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Phylogeny, divergence times, and historical biogeography of New World *Dryopteris* (Dryopteridaceae)[1]

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- *Premise of the study: Dryopteris* is a large, cosmopolitan fern genus ideal for addressing questions about diversification, biogeography, hybridization, and polyploidy, which have historically been understudied in ferns. We constructed a highly resolved, well-supported phylogeny for New World *Dryopteris* and used it to investigate biogeographic patterns and divergence times.
- *Methods:* We analyzed relationships among 97 species of *Dryopteris*, including taxa from all major biogeographic regions, with analyses based on 5699 aligned nucleotides from seven plastid loci. Phylogenetic analyses used maximum parsimony, maximum likelihood, and Bayesian inference. We conducted divergence time analyses using BEAST and biogeographic analyses using maximum parsimony, maximum likelihood, Bayesian, and S-DIVA approaches. We explored the monophyly of subgenera and sections in the most recent generic classification and of geographic groups of taxa using Templeton tests.
- *Key results:* The genus *Dryopteris* arose ca. 42 million years ago (Ma). Most of the Central and South American species form a well-supported clade which arose 32 Ma, but the remaining New World species are the result of multiple, independent dispersal and vicariance events involving Asia, Europe, and Africa over the last 15 Myr. We identified six long-distance dispersal events and three vicariance events in the immediate ancestry of New World species; reconstructions for another four lineages were ambiguous.
- *Conclusions:* New World *Dryopteris* are not monophyletic; vicariance has dominated the history of the North American species, while long-distance dispersal prevails in the Central and South American species, a pattern not previously seen in plants.

Key words: divergence time estimates; diversification; *Dryopteris*; ferns; historical biogeography; long-distance dispersal; neotropics; vicariance; phylogeny; polyploidy; Pteridophyta.

The last decade has seen a nearly exponential increase in the number of molecular phylogenies published at various taxonomic levels across the plant tree of life. Vast uncharted territory still remains, however, particularly for ferns. Ferns are sister to seed plants (Pryer et al., 2001) and are the second largest group of vascular land plants, with ca. 12 000 species (Smith et al., 2006). They inhabit a great variety of substrates, climates, and light regimes, both in habitats dominated by flowering plants and those where few angiosperms can survive. They also represent a critical evolutionary step, bridging the functional gap between nonvascular bryophytes and seed-bearing vascular plants. Despite their ubiquity and key position in land-plant evolution, however, ferns have generally received far less attention than the megadiverse flowering plants. Exhaustive phylogenetic studies of large fern genera, in particular, lag behind such studies for angiosperms, even though such studies can provide detailed insights into speciation, ecological diversification, morphological and physiological adaptation, and biogeographic patterns. Given that ferns are sister to the seed plants (Pryer et al., 2001), an increased understanding of these phenomena in ferns may help us to better understand how evolution has proceeded in angiosperms and gymnosperms.

The genus *Dryopteris*, the woodferns, is an ideal group for such inquiries. With ca. 225 species worldwide (Fraser-Jenkins, 1986), it is one of the largest genera in Dryopteridaceae, which is itself one of the largest families of leptosporangiate ferns (Smith et al., 2006). The genus encompasses species with a diverse set of ranges, habitats, and morphologies, and hybridization and polyploidy appear to be common (see below). *Dryopteris* can thus provide a model for exploring many questions that have long been understudied in ferns, including the relative importance of dispersal vs. vicariance in shaping geographic distributions, the adaptive significance of various morphological and physiological traits, and the relative importance of branching vs. reticulate evolution. Investigating such questions at a global scale will first require a highly resolved, well-supported phylogeny for *Dryopteris* independent of the morphological, physiological, and distributional traits under study.

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Systematic studies of *Dryopteris* date to the early 1900s, with Christensen's *Index Filicum* (1906) and two-part monograph on the tropical American taxa (1913, 1920). In this period, the genus largely served as a grab bag for numerous, tenuously related species; Christensen listed 735 species in *Dryopteris* (Christensen, 1906), with 280 in the Americas (Christensen, 1913, 1920). By the 1970s, the size and position of the genus had largely been clarified, as numerous taxa were sorted into other genera and families (Pichi-Sermolli, 1970, 1977). Today, on the basis of molecular data, *Dryopteris* is recognized as sister to the morphologically similar *Arachniodes*; together they are sister to *Polystichum* plus *Cyrtomium-Phanerophlebia* (Schuettpelz and Pryer, 2009). Classification within *Dryopteris* has been addressed in several major systems to date, all based exclusively on morphology: Ito (1935, 1936) treated the species of Japan and Taiwan; Ching (1938) considered the species of China, the Himalayas, India, and Sri Lanka; Wu (2000) revised the species of China; and Fraser-Jenkins (1986) provided a worldwide classification. The last is the currently accepted system for *Dryopteris*, including 208 species in four subgenera and 16 sections, as well as several species considered incertae sedis.

Dryopteris is nearly cosmopolitan, with individual species occurring on all continents except Antarctica, and on a number of oceanic islands (e.g., Hawaii), ranging through tropical, temperate, and boreal regions. Its apparent center of diversity lies in southern and eastern Asia (Hoshizaki and Wilson, 1999; Li and Lu, 2006b). Most species have distributions limited to one major biogeographic area (e.g., eastern North America or southeastern China), but are widespread locally in the region where they occur. A few species are known to inhabit quite restricted areas (e.g., *D. fragrans*), while several others occur across nearly the entire range of the genus (e.g., *D. wallichiana*).

Morphologically, *Dryopteris* is extremely diverse. Most species are terrestrial, though several epiphytes are known. Fronds of individual species vary substantially in height (1–20 dm), are one to four times pinnate, with or without glandular trichomes, and frequently bear dense scales on the stipe and rachis. Sori are circular, normally with reniform indusia, and borne ad- or abaxially. The base chromosome number of the genus is $n = 41$ (Liu et al., 2007), but whole-genome duplication (polyploidy) is common, and *Dryopteris* is considered extremely prone to hybridization (Manton, 1950). The potential roles of reticulate evolution and polyploidy in the genus have long been recognized and studied for the 13 species native to North America (Montgomery and Wagner, 1993; Stein et al., 2010).

The fossil origins of *Dryopteris* and Dryopteridaceae are somewhat clouded. Several genera outside *Dryopteris* have been described from supposed dryopteridaceous fossils, including *Allantodiopsis* and *Makopteris* (Collinson, 2001), *Cuyenopteris* (Vera, 2010), and *Wessiea* (Pigg and Rothwell, 2001; Serbet and Rothwell, 2006). None of these can be attributed unambiguously to Dryopteridaceae, however, with the first two instead being most likely Athyriaceae (Collinson, 2001), and the latter two being of uncertain placement in Blechnaceae or Dryopteridaceae (Serbet and Rothwell, 2006; Vera, 2010). The oldest fossils ascribed to *Dryopteris* are from 65 to 55 million years ago (Ma) in China (Wang et al., 2006). Supposed "*Dryopteris*" from the same age in the Fort Union formation of the west-central United States (Brown, 1962), and from the Eocene/Oligocene of Alaska (Wolfe, 1977) appear to be misidentified and more likely to be Thelypteridaceae (Collinson, 2001). A number of more securely identified, but much more recent fossil *Dryopteris* are known from the Middle to Late Miocene in Russia (Akhmetiev, 2009), Alaska (Wolfe et al., 1966; Wolfe and Tanai, 1980; Reinink-Smith and Leopold, 2005), and Iceland (Grímsson and Denk, 2007).

Molecular phylogenetic studies of *Dryopteris* so far have focused on the Hawaiian taxa (Geiger and Ranker, 2005), a number of Asian species (Li and Lu, 2006b), and several small European complexes (Schneller et al., 1998; Jiménez et al., 2009; Jiménez et al., 2010; Schneller and Krattinger, 2010; Ekrt et al., 2010). Some of these studies have included North American species as placeholders. As yet, however, no comprehensive phylogenetic study has been undertaken for *Dryopteris* of the New World, which has the highest number of species after Asia. As a consequence, little is known about how these taxa are related to each other and to species from other regions, or about how or when they may have reached the Americas. We thus also know almost nothing about relationships or historical biogeographic patterns of *Dryopteris* at a global scale, given that data from nearly an entire hemisphere are missing. To date, no sequence data have been produced for the 18 Central and South American species of *Dryopteris*, and only a handful of the North American taxa have been analyzed as part of broader studies (e.g., Geiger and Ranker, 2005).

Based on the dispersal ability of fern spores and the ability of some ferns to reproduce from single gametophytes via inbreeding, long-range dispersal should play an important role in the diversification and historical biogeography of many fern groups (Tryon, 1986; Moran, 2008). Such dispersal has been demonstrated to be a pervasive phenomenon in the polyphyletic Hawaiian *Dryopteris* (Geiger and Ranker, 2005) and the Hawaiian fern flora more generally (Geiger et al., 2007), as expected on oceanic islands with no physical connection to mainland source areas. It is also consistent with the relatively high incidence of fern lineages on tall, rainy oceanic islands, the relatively low levels of regional endemism in ferns at various taxonomic levels, and the relatively broad geographic ranges seen in many fern species and genera (Smith, 1972; Wagner, 1972; Kramer, 1993; Wolf et al., 2001). However, genetic data for several species, including *Dryopteris expansa* (Soltis and Soltis, 1987), indicates that some ferns may maintain primarily mixed or outcrossing mating systems (Soltis and Soltis, 1992) and thus would require the simultaneous arrival of at least two spores for their origin via long-distance dispersal. Thus, vicariance and short-distance dispersal might play a predominant role in the historical biogeography of at least some fern groups (Haufler, 2007). In addition, Raynor et al. (1976) demonstrated that ferns primarily of temperate forests, including *Dryopteris*, actually have relatively limited dispersal ability in these habitats, and if spores *are* able to escape the forest canopy, to disperse over longer ranges (more than ca. 1000 km), they will need to reach currents higher in the atmosphere (Puentha, 1991). Gradstein and van Zanten (1999) demonstrated that spores of most species will sustain sterilizing UV-damage from traveling at this height. To the extent that long-distance dispersal, facilitated by microscopic spores, does dominate the biogeographic history of many fern groups, it may obscure the importance of vicariance (Wolf et al., 2001). However, sophisticated models for testing between vicariance and long-distance dispersal (e.g., LaGrange [Ree and Smith, 2007] and S-DIVA [Yu et al., 2010]) have yet to be applied in any large fern group, although a recent study on *Nephrolepis* confirms the value of such approaches (Hennequin et al., 2010).

Motivated by the issues outlined, we embarked on constructing a phylogeny for New World *Dryopteris*, to provide the basis for addressing questions about the group's origins, historical biogeography, relative importance of vicariance vs.

long-distance dispersal, and—in the future—patterns of reticulate vs. branching evolution and the adaptive value of various morphological and physiological traits that vary across species. We employed plastid sequence data here as a first step, and subsequent analyses will incorporate nuclear genomic data. We have included an extensive sampling of species from other biogeographic regions, making our study the closest approach yet to a worldwide phylogeny for *Dryopteris*. We derived this phylogeny based on plastid DNA sequences from one gene and six intergenic spacers, calibrated against the age of several non-*Dryopteris* fossils, to address the following questions: (1) How many clades include New World *Dryopteris*, when did they arise, and what was their likely place of origin? (2) Do the species native to any geographic region form a clade? (3) Are the subgenera and sections recognized by Fraser-Jenkins (1986) monophyletic, or are they unnatural groups defined by morphological traits with multiple origins? (4) How many instances of vicariance vs. long-distance dispersal led to the distribution of species in the New World, and which of these phenomena best accounts for present-day distributions at a global scale?

MATERIALS AND METHODS

***Taxon sampling*—**The taxonomic sampling in this study includes representatives from each of the subgenera and sections of *Dryopteris* identified in the treatment by Fraser-Jenkins (1986), and representatives from all major geographic regions of the world. *Dryopteris* includes an estimated 225 species worldwide, with ca. 160 species in eastern and southern Asia, 13 species in North America north of Mexico, 18 in Hawaii, and from 15 to 30 species each in Europe, Africa, Australasia, and Central–South America (Fraser-Jenkins, 1986; Montgomery and Wagner, 1993; Mickel and Smith, 2004). Ninety-seven species of *Dryopteris* were included in the current study, including all species found in North, Central, and South America, as well as multiple species from Asia, Eastern and Western Europe, Hawaii, and other areas (Table 1). Material of the 13 North American species was collected in the field. Material of other species was obtained from herbarium specimens or from collaborators who provided either silica-dried material or DNAs.

Numerous outgroup taxa are included and were selected based on a family-level analysis of Dryopteridaceae by Liu et al. (2007) and an extensive survey of leptosporangiate ferns by Schuettpelz and Pryer (2009). Eighteen taxa in 13 genera are included. Appendix 1 provides voucher information and GenBank accession numbers for all taxa included in this study.

***DNA extraction, amplification, and sequencing*—**Total genomic DNA was extracted from silica-dried leaf material using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) and the manufacturer's protocol. For each species, one protein-coding region (*rbcL*) and six intergenic spacers (*psbA-trnH*, *trnP-petG*, *rps4-trnS*, *trnL-F*, *trnG-trnR*, and *rbcL-accD*) in the chloroplast genome were amplified using the polymerase chain reaction (PCR). Primers used for PCR and cycle sequencing were based on previous studies (Table 2). Regions were selected based on their utility and successful amplification in *Dryopteris* in previous fern studies, including those by Small and colleagues (2005: *trnP-petG*), and Korall and colleagues (2006: *rbcL*; 2007: *rbcL-accD*, *trnG-trnR*). All regions were amplified in 25-μL reactions containing 10 μL ddH_20, 2.5 μL 5× Colorless GoTaq Flexi buffer (Promega, Madison, Wisconsin, USA), 2.5 μL 2.5 mmol/L dNTP, 2 μL bovine serum albumin, 1 μL dimethylsulfoxide, 1 μL 25 mmol/L $MgCl_2$, 0.5 μL of each primer at 20 mmol/L, 0.25 μL GoTaq Flexi DNA polymerase (Promega), and 2 μL template DNA. Amplifications were carried out on MJ Research DNA Engine (Bio-Rad; Hercules, California) or Eppendorf MasterCycler Pro S (Eppendorf Scientific, Hamburg, Germany) thermal cyclers, following published cycling protocols for each region (Table 2).

PCR products were purified using ExoSAP-IT (USB Corp., Cleveland, Ohio, USA), and cycle-sequencing reactions carried out directly on the purified PCR products using BigDye Terminator 3.1 (Applied Biosystems, Foster City, California). Sequencing products were purified via gel filtration chromatography using Sephadex columns (Sigma-Aldrich, St. Louis, Missouri, USA) according to the manufacturer's protocols. All regions were sequenced in both directions to ensure unambiguous base calls, and sequencing was carried out at either the University of Wisconsin-Madison Biotechnology Center (Madison, Wisconsin) or the Smithsonian Institution Museum Support Center (Suitland, Maryland, USA).

***Sequence alignment and phylogenetic analyses*—**Sequences were edited and assembled in the program Sequencher 4.1 (Gene Code Corp., Ann Arbor, Michigan, USA), aligned with the program ClustalX v.2 (Larkin et al., 2007), and alignments adjusted manually in the program Se-Al v2.0a11 Carbon (Rambaut Research Group, University of Edinburgh, Edinburgh, UK). Gaps in the alignments due to insertion/deletion events (indels) were coded as present or absent using the approach of Simmons and Ochoterena (2000) as implemented in the program FastGap (Borschsenius, 2009) and appended to the nucleotide data as additional characters.

Incongruence between the data partitions representing different regions of the plastid genome was assessed via the incongruence length difference (ILD) test (Farris et al., 1996), implemented as the partition homogeneity test in the program PAUP* version 4.0b10.0 (Swofford, 2002). The test was conducted for each pair of regions included in the study. When incongruence was detected, we attempted to resolve conflicts by constraining the outgroups based on a priori knowledge of their relative positions. The amount of homoplasy in the data were evaluated using consistency indices, both including (CI) and excluding (CI′) autapomorphies (Givnish and Sytsma, 1997).

Phylogenetic relationships of *Dryopteris* were investigated using maximum parsimony (MP) in the program PAUPRat (Sikes and Lewis, 2001) and PAUP* (Swofford, 2002), maximum likelihood (ML) in the programs Garli 2.0 (Zwickl, 2006) and RAxML 7.2.8 (Stamatakis, 2006; Stamatakis et al., 2008), and Bayesian inference (BI) in the program MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). PAUPRat, RAxML, and MrBayes analyses were done within the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal 2 (http://www.phylo.org/portal2/) (Miller et al., 2010).

The MP analyses with PAUPRat (Sikes and Lewis, 2001), based on parsimony ratchet (Nixon, 1999), were conducted using 1000 ratchets with 200 iterations per replicate, following Sundue and colleagues (2010). Support for clades was estimated using parsimony bootstrap analysis in PAUP* (Swofford, 2002) with 1000 replicates, tree-bisection-reconnection (TBR) branch swapping, simple taxon addition with one tree held at each step, and a maximum of 100 trees saved per replicate to decrease the time needed to run large bootstrap replicates. All MP analyses were run both with and without the indel data included, to assess their effects on topology and clade support. These data were not included in the ML and BI analyses, as CIPRES does not provide a way to model standard (nonnucleotide) variables in its analyses.

For ML and BI analyses, the optimal model of molecular evolution for each data set was identified using hierarchical likelihood ratio tests and the Akaike information criterion in the program MrModeltest 2.3 (Nylander, 2004). The most likely phylogeny for the data set was produced in Garli 2.0 (Zwickl, 2006), using the optimal model of evolution for each gene partition. ML bootstrapping was executed in RAxML v. 7.2.8 (Stamatakis, 2006; Stamatakis et al., 2008). The CIPRES portal allows only one model to be in place in RAxML analyses, though the data set can be partitioned so that parameters for each partition may vary freely. The most complex model for the set of regions was employed, and 1000 bootstrap replicates were completed. The BI analyses were completed in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) on CIPRES, with different (optimal) models allowed for each region. Four independent runs of 5 000 000 generations were completed with four chains each (three heated, one cold), with a chain temp of 0.2 and uniform priors. Trees were sampled every 1000 generations, and the first 25% discarded as burn-in. A majority-rule consensus of the remaining trees was produced using PAUP* and used as the BI tree with posterior probabilities (PP).

Taxonomic and biogeographic hypotheses were evaluated using the Templeton test in PAUP*. We examined support for (1) monophyly of species from major geographic regions based on shared geography and (2) monophyly of subgenera and sections in the current classification (Fraser-Jenkins, 1986) (Table 1). These clades (based on shared geography or classification) were loaded as topological constraints to PAUP*, and the optimal most parsimonious tree for the unconstrained data set was compared to trees obtained with each constraint in place. Significance was evaluated at $P = 0.05$. Monophyly of the subgenus *Pycnopteris* and of several sections (*Remotae* and *Splendentes* in subgenus *Dryopteris*, *Nephrocystis* and *Purpurascentes* in subgenus *Nephrocystis*, and *Politae* in subgenus *Erythrovariae*) could not be assessed because only one representative from each was included in this study.

TABLE 1. Accessions of *Dryopteris* included in this study; their subgenera and sections (Fraser-Jenkins (1986)), and general geographic ranges (as used in biogeographic analyses—see Materials and Methods) are indicated. Europe includes taxa from the Caucasus region; Pacific includes taxa from Hawaii and the Marquesas. See Appendix 1 for voucher information.

Species	Subgenus	Section	Distribution
D. abbreviata	—	—	Europe
D. aemula	*Dryopteris*	*Aemulae*	Europe
D. affinis	—	—	Europe
D. alpestris	*Dryopteris*	*Dryopteris*	Asia
D. antarctica	*Dryopteris*	*Lophodium*	Africa
D. aquilinoides	*Dryopteris*	*Marginatae*	Europe
D. ardechensis	*Dryopteris*	*Dryopteris*	Europe
D. arguta	*Dryopteris*	*Pallidae*	Western North America, Central America
D. assimilis	—	—	Europe
D. athamantica	*Dryopteris*	*Cinnamomeae*	Africa
D. austriaca	—	—	Europe
D. barberigera	*Dryopteris*	*Dryopteris*	Asia
D. bissetiana	*Erythrovariae*	*Variae*	Asia
D. campyloptera	*Dryopteris*	*Lophodium*	Eastern North America
D. carthusiana	*Dryopteris*	*Lophodium*	Asia, Europe, Europe, Eastern North America, Western North America
D. caucasica	*Dryopteris*	*Dryopteris*	Europe
D. celsa	*Dryopteris*	*Pandae*	Eastern North America
D. championii	*Erythrovariae*	*Erythrovariae*	Asia
D. chinensis	*Dryopteris*	*Aemulae*	Asia
D. chrysocoma	*Dryopteris*	*Pandae*	Asia
D. cinnamomea	*Dryopteris*	*Cinnamomeae*	Central America
D. clintoniana	*Dryopteris*	*Pandae*	Eastern North America
D. costalisora	*Dryopteris*	*Pandae*	Asia
D. crassirhizoma	*Dryopteris*	*Fibrillosae*	Asia
D. crispifolia	*Dryopteris*	*Lophodium*	Europe
D. cristata	*Dryopteris*	*Pandae*	Asia, Europe, Eastern North America
D. cycadina	*Dryopteris*	*Hirtipedes*	Asia
D. cystolepidota	*Erythrovariae*	*Erythrovariae*	Asia
D. dickinsii	*Dryopteris*	*Hirtipedes*	Asia
D. dilatata	*Dryopteris*	*Lophodium*	Europe
D. erythrosora	*Erythrovariae*	*Erythrovariae*	Asia
D. expansa	*Dryopteris*	*Lophodium*	Western North America
D. fatuhivensis	*Dryopteris*	*Hirtipedes*	Pacific
D. filix-mas	*Dryopteris*	*Dryopteris*	Asia, Europe, Western North America
D. flaccisquama	—	—	South America
D. formosana	*Erythrovariae*	*Variae*	Asia
D. fragrans	*Dryopteris*	*Dryopteris*	Asia, Eastern North America, Western North America
D. futura	*Nephrocystis*	*Purpurascentes*	Central America
D. goeringianum	*Dryopteris*	*Marginatae*	Asia
D. goldiana	*Dryopteris*	*Dryopteris*	Eastern North America
D. guanchica	*Dryopteris*	*Lophodium*	Africa
D. gymnosora	*Erythrovariae*	*Erythrovariae*	Asia
D. hawaiiensis	*Dryopteris*	*Fibrillosae*	Pacific
D. hendersonii	—	—	Asia
D. hondoensis	*Erythrovariae*	*Erythrovariae*	Asia
D. huberi	*Dryopteris*	*Cinnamomeae*	South America
D. inequalis	*Dryopteris*	*Marginatae*	Africa
D. intermedia	*Dryopteris*	*Lophodium*	Eastern North America
D. juxtaposita	*Dryopteris*	*Pallidae*	Asia
D. karwinskyana	*Dryopteris*	*Cinnamomeae*	Central America
D. knoblochii	—	—	Central America
D. komarovii	—	—	Asia
D. labordei	—	—	Asia
D. lacera	*Dryopteris*	*Pallidae*	Asia
D. ludoviciana	*Dryopteris*	*Pandae*	Eastern North America
D. maderensis	—	—	Europe, Africa
D. marginalis	*Dryopteris*	*Pallidae*	Eastern North America
D. maxonii	*Dryopteris*	*Cinnamomeae*	Central America
D. monticola	—	—	Asia
D. muenchii	*Dryopteris*	*Cinnamomeae*	Central America
D. nubigena	—	—	Central America
D. odontoloma	*Dryopteris*	*Pallidae*	Asia
D. oligodonta	*Dryopteris*	*Marginatae*	Africa
D. oreades	*Dryopteris*	*Dryopteris*	Europe
D. pacifica	*Erythrovariae*	*Variae*	Asia
D. pallida	*Dryopteris*	*Pallidae*	Europe
D. pandae	*Dryopteris*	*Pandae*	Asia
D. patula	*Dryopteris*	*Cinnamomeae*	Central America, South America
D. pentheri	*Dryopteris*	*Marginatae*	Europe
D. polita	*Erythrovariae*	*Politae*	Asia
D. polylepis	*Dryopteris*	*Fibrillosae*	Asia
D. pseudofilix-mas	*Dryopteris*	*Fibrillosae*	Central America
D. pulcherrima	*Dryopteris*	*Fibrillosae*	Asia
D. pycnopteroides	*Dryopteris*	*Hirtipedes*	Asia
D. reflexosquamata	*Dryopteris*	*Splendentes*	Asia
D. remota	*Dryopteris*	*Remotae*	Asia
D. rosea	—	—	Central America
D. rossii	—	—	Central America
D. sacrosancta	*Erythrovariae*	*Variae*	Asia
D. saffordii	*Dryopteris*	*Cinnamomeae*	South America
D. salvinii	—	—	South America
D. scottii	*Dryopteris*	*Hirtipedes*	Asia
D. sieboldii	*Pycnopteris*	no section	Asia
D. simplicior	—	—	Central America
D. sordidipes	*Erythrovariae*	*Variae*	Asia
D. sparsa	*Nephrocystis*	*Nephrocystis*	Asia
D. spinosa	—	—	Asia
D. stenolepis	*Dryopteris*	*Hirtipedes*	Asia
D. stewartii	*Dryopteris*	*Pallidae*	Asia
D. subbipinnata	—	—	Pacific
D. sublacera	*Dryopteris*	*Pallidae*	Asia
D. tokyoensis	*Dryopteris*	*Pandae*	Asia
D. triangularis	—	—	Asia
D. uniformis	*Dryopteris*	*Pallidae*	Asia
D. varia	*Erythrovariae*	*Variae*	Asia
D. wallichiana	*Dryopteris*	*Fibrillosae*	Asia, Africa, Central America, South America, Pacific
D. xanthomelas	—	—	Africa

Notes: Species unplaced in the classification are indicated with a dash.

TABLE 2. Information on cpDNA regions sequenced for this study

Region	Primer source	Aligned bases	With outgroups		No. *Dryopteris* species	Just *Dryopteris*		Indels		
			Variable bases	PIC		Variable bases	PIC*	No.	PIC in *Dryopteris*	PIC in outgroups
rbcL	Korall et al., 2006	1372	430 (31%)	305 (22%)	92	289 (21%)	159 (12%)	54	6 (11%)	0
rbcL-accD	Korall et al., 2007	907	469 (52%)	370 (41%)	93	240 (25%)	148 (15%)	102	25 (25%)	22 (22%)
trnG-trnR	Korall et al., 2007	1288	692 (54%)	552 (43%)	90	326 (25%)	235 (18%)	182	37 (20%)	77 (42%)
psbA-trnH	Kress et al., 2005	563	262 (47%)	199 (35%)	92	110 (20%)	55 (10%)	54	9 (17%)	16 (30%)
trnP-petG	Small et al., 2005	624	397 (64%)	338 (54%)	94	226 (36%)	171 (27%)	111	29 (26%)	32 (29%)
rps4-trnS	Rouhan et al., 2004	593	369 (62%)	301 (51%)	92	175 (30%)	123 (21%)	96	10 (10%)	38 (40%)
trnL-F	Taberlet et al., 1991	325	202 (62%)	175 (54%)	94	96 (30%)	69 (21%)	55	8 (15%)	15 (27%)

Notes: PIC = parsimony informative characters

***Divergence time estimates*—**Divergence times were estimated using a Bayesian method (Drummond et al., 2006) implemented in the program BEAST 1.5.4 (Drummond and Rambaut, 2007). This method simultaneously estimates phylogeny and molecular rates using a Markov chain Monte Carlo (MCMC) strategy. The data set was partitioned by plastid region, and the optimal model for each region was specified. We implemented a Yule process speciation prior and an uncorrelated lognormal (UCLN) model of rate change, with clock models unlinked between partitions. Analyses were run for 50 000 000 generations, with parameters sampled every 1000 generations. Tracer v1.4 (Rambaut and Drummond, 2007) was used to examine the posterior distribution of all parameters and their associated statistics, including estimated sample sizes (ESS) and 95% highest posterior density (HPD) intervals. The program TreeAnnotator v1.5.4 (Drummond and Rambaut, 2007) was used to summarize the set of post burn-in trees and their parameters, to produce a maximum clade credibility (MCC) chronogram showing mean divergence time estimates with 95% HPD intervals.

We employed secondarily derived age estimates to calibrate our divergence time analyses. The uncertainty regarding the familial placement of four fossil genera originally placed in Dryopteridaceae, and of early "*Dryopteris*" fossils from North America (see Brown, 1962; Wolfe, 1977; Collinson, 2001; Pigg and Rothwell, 2001; Serbet and Rothwell, 2006; Vera, 2010) preclude these from being used to calibrate any phylogeny. We excluded the oldest fossil *Dryopteris*, from 66–55 Ma in China (Wang et al., 2006), to test the outcome of our secondarily derived age estimates for the origin of the genus; we also excluded more recent *Dryopteris* fossils from Russia (Akhmetiev, 2009), Alaska (Wolfe et al., 1966; Wolfe and Tanai, 1980; Reinink-Smith and Leopold, 2005), and Iceland (Grímsson and Denk, 2007) as checks on calculated dates of biogeographic spread. We note that none of the recent calibrations of broad-scale fern phylogenies (Schneider et al., 2004; Schuettpelz and Pryer, 2009) use any dryopteridaceous fossils for calibration. Although there is uncertainty inherent in relying on secondary calibrations, this approach has produced results congruent to fossil-based studies in several plant groups (Hennequin et al., 2010; Givnish et al., 2011).

Three calibration points were used, based on two previous studies of diversification in leptosporangiates (Schneider et al., 2004; Schuettpelz and Pryer, 2009). We used two schemes for modeling the distributions of these priors, to explore the effects on the results of using uniform vs. lognormal and normal priors. For the first analysis, we modeled the calibration points as uniform priors corresponding to the ranges of dates provided by previous authors; in two cases (calibration points A and B) this was the mean ± 1 SD (Schneider et al., 2004), and in the third (calibration point C) it was the range corresponding to 25–75% of the previously published age estimate (Schuettpelz and Pryer, 2009). These particular points were chosen because information was available about the range of ages estimated for each (e.g., mean ± SD or 25–75% age interval), rather than a single point estimate. For the second analysis, we used the same calibration points but modeled A and B as normal distributions with mean and SD equal to that given by Schneider et al. (2004), and C as a lognormal distribution with mean = 2.0, SD = 1.0, and offset = 81.8, which gave approximately the 25–75% age interval of Schuettpelz and Pryer (2009).

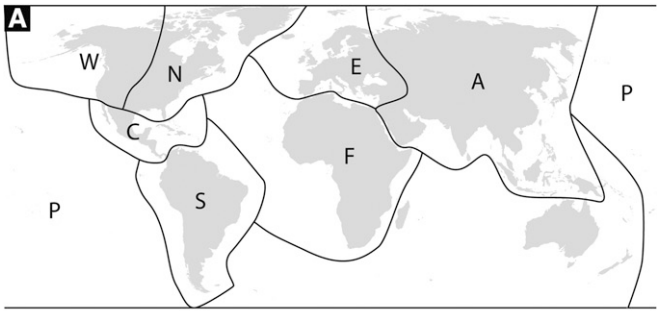
***Ancestral area reconstructions*—**Four contrasting methods were used for ancestral area reconstruction (AAR): MP using Mesquite 2.7.2 (Maddison and Maddison, 2009), BI using the BayesMultiState module in BayesTraits (Pagel and Meade, 2007), statistical dispersal–vicariance analysis in S-DIVA (Yu et al., 2010), and a model-based, parametric ML method (Ree et al., 2005; Ree and Smith, 2008) implemented in LaGrange (snapshot.20110117; Ree and Smith, 2007). Mesquite and BayesTraits can be used to reconstruct numerous types of trait data, while S-DIVA and LaGrange are designed explicitly for AAR.

Geographic distributions were coded as unordered character states corresponding to broad regions: Asia, Africa, Europe, Pacific (including taxa from Hawaii and the Marquesas), eastern North America (ENA), Western North America (WNA), Central America (CA), and South America (SA) (Fig. 1). Each species was coded according to its location of collection, and taxa known to have broad ranges were coded with multiple states (see Table 1). Outgroups were likewise coded according to their location of collection and additional regions where they are known to occur. Analyses were conducted with and without the outgroups coded to assess the effects on AAR for the stem of *Dryopteris*.

The MP analyses were conducted in Mesquite, with characters mapped onto the MCC chronogram from the divergence time analysis using the parsimony criterion. Several taxa occur in multiple locations, but this method cannot accommodate polymorphism, and so the single location of each sequenced collection was coded for each species in the MP analysis.

BayesTraits and S-DIVA allow multiple trees to be examined to incorporate phylogenetic uncertainty, and a random sample of 1000 trees from the post burn-in BI set of trees was input into these analyses. In S-DIVA, vicariance between several nonadjacent regions was excluded if these regions have not been in contact during the time in which *Dryopteris* is inferred to have been extant: between CA and Asia, Europe, and Africa; between SA and Asia, Europe, and Africa; between Africa and ENA and WNA; and between the Pacific islands and all other regions. We explored the effects of restricting the number of areas allowed in ancestral reconstructions by using the maxareas option with two, four, and all possible areas allowed. To reduce some of the uncertainty and arbitrariness of choosing priors under the Bayesian MCMC in BayesTraits, we used the hyperprior approach (the rjhp command) as recommended by the program's authors (Pagel et al., 2004; Pagel and Meade 2007). Combinations of hyperprior (exponential or gamma, mean and variance) and rate parameter values were explored to find acceptance rates between 20 and 40% (as recommended by Pagel and Meade, 2007). Subsequent analyses used the reversible-jump hyperprior command with a gamma distribution whose mean and variance were both seeded from a uniform distribution on the interval 0 to 10 (rjhp gamma 0 10 0 10). A rate parameter of 2 was employed, and analyses run for 10 000 000 million iterations with a burn-in of 1 000 000.

In contrast to the other AAR methods used, LaGrange (Ree and Smith, 2007) allows an explicit model of dispersal through time, depending on which routes were available during different historical intervals (Ree et al., 2005; Ree and Smith, 2008). In these stratified models, the phylogeny is divided into time slices, each of which has a separate Q matrix of dispersal rates between regions, with the rates ranging from zero to 1.0 and dependent on the extent to which regions are thought to have been connected in a given time interval (Ree and Sanmartín, 2009; Buerki et al., 2010). We performed analyses with two different dispersal-extinction-cladogenesis (DEC) models. In the first (DEC-vic), we emphasized paleogeographic history and constructed a DEC model similar to those used in studies of angiosperms, where vicariance is thought to play an important role and movement is limited by distance and geography. We divided the phylogeny into four time slices and assigned a *Q* matrix to each, the rates of which were based on the extent to which areas were connected geographically during each period (e.g., due to plate tectonics, land bridges) (Fig. 1). Time slices and rates were chosen based on a survey of the literature relevant to global geologic and geographic



B

	A	E	N	W	C	S	F	P
A	1	1	0.1	0.1	0.1	0.1	1	1
E	1	1	0.1	0.1	0.1	0.1	1	1
N	0.1	0.1	1	1	1	1	0.1	1
W	0.1	0.1	1	1	1	1	0.1	1
C	0.1	0.1	1	1	1	1	0.1	1
S	0.1	0.1	1	1	1	1	0.1	1
F	1	1	0.1	0.1	0.1	0.1	1	1
P	1	1	1	1	1	1	1	1

C

	A	E	N	W	C	S	F	P
A	1	1	1	1	0.1	0.1	1	0.0
E	1	1	0.5	0.5	0.1	0.1	1	0.0
N	1	0.5	1	1	1	1	0.1	0.0
W	1	0.5	1	1	1	1	0.1	0.0
C	0.1	0.1	1	1	1	1	0.1	0.0
S	0.1	0.1	1	1	1	1	0.1	0.0
F	1	1	0.1	0.1	0.1	0.1	1	0.0
P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1

D

	A	E	N	W	C	S	F	P
A	1	1	1	1	0.1	0.1	0.75	0.0
E	1	1	1	1	0.1	0.1	0.75	0.0
N	1	1	1	1	1	0.5	0.1	0.0
W	1	1	1	1	1	0.5	0.1	0.0
C	0.1	0.1	1	1	1	0.5	0.1	0.0
S	0.1	0.1	0.5	0.5	0.5	1	0.1	0.0
F	0.75	0.75	0.1	0.1	0.1	0.1	1	0.0
P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1

E

	A	E	N	W	C	S	F	P
A	1	1	1	1	0.1	0.1	0.1	0.0
E	1	1	1	1	0.1	0.1	0.1	0.0
N	1	1	1	0.1	1	1	0.1	0.0
W	1	1	0.1	1	1	1	0.1	0.0
C	0.1	0.1	1	1	1	0.1	0.1	0.0
S	0.1	0.1	1	1	0.1	1	0.75	0.0
F	0.1	0.1	0.1	0.1	0.1	0.75	1	0.0
P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1

Fig. 1. (A) Map showing biogeographic regions used in this study. A, Asia; E, Europe; N, Eastern North America; W, Western North America; C, Central America; S, South America; F, Africa; P, Pacific. (B–E) Q matrices corresponding to the four time slices used in the LaGrange-str analysis. Transition rates reflect degree of connectivity of geographic regions during that time period. (B) 5 Ma–present. (C) 30–5 Ma. (D) 60–30 Ma. (E) Before 60 Ma.

changes over the past 150 Myr (McKenna, 1975; Tiffney, 1985; Tiffney and Manchester, 2001; Morley, 2003; Donoghue and Smith, 2004; Sanmartin and Ronquist, 2004; Smith, 2009; Denk et al., 2010), and on previous studies of plant distributions which used LaGrange or its predecessor, AReA, for similar time periods and regions (Moore and Donoghue, 2007; Clayton et al., 2009; Buerki et al., 2010). In the second analysis (DEC-dis), we allowed unlimited dispersal between all areas for all time periods, with the exception of the Pacific islands, which are volcanic and recent in origin; dispersal to and from this region was therefore set to zero prior to 5 Ma, the approximate age of formation of extant islands (Buerki et al., 2010). We used the MCC chronogram from the divergence time analysis, and restricted the number of areas allowed in ancestral reconstructions by performing analyses with max-areas of two and four.

RESULTS

Phylogenetic analyses and hypothesis testing—The combined plastid data set consisted of 5699 aligned nucleotides, of which 2819 (50%) were variable and 2239 (39%) were parsimony-informative. Indels added an additional 657 characters, of which 328 (50%) were parsimony-informative. There were no unalignable regions, and the number of informative nucleotides varied between regions from 22% (*rbcL*) to 54% (*trnP-petG* and *trnL-F*) (Table 2). Pairwise ILD tests indicated marginally significant conflict between several pairs of regions ($P = 0.04$ for *rbcL-accD* vs. *trnG-trnR*, and *rps4-trnS* vs. *psbA-trnH*; $P = 0.03$ for *rbcL-accD* vs. *rbcL*). We suspected that this conflict might have been caused by homoplasy among the outgroup taxa. As expected, constraining the positions of the outgroups a priori and rerunning the ILD tests produced non-significant results. All subsequent analyses were able to recover the correct topology for the outgroups with no constraint employed.

The MP analysis of the plastid data set (without indels) identified 911 most-parsimonious trees of length 7564 steps. These shortest trees had a consistency index (CI) of 0.56, and CI′ (excluding autapomorphies) of 0.50. The MP bootstrap (BS) analysis resulted in a strict consensus tree that was highly resolved (101 out of 114 nodes). Inclusion of indels in the MP and BS analyses did not significantly alter topology, resolution, or clade support. These data were not included in subsequent ML and BI analyses because CIPRES does not provide a way to model them; however, the MP results indicate that additional informative characters provided by the indel data likely would not have led to additional resolution or increased support values.

The topology and support values produced by the MP analyses were highly congruent with the results of the ML and BI analyses (Fig. 2). Relative to MP, ML and BI provided increased resolution in some small clades at the tips of the tree, but there were no major topological differences between the analyses, and in general support values were similar. MrModelTest identified the following models of evolution as optimal for the plastid regions included here: HKY+Γ for *psbA-trnH*, *trnL-F*, and *rps4-trnS*; HKY+I+Γ for *rbcL-accD* and *trnP-petG*; and GTR+I+Γ for *rbcL* and *trnG-trnR*. ML analysis in Garli yielded a single best tree with -ln 45767.2102 (Fig. 2). In all analyses, *Dryopteris fragrans* was resolved as sister to the rest of the genus with very strong support (MP-BS/ML-BS/BI-PP = 93/98/1.0). The remaining *Dryopteris* fell into five moderately to well-supported major clades (labeled clades I–V), and relationships within each were generally resolved, though not necessarily with strong support. For clade I in particular, the placement of several taxa, including two New World species, differed between the best ML tree and the majority-rule consensus trees from MP, ML, and BI analyses, resulting in several nodes with low or zero support (labeled # in Fig. 2). The consensus topologies from the three analyses were identical to each other for this clade, and to the chronogram from the divergence time analysis; this chronogram (Fig. 3) thus reflects the majority-rule consensuses of the MP, ML, and BI analyses for clade I.

Templeton tests indicated a lack of support in the dataset for any of the hypotheses tested (Table 3). A phylogenetic basis for the current classification of *Dryopteris*, either at the subgenus or section level, was rejected ($P = 0.0001$ in all cases), and monophyly of taxa from any geographic region based only on shared geography was also rejected, for each region tested ($P = 0.0001$ in all cases). New World *Dryopteris* as a whole are also not monophyletic. Species from ENA and WNA fall into three of the five major clades (I, II, and III), plus the circumboreal *D. fragrans*, which is sister to the rest of the genus. Most of the CA and SA *Dryopteris* taxa form a monophyletic clade (IV), though several additional species are closely related to Asian, European, or Pacific taxa (Figs. 4, 5).

Divergence time estimations— After 50 million generations, all ESS values for the divergence time analyses (as viewed in Tracer) were well above the recommended threshold of 200, indicating that parameter space had been sufficiently sampled (Drummond and Rambaut, 2007). The coefficients of variation indicated that the data were not evolving in a clock-like fashion (values above 0.5), and the UCLN model was thus the most appropriate model of rate variation for this data set. The age estimates from our two analyses, which incorporated different prior distributions for the fossils, were nearly identical, differing on average by less than 1 Myr, and our divergence-time estimates agree well with previous estimates for the uncalibrated nodes in the outgroups We show only the results from the first analysis, with uniform priors, since the results of the two analyses were not significantly differently from one other.

We infer that the ancestors of *Dryopteris* and *Arachniodes* diverged approximately 63 Ma (Fig. 3). Cladogenetic events began within *Dryopteris* ca. 42 Ma, with a divergence between the ancestors of modern *D. fragrans* and the rest of the genus. Diversification within the major clades identified in this study (I–V) began between 25.5 and 14.6 Ma (Fig. 3). Most of the CA and SA taxa fall into one clade (IV), which underwent diversification throughout the Neogene and into the Quaternary (21.1–0.5 Ma), while several additional Latin American species are very recent in origin. The North American species are decidedly nonmonophyletic, and all except *D. fragrans* are less than 10 million years old.

Ancestral area reconstructions— Analyses were conducted both with and without outgroups, and inclusion of outgroup coding did not affect reconstructions for the stem of *Dryopteris* or within the genus. Since our goal was to focus on ancestral areas within *Dryopteris*, not at deep nodes leading to the outgroups, those branches are collapsed in Figs. 4 and 5, and AARs for the outgroup genera are not reported. Reconstructions of ancestral distributions of *Dryopteris* by MP, ML, BI, and S-DIVA generally agree with each other, except in cases where vicariance or dispersal are clearly implicated. The MP and BI reconstructions are ambiguous in such places, while S-DIVA and LaGrange provide specific AAR scenarios. Given that the MP and BI methods employed are applicable to nearly any type of trait data and that S-DIVA and LaGrange gave better-resolved and more plausible results in many instances of ambiguity, we will focus mainly on the results obtained with these latter, explicitly geography-based methods. For most nodes, S-DIVA reconstructed either a single ancestral area (e.g., Asia), or one to several vicariance scenarios (e.g., Asia-Europe or Asia-Europe-ENA). Movement between two separate regions generally occurs by way of vicariance between the two regions at an intervening node. There were only four nodes at which S-DIVA produced no reconstruction, indicating a long-distance dispersal event in one of the daughter lineages with no vicariance between the ancestral and daughter ranges (indicated with an asterisk in Fig. 4). Analyses performed with a maximum of two, four, and all possible areas allowed produced different results only at nodes where many possible vicariance scenarios were suggested. Figure 4 shows the results from max 4 areas at these nodes, which agreed with the results when all areas were allowed.

In the LaGrange analyses, the global log likelihood was higher under the DEC-dis model than under DEC-vic ($-\ln L$ $297.4 > 308.1$), though the analyses produced the same AAR for all but 12 nodes that are ancestral to New World *Dryopteris*. At several nodes where the models differ, DEC-vic describes a wider ancestral range, including more regions and with movement between distant areas occurring by way of vicariance through these widespread ranges at intervening nodes (similar to S-DIVA results), while DEC-dis constructed smaller ancestral ranges and implied direct dispersal between distant regions with no intervening occupation of the intermediate geographic areas. For nodes with more than one possible AAR in the LaGrange analyses, the reconstruction with the highest likelihood is given in Figs. 5 and 6, with the relative probability of that optimal reconstruction indicated. When these probabilities are low, it is generally because many ranges with various combinations of the same regions were suggested. However, the reconstruction shown always differed by at least $-\ln 1.0$ from the next-most-likely scenario. There were no significant differences between the analyses when different maximum area constraints were employed, except in computing power required to execute the analyses. With two areas allowed, there were 39 possible ancestral ranges; with four areas allowed, there were 157 possible ancestral ranges, which required significantly more computing time and power to process.

We identified 13 lineages that include New World *Dryopteris*, and our results implicate long-dispersal and vicariance for six and three of them, respectively (Table 4). AARs at another four nodes that are immediately ancestral to New World species remain ambiguous, with the different analytical approaches favoring different scenarios. Long-distance dispersal occurs at nodes 1-4 and 6-7, from Asia, Europe, and Africa to CA, SA, and ENA. Vicariance occurs at nodes 8-10, between Asia,

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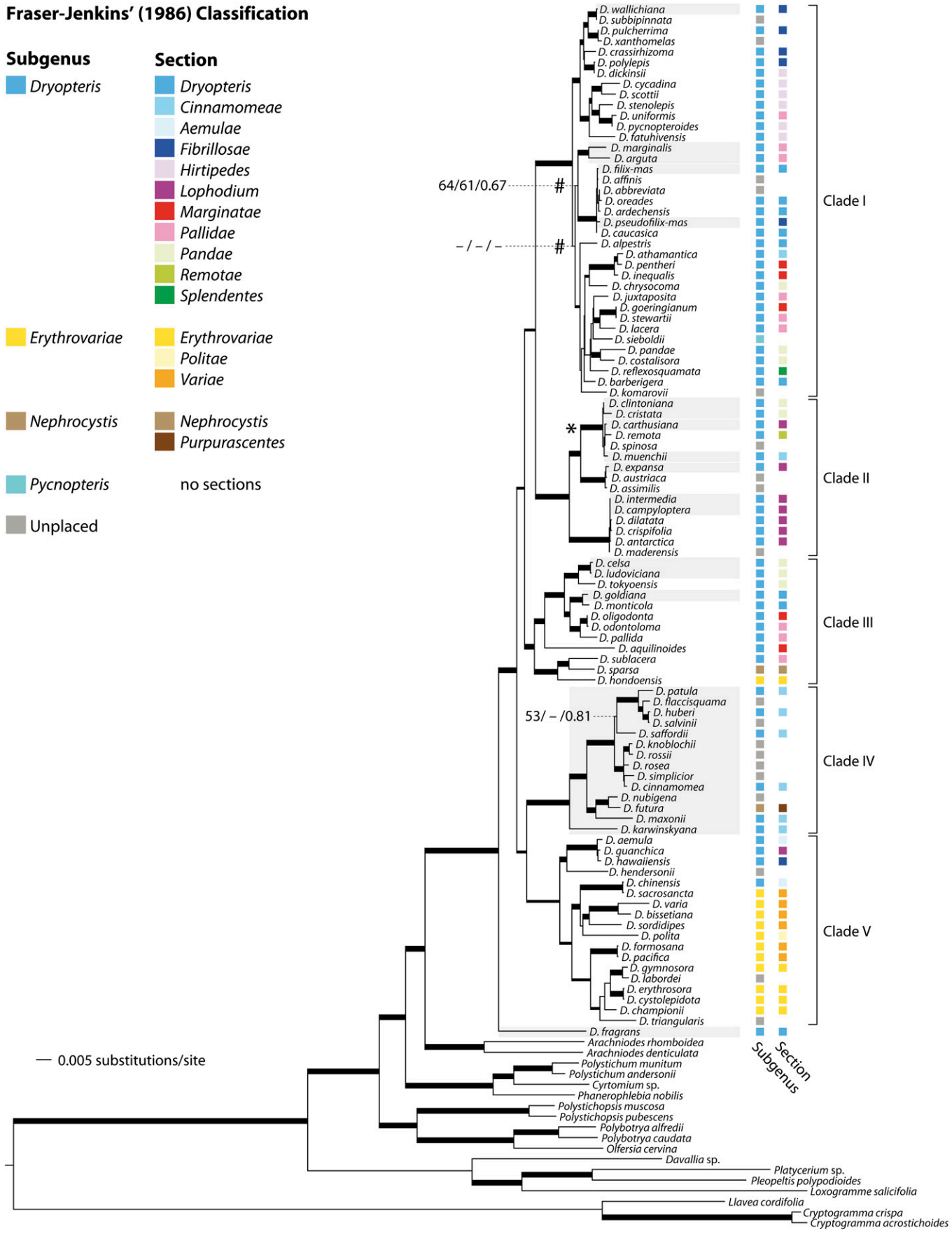
Fig. 2. Best maximum likelihood (ML) topology for all *Dryopteris* and outgroups included in this study ($-\ln = 45\,767.2102$). Thickest lines indicate strong support (MP BS ≥ 70%, ML BS ≥ 70% and BI PP ≥ 95%), medium lines indicate moderate support (either ML BS ≥ 70% or BI PP ≥ 95%), and thin lines indicate weak support (ML BS ≤ 70% and BI PP ≤ 95%). Support values are given as MP BS/ML BS/ BI PP. Weakly supported nodes are annotated only if they are ancestral to clades containing New World *Dryopteris* taxa. Gray boxes indicate the 30 New World taxa. Five major clades resolved in this study are indicated (note that *D. fragrans* is external to these clades). Colored boxes denote placement of taxa into subgenera and sections based on the most recent classification of *Dryopteris* (Fraser-Jenkins, 1986). Taxa unplaced in that system are indicated with gray boxes in the subgenus column. Two nodes with low or zero support in the MP, ML, and BI majority rule consensus trees are indicated with # (see text for discussion). An asterisk (*) indicates the small clade which contains putative descendants of "*D. semicristata*".


Fraser-Jenkins' (1986) Classification
Subgenus
Dryopteris
Erythrovariae
Nephrocystis
Pycnopteris
Unplaced
Section
Dryopteris
Cinnamomeae
Aemulae
Fibrillosae
Hirtipedes
Lophodium
Marginatae
Pallidae
Pandae
Remotae
Splendentes
Erythrovariae
Politae
Variae
Nephrocystis
Purpurascentes
no sections
64/61/0.67
– / – / –
53/ – /0.81
Clade I
Clade II
Clade III
Clade IV
Clade V
Section
Subgenus
0.005 substitutions/site

Europe, and North America, and nodes 5 and 11-13 are ambiguous (Table 4; Figs. 4–6). Figure 7 summarizes movements to the New World.

DISCUSSION

Phylogeny—Our analyses, which are based on a more extensive sampling of taxa and loci than any previous study of *Dryopteris*, provide the best resolution of relationships within the genus to date and demonstrate the complex evolutionary history of *Dryopteris* in the New World for the first time. Our phylogenetic results largely agree with those from previous studies and have important implications for reticulate evolution and historical biogeography of the New World taxa, which are highly polyphyletic with regard to region of origin. Five major clades were resolved with strong support (MP-BS ≥ 90, ML-BS ≥ 95, and BI-PP ≥ 0.84 for all), and New World species belong to four of them (I-IV; Figs. 4–6). At a finer scale, we define a total of 13 lineages that contain New World *Dryopteris* species; these arose between ≤1 and 42 Ma (Figs. 4–6). New World species or clades are sister to Asian taxa at eight of these nodes; European taxa at three; and African and Pacific taxa each at one (Table 4). Multiple vicariance and transoceanic dispersal events are responsible for these disjunctions, and our results indicate that both phenomena have been important in shaping the relationships and biogeographic history of *Dryopteris*.

Two previous molecular studies of *Dryopteris* were based on either one (*rps4-trnS*; Li and Lu, 2006b) or two (*rbcL*, *trnL-F*; Geiger and Ranker, 2005) plastid loci. Our analyses, based on seven plastid loci, provide greater resolution throughout much of the phylogeny. Clades I–V are moderately (ML BS ≥ 70% or BI PP ≥ 95%) to strongly (MP BS ≥ 70%, ML BS ≥ 70% *and* BI PP ≥ 95%) supported in our analyses (Fig. 2), agreeing broadly with the results of the two previous studies. Li and Lu (2006b) identified six major clades in their study of 60 Chinese *Dryopteris*, which included 20 species in common with ours. Their clade I is largely congruent with our clade I, and their clades III-IV-V together are monophyletic and correspond to our clade V. They were, however, unable to resolve relationships between these groups and the two other clades they identified. Geiger and Ranker's (2005) study of the Hawaiian *Dryopteris* taxa had 39 species in common with ours, almost all of which fell into monophyletic groups corresponding to our clades I, II, III, and V, with only *D. patula* as a representative of our clade IV. Their results do differ from ours in the placement of *D. fragrans*, putting it in a poorly supported clade corresponding to our clade V, while we placed it sister to the rest of the genus with 100% bootstrap and posterior probability support.

Given the tendency toward hybridization and polyploidy in *Dryopteris* (Manton, 1950), future studies will need to incorporate nuclear genomic data to identify such events and the extent to which they have influenced *Dryopteris*' evolutionary history. Plastids are maternally inherited in ferns (Gastony and Yatskievych, 1992; Vogel et al., 1998). Therefore, the current phylogeny will permit identification of one parent of hybrids or polyploids, but must be complemented in the next set of analyses with biparentally inherited nuclear DNA markers to identify paternal progenitors. Particular targets in the New World should include the reticulate complex in North America and the members of our clade IV in CA and SA (Montgomery and Wagner, 1993; Mickel and Smith, 2004). The North American group has been studied extensively (Walker, 1959, 1961; Petersen and Fairbrothers, 1983; Werth, 1991; Hutton and Stein, 1992; Stein et al., 2010), though not yet using the approach of contrasting plastid and nuclear DNA sequences. Morphological, cytological, genetic, and chemical research over the past century culminated in an explanation of the group's reticulate evolutionary history, which is one of the classics of such hypotheses in plant biology (Montgomery and Wagner, 1993). This hypothesis involves eight extant diploid species, four sexual allopolyploids, and a hypothetical diploid, "*D. semicristata*," whose existence was first postulated by Walker (1955) to explain the origin of two of the allotetraploids. Our plastid data provide the first partial support for this reticulation hypothesis based on DNA sequence data, as each of the polyploids is strongly supported as sister to one of its putative parents, with the supposed offspring of "*D. semicristata*" (Table 5) falling in a strongly supported clade (labeled with an asterisk in Fig. 2). In Central America, Mickel and Smith (2004) described the "*D. patula* complex", including *D. patula* and several other CA species that fall into our clade IV (*D. cinnamomea*, *D. rossii*, *D. rosea*, *D. simplicior*). Our results support a close relationship among most members of this group, with the addition of *D. knoblochii* but, ironically, the exclusion of *D. patula*, for which the complex was named (Fig. 2). In contrast, a recent morphometric analysis (Hernández-Hernández et al., 2009) found that *D. patula* was closely related to the four species mentioned above (this study did not include *D. knoblochii*). This incongruence between morphological and molecular results strongly suggests the possibly of hybridization and/or reticulate evolution within clade IV. Nuclear genomic data will be essential for testing these hypotheses about the North and Central-South American groups, and we are currently assembling such data.

Though we exhaustively sampled New World *Dryopteris* in the current study, there are still many taxa from other regions of the world, particularly Asia, which were not included here. Further approaches toward a worldwide phylogeny must focus on increasing sampling of Asian, European, and African taxa. Future studies should also attempt to resolve the positions of several small genera that are closely related to *Dryopteris* and at times have been suggested to render it paraphyletic (though with low support), including *Acrorumohra* and *Nothoperanema* (Geiger and Ranker, 2005; Li and Lu, 2006a, b).

Classification—Our results suggest that the current worldwide classification of *Dryopteris* (Fraser-Jenkins, 1986), based

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Fig. 3. Maximum clade credibility (MCC) chronogram from BEAST analysis showing mean divergence time estimates with 95% highest posterior density (HPD) intervals (blue bars). Clades I–V are as in Fig. 2. Mean age plus 95% HPD estimates are given for *Dryopteris*, the crown group of *Dryopteris* (minus *D. fragrans*), and clades I–V. Black circles indicate calibration points A–C, which were secondary estimates modeled as uniform priors with the following distributions: A, 148.56 ± 9.44 Ma; B: 49.57 ± 4.84 Ma; C: 93.8–83.9 Ma. A and B were derived from Schneider et al. (2004) and correspond to the mean ± 1 SD of their estimated dates for those nodes; C was derived from Schuettpelz and Pryer (2009) and corresponds to 25–75% of their estimated age range for that node. Tan boxes correspond to time slices in the DEC-vic analysis.

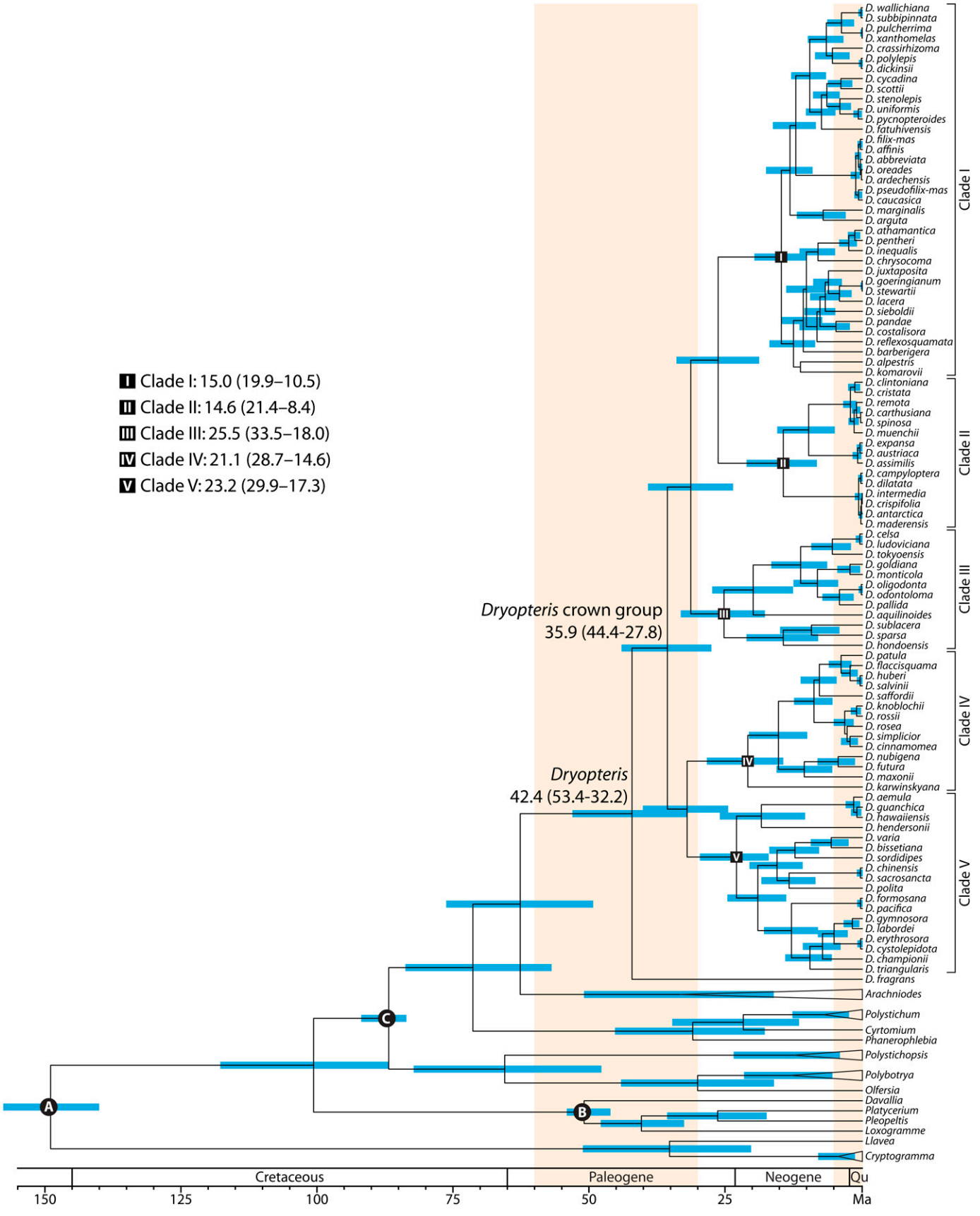
I Clade I: 15.0 (19.9–10.5)
II Clade II: 14.6 (21.4–8.4)
III Clade III: 25.5 (33.5–18.0)
IV Clade IV: 21.1 (28.7–14.6)
V Clade V: 23.2 (29.9–17.3)
Dryopteris crown group
35.9 (44.4-27.8)
Dryopteris
42.4 (53.4-32.2)
A
B
C
D. wallichiana
D. subbipinnata
D. pulcherrima
D. xanthomelas
D. crassirhizoma
D. polylepis
D. dickinsii
D. cycadina
D. scottii
D. stenolepis
D. uniformis
D. pycnopteroides
D. fatuhivensis
D. filix-mas
D. affinis
D. abbreviata
D. oreades
D. ardechensis
D. pseudofilix-mas
D. caucasica
D. marginalis
D. arguta
D. athamantica
D. pentheri
D. inequalis
D. chrysocoma
D. juxtaposita
D. goeringianum
D. stewartii
D. lacera
D. sieboldii
D. pandae
D. costalisora
D. reflexosquamata
D. barberigera
D. alpestris
D. komarovii
D. clintoniana
D. cristata
D. remota
D. carthusiana
D. spinosa
D. muenchii
D. expansa
D. austriaca
D. assimilis
D. campyloptera
D. dilatata
D. intermedia
D. crispifolia
D. antarctica
D. maderensis
D. celsa
D. ludoviciana
D. tokyoensis
D. goldiana
D. monticola
D. oligodonta
D. odontoloma
D. pallida
D. aquilinoides
D. sublacera
D. sparsa
D. hondoensis
D. patula
D. flaccisquama
D. huberi
D. salvinii
D. saffordii
D. knoblochii
D. rossii
D. rosea
D. simplicior
D. cinnamomea
D. nubigena
D. futura
D. maxonii
D. karwinskyana
D. aemula
D. guanchica
D. hawaiiensis
D. hendersonii
D. varia
D. bissetiana
D. sordidipes
D. chinensis
D. sacrosancta
D. polita
D. formosana
D. pacifica
D. gymnosora
D. labordei
D. erythrosora
D. cystolepidota
D. championii
D. triangularis
D. fragrans
Arachniodes
Polystichum
Cyrtomium
Phanerophlebia
Polystichopsis
Polybotrya
Olfersia
Davallia
Platycerium
Pleopeltis
Loxogramme
Llavea
Cryptogramma
Clade I
Clade II
Clade III
Clade IV
Clade V
Cretaceous
Paleogene
Neogene
Qu
150
125
100
75
50
25
Ma

TABLE 3. Results of hypothesis testing for monophyly based on classification system (Fraser-Jenkins, 1986) and geography.

Hypothesis	*P*	Tree length (steps)
Best, unconstrained tree		7564
Monophyletic subgenus *Dryopteris*	0.0001**	8334
Monophlyetic section *Aemulae*	0.0001**	7682
Monophlyetic section *Cinnamomeae*	0.0001**	7934
Monophlyetic section *Dryopteris*	0.0001**	7727
Monophlyetic section *Fibrillosae*	0.0001**	7805
Monophlyetic section *Hirtipides*	0.0001**	7620
Monophlyetic section *Lophodium*	0.0001**	7870
Monophlyetic section *Marginatae*	0.0001**	7772
Monophlyetic section *Pallidae*	0.0001**	7843
Monophlyetic section *Pandae*	0.0001**	7772
Monophyletic subgenus *Erythrovariae*	0.0001**	7803
Monophlyetic section *Erythrovariae*	0.0001**	7731
Monophlyetic section *Variae*	0.0001**	7659
Monophyletic subgenus *Nephrocystis*	0.0001**	7713
Monophyletic African taxa	0.0001**	8001
Monophyletic Asian taxa	0.0001**	7996
Monophyletic Pacific taxa	0.0001**	7740
Monophyletic E. European taxa	0.0001**	7792
Monophyletic W. European taxa	0.0001**	8005
Monophyletic Central American taxa	0.0001**	7945
Monophyletic South American taxa	0.0001**	7773
Monophyletic E. North American taxa	0.0001**	7884
Monophyletic W. North American taxa	0.0001**	7802

Notes: P-values from Templeton tests are given; ** highly significant values against monophyly ($P = 0.05$). Subgenus *Pycnopteris*, sections *Nephrocystis* and *Purpurascentes* in subgenus *Nephrocystis*, section *Politae* in subgenus *Erythrovariae*, and sections *Splendentes* and *Remotae* in subgenus *Dryopteris*, were each represented in this study by only one accession, and their monophyly therefore could not be tested.

solely on morphological traits, requires extensive revision to accurately reflect phylogenetic relationships. The current system divides the genus into the subgenera *Dryopteris*, *Erythrovariae*, and *Nephrocystis*, which contain respectively eleven, three, and two sections, and subgenus *Pycnopteris*, which includes only four species. Subgenus *Dryopteris* is by far the largest of the subgenera, including 141 of the species in the treatment, followed by *Erythrovariae* with 36, *Nephrocystis* with 27, and then *Pycnopteris* (Fraser-Jenkins, 1986). Templeton tests rejected the monophyly of all 14 subgenera and sections for which our sampling permitted such tests (Table 3). However, at the subgeneric level the two groups for which we had adequate sampling did seem to approach monophyly; these included subgenus *Dryopteris*, which corresponds largely to our clades I-II-III (though this grouping has only moderate support: MP-BS/ML-BS/BI-PP = 78/70/0.84), and subgenus *Erythrovariae*, which made up most of the strongly supported clade V (100/98/0.84) (Fig. 2). Li and Lu (2006b) also evaluated Fraser-Jenkins' (1986) classification in their phylogenetic study of 60 Chinese *Dryopteris* (discussed above). They too found support for subgenus *Dryopteris*, but not for *Erythrovariae*, for which their sampling was much better than ours.

Our clade IV contains additional members of subgenus *Dryopteris*, but is more closely related to clade V/subgenus *Erythrovariae* than to clades I-II-III (Fig. 2). Most of these taxa are in section *Cinnamomeae* within subgenus *Dryopteris*, or are considered *incertae sedis*, and all occur in CA or SA. Based on our results, circumscription of clade IV as a new subgenus may be merited. Our results also suggest that *D. fragrans*, which was placed in subgenus *Dryopteris* by Fraser-Jenkins (1986) but is strongly supported as sister to the rest of the genus here (93/98/1.0), should be recircumscribed as the sole species in an additional, monotypic subgenus. Subgenera *Nephrocystis* and *Pycnopteris* were poorly represented in our sampling (Table 1, Fig. 2), but the species included were embedded in our phylogeny, and in Li and Lu's study (2006b), among members of the other subgenera, indicating that these subgenera may need to be recircumscribed.

More thorough taxon sampling and inclusion of nuclear DNA sequence data will be needed before a reclassification of *Dryopteris* can be undertaken. However, based on the congruence between our molecular results and those of Geiger and Ranker (2005) and Li and Lu (2006b), it seems likely that an extensive revision of the infrageneric classification of *Dryopteris* is needed.

***Divergence times and historical biogeography of New World* Dryopteris—** On the basis of our divergence time analyses, we infer that the ancestors of *Dryopteris* and *Arachniodes* diverged ca. 63 Ma, and that the *Dryopteris* lineage at that point was confined to Asia (Fig. 3). Wang et al. (2006) dated a putative *Dryopteris* fossil from northeastern China to this period, the upper Paleocene (ca. 65–55 Ma), and several *Dryopteris* fossils dating to the middle Eocene (ca. 48–37 Ma) have been reported from northeastern Russia (Ahkmetiev, 2009), supporting the presence of early ancestors of the genus in this region at that time. An extinct species, *Dryopteris alaskana* from Alaska, has been dated to the late middle Eocene or early Oligocene (ca. 40–30 Ma) in Alaska (Wolfe, 1977), indicating that the genus may have extended somewhat into extreme northwestern North America at this point. There are also reports of *Dryopteris* fossils from western-central North America from the Paleocene, which would have been an earlier and more significant incursion; however, the placement of these fossil taxa in *Dryopteris* is dubious. Brown (1962, p. 42) examined several specimens of "*Dryopteris*" from the Fort Union Formation of the western United States and noted that they were either sterile or contained "misleading features", and about one he wrote, "No comparison with a living species is sugested [sic], and the reference to *Dryopteris* is entirely nominal." Collinson (2001) later called for a complete revision of all *Dryopteris* from this period in North America, specifically including those fossils described by Brown (1962) and Wolfe (1977). We therefore lack any

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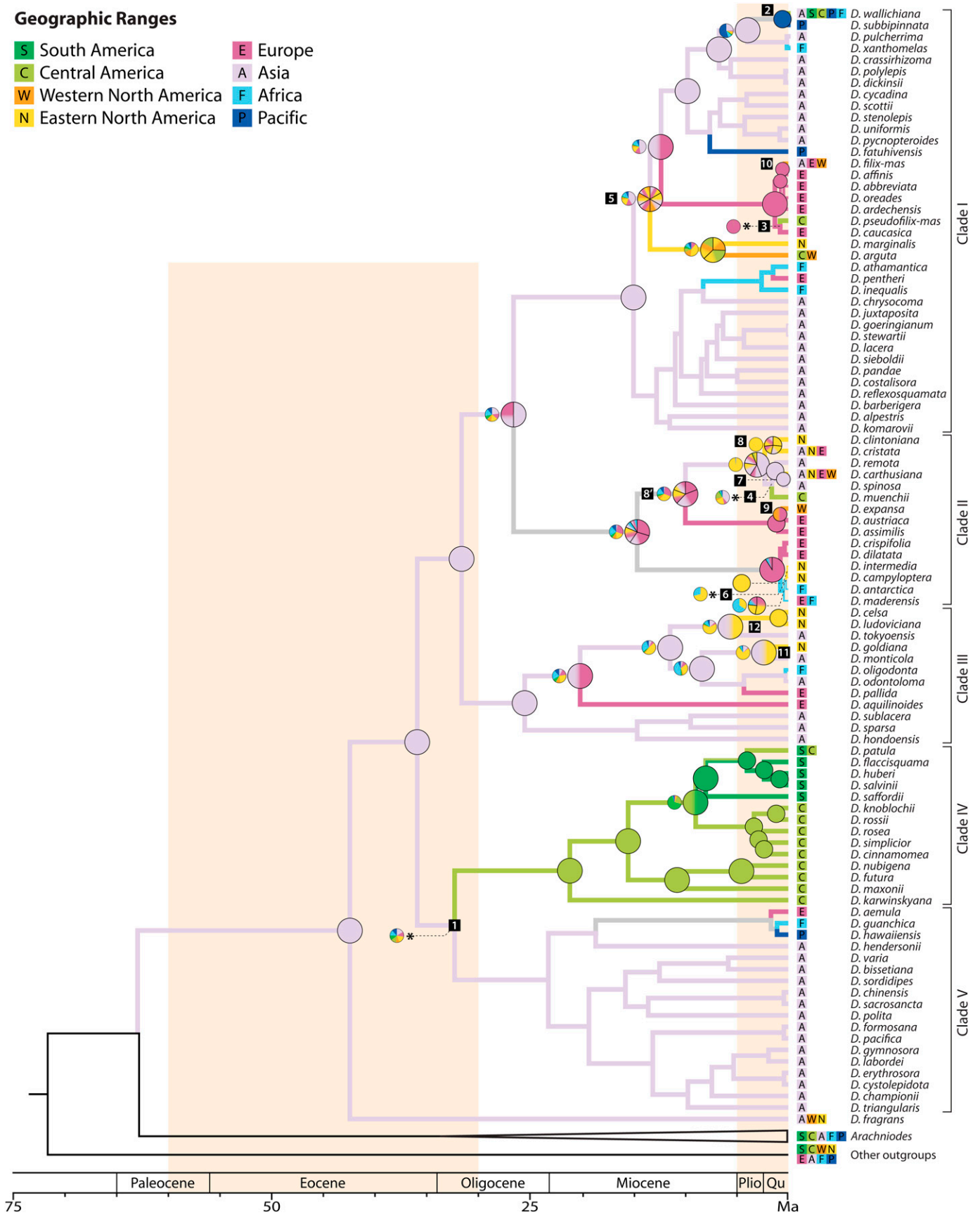
Fig. 4. Results of ancestral area reconstructions from MP, BI, and S-DIVA analyses, overlaid on the chronogram from the divergence time analysis. Present-day distributions of individual *Dryopteris* species and outgroups are indicated by colored boxes. Branch colors indicate inferred ancestral distributions under MP; gray indicates ambiguity. Large pie charts at nodes show ancestral distributions inferred by S-DIVA, with wedges showing the relative likelihood of alternative scenarios. Color gradients indicate vicariance events involving the regions indicated by those colors. Asterisks indicate nodes where dispersal is inferred (i.e., S-DIVA produced no reconstruction). Smaller pie charts to the left of S-DIVA pie charts indicate ancestral reconstructions by BayesTraits; BayesTraits results are only shown where they conflict with results from S-DIVA, or where there is substantial ambiguity between regions. Only nodes ancestral to clades containing New World *Dryopteris* taxa are annotated. Outgroups are collapsed, as their inclusion or exclusion from these analyses did affect results within *Dryopteris*. See text for discussion of numbered nodes.


Geographic Ranges
S South America
C Central America
W Western North America
N Eastern North America
E Europe
A Asia
F Africa
P Pacific
D. wallichiana
D. subbipinnata
D. pulcherrima
D. xanthomelas
D. crassirhizoma
D. polylepis
D. dickinsii
D. cycadina
D. scottii
D. stenolepis
D. uniformis
D. pycnopteroides
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D. assimilis
D. crispifolia
D. dilatata
D. intermedia
D. campyloptera
D. antarctica
D. maderensis
D. celsa
D. ludoviciana
D. tokyoensis
D. goldiana
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D. pallida
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D. nubigena
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D. aemula
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D. bissetiana
D. sordidipes
D. chinensis
D. sacrosancta
D. polita
D. formosana
D. pacifica
D. gymnosora
D. labordei
D. erythrosora
D. cystolepidota
D. championii
D. triangularis
D. fragrans
Arachniodes
Other outgroups
Clade I
Clade II
Clade III
Clade IV
Clade V
Paleocene
Eocene
Oligocene
Miocene
Plio
Qu
75
50
25
Ma

concrete fossil evidence that would place *Dryopteris* in the Americas prior to the dates indicated by our divergence time analyses.

Within *Dryopteris*, cladogenetic events began ca. 42 Ma, and the five major clades identified in this study began to diversify between 25.5 and 14.6 Ma. New World *Dryopteris* species belong to 13 lineages, and our AAR results for these taxa indicate that long-distance dispersal has dominated the history of the Central and South American taxa, while vicariance has played a larger role in the history of the North American taxa. In all, we identified a minimum of three vicariance events involving Europe and Asia and six transoceanic dispersal events, including four from Asia and one each from Africa and Europe. These events occurred between 42.4 and 0.2 ma (Table 4; Fig. 7). For four additional lineages, the historical scenario is ambiguous and either long-distance dispersal or vicariance can account for the observed distributions, depending on the AAR approach taken. We will examine the relative importance of vicariance vs. long-distance dispersal for each lineage of New World *Dryopteris*, and then consider the patterns that emerge in the context of fern biogeography generally.

Our analyses indicate that 17 of the 18 Central (CA) and South (SA) American *Dryopteris* species are derived from ancestors that arrived via four separate long-distance dispersal events over the last ca. 32 Myr (Table 4). Three of these events are relatively recent and account for just one species each, but the fourth was more ancient and resulted in the subsequent radiation of the remaining fourteen CA and SA taxa. This latter group forms the strongly supported clade IV (MP BS/ML BS/BI PP = 100/98/1.0), whose ancestor we infer to have dispersed to CA from Asia between 35.9 and 32.2 Ma (node 1; all node references refer to Table 4 and Figs. 4–6). The timing of this event is also consistent with the boreotropics hypothesis of a widespread early Tertiary flora in the northern hemisphere, though its apparent timing is near the end of the appropriate period (Lavin and Luckow, 1993; Morley, 2003). Subsequent movement of taxa between CA and SA likely reflects the increasing proximity of these landmasses in the late Miocene, culminating in the closing of the Isthmus of Panama in the Pliocene roughly 5 Ma (Morley, 2003).

Our analyses support three additional dispersal events to CA originating in Europe and Asia within the last 2 Myr (Table 4). Pantropical *D. wallichiana* is most closely related to Hawaiian *D. subbipinnata* (node 2) in our phylogeny, consistent with the results of Geiger and Ranker (2005). Our results indicate that these two species are descended from an ancestor in Asia and subsequently dispersed to the Pacific and Americas in the last 3.9 Myr. There was no overland connection between Asia and Central or South America at this point, and the Pacific Islands have never been connected to a continental landmass. However, we did not include separate accessions of this uniquely widespread species from East Asia, West Asia, and Africa in our analysis, and so are unable to test directly whether sequence data would support its long-distance dispersal from just one of those areas to the Americas. Vicariance is also ruled out for *D. pseudofilix-mas*, which is most closely related to European *D. caucasica* (node 3), from which it diverged ca. 0.8 Ma. This is far too recent for a vicariant origin, as the last available overland connection between Europe and the Americas, the North Atlantic Land Bridge (NALB), would not have been available after the late Miocene (Tiffney and Manchester, 2001; Denk et al., 2010); 32 Ma is the latest that a tropical taxon has been shown to have migrated via this route (Davis et al., 2004; Smedmark et al., 2010). The final dispersal event to CA was by the ancestor of *D. muenchii*, a Mexican cloud forest endemic (Reyes-Jaramillo et al., 2008) that is closely related in our phylogenies to members of the North American reticulate complex and several Asian species (node 4). AARs for this group conflict, but we hypothesize that *D. muenchii* descended from an Asian ancestor (Fig. 6) and dispersed independently to Central America in the last ca. 1.6 Myr.

For the one remaining CA species, *D. arguta*, which is sister to ENA species *D. marginalis*, (node 5), our various AARs present conflicting scenarios, consistent with both vicariance and dispersal. We reject one hypothesis, proposed by our DEC-dis analysis, because it reconstructs vicariance between Asia and CA ca. 13 Ma, which is improbable (Davis et al., 2004). The remaining two scenarios suggest, respectively, vicariance between various combinations of Asia, Europe, ENA, and WNA, or long-distance dispersal from Asia to ENA. Both of these scenarios are viable; we cannot rule out long-distance dispersal, and vicariance between Asia, Europe, and North America in this time period, the mid-Miocene, is plausible via either the NALB (Denk et al., 2010) or the Bering Land Bridge (BLB) (Tiffney and Manchester, 2001; Cook et al., 2005).

Compared to the CA and SA taxa, for most of which long-distance dispersal appears to be ultimately responsible, vicariance is implicated in the immediate history of several of the North American species, although we do find evidence for dispersal in two lineages. ENA species *D. intermedia* diverged from its African sister species *D. antarctica* too recently, within the last million years (node 6), for vicariance to account for it, and we therefore hypothesize that *D. intermedia* dispersed to ENA, where *D. campyloptera* subsequently arose via allopolyploid hybridization (Table 5). We also infer long-distance dispersal for *D. carthusiana* (node 7), which is widespread in ENA, WNA, Europe, and northern Asia (Carlson and Wagner, 1982) and whose closest relative in our phylogeny is Asian *D. spinosa*. We hypothesize that *D. carthusiana* descended from an Asian ancestor, and dispersed to Europe, ENA, and WNA within the last half million years. Short-distance dispersal can account for its movement to Europe from Asia, and between ENA and WNA, but vicariance between Asia and North America at this point, during the Pleistocene glaciations (Denk et al., 2010), is unlikely.

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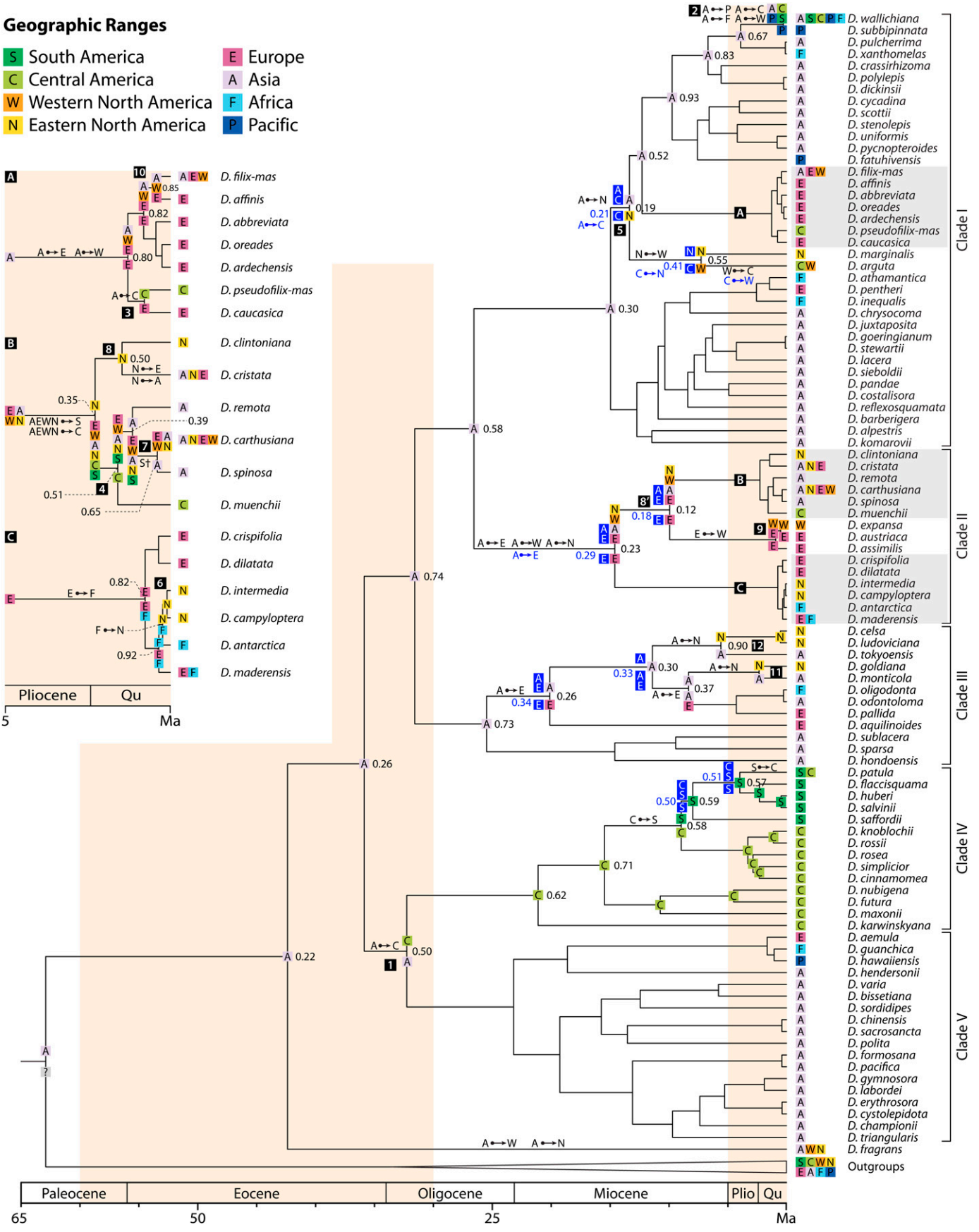
Fig. 5. Results of ancestral area reconstructions from LaGrange analyses, overlaid on the chronogram from the divergence time analysis. Inset enlarges several clades (A, B, and C) containing New World *Dryopteris*, which correspond to gray boxes at right. Results from the DEC-vic model are presented. This and the DEC-dis model produced the same ancestral area reconstructions (AARs) at all but 12 nodes; the alternative reconstructions from DEC-dis are indicated at eight of these nodes in this figure by blue boxes with white text. The remaining four nodes fall within clade B, and its DEC-dis reconstruction is given in Fig. 6. The AARs shown are those with the highest likelihoods, and for nodes with more than one reconstruction, the relative probability for the optimal reconstruction is given. Nodes with only one box indicate an ancestor in a single geographic region; combined boxes indicate an ancestor distributed through multiple regions. Regions separated toward the top and bottom of a node indicate separate ranges inherited by the daughter lineages. Inferred dispersal events are indicated by arrows connecting the source and destination ranges; † indicates one local extinction event in clade B. Dotted lines connect relatively probabilities or inferred movements with the appropriate node or branch in several locations.


Geographic Ranges
S South America
C Central America
W Western North America
N Eastern North America
E Europe
A Asia
F Africa
P Pacific
D. filix-mas
D. affinis
D. abbreviata
D. oreades
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D. gymnosora
D. labordei
D. erythrosora
D. cystolepidota
D. championii
D. triangularis
D. fragrans
Outgroups
Clade I
Clade II
Clade III
Clade IV
Clade V
Pliocene
Qu
5
Ma
Paleocene
Eocene
Oligocene
Miocene
Plio
Qu
65
50
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Ma

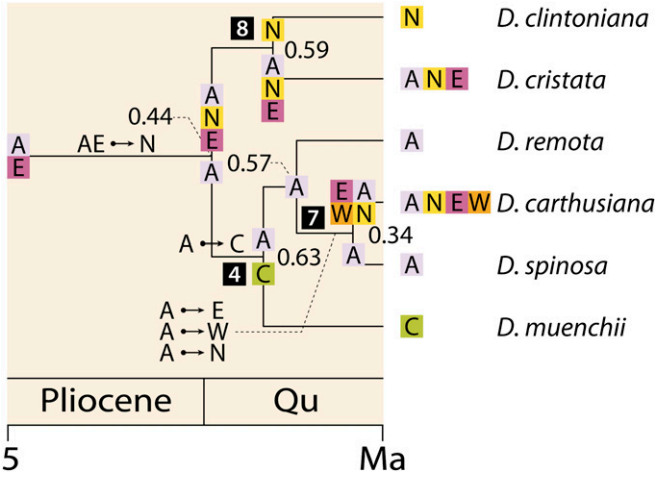

Fig. 6. Results of ancestral area reconstructions from LaGrange DEC-dis analysis for clade B (see Fig. 5). All notations follow those in Fig. 5.

Dryopteris carthusiana is a member of the small clade B, which also includes two other ENA taxa, *D. clintoniana* and *D. cristata* (node 8). Our AARs conflict on the ancestral scenario for clade B as a whole, but we infer that these taxa descended from an ancestor that had achieved a widespread range in Europe and Asia (node 8′) by at least 10 Ma. One daughter lineage, from which *D. carthusiana* descended, remained in Asia, while the other, the ancestor of *D. cristata*, expanded into ENA ca. 2.5 Ma, a movement consistent with availability of the BLB (Tiffney and Manchester, 2001). *Dryopteris clintoniana* is a polyploid hybrid derivative of *D. cristata* and is endemic to ENA, where it originated. An alternative scenario for clade B involves an ancestor with a range encompassing Asia, Europe, WNA, ENA, CA, and SA in the last 2.5 Ma. We reject this hypothesis, as it is extremely unlikely that a single ancestral group could have maintained a distribution through such a wide range of latitudes in the late Pliocene and Quaternary (Tiffney and Manchester, 2001; Milne, 2006; Denk et al., 2010).

We also hypothesize vicariance for the ranges of WNA taxa *D. expansa* (node 9) and *D. filix-mas* (node 10). Both diverged from their closest relatives, in Europe and Eurasia, respectively, ca. 0.5 ma, and ancestors of both are inferred to have occupied wide ranges spanning Europe-WNA and Eurasia-WNA at some point in the last 10–13 Myr. Movement between Europe, Asia, and North America during this period has been demonstrated for numerous plant groups (Milne, 2004; Ickert-Bond and Wen, 2006), in a primarily east-to-west direction (Tiffney and Manchester, 2001; Cook et al., 2005; Denk et al., 2010), and fossils assigned to *Dryopteris* have been found in the Kenai Peninsula and Cook Inlet region of Alaska (Wolfe et al., 1966; Wolfe and Tanai, 1980; Reinink-Smith and Leopold, 2005) and in Iceland (Grímsson and Denk, 2007) that date to this period, roughly the middle to late Miocene (ca. 14–6 Ma), in agreement with our results. *Dryopteris filix-mas* has remained widespread in WNA, Europe, and Asia, while speciation accompanied the breakup of the range of the ancestor of *D. expansa*; it is confined to WNA while its closest relative is in Europe.

For three final North American lineages our AARs are ambiguous, though we believe the balance of evidence leans toward vicariance for two of them, and dispersal for the third. ENA species *D. goldiana* (node 11) and *D. ludoviciana* (plus its putative tetraploid offspring *D. celsa*; node 12), each display a disjunct relationship with sister species in Asia, a pattern which has long been recognized in temperate plant groups in these regions (Wen, 1999, 2001; Donoghue and Smith, 2004). These ENA–Asian pairs diverged ca. 2.4 and 5.6 Ma, respectively, making vicariance via the BLB plausible in both cases (Tiffney and Manchester, 2001; Cook et al., 2005). Although we cannot rule out long-distance dispersal, the repeated, shared pattern of movement favors vicariance (Milne, 2006). Finally, circumboreal *D. fragrans* occurs today in Asia, WNA, and ENA, including along the eastern and western coasts of Greenland. It has had approximately the last 42 Myr to arrive at this widespread range, but until the last 5–10 kyr, almost all of its current range in North America would have been glaciated. Kalliola (1937) speculated that *D. fragrans* may have been able to live extremely close to the margins of the ice sheets, but even so, most of its range would have been under ice during the Pleistocene. Postglaciation recolonization, perhaps from scattered refugia in Beringia or elsewhere (Shafer et al., 2010), would likely have involved cumulative dispersal over thousands of kilometers in the last 5–10 kyr, as *D. fragrans* is restricted in habitat to sheer cliffs (Montgomery and Wagner, 1993) that are patchily distributed throughout its range in North America, from 44° to 71°N. Such dispersal capacity would have made its arrival from the Old World during the last 42 Myr almost inevitable.

Our results suggest that long-distance dispersal and vicariance have both played important roles in shaping the historical biogeography of New World *Dryopteris*. While it is nearly impossible to completely rule out long-distance dispersal (Milne, 2006), the congruence of climatic and paleogeographic factors support vicariance for several lineages, consistent with accepted scenarios in angiosperms (Wen et al., 1998, Denk et al., 2010). The main pattern emergent in our analyses is that long-distance dispersal has dominated the origins of the Central and South American species, while the opposite appears to be true for the species of temperate North America. We attribute the arrival of four to (more likely) seven of the 12 North American species to vicariance, and only three to long-distance dispersal. This pattern has not previously been demonstrated in any plant group, but we hypothesize that other genera that diversified in the same time period as *Dryopteris*, starting in the mid to late Eocene, may also show it. The migration of the Boreotropical flora, which moved across latitudes and provided the last overland floristic connection between the tropics of Asia and the Americas, had largely subsided by this time (Lavin and Luckow, 1993; Morley, 2003; Davis et al., 2004), even though land connections in the northern hemisphere remained relatively abundant, albeit sporadic, until recently (Tiffney and Manchester, 2001; Denk et al., 2010). Together these factors can account for the apparent influence of vicariance on the temperate members of the North American flora but not on the Central and South American floras.

For ferns with widespread ranges, long-distance dispersal has traditionally been seen as the likely explanation for their diversification (Tryon, 1985, 1986; Moran, 2008), simply because most ferns have dust-like, easily dispersed spores (Tryon and Lugardon, 1991). Wide disjunctions in the ranges of many genera have been attributed to dispersal (e.g., *Asplenium* [Wagner et al., 1993], *Polystichum* [Perrie et al., 2003]), and it has been supposed that frequent dispersal would weaken any signal of vicariance that may have taken place (Wolf et al., 2001). As mentioned earlier, however, vicariance is increasingly being seen as an important phenomenon in determining fern distributions (Kato, 1993; Wolf et al., 2001; Haufler,

TABLE 4. Summary of ancestral area reconstruction (AAR) results for nodes in Figs. 4–6 that are ancestral to species or groups of New World (NW) *Dryopteris*. For long-distance dispersal, the minimum age of the inferred dispersal event is indicated (Ma). For vicariance, the minimum age at which the ancestral range was achieved is given (Ma); note that these ranges may have been occupied prior to the node at which the New World species diverged. Both possible scenarios are indicated for four ambiguous nodes; we favor vicariance for two and dispersal for one, as noted in the text.

Node	NW Species	Range in NW	At node, NW lineage sister to...	LDD or Vicariance	Minimum age of dispersal event (95% HPD)	Minimum age of ancestral range	Ancestral range or direction of inferred movement
1	14 spp. in clade IV	CA, SA	Asia	LDD	32.3 (40.4–24.8)	–	Asia → CA
2	*D. wallichiana*	CA, SA	Pacific	LDD	3.9 (6.5–1.6)	–	Asia → CA, SA
3	*D. pseudofilix-mas*	CA	Europe	LDD	0.8 (1.6–0.1	–	Europe → CA
4	*D. muenchii*	CA	Asia	LDD	1.6 (2.7–0.7)	–	Asia → CA
5	*D. arguta, D. marginalis*	CA, WNA; ENA	Asia	Ambiguous	13.4 (17.8–9.2)	13.4 (17.8–9.2)	Vicariance between Asia, ENA, WNA, CA **or** dispersal from Asia → ENA
6	*D. intermedia, D. campyloptera*	ENA; ENA	Africa	LDD	0.2 (0.7–0.002)	–	Africa → ENA
7	*D. carthusiana*	ENA, WNA	Asia	LDD	0.4 (0.9–0.005)	–	Asia → Europe, ENA, WNA
8	*D. cristata, D. clintoniana*	ENA; ENA	Asia	Vicariance	–	2.3 (3.6-1.1)	Asia, Europe, ENA
9	*D. expansa*	WNA	Europe	Vicariance	–	0.9 (1.9–0.3)	Europe, WNA
10	*D. filix-mas*	WNA	Europe	Vicariance	–	1.3 (2.2-0.5)	Asia, Europe, WNA
11	*D. goldiana*	ENA	Asia	Ambiguous (vicariance)	2.4 (4.7–0.5)	2.4 (4.7–0.5)	Vicariance between Asia, ENA **or** dispersal from Asia → ENA
12	*D. ludoviciana, D. celsa*	ENA; ENA	Asia	Ambiguous (vicariance)	5.6 (9.4–2.2)	5.6 (9.4–2.2)	Vicariance between Asia, ENA **or** dispersal from Asia → ENA
13	*D. fragrans*	ENA, WNA	Asia	Ambiguous (dispersal)	42.4 (53.3–32.2)	0	Vicariance b/w Asia, ENA, WNA **or** dispersal from refugia → ENA, WNA, Asia

Notes: CA = Central America, SA = South America, ENA = eastern North America, WNA = western North America, HPD = highest posterior density interval, LDD = long-distance dispersal.

2007), and continental-scale geographic structure consistent with vicariance has previously been suggested for several widespread genera (e.g., *Adiantum* [Paris and Windham, 1988]; *Onoclea* [Gastony and Ungerer, 1997]; *Pteridium* [Der et al., 2009]; *Nephrolepis* [Hennequin et al., 2010]). Our study is the first to employ sophisticated methods (e.g., LaGrange, S-DIVA) to reconstruct the historical biogeography of a fern genus on a global scale, and our results provide additional

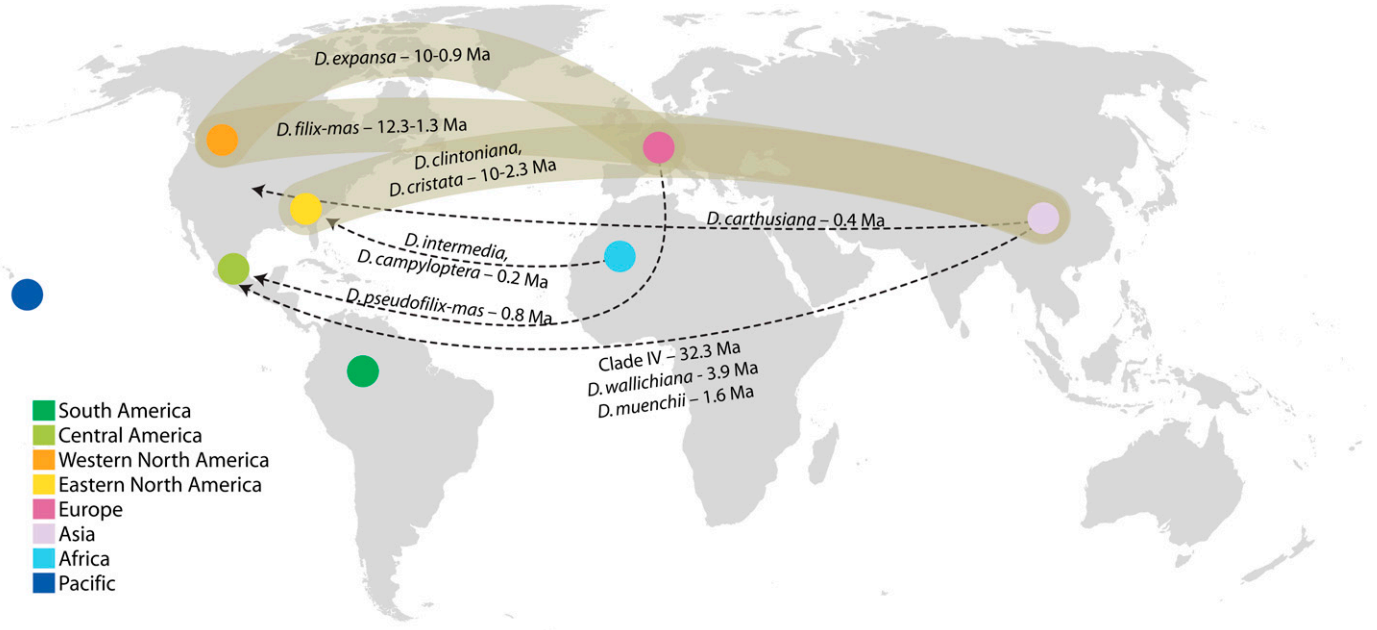

Fig. 7. Hypothesized movements of nine *Dryopteris* species or lineages to the New World. New World descendants of each event are given. Dotted lines indicate long-distance dispersal, and brown areas indicate widespread ranges prior to vicariance. The minimum age of each dispersal event is given, and for vicariance scenarios the approximate time period during which the indicated species' ancestor could have occupied that range is given. Brown shading is not meant to represent the exact range of an ancestral taxon, but indicates which regions were connected by that range. Six species in four lineages with ambiguous histories are not illustrated, including *D. marginalis*, *D. arguta*, *D. goldiana*, *D. ludoviciana*, *D. celsa*, and *D. fragrans*.

TABLE 5. Allopolyploids and their putative diploid parents, with ploidy indicated, in the North American reticulate complex. The ploidy of "*D. semicristata*" is unknown, but it is hypothesized to be diploid (Montgomery and Wagner, 1993).

Allopolyploid	Putative parents
D. campyloptera (4*x*)	*D. expansa* (2*x*), *D. intermedia* (2*x*)
D. carthusiana (4*x*)	*D. intermedia* (2*x*), "*D. semicristata*" (2*x*?)
D. celsa (4*x*)	*D. goldiana* (2*x*), *D. ludoviciana* (2*x*)
D. clintoniana (6*x*)	*D. cristata* (4*x*), *D. goldiana* (2*x*)
D. cristata (4*x*)	*D. ludoviciana* (2*x*), "*D. semicristata*" (2*x*?)

support for the importance of vicariance, particularly in the northern hemisphere.

Finally, our results have interesting implications for the North American reticulate complex (Montgomery and Wagner, 1993), and particularly for the missing diploid ancestor in this group, "*D. semicristata*". *Dryopteris cristata* and *D. carthusiana* are both putative tetraploid offspring of this species (Table 5) and occur in northern Asia and Europe as well as North America (Carlson and Wagner, 1982). *Dryopteris clintoniana* is a putative hexaploid between *D. cristata* and *D. goldiana* and is endemic to ENA (Montgomery and Wagner, 1993). The close relationship of these three species in our plastid phylogeny provides support for their shared ancestry, though *D. muenchii*, *D. remota*, and *D. spinosa* are also closely related to them. Our biogeographic results imply a likely Asian or Eurasian range for the hypothetical "*D. semicristata*", but this becomes problematic considering that the other putative parents of the tetraploids, *D. intermedia* and *D. ludoviciana* (Table 5), are endemic to eastern North America. The ranges of the parental taxa would have had to overlap, or be near enough that spores from the parents could intermingle. Did "*D. semicristata*" in fact occur in North America? Or was it Eurasian, with two separate dispersal events carrying its spores to North America, where the hybridizations occurred? A critical missing piece of this puzzle is a nuclear phylogeny for all taxa involved, including those newly identified here, for the first time, as close relatives of the complex. We currently have such a study underway, and hope to answer these questions in the near future.

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APPENDIX 1. Voucher information and GenBank accession numbers for species of *Dryopteris* and outgroups. All sequences were newly generated for this study. Herbarium abbreviations: WIS = University of Wisconsin-Madison, Madison, Wisconsin, USA; NY = New York Botanical Garden, Bronx, New York, USA; DUKE = Duke University, Durham, North Carolina, USA; MO = Missouri Botanical Garden, St. Louis, Missouri, USA; UC = University of California-Berkeley, Berkeley, California, USA; COLO = University of Colorado Museum, Boulder, Colorado, USA. P = Muséum National d'Histoire Naturelle, Paris, France; BM = The Natural History Museum, London, UK; REU = Université de la Réunion, Saint-Clotilde, Réunion. AFSSE and BPSSE indicate taxa that were obtained as spores from the American Fern Society Spore Exchange and British Pteridological Society Spore Exchange, respectively. These spores were germinated and grown by Geiger and Ranker (2005), and DNA material later provided to us. Missing sequences are indicated by a dash (—).

Taxon; *Voucher specimen,* Herbarium; GenBank accessions: *trnL-trnF*; *rbcL-accD*; *rbcL*; *psbA-trnH*; *rps4-trnS*; *trnG-trnR*; *trnP-petG*

Dryopteris Dryopteris abbreviata (C.Presl) Kuntze; *Christenhusz 4290*, UC; JN189126; JN189664; JN189557; JN189448; JN189231; JN189019; JN189342. ***Dryopteris aemula*** (Ait.) Kuntze; *Alejandre 83-89*, NY; JN189084; JN189625; —; JN189407; JN189189; JN188979; JN189301. ***Dryopteris affinis*** (Lowe) Fraser Jenk.; *Christenhusz 4281*, UC; JN189085; JN189626; JN189516; JN189408; JN189190; JN188980; JN189302. ***Dryopteris alpestris*** Tagawa ex Ching & S.K.Wu; *Heng 32147*, UC; JN189105; JN189645; JN189536; JN189428; JN189210; JN189000; JN189322. ***Dryopteris antarctica*** (Baker) C.Chr.; *Hennequin 2009 R109*, REU; JN189141; JN189682; JN189577; JN189467; JN189250; JN189038; JN189356. ***Dryopteris aquilinoides*** (Desv.) C.Chr.; *Kessler 13855*, UC; JN189106; JN189646; JN189537; JN189429; JN189211; JN189001; JN189323. ***Dryopteris ardechensis*** Fraser Jenk.; *BPSSE*; JN189163; JN189702; JN189596; JN189487; JN189271; JN189056; JN189377. ***Dryopteris arguta*** (Kaulf.) Maxon; *EBS35*, WIS; JN189077; JN189619; JN189509; JN189400; —; JN188972; JN189294. ***Dryopteris assimilis*** S.Walker; *Skvortsov 1.VIII 1982*, NY; JN189086; JN189627; JN189517; JN189409; JN189191; JN188981; JN189303. ***Dryopteris athamantica*** (Kunze) Kuntze; *Bodenghien 2037*, UC; JN189107; —; JN189538; JN189430; JN189212; JN189002; JN189324. ***Dryopteris austriaca*** (Jacq.) Woyn. ex Schinz & Thell.; *Degn 25*, NY; JN189087; JN189628; JN189518; JN189410; JN189192; JN188982; JN189304. ***Dryopteris barberigera*** Moore; *Miehe 94-191-14*, UC; JN189108; JN189647; JN189539; JN189431; JN189213; JN189003; JN189325. ***Dryopteris bissetiana*** (Baker) C.Chr.; *Moran, COLO*; JN189153; JN189693; JN189587; JN189479; JN189261; JN189048; JN189367. ***Dryopteris campyloptera*** (Kunze) Clarkson; *EBS22*, WIS; JN189072; JN189614; JN189504; JN189395; —; JN188967; JN189289. ***Dryopteris carthusiana*** (Vill.) H.P.Fuchs; *EBS41*, WIS; JN189079; JN189621; JN189511; JN189402; JN189184; JN188974; JN189296. ***Dryopteris caucasica*** (A.Braun) Fraser Jenk. & Corley; *Christenhusz 4309*, UC; JN189109; JN189648; JN189540; JN189432; JN189214; JN189004; JN189326. ***Dryopteris celsa*** (W.Palmer) Knowlt., T.S.Palmer & Pollard ex Small; *EBS27*, WIS; JN189069; JN189609; JN189499; JN189390; JN189175; JN188962; JN189284. ***Dryopteris championii*** (Benth.) C.Chr.; *Moran, COLO*; JN189154; JN189694; JN189588; JN189480; JN189262; JN189049; JN189368. ***Dryopteris chinensis*** Koidz.; *Zhang 2399*, UC; JN189110; JN189649; JN189541; JN189433; JN189215; JN189005; JN189327. ***Dryopteris chrysocoma*** (Christ) C.Chr.; *Unknown 188*, UC; JN189111; JN189650; JN189542; JN189434; JN189216; JN189006; JN189328. ***Dryopteris cinnamomea*** (Cav.) C.Chr.; *Rothfels 3099*, DUKE; JN189097; JN189638; JN189528; JN189420; JN189202; JN188992; JN189314. ***Dryopteris clintoniana*** (D.C.Eaton) Dowell; *EBS16*, WIS; JN189068; JN189608; JN189498; JN189389; JN189174; JN188961; JN189283. ***Dryopteris costalisora*** Tagawa; *Ranker 2029*, COLO; JN189170; JN189710; JN189603; JN189493; JN189278; JN189063; JN189384. ***Dryopteris crassirhizoma*** Nakai; *van der Werff 14065*, UC; JN189112; JN189651; JN189543; JN189435; JN189217; JN189007; JN189329. ***Dryopteris crispifolia*** Rasbach, Reichst. & G.Vida; *BPSSE*; JN189164; JN189703; JN189597; JN189488; JN189272; JN189057; JN189378. ***Dryopteris cristata*** (L.) A.Gray; *EBS51*, WIS; JN189082; JN189623; JN189514; JN189405; JN189187; JN188977; JN189299. ***Dryopteris cycadina*** (Franch. & Sav.) C.Chr.; *RBC TW 078*, UC; JN189113; JN189652; JN189544; JN189436; JN189218; JN189008; JN189330. ***Dryopteris cystolepidota*** (Miq.) C.Chr.; *AFSSE*; JN189160; JN189699; JN189593; JN189485; JN189268; JN189053; JN189374. ***Dryopteris dickinsii*** (Franch. & Sav.) C.Chr.; *BPSSE*; JN189165; JN189704; JN189598; JN189489; JN189273; JN189058; JN189379. ***Dryopteris dilatata*** (Hoffm.) A.Gray; *Hennequin 2010 B1*, P; JN189139; JN189680; JN189575; JN189465; JN189248; JN189036; JN189354. ***Dryopteris erythrosora*** (D.C.Eaton) Kuntze; *Geiger 94*, COLO; JN189147; JN189687; JN189581; JN189473; JN189255; JN189042; JN189361. ***Dryopteris expansa*** (C.Presl) Fraser Jenk. & Jermy; *EBS30*, WIS; JN189074; JN189616; JN189506; JN189397; JN189180; JN188969; JN189291. ***Dryopteris fatuhivensis*** E.Brown; *Wood 10092*, COLO; JN189168; JN189707; —; JN189490; JN189275; JN189060; JN189381. ***Dryopteris filixmas*** (L.) Schott; *EBS32*, WIS; JN189075; JN189617; JN189507; JN189398; JN189181; JN188970; JN189292. ***Dryopteris flaccisquama*** A.Rojas; *Fay 3152*, NY; JN189088; JN189629; JN189519; JN189411; JN189193; JN188983; JN189305. ***Dryopteris formosana*** (Christ) C.Chr.; *RBC 181*, UC; JN189114; JN189653; JN189545; JN189437; JN189219; JN189009; JN189331. ***Dryopteris fragrans*** (L.) Schott; *EBS47*, WIS; JN189080; —; JN189512; JN189403; JN189185; JN188975; JN189297. ***Dryopteris futura*** A.R.Sm.; *Quedensley 754*, UC; JN189103; JN189643; JN189534; JN189426; JN189208; JN188998; JN189320. ***Dryopteris goeringianum*** Koidz.; *Moran, COLO*; JN189148;

JN189688; JN189582; JN189474; JN189256; JN189043; JN189362. ***Dryopteris goldiana*** (Hook.) A.Gray; *EBS29*, WIS; JN189073; JN189615; JN189505; JN189396; JN189179; JN188968; JN189290. ***Dryopteris guanchica*** Gibby & Jermy; *Hennequin 2010 C2*, P; JN189137; JN189678; JN189573; JN189463; JN189246; JN189034; JN189352. ***Dryopteris gymnosora*** (Makino) C.Chr.; *Unknown 94.0752*, UC; JN189115; JN189654; JN189546; JN189438; JN189220; JN189010; JN189332. ***Dryopteris hawaiiensis*** (Hillebr.) W.J.Rob.; *Geiger 74*, COLO; JN189144; JN189685; JN189580; JN189470; JN189253; JN189041; JN189359. ***Dryopteris hendersonii*** (Bedd.) C.Chr.; *Kramer 7731*, UC; JN189116; —; JN189547; JN189439; JN189221; —; —. ***Dryopteris hondoensis*** Koidz.; *Moran, COLO*; JN189149; JN189689; JN189583; JN189475; JN189257; JN189044; JN189363. ***Dryopteris huberi*** (Christ) C.Chr.; *Sperling 5841*, NY; JN189089; JN189630; JN189520; JN189412; JN189194; JN188984; JN189306. ***Dryopteris inequalis*** (Schlecht.) Kuntze; *Unknown 7749*, UC; JN189117; JN189655; JN189548; JN189440; JN189222; JN189011; JN189333. ***Dryopteris intermedia*** Kuntze; *EBS18*, WIS; —; JN189613; JN189503; JN189394; JN189178; JN188966; JN189288. ***Dryopteris juxtaposita*** Christ; *Heng 24049*, UC; JN189118; JN189656; JN189549; JN189441; JN189223; JN189012; JN189334. ***Dryopteris karwinskyana*** (Mett.) Kuntze; *Marcos 354*, NY; JN189090; JN189631; JN189521; JN189413; JN189195; JN188985; JN189307. ***Dryopteris knoblochii*** A.R.Sm.; *Devender 98-1566*, NY; JN189091; JN189632; JN189522; JN189414; JN189196; JN188986; JN189308. ***Dryopteris komarovii*** Kossinsky; *Wundisch 94-453-19*, UC; JN189119; JN189657; JN189550; JN189442; JN189224; JN189013; JN189335. ***Dryopteris labordei*** (Christ) C.Chr.; *Ranker 2006*, COLO; JN189169; JN189709; JN189602; JN189492; JN189277; JN189062; JN189383. ***Dryopteris lacera*** (Thunb.) Kuntze; *Moran, COLO*; JN189151; JN189691; JN189585; JN189477; JN189259; JN189046; JN189365. ***Dryopteris ludoviciana*** (Kunze) Small; *EBS48*, WIS; JN189081; JN189622; JN189513; JN189404; JN189186; JN188976; JN189298. ***Dryopteris maderensis*** Alston; *BPSSE*; JN189166; JN189705; JN189599; —; JN189274; JN189059; JN189380. ***Dryopteris marginalis*** (L.) A.Gray; *EBS17*, WIS; JN189071; JN189612; JN189502; JN189393; JN189177; JN188965; JN189287. ***Dryopteris maxonii*** Underw. & C.Chr.*; Rothfels 3197*, DUKE; JN189098; JN189639; JN189529; JN189421; JN189203; JN188993; JN189315. ***Dryopteris monticola*** (*goldiana* subsp. *monticola*) (Makino) C.Chr.; *Togasi, COLO*; JN189156; —; —; JN189482; JN189264; —; JN189370. ***Dryopteris muenchii*** A.R.Sm.; *EBS54*, WIS; JN189104; JN189644; JN189535; JN189427; JN189209; JN188999; JN189321. ***Dryopteris nubigena*** Maxon & C.V.Morton; *Sundue 1363*, NY; JN189070; JN189611; JN189501; JN189392; —; JN188964; JN189286. ***Dryopteris odontoloma*** (Moore) C.Chr.; *AFSSE*; JN189157; JN189696; JN189590; JN189483; JN189265; JN189051; JN189371. ***Dryopteris oligodonta*** (Desv.) Pic.Serm.; *Hennequin 2010 C11*, P; JN189138; JN189679; JN189574; JN189464; JN189247; JN189035; JN189353. ***Dryopteris oreades*** Fomin; *Vasak 427039*, COLO; JN189146; —; —; JN189472; JN189254; —; JN189360. ***Dryopteris pacifica*** (Nakai) Tagawa; *AFSSE*; JN189161; JN189700; JN189594; JN189486; JN189269; JN189054; JN189375. ***Dryopteris pallida*** Fomin; *AFSSE*; JN189158; JN189697; JN189591; —; JN189266; —; JN189372. ***Dryopteris pandae*** (Clarke) C. Chr.; *Unknown 11514*, UC; JN189120; JN189658; JN189551; JN189443; JN189225; JN189014; JN189336. ***Dryopteris patula*** (Sw.) Underw.; *EBS2*, WIS; —; JN189610; JN189500; JN189391; JN189176; JN188963; JN189285. ***Dryopteris pentheri*** (Krasser) C.Chr.; *Hennequin 2009 R2*, BM; JN189140; JN189681; JN189576; JN189466; JN189249; JN189037; JN189355. ***Dryopteris polita*** Rosenst.; *Ranker 2003*, COLO; JN189173; JN189713; JN189606; JN189496; JN189281; JN189066; JN189387. ***Dryopteris polylepis*** (Franch. & Sav.) C.Chr.; *Moran, COLO*; JN189155; JN189695; JN189589; JN189481; JN189263; JN189050; JN189369. ***Dryopteris pseudofilix-mas*** (Fée) Rothm.; *Montgomery 04-171*, NY; JN189092; JN189633; JN189523; JN189415; JN189197; JN188987; JN189309. ***Dryopteris pulcherrima*** Ching; *27540*, MO; JN189083; JN189624; JN189515; JN189406; JN189188; JN188978; JN189300. ***Dryopteris pycnopteroides*** (Christ) C.Chr.; *Moran, COLO*; JN189150; JN189690; JN189584; JN189476; JN189258; JN189045; JN189364. ***Dryopteris reflexosquamata*** Hayata; *Ranker 2040*, COLO; JN189171; JN189711; JN189604; JN189494; JN189279; JN189064; JN189385. ***Dryopteris remota*** Hayata; *Schuettpelz 528*, DUKE; JN189099; JN189640; JN189530; JN189422; JN189204; JN188994; JN189316. ***Dryopteris rosea*** Mickel & Beitel; *Mickel 4428A*, NY; JN189093; JN189634; JN189524; JN189416; JN189198; JN188988; JN189310. ***Dryopteris rossii*** C.Chr.; *Rothfels 3182*, DUKE; JN189100; JN189641; JN189531; JN189423; JN189205; JN188995; JN189317. ***Dryopteris sacrosancta*** Koidz.; *AFSSE*; JN189159; JN189698; JN189592; JN189484; JN189267; JN189052; JN189373. ***Dryopteris saffordii*** C.Chr.; *Sagastegui 15507*, NY; JN189094; JN189635; JN189525; JN189417; JN189199; JN188989; JN189311. ***Dryopteris salvinii*** (Baker) Kuntze; *Irwin 34351*, NY; JN189095; JN189636; JN189526; JN189418; JN189200; JN188990; JN189312. ***Dryopteris scottii*** (Bed) Ching; *RBC 202*, UC; JN189121; JN189659; JN189552; JN189444; JN189226; JN189015; JN189337. ***Dryopteris sieboldii*** (van Houtte) Kuntze; *AFSSE*; JN189162; JN189701; JN189595; —; JN189270; JN189055; JN189376. ***Dryopteris simplicior*** Mickel & Beitel; *Breedlove 21937*, NY; JN189096; JN189637; JN189527; JN189419; JN189201; JN188991; JN189313. ***Dryopteris sordidipes*** Tagawa; *Ranker 2061*, COLO; JN189172; JN189712; JN189605; JN189495; JN189280; JN189065; JN189386. ***Dryopteris sparsa*** (D.Don) Kuntze; *Ranker 2015*, COLO; —; JN189708; JN189601; JN189491; JN189276; JN189061; JN189382. ***Dryopteris spinosa*** Copel.; *Argus 9327*, COLO; JN189145; JN189686; —; JN189471; —; —; —. ***Dryopteris stenolepis*** (Baker) C.Chr.; *Unknown 99*, UC; JN189122; JN189660; JN189553; JN189445; JN189227; JN189016; JN189338. ***Dryopteris stewartii*** Fraser Jenk.; *Moran, COLO*; JN189152; JN189692; JN189586; JN189478; JN189260; JN189047; JN189366. ***Dryopteris subbipinnata*** W.H.Wagner & R.W. Hobdy; *Oppenheimer H50074*, COLO; JN189143; JN189684; JN189579; JN189469; JN189252; JN189040; JN189358. ***Dryopteris sublacera*** Christ; *Yatskievych 02-55*, UC; JN189123; JN189661; JN189554; JN189446; JN189228; JN189017; JN189339. ***Dryopteris tokyoensis*** (Matsum.) C.Chr.; *Moran, COLO*; JN189142; JN189683; JN189578; JN189468; JN189251; JN189039; JN189357. ***Dryopteris triangularis*** Herter; *BPSSE*; JN189167; JN189706; JN189600; —; —; —; —. ***Dryopteris uniformis*** Makino; *Hoshizaki 84-10*, UC; JN189124; JN189662; JN189555; JN189447; JN189229; JN189018; JN189340. ***Dryopteris varia*** (L.) Kuntze; *RBC 105*, UC; JN189125; JN189663; JN189556; —; JN189230; —; JN189341. ***Dryopteris wallichiana*** (Spreng.) Hyl; *EBS1*, WIS; JN189067; JN189607; JN189497; JN189388; —; JN188960; JN189282. ***Dryopteris xanthomelas*** (Christ) C.Chr.; *Miehe 94-149-17*, UC; JN189127; JN189665; JN189558; JN189449; JN189232; JN189020; JN189343.

Outgroups ***Arachniodes denticulata*** (Sw.) Ching; *Kromer 2550*, UC; JN189102; JN189642; JN189533; JN189425; JN189207; JN188997; JN189319. ***Arachniodes rhomboidea*** (Wall. ex Mett.) Ching; *RBC TW 022*, UC; JN189101; —; JN189532; JN189424; JN189206; JN188996; JN189318. ***Cryptogramma acrostichoides*** R.Br.; *Pryer 06-04*, DUKE; JN189136; JN189676; JN189571; JN189461; JN189244; JN189032; JN189350. ***Cryptogramma crispa*** (L.) R.Br.; *Reeb VR 4VIII-02/1*, DUKE; JN189135; JN189675; JN189570; JN189460; JN189243; JN189031; JN189349. ***Cyrtomium falcatum*** (L.f.) C.Presl; *EBS76*, WIS; JN189129; JN189667; JN189561; JN189452; JN189235; JN189022; JN189344. ***Davallia fejeensis*** Hook.; *EBS77*, WIS; JN189130; JN189668; JN189562; JN189453; JN189236; JN189023; JN189345. ***Llavea cordifolia*** Lag.; *Rothfels 3025*, DUKE; JN189131; JN189671; JN189566; JN189456; JN189240; JN189027; JN189347. ***Loxogramme salicifolia*** Makino; *Schuettpelz 1199A*, DUKE; JN189132; JN189672; JN189567; JN189457; —; JN189028; —. ***Olfersia cervina*** Kunze; *Rothfels 2659*, DUKE; —; —; JN189565; JN189455; JN189239; JN189026; —. ***Phanerophlebia nobilis*** (Schlecht. & Cham.) C.Presl; *Rothfels 5*, DUKE; JN189134; JN189674; JN189569; JN189459; JN189242; JN189030; —. ***Platycerium superbum*** de Jonch. & Hennipman; *EBS78*, WIS; —; JN189669; JN189563; JN189454; JN189237; JN189024; —. ***Pleopeltis polypodioides*** (L.) E.G.Andrews & Windham; *Rothfels 2471*, DUKE; JN189133; JN189673; JN189568; JN189458; JN189241; JN189029; JN189348. ***Polybotrya alfredii*** Brade; *Moran 7612*, DUKE; —; JN189670; JN189564; —; JN189238; JN189025; JN189346. ***Polybotrya caudata*** Kunze; *Rothfels 2660*, DUKE; —; JN189677; JN189572; JN189462; JN189245; JN189033; JN189351. ***Polystichopsis muscosa*** (M.Vahl) Proctor; *Christenhusz 2675, UC*; JN189128; —; JN189559; JN189450; JN189233; —; —. ***Polystichopsis pubescens*** (L.) C.V.Morton; *Mickel 9027*, UC; —; JN189666; JN189560; JN189451; JN189234; JN189021; —. ***Polystichum andersonii*** Hopkins; *EBS39*, WIS; JN189078; JN189620; JN189510; JN189401; JN189183; JN188973; JN189295. ***Polystichum munitum*** (Kaulf.) C.Presl; *EBS34*, WIS; JN189076; JN189618; JN189508; JN189399; JN189182; JN188971; JN189293.