biogeography: Evolution on a Hot Spot Archipelago. Smithsonian Institution Press, Washington, DC.
Wagner, W.L., Weller, S.G., Sakai, A.K., 1995b. Phylogeny and biogeography in *Schiedea* and *Alsinidendron* (Caryophyllaceae). In: Wagner, W.L., Funk, V.A. (Eds.), Hawaiian Biogeography: Evolution on a Hot Spot Archipelago. Smithsonian Institution Press, Washington, DC, pp. 221–258.
Wagner, W.L., Herbst, D.R., Sohmer, S.H., 1999b. Manual of the Flowering Plants of Hawai'i. University of Hawai'i Press/Bishop Museum Press, Honolulu.
Wendel, J.F., Schnabel, A., Seelanan, T., 1995. An Unusual Ribosomal DNA-Sequence from *Gossypium* Gossypioides Reveals Ancient, Cryptic, Intergenomic Introgression. Mol. Phylogenet. Evol. 4, 298–313.
Witter, M.S., 1990. Evolution in the Madiinae: evidence from enzyme electrophoresis. Ann. Missouri Bot. Gard. 77, 110–117.
Witter, M.S., Carr, G.D., 1988. Adaptive radiation and genetic differentiation in the Hawaiian Silversword alliance (Compositae: Madiinae). Evolution 42, 1278–1287.
Wolf, P.G., Soltis, P.S., Soltis, D.E., 1994. Phylogenetic relationships of Dennstaedtioid ferns: evidence from *rbcL* sequences. Mol. Phylogenet. Evol. 3, 383–392.