

The Eastern Asian and Eastern and Western North American Floristic Disjunction: Congruent Phylogenetic Patterns in Seven Diverse Genera

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One of the most remarkable examples of intercontinental disjunction of the North Temperate Flora involves eastern Asia and eastern and western North America. Although there has been considerable interest in this phytogeographic pattern for over 150 years (e.g., Gray, 1859; Li, 1952; Graham, 1972; Boufford and Spongberg, 1983; Wu, 1983; Tiffney, 1985a, 1985b), relationships among taxa displaying the disjunction remain obscure. Understanding phylogenetic relationships is, however, a prerequisite for historical biogeographic analyses of this distributional pattern. To understand better the relationships of taxa displaying this intercontinental disjunction, phylogenetic analyses were conducted using a variety of DNA data sets for species of four genera (*Cornus*, *Boykinia*, *Tiarella*, and *Trautvetteria*) that occur in eastern Asia, eastern North America, and western North America. An area cladogram was constructed for each of the four genera, all of which show a similar pattern of relationship: the eastern Asian species are sister to all North American species. An identical phylogenetic pattern is also found in three other taxa exhibiting this disjunction (*Aralia* sect. *Aralia*, *Calycanthus*, and *Adiantum pedatum*). The congruent phylogenetic pattern found in these seven diverse genera raises the possibility of a common origin of the eastern Asia, eastern and western North America disjunction. The data are in agreement with the long-standing hypothesis that this well-known floristic disjunction represents the fragmentation of a once continuous Mixed Mesophytic forest community and suggest that the disjunction may have involved only two major vicariance events: an initial split between Eurasia and North America, followed by the isolation of floras between eastern and western North America. However, congruence between phylogenies and geographic distributions does not necessarily indicate an identical phytogeographic history. Taxa exhibiting the same phylogenetic pattern

may have originated at different geological times. Analysis of divergence times using the molecular clock indicates that species of *Cornus*, *Boykinia*, and *Calycanthus* may have diverged at different geological times, suggesting that the floristic disjunction involving eastern Asia and North America may not be simple; it may have involved multiple historical events at very different geological times in different genera. © 1998 Academic Press

INTRODUCTION

The flora of the North Temperate Zone exhibits striking intercontinental floristic similarities. This similarity is most remarkable between eastern Asia and eastern North America, and to a lesser extent in two or more of the following five areas: eastern Asia, western Asia, eastern North America, western North America, and southeastern Europe. Floristic elements displaying intercontinental disjunction in the North Temperate Zone have generally been considered Tertiary relicts that prior to the late Miocene were more widely distributed across Laurasia, forming part of a Mixed Mesophytic forest community (Li, 1952; Wolfe and Leopold, 1967; Wolfe, 1969, 1972, 1975; Wood, 1971, 1972; Graham, 1972). Following this hypothesis, the disjunction arose following geographic and climatic changes, including the separation of North America and Eurasia due to continental drift, the closing of the Turgai Straits in the Old World during the Oligocene, the Tertiary orogenies of western North America, and worldwide climatic fluctuations through the Tertiary. These events eliminated plants from many areas, particularly Europe and central North America (Graham, 1972; Leopold and MacGinitie, 1972; Tiffney, 1985a).

The disjunct distributional patterns of the North Temperate Flora, particularly those involving eastern Asia and North America, have been the subject of study for more than 150 years (e.g., Gray, 1859, 1878; Hu, 1935; Chaney, 1947; Li, 1952, 1972; Koyama and Kawano, 1964; Graham, 1972; Hara, 1972; Wolfe, 1972,

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1975, 1981, 1985; Boufford and Spongberg, 1983; Iltis, 1983; Hong, 1983, 1993; Hsu, 1983; Koyama, 1983; Tamura, 1983; Wu, 1983; Tiffney, 1985a, 1985b; Boufford, 1992). Despite long-standing interest, phylogenetic relationships among taxa displaying the disjunction have remained obscure, and the origin of the disjunction has been controversial. Major disagreements exist regarding: (1) the pattern of relationship (if any) among plants exhibiting the disjunction, (2) whether the disjunction had a single origin or involved multiple biogeographic events at different times in different taxa, and (3) the proposed geographic origin(s) of the disjunct taxa. Before these and other issues regarding this prominent biogeographic disjunction can be properly evaluated, a clear understanding of the phylogeny of taxa exhibiting the disjunct patterns is required.

We focused on taxa showing a disjunct distribution in eastern Asia and both eastern and western North America. Approximately 30 genera have closely related species occurring in these three areas (Wood, 1971, 1972; Li, 1972; Wu, 1983). There is no consensus of opinion as to how plants found in all three areas are related to one another; authors have suggested different patterns for different taxa. In *Trautvetteria*, Tamura (1983) suggested that the closest relationship is between the eastern Asian and western North American species. In contrast, in *Amsonia*, *Gaultheria*, *Osmorhiza*, and *Styrax*, Wood (1972) considered the closest relationship to be between the eastern Asian and eastern North American taxa. These hypotheses were based solely on morphological similarities, and no phylogenetic analyses were conducted.

Only a few genera occurring in eastern Asia and both eastern and western North America have been studied phylogenetically, including *Mitella* (Soltis *et al.*, 1991; Soltis and Kuzoff, 1995), *Hydrangea* (Soltis *et al.*, 1995), *Adiantum pedatum* L. (Paris, 1991; Paris and Hauffler, 1994), *Trillium* (Kato *et al.*, 1995), *Calycanthus* (Wen *et al.*, 1996), and *Aralia* sect. *Aralia* (Araliaceae) (Wen *et al.*, 1996). Phylogenetic studies have revealed that *Hydrangea* and *Mitella*, genera long considered "classic" examples of this floristic disjunction, are polyphyletic. Hence these genera are not useful models for the study of this disjunction. These findings clearly demonstrate the importance of a sound phylogenetic framework in inferring biogeographic history; comparison of taxa that are not truly closest relatives may lead to erroneous biogeographic conclusions. In contrast, phylogenetic analysis of *Trillium* indicated that, although monophyletic, phytogeographic hypotheses will be best considered within discrete subclades of the genus (Kato *et al.*, 1995), a task that requires greater taxon density than presently available. Analyses of *Calycanthus* (three species with one in each area) and *Aralia* sect. *Aralia* (nine species, one in eastern North America, one in western North

America, and seven in eastern Asia) suggest that species from eastern and western North America are sisters, which are in turn the sister of the Asian species. Clearly, more taxa showing this disjunct distributional pattern need to be examined phylogenetically to improve our understanding of the eastern Asia, eastern and western North America disjunction. We therefore conducted molecular phylogenetic analyses of four genera, the big-bracted dogwoods of *Cornus* (Cornaceae), *Boykinia*, and *Tiarella* (Saxifragaceae), and *Trautvetteria* (Ranunculaceae), groups that currently occur only in eastern Asia, eastern North America, and western North America. Our goals were to: (1) estimate phylogenies for these four genera, (2) determine whether there are general phylogenetic patterns for diverse genera showing this distributional pattern; (3) construct area cladograms based on the molecular phylogenies of these genera to elucidate relationships among eastern Asia, eastern North America, and western North America; (4) gain initial insight into the geographic origin of taxa exhibiting this disjunction to improve our understanding of the North Temperate Flora.

MATERIALS AND METHODS

Molecular Phylogenetic Analyses

Several DNA regions were sequenced to infer phylogeny, although not all regions were analyzed for each genus: the chloroplast genes *rbcl* and *matK* and the internal transcribed spacers of the nuclear ribosomal RNA genes (ITS regions). Methods of amplification and sequencing followed Morgan and Soltis (1993) and Xiang *et al.* (1993) for *rbcl*, Johnson and Soltis (1994, 1995) for *matK*, and Baldwin (1992) and Soltis and Kuzoff (1995) for ITS. Previously published cpDNA restriction site variation was also used for phylogeny estimation (Soltis *et al.*, 1991, 1993; Xiang *et al.*, 1996).

The general methods of phylogenetic analysis were as follows unless specified in the figure legends. For each genus, several outgroups were used. In addition, both broad phylogenetic analyses involving related genera and more focused analyses involving only the study genera were conducted. Outgroups for the narrow analyses were chosen based on results from broad analyses that identified sister taxa of the study group. The pattern of phylogenetic relationships within each genus was the same in all analyses.

Data were analyzed with PAUP 3.1.1 (Swofford, 1993) using Fitch parsimony. For *Cornus* and *Boykinia*, a branch-and-bound search was conducted, and for *Tiarella* and *Trautvetteria*, the exhaustive search option was used. To evaluate the relative support of relationships revealed in the most parsimonious trees, bootstrap analysis (Felsenstein, 1985) with 100 replicates was performed, and Bremer support (or decay index) (Bremer, 1988) was estimated following Eer-

nisse and Kluge (1993). Additional background data for each genus analyzed are presented below.

Cornus. *Cornus* (the dogwoods) is a genus of approximately 55 species, within which the big-bracted dogwoods form a monophyletic group (Xiang *et al.*, 1993, 1996; Xiang and Soltis, in press). This group consists of seven closely related but morphologically variable species, with *C. florida* L. distributed in eastern North America, *C. disciflora* Moc. & Sessé in Central America, *C. nuttallii* Audubon in western North America, and *C. capitata* Wall., *C. kousa* Hance, *C. hongkongensis* Hemsley, and *C. multinervosa* (Pojarkova) Q. Y. Xiang in eastern Asia (Xiang, 1987). The morphological variation among species of the big-bracted dogwoods parallels their geographic distributions. All eastern Asian species are morphologically very similar and produce compound fruits in heads. These species also form a distinct clade in a recent phylogenetic analysis using morphological characters (Murrell, 1993). All American species produce separate fruits in clusters. *Cornus florida* from eastern North America and the eastern Asian species have four large, showy bracts, whereas *C. nuttallii* from western North America has six large showy bracts; *C. disciflora* from Central America has four bracts that abscise early without having expanded.

Phylogenetic analyses employed cpDNA restriction sites and a combined data set of *rbcL*-*matK* sequences-cpDNA restriction sites (1440 bp of *rbcL*, 1212 bp of *matK*, and 242 restriction sites). All three American species, *C. florida*, *C. disciflora*, and *C. nuttallii*, and two representatives of the eastern Asian clade, *C. capitata* and *C. kousa*, were included in the analysis of cpDNA restriction sites. All of these species except *C. disciflora* (which was not included because *rbcL* and *matK* could not be amplified due to the degradation of DNA subsequent to the earlier restriction site study)

were then included in a combined analysis of *rbcL*-*matK* sequences and cpDNA restriction sites. All taxa included in this analysis of combined data sets have at least two of the three data sets available. On the basis of the results of previous studies of *Cornus* and its closest relatives (Xiang *et al.*, 1996, 1998a), two dwarf dogwoods (*C. canadensis* and *C. unalaschkensis*) and three cornelian cherries (*C. mas*, *C. officinalis*, and *C. sessilis*) were selected as outgroups. Molecular data were from our recent studies (Xiang *et al.*, 1993, for *rbcL* sequences; 1996, for restriction sites; and 1998a, for *matK* sequences), except for the *rbcL* sequence of *Cornus nuttallii*, which was generated in this study (Table 1).

Boykinia. *Boykinia* contains seven species, with *B. aconitifolia* Nutt. the only species from eastern North America, *B. lycoctonifolia* (Maxim.) Engl. the single species in eastern Asia, and five species, *B. intermedia* (Piper) G. N. Jones, *B. major* A. Gray, *B. occidentalis* Torrey & Gray, *B. rotundifolia* Parry, and *B. richardsonii* (Hook.) Rothrock, in western North America (Gornall and Bohm, 1985; Soltis *et al.*, 1993). The monophyly of *Boykinia* has been demonstrated in several previous phylogenetic analyses of Saxifragaceae s. s. (Soltis *et al.*, 1993, 1996; Johnson and Soltis, 1995). We focused on relationships within the genus and conducted phylogenetic analyses using cpDNA restriction sites and nuclear ITS sequences separately. All species of the genus except *B. richardsonii*, a high polyploid for which suitable DNA was not available (see Soltis *et al.*, 1993), were included. Chloroplast DNA restriction site data were from Soltis *et al.* (1993), and ITS sequences were from Soltis *et al.* (1996). *Suksdorfia* and *Bolandra* were used as outgroups based on the results of previous analyses of Saxifragaceae s. s. (Soltis *et al.*, 1993). Because the cpDNA and ITS trees revealed different

TABLE 1

Sources of Material and Molecular Data Generated in This Study for Phylogenetic Analyses

Taxa	Data	Voucher or sources	Genebank Accession Numbers
<i>Cornus</i> (Cornaceae)			
<i>C. nuttallii</i> Audubon	<i>rbcL</i>	Arn. Arb. No. 573-73-A (WS)	AF006833
<i>Tiarella</i> (Saxifragaceae)			
<i>T. polyphylla</i> D. Don	ITS-1, ITS-2	<i>Soltis 2555</i> (WS)	AF006834, AF015444
<i>Trautvetteria</i> (Ranunculaceae)			
<i>T. carolinensis</i> (Walt.) Vail	ITS-1, ITS-2 <i>matK</i>	<i>Hoot 92018</i> , F, Hoot Garden <i>Hoot 92018</i> , F, Hoot Garden	U96035, U96036 AF007946
<i>T. grandis</i> Nutt.	ITS-1, ITS-2 <i>matK</i>	UCBG 82.1322 UCBG 82.1322	U96039, U96040 AF007945
<i>T. japonica</i> Sieb. & Zucc.	ITS-1, ITS-2 <i>matK</i>	<i>Ohba A3018</i> , Nikko Bot. Gard., Japan <i>Ohba A3018</i> , Nikko Bot. Gard., Japan	UU96037, U96038 AF007944
<i>Myosurus</i> (Ranunculaceae)			
<i>M. minimus</i> L.	ITS-1, ITS-2 <i>matK</i>	<i>Chase 532</i> , K, RBG, Kew <i>Chase 532</i> , K, RBG, Kew	U96041, U96042 AF007947

Note. Abbreviations of UCBG, Arn. Arb., and RBG represent University of California Botanical Garden, the Arnold Arboretum, and the Royal Botanic Gardens, Kew, respectively. Xiang and Soltis vouchers are deposited at WS.

relationships among the North American taxa, the two data sets were not combined into a single analysis.

Tiarella. *Tiarella* has three species: *T. cordifolia* L. in eastern North America, *T. trifoliata* L. with three varieties (var. *trifoliata*, var. *unifoliata* (Hook.) Kurtz., and var. *laciniata* (Hook.) Wheel.) in western North America, and *T. polyphylla* D. Don in eastern Asia. Previous analyses of several chloroplast markers suggested that *Tiarella* is polyphyletic (Soltis *et al.*, 1991; Xiang, 1995). These analyses indicate that the eastern North American species (*T. cordifolia*) is more closely related to *Heuchera* than it is to the other species of *Tiarella*. Morphology and nuclear ITS sequence data suggest, in contrast, that *Tiarella* is monophyletic (Soltis and Kuzoff, 1995). The totality of data indicates that *T. cordifolia* has apparently captured the chloroplast of a species of *Heuchera* and that the cpDNA-based topologies do not reflect organismal relationships. As reviewed elsewhere (Soltis and Kuzoff, 1995), this chloroplast capture scenario is further suggested by the presence of naturally occurring intergeneric hybrids between *T. cordifolia* and species of *Heuchera*.

Because the chloroplast genome is providing an inaccurate assessment of organismal relationships in *Tiarella*, our phylogenetic hypothesis is based solely on nuclear sequences. In this study, we conducted phylogenetic analyses of the three species of *Tiarella* using ITS sequences. A broad analysis of ITS sequences for the entire *Heuchera* group (a clade comprising *Tiarella* and eight other genera of Saxifragaceae; Soltis *et al.*, 1991, 1993) was first performed using *Darmera* and *Rodgersia* as outgroups. Sequences of ITS were from Soltis and Kuzoff (1995), except that for *T. polyphylla*, which was obtained in this study. Because our broad analysis of ITS sequences indicates that *Tiarella* is monophyletic (tree not shown), a second narrow analysis of ITS sequences was conducted for *Tiarella* using an exhaustive search. *Conimitella williamsii*, *Mitella diversifolia*, and *Mitella stauropetala* were used as outgroups for this focused analysis because of their close phylogenetic relationship to *Tiarella*.

Trautvetteria. *Trautvetteria* consists of three species: *T. carolinensis* (Walt.) Vail from eastern North America, *T. grandis* Nutt. from western North America, and *T. japonica* Sieb. & Zucc. from eastern Asia. Sequences of *matK* and ITS were obtained for these three species, as well as for the outgroup *Myosurus minimus* L. *Myosurus* was chosen as the outgroup based on the results of broad phylogenetic analyses of Ranunculaceae (Hoot, 1995, personal communication) that indicate that *Myosurus* is a close relative of *Trautvetteria*. Of the sequencing primers designed by Johnson and Soltis (1994, 1995) for *matK*, only two, 1168R and 1470R, could be used in this study. These primers provided approximately 600 base pairs (bp) of *matK* sequence for the species studied. Sequences of

both the ITS-1 and ITS-2 regions were also obtained for *Trautvetteria* and *Myosurus*. Separate analyses of *matK* and ITS sequences were performed. Because ITS and *matK* sequences yielded identical topologies, we combined these data sets and conducted subsequent analyses on the combined data matrix.

Area Cladograms

Area cladograms were constructed for the big-bracted dogwoods of *Cornus*, *Boykinia*, *Tiarella*, and *Trautvetteria* by replacing terminal taxon names on a cladogram with the distributional area of the respective taxon (see Platnick and Nelson, 1978; Nelson and Platnick, 1981; Humphries and Parenti, 1986). The area cladograms for the four genera were compared, and a congruent general area cladogram was constructed to show the area relationships among eastern Asia, eastern North America, and western North America.

Center of Origin

Establishing the center of origin for a group without phylogenetic information (e.g., using the "age-and-area" hypothesis; see Brown and Gibson, 1983; Futuyma, 1986) is often problematic. We attempted to do this using a phylogenetic approach using MacClade 3.05 (Maddison and Maddison, 1992) to map the character "area" onto the topology in the most parsimonious manner to infer the geographic area of the ancestor (the root node) of each study group. In many cases, however, conducting this mapping exercise was so straightforward that it could be done by hand.

Topologies resulting from broad phylogenetic analyses that clearly depict the sister group of the study group (these trees are not shown here) were used as the basis for determining the outgroups used in the topology for "area" mapping. For *Cornus*, the topologies resulting from broad analyses of cpDNA restriction sites for the entire genus and a combined *rbcL*-*matK* data set representing the entire Cornaceae were used for determining the sister group of the big-bracted dogwoods (Xiang *et al.*, 1993, 1998a). For *Boykinia*, both the ITS and cpDNA restriction site topologies for the entire "*Boykinia* group" (a clade of six genera) (Soltis *et al.*, 1993, 1996), as well as cpDNA restriction site and *matK* topologies representing the entire Saxifragaceae s. s. (Soltis *et al.*, 1993, 1996), were used. For *Tiarella*, we used the ITS tree for the entire *Heuchera* group (a clade of nine genera) (Soltis and Kuzoff, 1995). For *Trautvetteria*, we used the combined *atpB*-*rbcL*-18S rDNA tree for Ranunculaceae (Hoot, 1995). The geographic distribution of the outgroup was assigned based on the known distribution of all members of the outgroup (not just the several outgroup species depicted in the figures). Therefore, geographic areas of species shown in the phylogenetic trees do not necessarily represent the geographic areas of the entire outgroup genera. For example, the sister group of the

big-bracted dogwoods was indentified as the dwarf dogwoods by the broad analysis for the genus. The dwarf dogwoods have four species distributed circum-boreally and extending to Burma (see Xiang *et al.*, 1996). In the phylogenetic trees (Fig. 1), only two North American species, *C. canadensis* and *C. unalaschensis*, are shown. In the geographic "area mapping" analysis, the geographic area of the outgroup of the big-bracted dogwoods was considered as circumboreal, instead of North America.

We also performed this same exercise for *Calycanthus* and *Aralia* sect. *Aralia*, taxa investigated phylogenetically by others (Wen *et al.*, 1996). For *Calycanthus*, the broad phylogenetic analysis of *rbcL* sequences representing magnoliids (Qiu *et al.*, 1993) was employed. For *Aralia* sect. *Aralia*, the cpDNA restriction site topology of the section, using *Aralia* sect. *Dimorphanthus* as the sister group (Wen *et al.*, 1996), was used.

RESULTS

Phylogenetic Relationships

Cornus. Phylogenetic analysis of cpDNA restriction sites for the big-bracted dogwoods reveals relationships (Fig. 1a) identical to those found in broad analyses of the entire genus *Cornus* (Xiang *et al.*, 1996). That is, the eastern Asian species sampled (*C. capitata*, *C. kousa*) form a clade and are the sister group of a clade containing all American species. Analysis of the combined *rbcL*-*matK*-cpDNA restriction site data set for the big-bracted dogwoods reveals the same general topology observed with restriction sites alone (although now *C. disciflora* is omitted; see above) (Fig. 1b). The

eastern Asian species (*C. capitata*, *C. kousa*) form a clade and are sister to the North American species analyzed, *C. florida* and *C. nuttallii*.

Boykinia. The phylogenetic analyses of cpDNA restriction sites and ITS sequences for *Boykinia* both place the eastern Asian species, *B. lycoctonifolia*, as sister to a clade that comprises all of the North American species (Figs. 2a and 2b). Within this North American clade, the ITS tree places *B. rotundifolia* from western North America as the sister to a clade containing *B. aconitifolia* from eastern North America and the remaining western North American species (Fig. 2b), whereas the cpDNA tree shows a trichotomy among *B. aconitifolia*, *B. rotundifolia*, and the remaining species (Fig. 2a).

Tiarella and Trautvetteria. The phylogenetic analysis of ITS sequences for *Tiarella* and analyses of ITS and *matK* sequences for *Trautvetteria* indicate that, for both genera, the two North American species are more closely related to each other than either is to the eastern Asian species (Figs. 3 and 4).

Area Cladograms

The four area cladograms based on the molecular phylogenies of the big-bracted species of *Cornus*, *Tiarella*, *Boykinia*, and *Trautvetteria* are congruent in showing that eastern and western North America are more closely related to each other than either is to eastern Asia (Fig. 5).

Center of Origin

Our attempts to infer the centers of origin of these four genera by mapping geographic area onto topo-

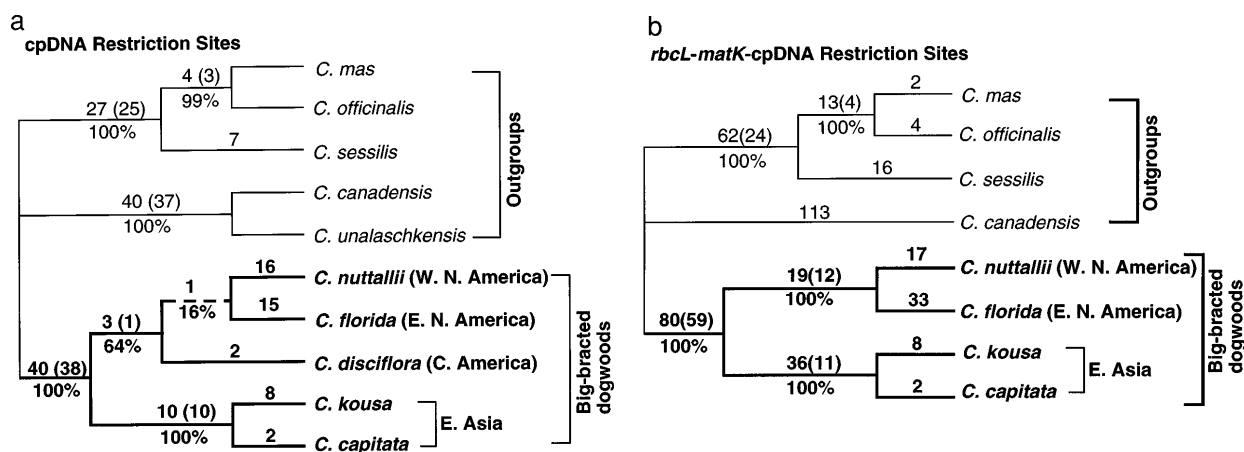


FIG. 1. Phylogenetic analyses of the big-bracted dogwoods of *Cornus*. (a) One of the three shortest trees resulting from exhaustive search of the cpDNA restriction site data using cornelian cherries (*C. mas*, *C. officinalis*, and *C. sessilis*) and the dwarf dogwoods (*C. canadensis* and *C. unalaschensis*) as outgroups (length = 175 steps; CI = 0.961, excluding uninformative characters, RI = 0.982). Ingroup is designated in bold. The number of restriction site mutations supporting each clade is given above each branch, along with the decay value (in parentheses); bootstrap values are given below branches. The dashed line represents the branch not recognized in all the three shortest trees. (b) The single shortest tree resulting from the combined analysis of *rbcL*-*matK* sequences and restriction sites of cpDNA for the big-bracted dogwoods (length = 405 steps; CI = 0.938, excluding uninformative characters, RI = 0.962). Ingroup are designated in bold. Numbers on branches are as designated for (a).

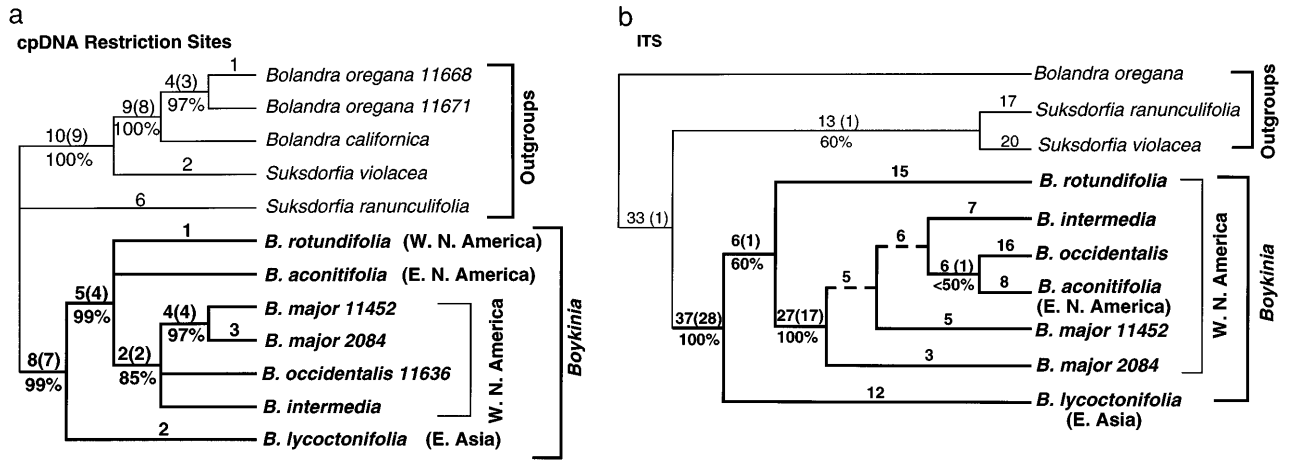


FIG. 2. Phylogenetic analyses of *Boykinia*. (a) The single shortest tree resulting from phylogenetic analysis of cpDNA restriction sites for *Boykinia* (length = 57 steps; CI = 0.911, excluding uninformative characters, RI = 0.965). Ingroup are designated in bold. Numbers on branches are as designated for Fig. 1a. (b) One of the two shortest trees resulting from phylogenetic analysis of ITS sequences for *Boykinia* (length = 236 steps; CI = 0.747, excluding uninformative characters, RI = 0.802). Ingroup are designated in bold. Numbers on branches are as designated for Fig. 1a. Dashed lines represent branches not recognized in both shortest trees; all other branches are present in both most parsimonious trees.

gies have produced several outcomes that are largely correlated with the geographic distribution of the sister group of the study group (see Figs. 1–4). For example, all broad phylogenetic analyses of the *Boykinia* group, as well as of Saxifragaceae s. s. (Soltis *et al.*, 1993, 1995, 1996), indicate that the sister taxa of *Boykinia* are *Bolandra* and *Suksdorfia*, genera confined to western North America. Hence, for *Boykinia*, the area-mapping suggests that the center of origin is western North America. In *Cornus*, in contrast, the immediate sister group of the big-bracted dogwoods is the dwarf dogwoods, taxa with a circumboreal distribution. Thus, the place of origin for the big-bracted dogwoods is equivocal. Similarly, for *Trautvetteria*, the sister genera, *Myosurus* and *Ranunculus* (Hoot, 1995), are both widely distributed in the Northern Hemisphere. Thus, once again the geographic origin for the genus is indicated as uncertain. For *Tiarella*, no well-supported sister group has been identified (Soltis and Kuzoff, 1995). Finding

the sister group of *Tiarella* is complicated because extensive chloroplast capture in the clade of genera to which it belongs has rendered cpDNA data useless for phylogenetic inference, and ITS sequence data provide insufficient resolution of the relationships of *Tiarella*. Thus, the center of origin for the genus also remains uncertain.

For *Calycanthus*, the sister group is *Chimonanthus* (the only other member of Calycanthaceae), a genus restricted to eastern Asia (Qiu *et al.*, 1993). Thus, Asia is implicated as the center of origin for *Calycanthus*. The sister group of *Aralia* sect. *Aralia* is *Aralia* sect. *Dimorphanthus* (Wen *et al.*, 1996), which occurs in both eastern Asia and eastern North America. Although the site of origin of section *Aralia* is equivocal based on the

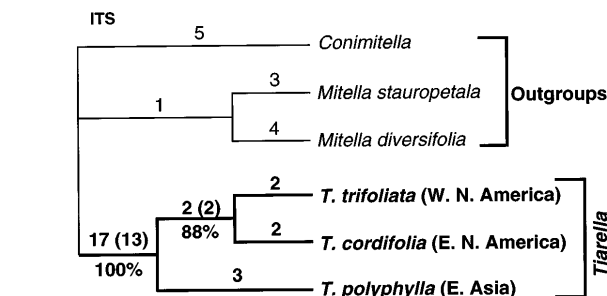


FIG. 3. The single shortest tree resulting from phylogenetic analysis of ITS sequences of *Tiarella* (length = 39 steps, CI = 0.952, excluding uninformative characters, RI = 0.971). Ingroup are designated in bold. Numbers on branches are as designated for Fig. 1a.

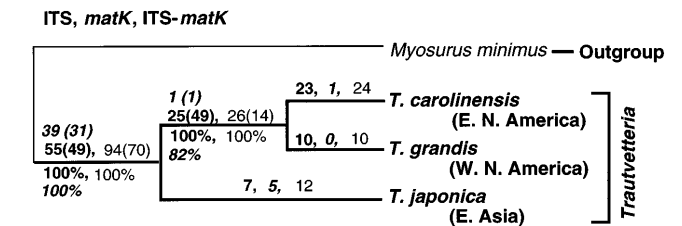


FIG. 4. The identical shortest trees resulting from phylogenetic analyses of ITS, *matK*, and combined ITS–*matK* sequences, respectively, for *Trautvetteria*. The single ITS shortest tree has a length of 120 steps, a CI of 0.974, excluding uninformative characters, and a RI of 0.971; the single *matK* shortest tree has a length of 46 steps, a CI of 1.000, excluding uninformative characters, and a RI of 1.000; the single ITS–*matK* tree has a length of 166 steps, a CI of 0.983, excluding uninformative characters, and a RI of 0.982. Ingroup are designated in bold. Numbers on branches are as designated for Fig. 1a; those in italic are for the *matK* tree and those in roman are for the ITS–*matK*.

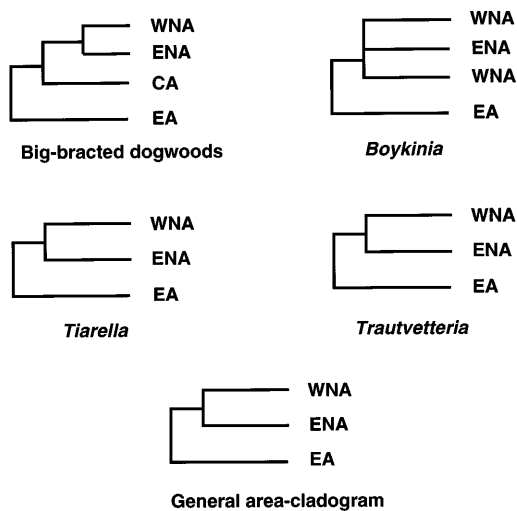


FIG. 5. Area cladograms derived by replacing terminal taxon names on a phylogenetic tree with the distributional area of the respective taxon. WNA, western North America; ENA, eastern North America; CA, Central America; and EA, eastern Asia.

distribution of its sister group, an origin in Asia is suggested by the distributions of the basal lineages within section *Aralia*. The first-branches of section *Aralia* terminate in species from eastern Asia.

DISCUSSION

Pattern of relationships. Phylogenetic analyses for four genera (the big-bracted species of *Cornus*, *Boykinia*, *Tiarella*, and *Trautvetteria*) occurring disjunctly in eastern Asia, eastern North America, and western North America reveal a single pattern of biogeographic relationships among species (Figs. 1–4). In all four genera, species from eastern North America are the sister group to species from western North America, and the Asian species are the sister group to all North American species. In contrast, intuitive inferences (i.e., nonphylogenetic) based on other sources of data suggested alternative relationships or did not resolve relationships within each genus. For example, the three species of *Tiarella* have very similar karyotypes, and each has a unique set of flavonoid constituents (Soltis and Bohm, 1984). These characters did not provide any synapomorphies within *Tiarella* and suggested that all three species are equally distinct. Noncladistic inferences of relationships in *Trautvetteria* based on morphological characters suggested that the eastern Asian species (*T. japonica*) and the western North American species (*T. grandis*) are more closely related to each other than either is to the eastern North American species (*T. carolinensis*) (Tamura, 1983). This conclusion conflicts with the phylogenetic analyses presented here.

The pattern of phylogenetic relationships reported

here for four genera is similar to that for *Aralia* sect. *Aralia* (Araliaceae) (Wen *et al.*, 1996), *Calycanthus* (Calycanthaceae) (Wen *et al.*, 1996), and *Adiantum pedatum* L. (Adiantaceae) (Paris, 1991; Paris and Haufler, 1994), all with species or populations occurring in eastern Asia, eastern North America, and western North America. Therefore, phylogenetic analyses indicate that the big-bracted species of *Cornus*, *Boykinia*, *Tiarella*, *Trautvetteria*, *Aralia*, *Calycanthus*, and *Adiantum pedatum*, a diverse array of taxa, all show a similar pattern of relationships among species: the eastern and western North American species consistently are sisters, with the Asian representative(s) in turn sister to the North American clade.

It is not clear how general the phylogenetic pattern observed here may be among the approximately 30 genera that exhibit this disjunction. Additional phylogenetic analyses of taxa exhibiting this disjunction are therefore encouraged. It is also noteworthy that a large number of genera exhibit a slightly more complex disjunction pattern than that discussed here. That is, in addition to having representatives in eastern Asia and both eastern and western North America, some genera also have member taxa in Europe (e.g., *Aesculus*, *Chrysosplenium*). Nonetheless, several recent phylogenetic analyses indicate that a phylogenetic pattern similar to that noted here may exist for both *Aesculus* L. (Hippocastanaceae; ITS and *matK* sequence data; Xiang *et al.*, in 1998b) and *Chrysosplenium* (Hibsch-Jetter *et al.*, unpublished) in that the North American species form a clade that is sister to the Old World taxa.

Congruent biogeographic pattern versus shared phylogeographic history. Congruence between phylogenetic topologies and geographic distribution of lineages has been used as a basis for inference of shared historical biogeography (Platnick and Nelson, 1978; Nelson and Platnick, 1981; Humphries and Parenti, 1986; Sober, 1988; Oosterbroek and Arntzen, 1992). Based on this principle, the consistent pattern of phylogenetic relationship observed among the seven diverse taxa compared here may indicate a common biogeographic history. These data can, for example, be viewed as support for the long-standing hypothesis that the disjunction in eastern Asia, eastern North America, and western North America represents the fragmentation of a once-continuous plant community (e.g., Gray, 1878; Hu, 1935; Chaney, 1947, 1959; Li, 1952; Wood, 1971, 1972). For example, Gray (1878) proposed that a continuous flora existed across the high latitudes of the Northern Hemisphere through the Bering Strait. This flora was subsequently broken up by Pleistocene glaciation, resulting in the disjunction of taxa on different continents. Gray's hypothesis was further developed by Chaney (1947, 1959) who proposed the "Arcto-Tertiary Geoflora" concept to explain the floristic similarities between eastern Asia and North America. Chaney envisioned a common biota

with a taxonomic composition similar to that of the modern North Temperate Flora occupied the high latitudes of Eurasia and North America in the early Tertiary. This Geoflora subsequently spread southward with little changes in its composition. During the late Tertiary and Quaternary, most of this flora became extinct in Europe, western Asia, and central North America, resulting in the disjunctions in the Northern Hemisphere observed today. Updated paleontological evidence, however, contradicts the Arcto-Tertiary Geoflora concept (Wolfe, 1969, 1972, 1975, 1985). The new paleontological evidence suggests the presence of an early Tertiary "boreotropic flora" with dynamic taxonomic composition in the Northern Hemisphere (Wolfe, 1969, 1972, 1975, 1985; see also Tiffney, 1985a, 1985b) regarded the intercontinental similarities of the North Temperate Flora to be the result of the spreading of the boreotropic flora. This boreotropic flora developed into what has been termed a "Mixed Mesophytic forest" (a warm-temperate type of vegetation) in the mid-Tertiary (Wang, 1961; Tiffney, 1985a); this forest was once more or less continuously distributed throughout the Northern Hemisphere, but later climatic and geological changes resulted in its range restriction and ultimately the disjunction observed today (Li, 1952; Wood, 1971, 1972; Graham, 1972; Wolfe, 1972, 1981; Hsu, 1983; Tiffney, 1985a, 1985b). Thus, the extant eastern Asian-eastern and western North American disjuncts are considered as some of the remnants of this mixed mesophytic forest (Li, 1952; Wood, 1971, 1972; Graham, 1972; Hsu, 1983). Under this hypothesis, the pattern of phylogenetic relationship observed suggests that the eastern and western North American disjunction occurred after the initial eastern Asian-North American isolation. Thus, one of the first disruptions in the continuous Mixed Mesophytic forest would have been the separation of Eurasia from North America due to continental drift. Various geologic and climatic changes later eliminated this flora from much of Europe, central Asia, and central North America (Graham, 1972; Leopold and MacGinitie, 1972; Tiffney, 1985a, 1985b), resulting in the disjunction in eastern Asia, eastern and western North America.

We stress, however, that congruence between phylogenies and geographic distributions does not necessarily indicate an identical phytogeographic history. The floristic disjunction in eastern Asia, eastern North America, and western North America might have originated at very different geological times in different genera, a phenomenon known as "pseudocongruence" (Cunningham and Collins, 1994), as pointed out by Tiffney (1985a; see also Wolfe, 1969, 1972, 1981; Li, 1972). In this regard, information on time of divergence between species in different genera is critical to determine whether the observed identical phylogenetic pat-

tern among the diverse seven taxa represents pseudocongruence or a shared floristic history.

Divergence time between lineages can be estimated using fossil evidence or a molecular clock (although difficulties with a molecular clock approach are well-known; see below). All seven taxa discussed herein except *Cornus* are either unknown as fossils (*Boykinia*, *Tiarella*, *Trautvetteria*, and *Adiantum pedatum*) or have an insufficient fossil record (*Aralia* sect. *Aralia* and *Calycanthus*) to estimate the divergence times between disjunct species (Collinson *et al.*, 1993; Taylor and Taylor, 1993; Friis *et al.*, 1994; Lang, 1994; Mai, 1994). Fossil fruit stones of separate-fruited, big-bracted dogwoods (like the extant North American species) appear in the mid-Oligocene and Miocene deposits in Europe, and fruit stones of compound-fruited, big-bracted dogwoods (like the extant eastern Asian species) were found in Europe in the Pliocene and in Japan in the Pleistocene (see Eyde, 1988). This fossil evidence suggests that the big-bracted dogwoods evolved approximately 30–32 million years before present (BP), during the Oligocene; fossil evidence also suggests that the eastern Asian and North American big-bracted dogwoods diverged at least five million years BP.

The molecular clock is based on the neutral theory of molecular evolution and assumes a constant rate of molecular evolution across lineages (Zuckerkandle and Pauling, 1965; Kimura, 1983). Although there is controversy regarding the neutral theory, and heterogeneous rates of molecular evolution have been documented in different lineages (e.g., Wilson *et al.*, 1990; Gaut *et al.*, 1992, 1993, 1996), a molecular clock may be useful for estimating divergence times if the clock can be calibrated with some confidence. An overall divergence rate of approximately 10^{-9} nucleotide substitutions per site per year has been estimated for the chloroplast genome (Zurawski *et al.*, 1984; Zurawski and Clegg, 1987). This molecular clock was used to estimate of time of divergence between the eastern Asia-eastern North America disjunct species pair in *Liriodendron* (Magnoliaceae) based on cpDNA restriction site data (Parks and Wendel, 1990). The estimate was congruent with that from fossil evidence (11–14 million years BP) (Parks and Wendel, 1990).

With the aim of gaining some insight into the time of divergence between species from eastern Asian and both eastern and western North America, we applied this molecular clock for three genera, *Cornus*, *Boykinia*, and *Calycanthus*, that have cpDNA restriction site data available. Assuming a sequence divergence rate of 0.1% per million years, the divergence time between the eastern Asian and North American big-bracted dogwoods is estimated to be 13.1 million years BP (in the mid-Miocene), based on a 2.61% sequence divergence estimated from restriction site mutations using the computer program SDE 1.2 (Wolfe and Wolfe,

1993) [The SDE program estimates sequence divergence using the Jukes and Cantor (1969) method with correction for multiple hits. Equations 5.3, 5.38, and 5.41 of Nei (1987), and 3.19, 3.32, and 3.33 of Li and Graur (1991) were implemented in the calculation.] This estimate falls in the time range estimated based on fossil evidence of *Cornus* as discussed above. Divergence time for the big-bracted dogwoods within the North American continent is estimated to be 9.7 million years BP (in the mid-Miocene) based on an average of 1.94% sequence divergence between species.

Using the same molecular clock and cpDNA restriction site data, the divergence time between the eastern Asian and North American species of *Boykinia* is estimated to be only 2.6 million years BP (end of the Pliocene) (0.51% sequence divergence between the two lineages); between the eastern and western North American species, the divergence time is 1.2 million years BP (in the Pleistocene) (0.23% sequence divergence). Similarly, for *Calycanthus*, the divergence time between the eastern Asian and North American species is estimated at 3.1 million years BP, in the Pliocene (0.61% sequence divergence), and that between the eastern and western North American species is 2.6 million years BP, or the end of the Pliocene (0.52% sequence divergence) (Wen *et al.*, 1996). Divergence times for *Trautvetteria*, *Tiarella*, and *Aralia* sect. *Aralia* cannot be estimated using this same clock because the occurrence of cpDNA capture involving *Tiarella* and *Heuchera* would distort the estimate, and cpDNA restriction site data are not available for *Trautvetteria* and *Aralia* sect. *Aralia*.

This analysis of divergence times suggests that species of *Cornus* diverged much earlier than species of *Boykinia* and *Calycanthus*, with species of *Boykinia* diverging most recently. The different divergence times estimated for species of these genera may indicate that either the isolation of species in these genera occurred at different geological times, or, alternatively, these genera simply have experienced unequal rates of molecular evolution.

To distinguish between these two possibilities, relative rate tests of cpDNA evolution need to be performed for *Cornus*, *Boykinia*, and *Calycanthus*. If the different divergence times estimated for *Cornus*, *Boykinia*, and *Calycanthus* were totally attributable to the rate differences, we would expect to see a much higher rate in *Cornus*, but similar rates in *Boykinia* and *Calycanthus*. Because assessment of homology of restriction site mutations in distantly related taxa becomes problematic, such tests are not feasible with restriction site data for these three genera that belong to three different subclasses of flowering plants (Asteridae, Rosidae, and Magnoliidae, respectively). We therefore conducted relative rate tests for the chloroplast gene *rbcl* in these genera. The tests were performed for both synonymous and nonsynonymous substitutions using the method of

Li and Bousquet (1992) and following Gaut *et al.* (1996) and Xiang *et al.* (1998a). The *rbcl* sequence of *Ceratophyllum*, a genus that is the sister to all other flowering plants in the *rbcl* sequence analysis of Chase *et al.* (1993), was first used as the reference sequence for the tests. Because *Ceratophyllum* has a relatively long branch (44) in the *rbcl* tree of Chase *et al.* (1993), its sister relationship to all other flowering plants may be questionable. We therefore performed the tests using a second reference sequence, the *rbcl* of *Nymphaea*, a member of "Paleoherb II" in the *rbcl* analysis of Chase *et al.* (1993), to compare the results.

When *Ceratophyllum* was used as the reference taxon, the rates of evolution of *rbcl* do not differ significantly between *Cornus* and *Boykinia* for either synonymous or nonsynonymous substitutions (Table 2), but do differ significantly between *Calycanthus* and *Boykinia* for both synonymous and nonsynonymous substitutions (Table 2). The rate of synonymous substitutions in *Calycanthus* is lower than that in *Boykinia*, but the rate of nonsynonymous substitutions is the reverse (Table 2). A similar situation exists between *Cornus* and *Calycanthus* (see Table 2). *Cornus* has a higher synonymous substitution rate, but a lower nonsynonymous substitution rate compared to *Calycanthus*, suggesting that the total substitution rates between these genera may not be significantly different.

TABLE 2

Results of Relative Rate Tests at *rbcl* for *Cornus*, *Boykinia*, and *Calycanthus*

Taxa compared		Ks		Ka		Kt	
1	2	K1-K2	Test statistic	K1-K2	Test statistic	K1-K2	SE
Reference taxon: <i>Ceratophyllum</i>							
A	B	0.092	2.288	-0.005	-1.083	0.0124	0.00845
A	C	-0.001	-0.261	0.006	1.569	0.0032	0.00870
B	C	-0.102	-2.691	0.011	2.597	-0.0092	0.00835
Reference taxon: <i>Nymphaea</i>							
A	B	0.078	1.917	0.004	0.763	0.0180	0.00875
A	C	0.008	0.750	0.009	2.127	0.0121	0.00895
B	C	-0.050	-1.349	0.005	1.167	-0.0059	0.00840

Note. *Ceratophyllum demersum* L. and *Nymphaea odorata* Aiton were used as reference taxa for the tests. A, *Cornus canadensis*; B, *Calycanthus floridus*; and C, *Boykinia rotundifolia*. The *rbcl* sequences of taxa used in the tests were from Xiang *et al.* (1993) for *Cornus*, from Chase *et al.* (1993) for *Ceratophyllum*, *Nymphaea*, and *Calycanthus*, and from Soltis *et al.* (1993) for *Boykinia*. K1-K2, Difference in the weighted number of substitutions per site between lineage 1 and lineage 2; Ks, synonymous substitutions; Ka, nonsynonymous substitutions; and Kt, Total substitutions. For Ks and Ka, a test statistic with absolute value >1.96 (indicated in bold) is significant at the 0.05 level. For Kt, a value of K1-K2 greater than two times of the standard error is considered significant at the 0.05 level.

Our subsequent tests for total substitution rates using the Kimura-2-parameter distance method of MEGA 1.0 (Kumar *et al.*, 1993) following Li (1997, pages 218–219) indeed showed no significant differences among the three genera (Table 2). When *Nymphaea* was used as the reference taxon, both synonymous and nonsynonymous substitution rates do not differ significantly among the three genera except for the nonsynonymous substitution rate between *Cornus* and *Boykinia*, which differ significantly (Table 2). These results apparently do not fit the expectation that the longer divergence estimate for *Cornus* is due to an elevated rate of *rbcL* evolution. Instead, the different divergence times obtained for *Cornus*, *Boykinia*, and *Calycanthus* may provide evidence for pseudocongruence; that is, these three genera obtained their disjunct distributions in eastern Asia, eastern North America, and western North America at different geological times.

Therefore, the seven diverse genera examined in this study do not necessarily share an identical biogeographic history although they exhibit the same pattern of phylogenetic relationship among species. Some genera, such as the big-bracted dogwoods, may be relicts of the Mixed Mesophytic forest and were once continuously distributed in the Northern Hemisphere, given that the species diverged near the middle Miocene. The big-bracted dogwoods probably obtained their distribution in the two continents via the Bering land bridge or via a series of island "stepping stones" in the North Atlantic (see Tiffney, 1985a, 1985b). Others, such as *Calycanthus* (producing seeds enclosed in fleshy receptacles that may be eaten by birds) and *Boykinia* (bearing tuberculate seeds that may become caught in feathers of birds), may have obtained their disjunct distributions through long-distance dispersal, given that species in these genera were isolated relatively recently based on the molecular clock. Alternatively, these two genera may have obtained their disjunct distributions through gradual migration across the Bering land bridge, followed by long-distance dispersal within North America. The Bering land bridge was periodically available for exchanges of plants between eastern Asia and western North America almost throughout the Tertiary (until 3.5 million years BP; see Allen, 1983; Tiffney, 1985a, 1985b; Cunningham and Collins, 1994). The North Atlantic bridge was, in contrast, functional until only the late Eocene, although a series of island "stepping stones" existed in the North Atlantic until the early Oligocene (Tiffney, 1985a). Exchanges of elements between eastern and western North American floras ceased in the Miocene (Tiffney, 1985a). Our molecular data thus do not refute the view that the floristic similarities between eastern Asia and North America have a complex history involving multiple historical events (both vicariant and dispersal) at different geological times in different taxa (Wolfe, 1969, 1972, 1975, 1981, 1985; Li, 1972; Tiffney, 1985a).

Center of origin. Although we have identified a consistent pattern of phylogenetic relationship among six genera of flowering plants and a fern species found in eastern Asia and both eastern and western North America, our analyses suggest that the continent of origin for these genera may differ or simply be ambiguous. We preface this discussion by stressing the vagaries and difficulties of inferring a center of origin based on the mapping of geographic distribution onto phylogenies. For *Calycanthus* and *Aralia* sect. *Aralia*, the continent of origin appears to be Asia. Conversely, broad phylogenetic analyses of the *Boykinia* group, as well as of the entire Saxifragaceae s. s. (Soltis *et al.*, 1993, 1995, 1996), indicate that for *Boykinia*, western North America is the center of origin. For many of the genera examined, however, the results are equivocal. For example, the sister group of *Trautvetteria* is a clade composed of *Ranunculus* and *Myosurus*, two genera that are widely distributed in both the Northern and Southern Hemispheres and for which phylogenetic relationships among species are poorly understood. Similarly, the sister group of the big-bracted dogwoods has a circumboreal distribution; thus the center of origin is equivocal. For *Tiarella*, no well-supported sister group has yet been identified in several phylogenetic analyses; hence, no center of origin can yet be determined.

Fossil evidence would permit further evaluation of the biogeographic hypothesis on the center of origin inferred from phylogenetic analyses of molecular data. As mentioned above, none of the seven taxa discussed herein except *Cornus* has an adequate fossil record.

CONCLUSIONS

Six genera of angiosperms, *Boykinia*, *Tiarella*, *Trautvetteria*, the big-bracted dogwoods of *Cornus*, *Aralia* sect. *Aralia*, and *Calycanthus*, and the fern species, *Adiantum pedatum*, show a similar pattern of biogeographic relationships: the eastern and western North American species/populations are sister groups; this clade is in turn the sister of the eastern Asian species/populations. Under the principle of historical biogeography (i.e., congruence between phylogenetic topologies and geographic distribution of lineages indicates shared biogeographic histories), the phylogenetic data presented here lend support to the vicariant hypothesis that this well-known floristic disjunction represents the fragmentation of a more or less continuous Mixed Mesophytic forest community that existed during the Tertiary. We caution, however, that congruence between phylogenies and geographic distributions does not necessarily indicate an identical phylogeographic history. The floristic disjunction involving eastern Asia, eastern North America, and western North America may have originated at very different geological times in different genera (e.g., Li, 1972; Tiffney,

1985b), a phenomenon known as "pseudocongruence" (Cunningham and Collins, 1994). Our initial attempts to evaluate this possibility using a molecular clock to estimate divergence times do not refute the hypothesis of pseudocongruence. Other taxa displaying this disjunction should be examined for phylogenetic relationships and time of divergence between disjunct species, and additional efforts should be made to evaluate the possibility of pseudocongruence.

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