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 (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,
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 Maule, 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, phylogenetic hypotheses will be best considered within discrete subspecies 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 Er-
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. unalaschakensis) 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 Bohn, 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>
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<th>Genebank Accession Numbers</th>
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<tr>
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<td>U96035, U96036</td>
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Note. Abbreviations of UCBG, Arn. Arb., and RBG represent University of California Botanical Garden, the Arnold Arboretum, and the Royal Botanical 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. lasiophylla (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 Hauchera 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 Hauchera 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. Conimicella williamsii, Mitella diversifolia, and Mitella stauroptala 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 minus 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 identified as the dwarf dogwoods by the broad analysis for the genus. The dwarf dogwoods have four species distributed circumferentially and extending to Burma (see Xiang et al., 1996). In the phylogenetic trees (Fig. 1), only two North American species, C. canadensis and C. unalaschakensis, are shown. In the geographic “area mapping” analysis, the geographic area of the outgroup of the big-bracted dogwoods was considered as circumferential, instead of North America.

We also performed the 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. nuttalii.

**Boykinia.** The phylogenetic analyses of cpDNA restriction sites and ITS sequences for Boykinia both place the eastern Asian species, B. lycocotonifolia, 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 topolo-

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**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. unalaschakensis) 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).
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 (Qi 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. 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.](image1)

![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.](image2)

![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.](image3)
Aralia. The first-branches of section Aralia terminate in species from eastern Asia. 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. (Adianthaceae) (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 phytogeographic 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). 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 pattern 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 (Zuckerkandl 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^{-8} 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 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,
and nonsynonymous substitutions using the method of Jukes and Cantor (1969) 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 are 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

<table>
<thead>
<tr>
<th>Taxa compared</th>
<th>Ks</th>
<th>Ka</th>
<th>Kt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference taxon: Ceratophyllum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A B</td>
<td>0.092</td>
<td>2.288</td>
<td>-0.005</td>
</tr>
<tr>
<td>A C</td>
<td>-0.001</td>
<td>-0.261</td>
<td>0.006</td>
</tr>
<tr>
<td>B C</td>
<td>-0.102</td>
<td>-2.691</td>
<td>0.011</td>
</tr>
<tr>
<td>Reference taxon: Nymphaea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A B</td>
<td>0.078</td>
<td>1.917</td>
<td>0.004</td>
</tr>
<tr>
<td>A C</td>
<td>0.008</td>
<td>0.750</td>
<td>0.009</td>
</tr>
<tr>
<td>B C</td>
<td>-0.050</td>
<td>-1.349</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Note. Ceratophyllum demersum L. and Nymphaea odorata Alton were used as reference taxa for the tests. A, Cornus canadensis; B, Calycanthus floridus; 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 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 relics 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 phyogeographic 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,

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PHylogenetic Patterns of Disjunct TAxA


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