

Phylogeny and Biogeography of *Cercis* (Fabaceae): Evidence from Nuclear Ribosomal ITS and Chloroplast *ndhF* Sequence Data

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Communicating Editor: John V. Freudenstein

ABSTRACT. The phylogeny of *Cercis* (Fabaceae) was estimated with DNA sequences of the nuclear ribosomal ITS region and the 3' end of the chloroplast gene *ndhF*. The combined analysis recovers three trees in which a well supported clade of North American and western Eurasian species is nested within a paraphyletic group of Chinese species. In the single most unambiguously resolved topology from these trees, *C. canadensis* from eastern North America is more closely related to *C. siliquastrum* from western Eurasia than to *C. occidentalis* from western North America. DIVA and character optimizations based on this topology suggest that the initial intercontinental divergence events in *Cercis* involved mesophytic ancestors. Subsequent inferred intercontinental divergence events involving xerophytic ancestors are consistent with the Madrean-Tethyan hypothesis, which postulates an early Tertiary floristic link between the arid regions of western North America and western Eurasia. Calibration of branch lengths with the fossil record suggests that the North American and western Eurasian lineages diverged between 6 and 32 million years ago. The oldest of these values is consistent with both the Madrean-Tethyan hypothesis (>23 million years ago) and dispersion across a North Atlantic land bridge connecting Europe to North America (>13 million years ago), whereas the youngest requires an explanation involving long-distance dispersal.

Numerous groups of plants are known to be discontinuously distributed between two or more of the temperate mesophytic forested regions of the northern hemisphere. These regions include eastern and far western North America, parts of Europe and western Asia, and much of eastern Asia. There is now ample evidence that these disjunctions have originated largely through Tertiary range restrictions of formerly pan-hemispheric forest communities as a response to gradually cooling and drying climates (Wood 1972; Axelrod 1975; Tiffney 1985a, b, 2000; Boulter 1994; Collinson 1994; Manchester 1999; Wen 1999; Tiffney and Manchester 2001). Paleontological and molecular phylogenetic studies suggest that these disjuncts neither arose at the same time nor under the same set of geophysical and climatic conditions, but rather have multiple geographic origins, dispersion pathways, and divergence times (Tiffney 1985b; Manchester 1999; Wen 1999; Donoghue et al. 2001). The challenge that lies ahead for the study of Northern Hemisphere historical biogeography is to attempt to reconstruct an apparently complex history through the identification of subpatterns, perhaps associated with ecophysiological differences.

Two such subpatterns may differentiate groups of northern hemisphere Tertiary relict taxa that have evolved since the development of arid climates beginning in the middle Tertiary. In attempting to account for the presence of similar taxa occurring in the arid zones of western North America and parts of western Eurasia, Axelrod (1975) postulated a once nearly con-

tinuous low- to middle-latitude broadleaved sclerophyllous flora under semi-arid climate that spanned the two regions when the Atlantic Ocean was narrower in the Paleogene. These "Madrean-Tethyan links" were thought to have evolved from laurophyllous forests that are now restricted to the south of the past and current arid zones. Intercontinental floristic continuity was considered to have been disrupted by the end of the Paleogene (~23 million years ago [Mya]). In contrast, Raven (1971, 1973) and Wolfe (1975) suggested that plants common to the arid regions of western North America and Eurasia are the products of convergent evolution to arid climates from once more widespread mesophytic ancestors or are the result of long-distance dispersal. According to Wolfe (1975), the ancestors of these groups were members of the boreotropical flora or its mesophytic descendants. In the simplest scenario of this sort, a mesophytic ancestor would give rise to a mesophytic and xerophytic descendant on each continent. Integral to this explanation is the existence of mesophytic to thermophilic floristic connections across one or both of the high-latitude land bridges (Beringian and North Atlantic) corresponding to a boreotropical historical pattern (Wolfe 1975), for which there is strong evidence (e.g., Wolfe 1967, 1975, 1977; Tiffney 1985a, b, 2000; Manchester 1999; Graham 1999; Tiffney and Manchester 2001). These connections are thought to have been severed by the mid Miocene, i.e., ~ 13 Mya (Parks and Wendel 1990).

One group that can potentially provide insight into

the predictive value of these hypotheses is *Cercis* L. (Fabaceae: Cercideae: Cercidinae). This group consists of approximately ten species widely scattered across the north temperate zones in four areas: the eastern United States to northeastern Mexico, western North America, the Mediterranean region to central Asia, and eastern Asia (Li 1944; Robertson 1976). The distribution of *Cercis* thus extends from the mesic through arid climatic zones in both North America and Eurasia. Currently, there are thought to be two species in North America (Isley 1975). One of these, *C. canadensis* L., consists of three varieties. Variety *canadensis* is widespread in the mesophytic forests of eastern North America (Connecticut to Nebraska, south to Florida and the eastern slopes of the northern Sierra Madre Oriental), and is typically found in rich woods, bottomlands, and forest edges. Varieties *mexicana* (Rose) M. Hopkins and *texensis* (S. Watson) M. Hopkins occur in the semi-arid region of the Edwards Plateau and southward along the western slopes of the northern Sierra Madre Oriental (Isley 1975; Ballenger 1992). The other North American species, *C. occidentalis* Torr. ex A. Gray, occurs in California, Arizona, Utah, and Nevada, where it grows in chaparral, foothill woodland, and exposed dry washes and protected valleys (Barneby 1989; Ballenger 1992; Hickman 1993). *Cercis siliquastrum* L. is distributed in the Mediterranean region from France to Turkey and Afghanistan (possibly native only from Turkey eastward; Robertson and Lee 1976; Tutin et al. 1968; Rechinger 1986), occurring in the maquis in the Mediterranean region and dry lower montane forests in west-central Asia (Rechinger 1986; Davis 1970; Zohary 1972; Townsend 1974). It is similar to *C. griffithii* Boiss., which is endemic to central Asia (Rechinger 1986). All six eastern Asian species are restricted to temperate China, with *C. chinensis* Bunge and *C. glabra* Pamp. widely distributed throughout southern China; *C. chingii* Chun, *C. chuniana* F. P. Metcalf, and *C. gigantea* F. C. Cheng and Keng f. restricted to southeastern China; and *C. racemosa* Oliv. restricted to central-southwestern China (Li 1944; Wei 1988; Wei and He 1993). *Cercis funiushanensis* S. Y. Wang and T. B. Chao, and *C. pubescens* S. Y. Wang, two other species described from Henan Province (Zhang and Wang 1988), appear to be only minor variants of *C. chinensis* and *C. glabra*, respectively. The Chinese species occur in a variety of mixed deciduous forests (Li 1944; Robertson and Lee 1976) under humid, temperate to warm-temperate climates, but apparently avoid the subtropical zone of the extreme south (Li 1944).

Cercis species appear to have become adapted to the climatic regimes under which they occur through modifications of leaf morphology (Isley 1975; Wunderlin et al. 1981). The leaf blades of *C. canadensis* var. *canadensis* of eastern North America and all Chinese species are typically thin, acuminate, and dull on the

upper surface. In contrast, the leaves of the (mostly) Mexican and Texan varieties of *C. canadensis* (*C. canadensis* vars. *mexicana* and *texensis*), the western North American species *C. occidentalis*, the Mediterranean species *C. siliquastrum*, and the central Asian species *C. griffithii* are coriaceous, rounded at the apex and often pubescent, glossy, or glaucous on the upper surface (Hopkins 1942; Isley 1975). These differences have been maintained in a common garden setting (Raulston 1990), suggesting that they are genetically based. The only significant exception to the distinctness of these forms occurs in Texas and Oklahoma, where *C. canadensis* vars. *canadensis* and *texensis* are sympatric and populations often exhibit morphological intermediacy. This has been attributed either to introgression between the two varieties (Anderson 1953; Isley 1975 ["Isely hypothesis no. 1"]) or clinal variation of characters along environmental gradients (Hopkins 1942; Isley 1975 ["Isely hypothesis no. 2"]).

Several historical scenarios have been posited to account for the correlation between climate and morphology across the current distribution of *Cercis*. Axelrod (1975) suggested that the distribution of *Cercis* in both the arid regions of North America and Eurasia provides evidence for the Madrean-Tethyan hypothesis. In contrast, Raven (1971, 1973) suggested that *Cercis* taxa in the arid regions of North America and Eurasia are the product of convergence to arid conditions of once more widespread mesophytic ("Arcto-Tertiary") ancestors. The latter explanation is consistent with the most comprehensive taxonomic treatments of the North American species (i.e., Hopkins 1942; Isley 1975; Barneby 1989), all of which have considered all *Cercis* in North America to form a cohesive group. Hopkins (1942), in particular, hypothesized that western North American populations of *Cercis* were derived from those in the east. McVaugh (1952), on the other hand, hypothesized a xerophytic North American ancestor that originated in Mexico and subsequently adapted to mesic conditions in eastern North America.

A related issue concerns the extent to which each of the northern hemisphere high-latitude land bridges may have served as a migration route for currently intercontinentally disjunct plant groups, particularly those comprising the presumed descendants of the boreotropical flora (Wolfe 1975; Tiffney 1985b; Wen 1999). The Beringian bridge was available as a route of intercontinental exchange throughout much of the Tertiary, although its extremely high latitude (between 69° and 75° N; Wen 1999) may have limited the migration of thermophilic elements. In contrast, the North Atlantic bridge was farther south (60° N; Taylor 1990), which would have allowed the exchange of both deciduous and more thermophilic evergreen elements. The major factor limiting the exchange capacity of the North Atlantic bridge is the relatively short interval (Eocene) in

TABLE 1. DNA vouchers with taxon, locality, voucher information, and GenBank accession number. “—” = not sampled.

| Species | Voucher | Source | ITS | <i>ndhF</i> |
|--|----------------------------------|--|----------|-------------|
| <i>Bauhinia faberi</i> Oliv. | <i>C. Davis s.n.</i> (GH) | UC-Berkeley Botanical Garden, U.S.A. (88.1198) | AF390195 | AF390209 |
| <i>Cercis canadensis</i> L. var. <i>canadensis</i> (Accession 1) | <i>P. W. Fritsch 1468</i> (CAS) | Rancho El Cielo, Tamaulipas, Mexico | AF390181 | — |
| <i>Cercis canadensis</i> L. var. <i>canadensis</i> (Accession 2) | <i>P. W. Fritsch 1447</i> (CAS) | Durham, North Carolina, U.S.A. | AF390188 | AF390204 |
| <i>Cercis canadensis</i> L. var. <i>mexicana</i> (Rose) M. Hopkins | <i>P. W. Fritsch 1466</i> (CAS) | NCSU Arboretum, U.S.A. | AF390186 | AF390202 |
| <i>Cercis canadensis</i> var. <i>texensis</i> (S. Watson) M. Hopkins (Accession 1) | <i>P. W. Fritsch 1458</i> (CAS) | NCSU Arboretum, U.S.A. | AF390180 | AF390197 |
| <i>Cercis canadensis</i> var. <i>texensis</i> (S. Watson) M. Hopkins (Accession 2) | <i>P. W. Fritsch 1465</i> (CAS) | NCSU Arboretum, U.S.A. | AF390187 | AF390203 |
| <i>Cercis chinensis</i> Bunge | <i>P. W. Fritsch 1460</i> (CAS) | NCSU Arboretum, U.S.A. (940598) | AF390189 | AF390205 |
| <i>Cercis chingii</i> Chun | <i>P. W. Fritsch 1454</i> (CAS) | Hangzhou Botanical Garden, China | AF390193 | AF390206 |
| <i>Cercis gigantea</i> F. C. & Keng f. (Accession 1) | unvouchered | NCSU Arboretum, U.S.A. | AF390194 | AF390207 |
| <i>Cercis gigantea</i> F. C. Cheng & Keng f. (Accession 2) | unvouchered | NCSU Arboretum, U.S.A. | — | AF390208 |
| <i>Cercis glabra</i> Pamp. (Accession 1) | <i>P. W. Fritsch 1451</i> (CAS) | Hangzhou Botanical Garden, China | AF390190 | — |
| <i>Cercis glabra</i> Pamp. (Accession 2) | <i>P. W. Fritsch 1464</i> (CAS) | NCSU Arboretum, U.S.A. | — | AF390196 |
| <i>Cercis occidentalis</i> Torr. ex A. Gray (Accession 1) | <i>C. R. Parks 9-28-95</i> (CAS) | California, U.S.A. | AF390182 | AF390198 |
| <i>Cercis occidentalis</i> Torr. ex A. Gray (Accession 2) | <i>P. W. Fritsch 1749</i> (CAS) | Sonoma Co., California, U.S.A. | AF390183 | AF390199 |
| <i>Cercis racemosa</i> Oliv. (Accession 1) | <i>P. W. Fritsch 1452</i> (CAS) | Hangzhou Botanical Garden, China | AF390191 | — |
| <i>Cercis racemosa</i> Oliv. (Accession 2) | <i>P. W. Fritsch 1459</i> (CAS) | NCSU Arboretum, U.S.A. | AF390192 | — |
| <i>Cercis siliquastrum</i> L. (Accession 1) | <i>P. W. Fritsch 1467</i> (CAS) | Lisbon Botanical Garden, Portugal | AF390185 | AF390201 |
| <i>Cercis siliquastrum</i> L. (Accession 2) | <i>P. W. Fritsch 1469</i> (CAS) | Olympia, Greece | AF390184 | AF390200 |

which it is thought to have been a feasible passageway. However, several recent lines of evidence from geology, paleofloristics, and molecular divergence values imply the existence of a North Atlantic floristic connection for some temperate-deciduous elements as late as the Miocene (Tiffney 1985a; Hoey and Parks 1991, 1994; Li et al. 1997).

From the study by one of us (PWF) of herbarium specimens collected throughout the range of *Cercis*, it appears that morphological differences among the species are largely quantitative and continuous, and of limited utility for phylogenetic estimation. Here we use phylogenetic patterns and divergence times estimated from DNA sequences of the nuclear ribosomal ITS region and the 3' end of the chloroplast gene *ndhF* to test the various scenarios of historical biogeography developed for *Cercis*. ITS sequences have been routinely used to study genetic variation and phylogenetic relationships at the infrageneric level (reviewed in Baldwin et al. 1995). The gene *ndhF* encodes a subunit of the nicotinamide dehydrogenase complex and shows approximately twice the average mutation rate of *rbcl* (Sugiura 1989; Olmstead and Sweere 1994). The 3' region of *ndhF* appears to be particularly variable (see

Ferguson 1999), and may therefore be useful in inferring relationships among closely related species.

MATERIALS AND METHODS

Taxonomic Sampling. *Cercis* is easily distinguished from other members of subtribe Cercidinae by its pseudopapilionoid corolla (Wunderlin et al. 1987) and appears on this basis to be monophyletic. Seventeen accessions from eight species of *Cercis*, representing all major geographical areas except for Central Asia, were sequenced for the ITS region and/or the 3' region of *ndhF* (Table 1). Two individuals each of the species *C. gigantea*, *C. glabra*, *C. occidentalis*, *C. racemosa*, and *C. siliquastrum* were sampled. Samples of *C. canadensis* included all three infraspecific taxa: var. *canadensis* (one individual from northeastern Mexico and one from the southeastern United States), var. *mexicana* (one individual), and var. *texensis* (two individuals). *Cercis griffithii* from central Asia and *C. chuniana* from eastern Eurasia were not sampled in this study. The former species is most likely a minor variant of *C. siliquastrum* from western Eurasia, and the latter is probably closely related to the eastern Eurasian species *C. chinensis*, *C. glabra*, and *C. racemosa*.

The tribe Cercideae Bronn, which also includes *Bauhinia* L., appears to be monophyletic (Doyle et al. 1997; Bruneau et al. 2001). *Bauhinia faberi* Oliv., from Yunnan, China, served as the outgroup in our analyses. Other groups traditionally recognized in the Cercideae are *Adenolobus* (Harv. ex Benth.) Torre & Hillcoat and *Griffonia* Baill. (constituting, with *Cercis*, subtribe Cercidinae), and *Brenierea* Humbert (constituting, with *Bauhinia*, subtribe Bauhiniinae (Benth.) Wunderlin; Wunderlin et al. 1981). The internal taxonomy of the Cercideae suggests that *Adenolobus* and *Griffonia* are

more closely related to *Cercis* than either is to *Bauhinia* (Wunderlin et al. 1981). Representatives of the other members of the Cercideae were not included because we were unable to obtain genomic DNA for them.

DNA Sequencing. Total genomic DNA was extracted from silica-dried leaf material with the 2× CTAB method (Doyle and Doyle 1987) and purified with the GENECLEAN II kit (Qbiogene, Inc.). Polymerase chain reaction (PCR) conditions and visualization of PCR products generally followed Li et al. (1997), except that 10% DMSO was included in the ITS reactions, and the PCR program consisted of an initial 94°C heating step for three min, followed by 35 cycles at 94°C for 30 sec, 50°C (ITS) or 48°C (*ndhF*) for one min, and 72°C for 50 sec with an additional six min at 72°C following the final cycle.

The ITS region was amplified with the primers ITS4 (White et al. 1990) and ITSLEU (Baum et al. 1998). The 3' region of *ndhF* was amplified with the primers 5.5F (5'-TTAGTRAYAGTYGGTTGATTAC) and 10.2R (5'-RCCATCRATNACTCGCYATCAAAAA) (Davis et al. 2001). A single fragment was observed for all amplifications. Fragments were excised from the gel and cleaned with a QIAquick Gel Extraction Kit (Qiagen Corp.). In most cases, the cleaned ITS PCR products were cloned with the pGEM T-easy Cloning Vector System II (Promega Corp.). Plasmids were prepared with the QIAprep Spin Miniprep Kit (Qiagen Corp.). Sequences were obtained by using the ITS amplification primers and the internal sequence-specific primers ITS2 and ITS3B (Baum et al. 1998). Two clones were sequenced for each accession to check for polymorphisms (e.g., Wendel et al. 1995; Buckler and Holtsford 1996; Campbell et al. 1997). In one case (*C. occidentalis* 2), the ITS PCR product was used directly for sequencing. We sequenced 33 copies of the ITS region from eight species of *Cercis* (15 accessions) plus the outgroup. The cleaned *ndhF* PCR products were used directly for sequencing 13 accessions from seven species of *Cercis* plus the outgroup with the amplification primers from above.

Double-stranded PCR products were sequenced in both directions with dye-terminators and cycle sequencing protocols (DyePrimer and BigDye kits; Applied Biosystems, Inc.). Sequences were obtained with an ABI model 377 automated sequencer (Applied Biosystems, Inc.) and edited with the computer program Sequencher 3.0 (Gene Codes Corp.). Both strands were sequenced with a minimum overlap of ~80% of the total sequence length. All newly generated sequences were submitted to GenBank (Table 1).

Phylogenetic Analyses. Nucleotide sequences were aligned manually. The data matrix and trees are available in TreeBASE (www.herbaria.harvard.edu/treebase). Phylogenetic analyses were conducted with PAUP* (Swofford 1999; ver. 4.0b8). Parsimony analyses were conducted independently for the two data sets, followed by an analysis of the combined data that included only those accessions sampled for both data sets (twelve in all). Gapped positions were treated as missing by PAUP*, but we included potentially parsimony-informative indels in the analyses as binary characters, following the reasoning of Simmons and Ochterena (2000) that we implemented by using a gap-coding script distributed by R. Ree (Harvard University Herbaria). All searches for equally parsimonious trees were implemented with the branch-and-bound option selected and uninformative characters excluded. Characters were weighted equally and character states were unordered. Bootstrap support (Felsenstein 1985) for each clade was estimated from 1,000 heuristic search replicates with uninformative characters excluded and simple taxon addition, TBR branch swapping, and MulTrees selected.

To examine the extent of conflict between the nuclear and chloroplast data, we conducted two tests. The Templeton test (Templeton 1983) was used to assess congruence among the trees generated from each independent data set. The set of shortest trees in which the monophyly of each clade of interest is not supported was used to evaluate the significance of the extra steps required by these trees with a Wilcoxon sign-rank test. Analyses included only accessions with complete data. ITS and *ndhF* data sets were analyzed independently and the trees generated from the rival

data set were analyzed for congruence with the Templeton test in PAUP*. To assess the level of congruence between the ITS and *ndhF* data sets, we employed the incongruence length difference (ILD) test (Farris et al. 1994, 1995; implemented in PAUP* as the partition-homogeneity test). The ILD test compares the Mickevich and Farris index (Mickevich and Farris 1981) to a null distribution through multiple random repartitions of the data. We used 100 random taxon sequence-addition replicates (saving only 10 trees per replicate), TBR, and heuristic searches with 999 repartitions of the data.

Historical Biogeography. The biogeographic scenarios hypothesized for *Cercis* were tested with evidence from patterns of dispersal and vicariance, divergence times of vicariads, and the inferred evolution of leaf morphology. Patterns of dispersal and vicariance were reconstructed from the combined phylogeny with dispersal-variance analysis (DIVA; Ronquist 1997). This method assumes that geographic distributions can be the result of both dispersal and vicariance events. Ancestral states are reconstructed through minimizing the number of dispersal events needed to explain the distribution pattern. Speciation is assumed to be caused by vicariance, and vicariance events are not counted as steps in the equally optimal distributions. The data matrix was constructed by scoring terminals for presence or absence in each area and the analysis was performed with the aid of the DIVA 1.1 computer program (Ronquist 1996). Because DIVA requires a fully resolved topology for implementation, we combined *C. canadensis* vars. *mexicana* and *texensis* into a single terminal. These taxa occupy contiguous areas and exhibit only minor morphological differences, such as the quantity of leaf pubescence. To infer the number of intercontinental dispersal or vicariance events, four areas (eastern North America, western North America, eastern Eurasia, and western Eurasia) were delimited. The delimitation of these areas is based on the now well established concept of Tertiary mesophytic forest refugia in the northern hemisphere (Wood 1972; Szafer 1975; Tiffney 1985b; Wen 1999). Because *C. canadensis* vars. *mexicana* and *texensis* are distributed in an area that is nearly equidistant between eastern and western North America, two analyses were run, one assigning these taxa to eastern North America, the other assigning them to western North America.

In our initial unconstrained DIVA reconstructions, ancestral areas were estimated without restricting the number of inferred areas at each node. In this case, the basal ancestors of *Cercis* are likely to be inferred as widespread, a problem of DIVA highlighted by Ronquist (1996, 1997). Because none of the extant species of *Cercis* are widespread among these areas, we conducted another optimization in which the maximum number of areas assigned to each node was restricted to two (see Ronquist 1996, 1997). Two restricted analyses were conducted by assigning *C. canadensis* vars. *mexicana* and *texensis* to alternative geographic areas, as above.

Although caution is advisable when estimating divergence times from molecular data (Sanderson 1998), we can provide rough estimates of divergence to test hypotheses concerning the historical biogeography of *Cercis*. Likelihood ratios (Felsenstein 1981; Goldman 1993; Yang et al. 1995; Huelsenbeck and Rannala 1997) were calculated to test whether sequences in *Cercis* evolved in a clock-like fashion. We performed likelihood ratio tests with a pruned version of the most highly resolved parsimony tree from the combined ITS and *ndhF* data set using a series of models with increasing complexity, following the available options in PAUP* (see Table 2). The pruned tree includes only one accession per species, except for *C. canadensis*, which is represented by both a mesophytic and xerophytic representative (*C. canadensis* var. *canadensis* and *C. canadensis* var. *mexicana*, respectively). Branch lengths and an associated likelihood score were calculated for the best-fitting model of sequence evolution in the absence of a molecular clock. This tree was then rooted and a likelihood score was calculated for this tree under a molecular clock. If the likelihood ratio— $2\log L1/L2$ (where L1 is the clock log-likelihood and L2 is the nonclock log-likelihood) is not significant as determined from a χ -square test with six degrees of freedom (number of taxa—2), then the null hypothesis of rate homogeneity across the tree cannot be rejected, and estimates of optimal branch lengths can be considered pro-

TABLE 2. Comparison of likelihood scores for different models of evolution. In each comparison, model 1 is the simpler model and represents a special case of model 2. ** = $P < 0.005$. For other values $P > 0.05$. (c) = molecular clock enforced.

| Model 1 | Model 2 | -log L1 | -log L2 | df | -2log L1/L2 |
|--------------------|------------------------|------------|------------|----|-------------|
| JC | F81 | 2928.96417 | 2912.96194 | 3 | 32.00446** |
| F81 | HKY | 2912.96194 | 2889.19204 | 1 | 47.53980** |
| HKY | GTR | 2889.19204 | 2878.92676 | 4 | 20.53056** |
| GTR | GTR + Γ | 2878.92676 | 2874.66947 | 1 | 8.51458** |
| GTR + Γ | GTR + I + Γ | 2874.66947 | 2874.57856 | 1 | 0.18182 |
| GTR + I + Γ | GTR + I + Γ (c) | 2874.57856 | 2876.26840 | 6 | 3.37968 |

portional to time. Standard errors around nodes were estimated with the three-step nonparametric bootstrap procedure of Baldwin and Sanderson (1998).

To infer the historical pattern of adaptation to mesic and arid environments within *Cercis*, standard unweighted parsimony character optimization (Maddison and Maddison 1992) was conducted on a two-state leaf character (mesophytic versus xerophytic). The states of this character are meant to encapsulate the morphological differences in leaves associated with mesic (thin, acuminate, dull adaxially) versus arid (coriaceous, rounded at the apex, pubescent, glossy, or glaucous adaxially) climates. The analysis was conducted with MacClade 3.0 (Maddison and Maddison 1992), and took into account all possible resolutions of polytomies on the most resolved topology from the combined analysis.

RESULTS

ITS. ITS region sequences for *Cercis* range from 614 to 620 nucleotides long; the *Bauhinia faberi* sequence is 614 bp long. Limits of ITS 1, ITS 2, and the 5.8S gene were determined by comparison with published sequences in GenBank (AF287640 and AF218537). In *Cercis*, the lengths of ITS 1, 5.8S, and ITS 2 range from 242 to 244 bp, 159 to 160 bp, and 215 to 216 bp, respectively. All clones of each accession suggest that ITS polymorphisms are not present in *Cercis*. On this basis, we reduced all clones to one accession per sample to facilitate analyses. G + C content in each segment of the ITS region ranges from 57% to 59%. Pairwise sequence divergence is comparable among the two spacer regions (0% to 3.8% for both ITS 1 and ITS 2).

Sequence alignment produced a data matrix of 637 nucleotide characters, 29 (4.6%) of which are parsim-

ony-informative. The inclusion of four parsimony-informative gaps (ranging from one to three bp long) were coded and resulted in a final data matrix of 33 characters. The matrix includes two (0.31%) cells with missing values.

ndhF. The 3' region of *ndhF* that we sequenced begins at position 1,316 and ends at position 2,020 of the *ndhF* gene in *Nicotiana* (Olmstead et al. 1993). In *Cercis*, the *ndhF* fragment ranges from 728 to 746 bp long. Pairwise sequence divergence between the ingroup taxa and the outgroup ranges from 5.5% to 6.4%, and 0% to 2.2% among ingroup taxa. Sequence alignment produced a data matrix of 756 characters, six (0.79%) of which are parsimony-informative. The inclusion of four gap characters (two of which were potentially informative; Table 3) resulted in a final data matrix of eight characters, including three cells (0.39%) with missing values. We were unable to amplify *ndhF* for *C. racemosa*.

Phylogenetic Relationships. Ten equally parsimonious trees were recovered from the analysis of ITS sequences (length = 52; CI = 0.69; RI = 0.74; Fig. 1). *Cercis chingii* is resolved as the sister to all other *Cercis* species (bootstrap support = 68%). All accessions of *C. canadensis* form a clade (87%) except for *C. canadensis* var. *canadensis*-1 from northeastern Mexico, which forms part of a polytomy (87%) that also comprises the clade consisting of the rest of the *C. canadensis* accessions, a clade comprising the two *C. occidentalis* ac-

TABLE 3. Indel region in *ndhF*. Four characters were scored (shown at right) for the gapped region below.

| | | |
|--------------------------------|---|------|
| <i>Cercis chingii</i> | GAATAATAATGAAACTTTATTAAACAATGAATAATAATG | 1000 |
| <i>Cercis chinensis</i> | GAATAATAATAATAAT-----GAGAATAATAATG | 0001 |
| <i>Cercis glabra</i> -2 | GAATAATAATAATAAT-----GAGAATAATAATG | 0001 |
| <i>Cercis canadensis</i> CAN-2 | GAATAATAAT-----AATAATG | 000? |
| <i>Cercis canadensis</i> MEX | GAATAATAAT-----AATAATG | 001? |
| <i>Cercis canadensis</i> TEX-1 | GAATAATAAT-----AATAATG | 001? |
| <i>Cercis canadensis</i> TEX-2 | GAATAATAAT-----AATAATG | 001? |
| <i>Cercis gigantea</i> -1 | GAATAATAAT-----AATAATG | 001? |
| <i>Cercis gigantea</i> -2 | GAATAATAAT-----AATAATG | 001? |
| <i>Cercis siliquastrum</i> -1 | GAATAATAAT-----AATAATG | 001? |
| <i>Cercis siliquastrum</i> -2 | GAATAATAAT-----AATAATG | 001? |
| <i>Cercis occidentalis</i> -1 | GAAT-----AATAATG | 01?? |
| <i>Cercis occidentalis</i> -2 | GAAT-----AATAATG | 01?? |
| <i>Bauhinia faberi</i> | GAAT-----AATAATG | 01?? |

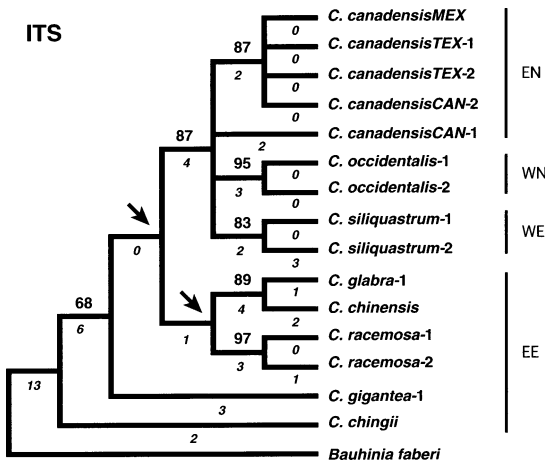


FIG. 1. One arbitrarily selected tree of ten equally parsimonious ITS trees for *Cercis* (length = 52; CI = 0.69; RI = 0.74). Arrows indicate clades not present in the strict consensus. Numerals above the branches are $\geq 50\%$ bootstrap estimates. Numerals below the branches are branch lengths under ACCTRAN optimization. EN = eastern North America, WN = western North America, EE = eastern Eurasia, WE = western Eurasia.

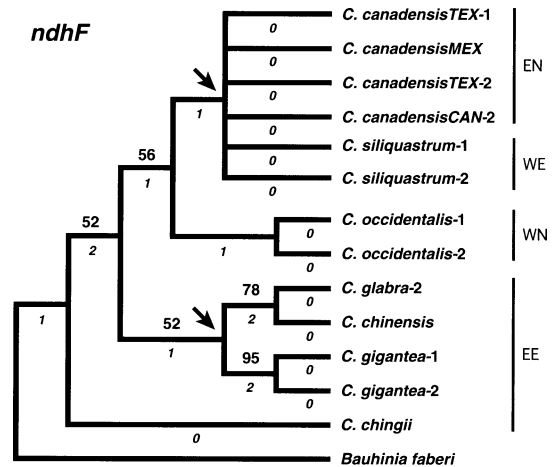


FIG. 2. One arbitrarily selected tree of three equally parsimonious *ndhF* trees for *Cercis* (length = 10; CI = 0.80; RI = 0.88). Arrows indicate clades not present in the strict consensus. Numerals above the branches are $\geq 50\%$ bootstrap estimates. Numerals below the branches are branch lengths under ACCTRAN optimization. EN = eastern North America, WN = western North America, EE = eastern Eurasia, WE = western Eurasia.

cessions (95%), and a clade comprising the two *C. siliquastrum* accessions (83%). *Cercis chinensis* and *C. glabra* form a clade (89%), as do the two accessions of *C. racemosa* (97%). The rest of the ITS strict consensus is unresolved.

Three equally parsimonious trees were recovered from the analysis of *ndhF* sequences (length = 10; CI = 0.80; RI = 0.88; Fig. 2). *Cercis chingii* is resolved as the sister to all other *Cercis* species (bootstrap support = 52%). All accessions of *C. canadensis* form a clade with *C. siliquastrum* and *C. occidentalis* (56%). The two accessions of *C. occidentalis* form a clade (<50%). The western Eurasian and North American clade (Fig. 2) forms a trichotomy with two clades of eastern Eurasian species. The first is a clade containing *C. chinensis* and *C. glabra* (78%). The second is a clade consisting of the two accessions of *C. gigantea* (95%).

As determined from the ILD test, the summed length of the trees generated from the individual data sets (ITS and *ndhF*) does not differ significantly from the sums of individual data sets partitioned randomly ($P = 1.00$), indicating that the data sets are congruent. As determined from the Templeton test, the trees generated from each individual reduced taxon data set (five equally parsimonious trees for ITS and three for *ndhF*; trees not shown) correspond to reduced versions of the trees recovered from the primary data sets (Figs. 1 and 2), and none are significantly different when compared with those generated from their rival data sets (Table 4), indicating that topologies do not conflict. The three equally parsimonious trees resulting from analysis of the combined data set comprising 12 ac-

cessions and 29 parsimony-informative characters (length = 54; CI = 0.74; RI = 0.69; Fig. 3) are nearly identical to both the *ndhF* and ITS strict consensus trees, except the combined topology provides better overall resolution and bootstrap support than either independent analysis. *Cercis canadensis*, *C. occidentalis*, and *C. siliquastrum* are all monophyletic. Moreover, the

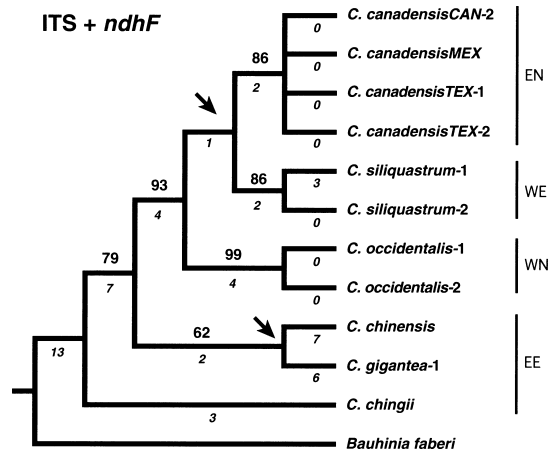


FIG. 3. The most resolved tree (length = 54; CI = 0.74; RI = 0.69) selected from among three of the equally parsimonious trees from the combined ITS + *ndhF* analysis for *Cercis*. Arrows indicate clades not present in the strict consensus. Numerals above the branches are $\geq 50\%$ bootstrap estimates. Numerals below the branches are branch lengths under DELTRAN optimization. EN = eastern North America, WN = western North America, EE = eastern Eurasia, WE = western Eurasia.

TABLE 4. Summary of Wilcoxon signed ranks test results for data combination. The cost equals the length of the shortest tree under the constraint minus the length of the constrained tree. Gain and Loss refer to the number of characters with extra steps on the constrained or unconstrained tree, respectively. N = number of ranks.

| Data set | Constraint | Cost | Gain | Loss | N | P (2-tailed) |
|-------------|-------------------|------|------|------|-----|-----------------------|
| ITS | <i>ndhF</i> trees | 6–8 | 6–8 | 0 | 4 | $P > 0.0633$ |
| <i>ndhF</i> | ITS trees | 0–1 | 0–1 | 0–1 | 1–2 | $0.3173 < P < 1.0000$ |

most highly resolved of the three trees groups *C. canadensis* as sister to *C. siliquastrum* (bootstrap support <50%; Fig. 3). The two other topologies are ambiguous with respect to the relationship between North American and western Eurasian *Cercis*.

Historical Biogeography. The topology that we

employed for use in historical biogeographical analyses (Fig. 4) is identical to a pruned version of that in Figure 3. The optimization of leaf type on the most resolved tree from the combined analysis (Fig. 3) consists of two steps (Fig. 5). The state “mesophytic” is assigned to the most recent common ancestor (MRCA)

ITS + *ndhF* pruned

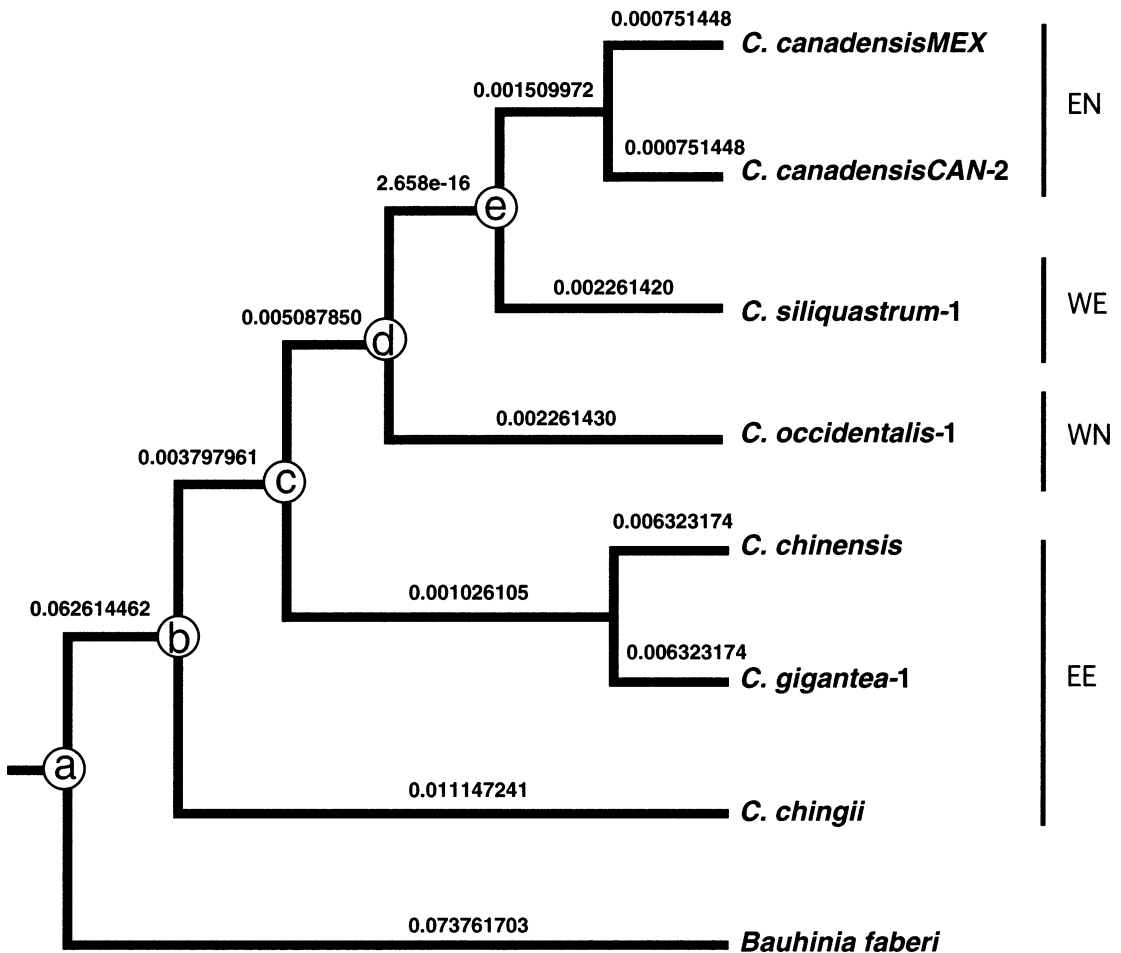


FIG. 4. The most resolved pruned tree for *Cercis*, selected from among the three equally parsimonious trees from the combined ITS + *ndhF* analysis for *Cercis*. Numerals above the branches are optimal maximum likelihood branch lengths imposed on this tree under the GTR+I+ Γ model of sequence evolution with the molecular clock enforced. Nodes a-e are labeled for text reference. EN = eastern North America, WN = western North America, EE = eastern Eurasia, WE = western Eurasia.

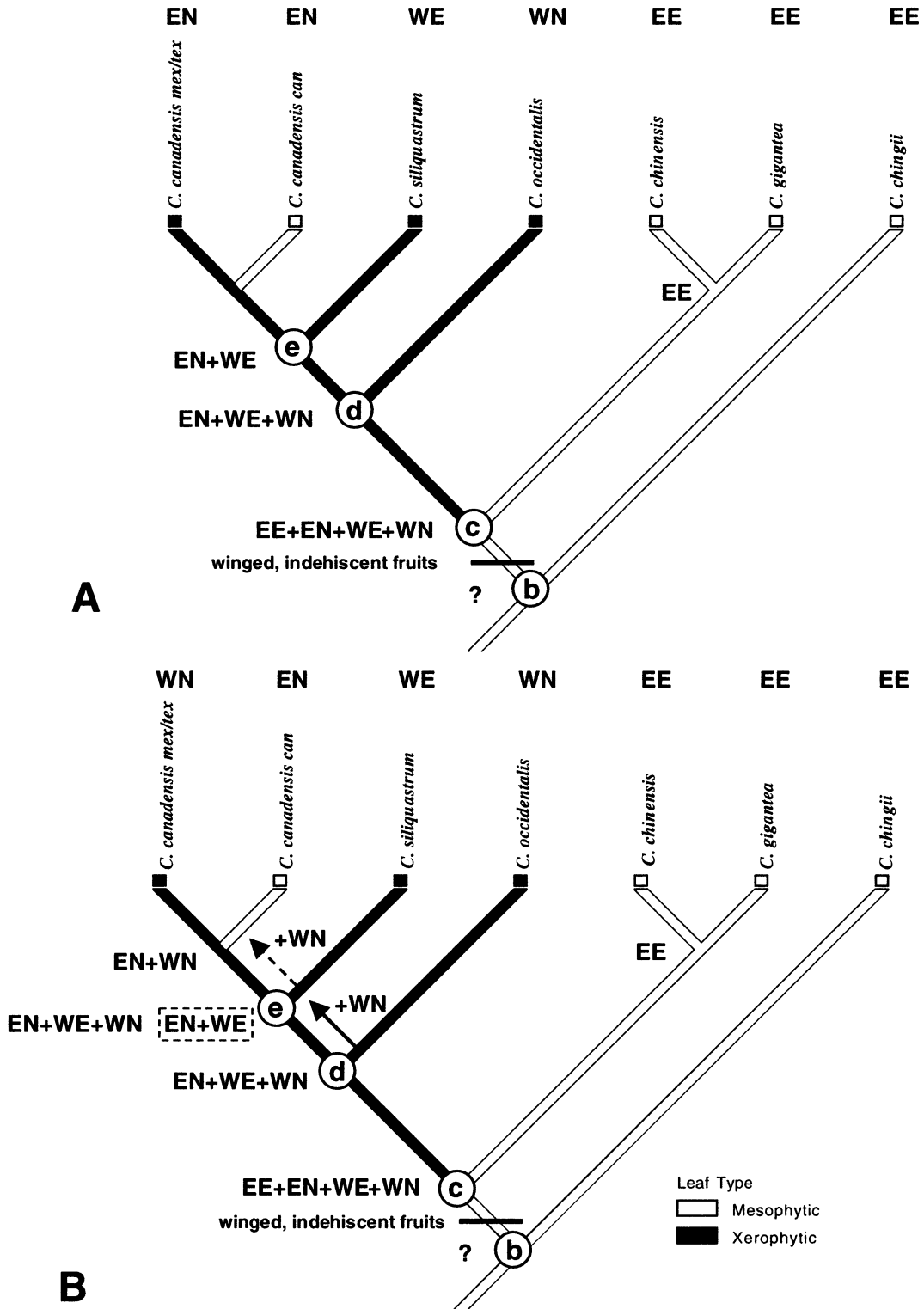


FIG. 5. Evolution of leaf type and DIVA optimization over the most resolved topology derived from the combined ITS + *ndhF* analysis, with *Cercis canadensis* vars. *mexicana* and *texensis* combined into a single terminal. Arrows indicate dispersal events. EN = eastern North America; WN = western North America; EE = eastern Eurasia; WE = western Eurasia. Nodes b–e are labeled for text reference. Ancestral node of *Cercis* (b) scored as “?” due to ambiguity associated with area reconstructions. A. Analysis with *C. canadensis* vars. *mexicana* and *texensis* scored for eastern North America. The optimal reconstruction requires

of *Cercis*. The state "xerophytic" is assigned to the MRCA of the North American + western Eurasian species, and "mesophytic" is independently derived in *C. canadensis* var. *canadensis* of eastern North America. Comparable results are obtained no matter how the accessions of *C. canadensis* are resolved in Fig. 3.

DIVA reconstructions in which ancestral nodes were constrained to two areas (not shown) were less parsimonious than those from the unconstrained analyses (Fig. 5). Because we inferred a diverse array of ancestral area reconstructions from these unconstrained and constrained analyses (ranging from Eastern Eurasia only to all four relevant areas), we have indicated the MRCA of *Cercis* as "?". For the purpose of our discussion we have chosen to focus only on the unconstrained reconstructions, beginning with the MRCA of *C. gigantea* and *C. canadensis* vars. *mexicana* and *texensis*.

DIVA with *Cercis canadensis* vars. *mexicana* and *texensis* scored for eastern North America and excluding the MRCA of *Cercis* resulted in a single optimal reconstruction that can be explained strictly by vicariance (Fig. 5A). When interpreted in conjunction with the evolution of leaf type, the following scenario can be inferred from the analysis. Vicariance of a widespread mesophytic ancestor resulted in the MRCA of *C. gigantea* and *C. chinensis* in eastern Eurasia, and a xerophytic ancestor spanning the other three areas {eastern North America + western North America + western Eurasia}. Vicariance involving this xerophytic ancestor resulted in *C. occidentalis* in western North America and a xerophytic MRCA of *C. canadensis* and *C. siliquastrum* spanning western Eurasia and eastern North America. This was followed by vicariance of a xerophytic ancestor that resulted in *C. siliquastrum* in western Eurasia and a xerophytic ancestor of *C. canadensis* in eastern North America. Finally, reversal to the mesophytic condition occurred in *C. canadensis* var. *canadensis*.

DIVA with *Cercis canadensis* vars. *mexicana* and *texensis* scored for western North America and excluding the MRCA of *Cercis* resulted in two optimal reconstructions that required one dispersal (Fig. 5B). The reconstructions are identical with that resulting from scoring *C. canadensis* vars. *mexicana* and *texensis* for eastern North America, until just after the divergence of *C. occidentalis* from {*C. canadensis* + *C. siliquastrum*}. At that point, either 1) dispersal from {eastern North America + western Eurasia} back into western North America was followed by vicariance of a xerophytic ancestor, resulting in *C. siliquastrum* in western Eur-

asia and *C. canadensis* in North America; or 2) vicariance of a xerophytic ancestor that resulted in *C. siliquastrum* in western Eurasia and *C. canadensis* in eastern North America was followed by dispersal from eastern North America to western North America. Finally, vicariance of a xerophytic ancestor resulted in *C. canadensis* var. *canadensis* in eastern North America, where it reverted to the mesophytic condition, and *C. canadensis* vars. *mexicana* and *texensis* in western North America.

The GTR+I+ Γ model has a higher likelihood than the other models of sequence evolution (Table 2) for the most resolved pruned tree (Fig. 4). On this basis, it was chosen for testing molecular rate constancy in *Cercis*. When clock and nonclock versions of the GTR+I+ Γ model were compared, a molecular clock could not be rejected (Table 2). Therefore, the branch lengths of the optimal rooted tree for the GTR+I+ Γ model under the enforcement of a molecular clock (Fig. 4) can be taken as approximately proportional to time.

After determining that a molecular clock could not be rejected, we calibrated the clock with the oldest reliable fossil identified as *Cercis* (fruiting material dated at ~32 million years ago [Mya] from the John Day Formation in Oregon [Manchester and Meyer 1987; Herendeen et al. 1992]) to estimate divergence times between vicariads in *Cercis*. The habitat of the original plant from which this fossil was derived was inferred to be dominated by broad-leaved deciduous trees as part of a community comparable to the mixed northern hardwood forest extant today in eastern Asia (Manchester and Meyer 1987). This fossil cannot be assigned with certainty to any extant species of *Cercis* (P. Herendeen, pers. comm.). Like all extant species of *Cercis* except *C. chingii*, however, it has winged fruits, a presumably derived feature within *Cercis* and the Cercideae (Fig. 5). The presence of the wing indicates that this fossil must lie at or above the divergence between *C. chingii* and the rest of *Cercis* (node b in Fig. 4; Fig. 5), exclusive of the branch leading to *C. chingii* itself. The upper bound on the placement of the fossil on the tree cannot be determined from morphology, but the inferred mesophytic habitat in which the fossil plant occurred suggests placement along the branch subtending *C. canadensis* var. *canadensis*, or anywhere between nodes b and d, inclusive, in Fig. 4 (see also Fig. 5). If the fossil is placed at the base of the branch subtending *C. canadensis* var. *canadensis/mexicana*, then the estimated time of divergence for node b (the basal di-

←

no dispersals. B. Analysis with *C. canadensis* vars. *mexicana* and *texensis* scored for western North America. The two equally optimal reconstructions each require one dispersal. The alternative reconstruction is indicated with the dashed box and dashed arrow.

vergence in *Cercis*) is 474.7 ± 39.9 Mya, an unrealistically early date (e.g., before the inferred origin of seed plants). If the fossil is placed at node b, then the estimated divergence times for the other labeled nodes in Fig. 4 are: a = 211.7 ± 26.0 Mya, c = 21.1 ± 1.5 Mya, d = 6.5 ± 0.47 Mya, and e = 6.5 ± 0.47 Mya. Possible divergence times for the last intercontinental divergence event (node e) range from 6 to 32 Mya, depending on where the 32 million-year-old fossil is placed between nodes b and d, inclusive. The youngest age estimate of node a (185.7 Mya) predates the origin of angiosperms, and substantially predates the inferred time of origin of the Leguminosae (70 Mya or somewhat earlier; Raven and Polhill 1981; Herendeen et al., 1992), suggesting that the branch between nodes a and b has undergone a differential rate increase despite the fact that the clock could not be rejected.

DISCUSSION

Phylogeny and Classification. In all analyses, *Cercis chingii* is sister to the rest of *Cercis*. This species is distinct from the others in its coriaceous, unwinged, and consistently dehiscent fruit (versus thin, winged, and indehiscent or tardily dehiscent fruits), with twisted valves at maturity (Li 1944; Robertson and Lee 1976). Fruits of the other members of subtribe Cercidinae are unwinged and dehiscent (Aubréville 1968; Wunderlin et al. 1987), suggesting that these character states are ancestral in *Cercis* (Fig. 5). Relatively few other discrete morphological characters for use in diagnosing species or species groups within *Cercis* exist (Isley 1975), thus limiting the utility of morphological characters for phylogenetic analysis.

Several workers have suggested that all *Cercis* from North America are monophyletic or even conspecific (Hopkins 1942; McVaugh 1952; Isley 1975 ["hypothesis no. 2"]; Barneby 1989). Our results suggest that *Cercis* species from North America do not form a monophyletic group; rather, *C. canadensis* from eastern North America is more closely related to *C. siliquastrum* from western Eurasia than to *C. occidentalis* from western North America. Bootstrap support and resolution for this relationship is weak, however, and more character support for this topology is desirable for confirmation of these results. The topology suggests that *C. occidentalis* should be considered a species distinct from *C. canadensis*. The alternative choice of merging *C. occidentalis* and *C. canadensis* into a single species would also require including *C. siliquastrum* in this species to be consistent with the phylogeny, a taxonomic arrangement that has never been proposed.

With the exception of *Cercis canadensis* var. *mexicana*, *Cercis* taxa from xerophytic habitats do not yield kaempferol glycosides in detectable amounts (Salatino et al. 2000), consistent with the treatment of North American *C. canadensis* and *C. occidentalis* as distinct

species, and the placement of *C. canadensis* var. *texensis* in synonymy under *C. occidentalis*. Our results agree with the former but not the latter. Instead, they suggest that kaempferol glycosides were primitively present in *Cercis* and lost once (along the branch subtending the North American and western Eurasian species), with at least one reversal to kaempferol glycoside production in *C. canadensis*.

Dispersed introgression between *Cercis canadensis* vars. *canadensis* and *texensis* has been hypothesized to extend from northeastern Texas to southwestern Illinois (Anderson 1953). No evidence, other than the geographic location of morphological intermediates, has existed to support or refute this hypothesis. It has been suggested that intergradation between nonsister taxa will almost certainly be secondary, whereas intergradation between sister taxa may be either primary or secondary (Rieseberg and Ellstrand 1993). In our analysis, all accessions of *C. canadensis* group together in both the ITS and *ndhF* trees (except the accession of *C. canadensis* var. *canadensis*-2, which is unresolved in ITS). Thus, no evidence of introgression was detected in this study. More intensive taxon and character sampling from the area of intermediacy is required to fully assess the possibility of introgression between these taxa in *Cercis*.

No detailed taxonomic study has been conducted on Mexican *Cercis*. All of the *C. canadensis* accessions form a clade in the ITS trees, with the exception of *C. canadensis* from Tamaulipas in northeastern Mexico, which is genetically distinct (Fig. 1). This accession appears to fall within the range of leaf morphology corresponding to *C. canadensis* var. *canadensis*. It was collected in the cloud forest along the eastern escarpment of the Sierra Madre Oriental. Varieties *canadensis* and *mexicana* both occur in this mountain range, where they reportedly tend toward ecological separation: var. *canadensis* is distributed on the wetter eastern slopes, var. *mexicana* on the drier western slopes (Ballenger 1992). Nonetheless, many collections from Mexico exhibit characters intermediate between all three varieties of *C. canadensis* (Ballenger 1992; Rzedowski and Rzedowski 1997).

Historical Biogeography. The limited fossil record for *Cercis* begins at 32 Mya (Oligocene) with the fruit from the John Day formation in Oregon (Manchester and Meyer 1987; Herendeen et al. 1992), as part of a flora dominated by broad-leaved deciduous trees comparable to a mixed northern hardwood forest. The only other confirmed records of *Cercis* fossils (reviewed in Guo and Zhou 1992; Herendeen et al. 1992; Owens et al. 1998) are leaf specimens from the Succor Creek flora (15 Mya; middle Miocene; Oregon), the Ellensburg, Washington flora (*C. buchananensis* Condit; 11.5 Mya; middle Miocene; Washington; Smiley, 1963), the Table Mountain flora (*C. buchananensis* Condit; 10.5 Mya;

middle Miocene; California; Condit, 1944), and *C. miochinensis* (late Miocene; China; Hu and Chaney 1940); and two fruit specimens from China (Oligocene; Guo and Zhou 1992). Only the basal half of the leaf is observable in the Succor Creek specimen. The apex is clearly rounded in both the Ellensburg and Table Mountain specimens, whereas in the Chinese specimens it is distinctly acuminate. The Succor Creek vegetation is inferred to have comprised warm-temperate forest with evergreen elements in the lowlands and cool-temperate deciduous and conifer forest at higher elevations (Graham 1999). The Table Mountain flora is thought to have been ecotonal between mesophytic and dry forests (Condit 1944). The early florules of the Ellensburg flora are dominated by plants of mesophytic affinity, whereas the late florules are dominated by those with semi-arid affinity (Smiley 1963). This mesophytic to xerophytic sequence in North American *Cercis* fossils agrees with the sequence of leaf evolution suggested by DIVA (Fig. 5). As inferred from the fossil record, the change from mesophytic to xerophytic *Cercis* occurred between 10.5 and 15 Mya, which falls within the range of the divergence estimates along the branch between nodes c and d in Fig. 4 (6 to 111.2 Mya). This is the branch along which character optimization recovers the change from the mesophytic to xerophytic condition (Fig. 5).

Our results suggest that the initial vicariance events in *Cercis* separating populations of eastern Eurasian *Cercis* from those in western Eurasia and North America (see Fig. 5) involved mesophytic ancestors, and thus are compatible with a scenario of a disruption in distribution across either Beringia or central Asia. We consider vicariance in Beringia less likely owing to two main factors. First, is the absence of *Cercis* from Japan, Korea, and northeastern China. This absence contrasts with other groups interpreted as having extended through Beringia, such as *Hamamelis* (Wen and Shi 1998; Li et al. 2000) and *Triosteum* (Gould and Donoghue 2000). Secondly, *Cercis* now occupies some warm-temperate regions in both North America and Eurasia, in contrast to the more cold-temperate distributions of most groups associated with the Beringian connection.

Our data suggest that the adaptation to arid climate originated once in *Cercis*, and that the most recent vicariance event in the group occurred among xerophytic ancestors in North America and western Eurasia (Fig. 5). This pattern implies a direct trans-Atlantic link between the arid floras of western North America and western Eurasia, as suggested by Axelrod (1975). Parallel adaptation to arid climates on each northern hemisphere continent, as would be predicted from the scenarios in Raven (1971, 1973) and Wolfe (1975), is not supported. The data further suggest a mesophytic reversal in *C. canadensis* var. *canadensis* in eastern North America from a xerophytic ancestor. This supports the

hypothesis of McVaugh (1952) of a xerophytic origin for some taxa including *Cercis* now occurring in the mesophytic forests of the eastern United States. Migration from southern North America, as specifically postulated by McVaugh (1952), cannot be assessed with our data. Hopkins' (1942) hypothesis of a derivation of *Cercis* populations in western North America from eastern North America is not supported by our data.

Of the two other genera of subtribe Cercidinae, *Adenolobus* has been suggested to be most closely related to *Cercis* (Brummitt and Ross 1977; Wunderlin et al. 1981). *Adenolobus* consists of two species endemic to southwestern Africa, both growing under xeric conditions. The leaves of *Adenolobus* resemble those of all species of *Cercis* in leaf venation pattern, and resemble the leaves of the xerophytic species of *Cercis* in their rounded shape with shallowly emarginate apices (Brummitt and Ross 1977). If *Adenolobus* is added to Fig. 5 as the sister group of *Cercis* and its leaf type is assigned "xerophytic", three equally optimal reconstructions of leaf type result, two of which assign "mesophytic" to the MRCA of *Cercis*, and the other of which assigns "xerophytic" to this ancestor. This leaves open the possibility that the MRCA of *Cercis* was adapted to xerophytic conditions, rather than mesophytic conditions as concluded from our data. This modification results in a historical scenario consistent with the Madrean-Tethyan hypothesis nonetheless, because the reconstructions of the nodes above the MRCA of *Cercis* are identical to those in our analyses. More detailed phylogenetic data on the phylogenetic relationships within tribe Cercidae are needed to allow a more rigorous test of the plesiomorphic state of *Cercis* leaves.

Several neontological studies of taxa with representatives in western North America and the Mediterranean region have demonstrated various levels of support for the Madrean-Tethyan hypothesis (Fritsch 1996, 2001; Liston 1997; Liston et al. 1989, 1992, 1999; Hileman et al. 2001), whereas others have not supported the hypothesis (Köhler and Brückner 1989; Ray 1995; Manos et al. 1999). One problematic feature of the Madrean-Tethyan hypothesis is the incorporation of middle- to low-latitude intercontinental dispersion as the means by which the arid-adapted floras on each continent were connected. The existence and serviceability of putative middle-latitude Atlantic volcanic island arcs hypothesized by Axelrod (1975) as a means for trans-continental migration is uncertain. The North Atlantic land bridge is more firmly established as an intercontinental migration route, particularly for evergreen and some deciduous elements of the Eocene boreotropical flora (Tiffney 1985b, 2000; Manchester 1999; Tiffney and Manchester 2001). If the North Atlantic land bridge served as a conduit after the Eocene, climatic factors would have limited passage to some

warm- to cool-temperate deciduous elements through the middle Miocene (~13 Mya; Hoey and Parks 1991, 1994; Tiffney and Manchester 2001), and to cool-temperate and boreal elements in the later Miocene (Tiffney and Manchester 2001). In any case, no direct (fossil or geological) evidence exists for the type of Tertiary high-latitude arid regions that could serve as an intercontinental migration corridor for the xerophytic species of *Cercis* (Axelrod 1975; Wolfe 1975).

Our estimates of the divergence time between North American and western Eurasian *Cercis* range from 6 to 32 Mya. The oldest of these estimates is consistent with either dispersion via the North Atlantic land bridge (> 13 Mya; Parks and Wendel 1990) or a low-latitude trans-Atlantic dispersion (> 23 Mya; Axelrod 1975). The youngest divergence times are incompatible with current geological and climatic interpretations, and would therefore require long-distance dispersal to explain the current distribution of *Cercis*. Further insight into the historical biogeography of *Cercis* will require the incorporation of additional fossil material assigned to particular lineages within *Cercis* as well as additional molecular sequence data to help resolve phylogenetic relationships within this group.

ACKNOWLEDGEMENTS. We thank J. Freudenstein, P. Herendeen, and J. Wen for comments on the manuscript. C. Parks, H. Forbes of the University of California-Berkeley Botanic Garden, and the late J. C. Raulston of the North Carolina State University Arboretum kindly provided material for this study. C. Bell provided assistance and discussion on the analysis of the molecular clock and divergence times.

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NOTE ADDED IN PROOF: As the present paper was going to press another study of *Cercis* phylogeny appeared: Hao, G., D.-X. Zhang, L.-X. Guo, M.-Y. Zhang, Y.-F. Deng, and X.-Y. Wen. 2001. A phylogenetic and biogeographic study of *Cercis* (Leguminosae). *Acta Botanica Sinica* 43: 1275–1278. Their parsimony analysis of ITS sequences yielded results that do not differ strongly from those presented here based on ITS plus *nhdF* sequences.