

## PHYLOGENETIC RELATIONSHIPS AND EVOLUTION IN *CHRYSOSPLENIUM* (SAXIFRAGACEAE) BASED ON *MATK* SEQUENCE DATA<sup>1</sup>

DOUGLAS E. SOLTIS,<sup>2,7</sup> MIYUKI TAGO-NAKAZAWA,<sup>3</sup> QIU-YUN XIANG,<sup>4</sup>  
SHOICHI KAWANO,<sup>5</sup> JIN MURATA,<sup>3</sup> MICHIO WAKABAYASHI,<sup>6</sup> AND  
CAROLA HIBSCH-JETTER<sup>2</sup>

<sup>2</sup>School of Biological Sciences, Washington State University, Pullman, Washington 99164-4236 USA;

<sup>3</sup>Botanical Gardens, University of Tokyo, Hatanaka, Bunkyo-ku, Tokyo 112-0001, Japan;

<sup>4</sup>Department of Biological Sciences, Idaho State University, Pocatello, Idaho 83209-8007 USA;

<sup>5</sup>Department of Botany, Kyoto University, Sakyo-ku, Kyoto-shi, Kyoto 606-8502, Japan; and

<sup>6</sup>Makino Herbarium, Faculty of Science, Tokyo Metropolitan University, Minami-Osawa, Hachioji-shi, Tokyo 192-0397, Japan

*Chrysosplenium* (Saxifragaceae) consists of 57 species widely distributed in temperate and arctic regions of the Northern Hemisphere, with two species restricted to the southern part of South America. Species relationships within the genus are highly problematic. The genus has traditionally been divided into two groups, sometimes recognized as sections (*Oppositifolia* and *Alternifolia*), based on leaf arrangement, or, alternatively, into 17 series. Based on morphological features, Hara suggested that the genus originated in South America and then subsequently migrated to the Northern Hemisphere. We conducted phylogenetic analyses of DNA sequences of the chloroplast gene *matK* for species of *Chrysosplenium* to elucidate relationships, test Hara's biogeographic hypothesis for the genus, and examine chromosomal and gynoecial diversification. These analyses revealed that both sections *Oppositifolia* and *Alternifolia* are monophyletic and form two large sister clades. Hence, leaf arrangement is a good indicator of relationships within this genus. Hara's series *Pilosa* and *Macrostemon* are each also monophyletic; however, series *Oppositifolia*, *Alternifolia*, and *Nepalensia* are clearly not monophyletic. MacClade reconstructions suggest that the genus arose in Eastern Asia, rather than in South America, with several independent migration events from Asia to the New World. In one well-defined subclade, species from eastern and western North America form a discrete clade, with Old World species as their sister group, suggesting that the eastern and western North American taxa diverged following migration to that continent. The South American species forms a clade with species from eastern Asia; this disjunction may be the result of ancient long-distance dispersal. Character mapping demonstrated that gynoecial diversification is dynamic, with reversals from inferior to half-inferior ovaries, as well as to ovaries that appear superior. Chromosomal evolution also appears to be labile with several independent origins of  $n = 12$  (from an original number of  $n = 11$ ) and multiple episodes of aneuploidy.

**Key words:** chromosomal evolution; *Chrysosplenium*; gynoecial diversification; *matK* sequences; phylogeography; Saxifragaceae.

*Chrysosplenium* (Saxifragaceae) is a distinctive genus of creeping perennials consisting of 57 species (Maximowicz, 1887; Franchet, 1890; Hara, 1957; Packer, 1963; Spongberg, 1972). The genus is primarily restricted to the Northern Hemisphere with species occurring in eastern North America (two species), western North America (four species), Europe (two species), and eastern Asia, where the greatest number of species are present; for example, 27 species are restricted to the Sino-Himalayan region, and 10 are native to Japan (Spongberg, 1972). Two species of the genus occur disjunctly in South America. Thus, *Chrysosplenium* exemplifies, in large part, a well-known floristic disjunction involving eastern and western North America, eastern Asia, and Europe (e.g., Gray, 1846, 1859; Hu, 1935; Chaney, 1947; Li, 1952, 1972; Graham, 1972; Hara, 1972; Wolfe, 1972, 1975, 1981, 1985; Wood, 1972; Raven and Axelrod, 1974; Boufford and Spongberg, 1983; Wu, 1983; Tiffney, 1985a, b; Boufford, 1992; Hong, 1993; Guo, Richlefs, and Cody, 1998; Guo, 1999). Based on

morphological data and his views of primitive and derived character states, Hara proposed that *Chrysosplenium* arose in South America with subsequent migration to North America and the Old World. Recent molecular phylogenetic analyses have provided important biogeographical insights into taxa exhibiting this pattern of disjunction (e.g., *Aesculus*; Xiang et al., 1998), as well as for taxa occurring only in the first three of these areas (e.g., Xiang, Soltis, and Soltis, 1998).

Relationships within *Chrysosplenium* are problematic. The genus was divided into two subgenera (*Gamosplenium* and *Dialysplenium*) and several series by Maximowicz (1877). Franchet (1890) divided the genus into two sections based on the presence of opposite vs. alternate leaves—sect. *Oppositifolia* and sect. *Alternifolia*. Hara (1957), in contrast, decided that the two sections recognized by Franchet were unnatural; he divided *Chrysosplenium* into 17 series.

Several broad phylogenetic studies of Saxifragaceae that included sequences of the chloroplast gene *matK* for species of *Chrysosplenium* suggested that *matK* sequence data would be useful for resolving relationships within the large, problematic genus *Chrysosplenium* (Johnson and Soltis, 1994, 1995; Soltis et al., 1996). More recently, Nakazawa et al. (1997) used *matK* sequences to resolve species relationships in a study focused on Japanese members of *Chrysosplenium*.

We constructed a *matK* sequence data set for *Chrysosplen-*

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<sup>7</sup> Current address: Department of Botany and the Genetics Institute, University of Florida, Gainesville, FL 32611-8526 USA.

*ium* and included species from throughout the geographic range of the genus and representing 13 of the 17 series recognized by Hara (1957). Our study had four basic objectives: (1) to provide an initial phylogenetic hypothesis of species relationships for this enigmatic genus and begin to evaluate the monophyly of Hara's series; (2) to determine whether the genus consists of two major lineages corresponding to sections *Alternifolia* and *Oppositifolia*; (3) to assess biogeographic patterns among species of *Chrysosplenium*, with particular interest in Hara's proposed South American origin for the genus and in those species occurring in eastern and western North America, eastern Asia, and Europe; and (4) to examine the evolution of ovary position and chromosome number in light of the resultant phylogenetic hypothesis.

## MATERIALS AND METHODS

**Plant materials**—We obtained *matK* sequences from 33 collections representing 28 species of *Chrysosplenium* and the outgroup, *Peltoboykinia tellimoides* (Table 1). Plant material was obtained from natural populations, botanical gardens, or herbarium specimens. Voucher specimens were deposited at the Marion Ownbey Herbarium (WS), Washington State University, and the Makino Herbarium (MAK), Tokyo Metropolitan University.

**DNA isolation and sequencing**—DNAs were isolated via either the miniprep procedure of Saghai-Marouf et al. (1984) and Doyle and Doyle (1987) as modified by Soltis et al. (1991) or by a microprep procedure (Cullings, 1992) that requires even smaller amounts (<0.1 g) of leaf tissue. The microprep procedure was used primarily to isolate DNA from herbarium specimens, which played a critical role as a source of DNA for several species.

Amplification of *matK* followed methods described earlier (e.g., Johnson and Soltis, 1994, 1995). For manual sequencing, preparation of single-stranded DNAs and dideoxy sequencing followed Johnson and Soltis (1994, 1995); preparation of samples for automated sequencing followed Mort et al. (in press). For some taxa we sequenced 1080 bp (base pairs) per taxon representing over two-thirds of the gene beginning at the 5' end; for other taxa 898 bp of sequence data were obtained (following Nakazawa et al., 1997). Several different PCR (polymerase chain reaction) primer combinations were used to amplify *matK*. The most commonly used combinations were: *trnK*-3914F and *trnK*-2R; *trnK*-253F and *matK*-2000R; *trnK*-710F and *matK*-2000R; *matK*-934 and *matK*-MR; *matK*-1412F and *matK*-1848R; *matK*-XF and *matK*-2200R. The sequencing primers used were: *trnK*-710JF, *matK*-1168, *matK*-1253R, *matK*-1470R, and *matK*-1412F. The sequences of all primers were provided previously (Johnson and Soltis, 1994, 1995; Soltis et al., 1996; Nakazawa et al., 1997).

Earlier phylogenetic analyses of Saxifragaceae indicated that the sister group of *Chrysosplenium* is *Peltoboykinia*. This relationship receives high bootstrap support and is revealed by the analysis of *rbcL*, *matK*, and ITS sequences (Soltis et al., 1993, 1996; Johnson and Soltis, 1994, 1995, 1998). *Peltoboykinia* was therefore used as the outgroup in our analyses. The relationships of the *Peltoboykinia/Chrysosplenium* clade within Saxifragaceae are unclear, however, with as many as seven clades possible close relatives; hence, in this study no other outgroups other than *Peltoboykinia* were employed.

**Phylogenetic analyses**—The *matK* sequences were easily aligned by eye and varied in length due to the presence of several short insertions and deletions (occurring, as expected, in multiples of three base pairs) ranging in length from 3 to 15 bp (Table 2). The aligned data set is available at <http://www.wsu.edu:8080/~soltislab/>. Phylogenetic analyses were conducted using both parsimony and maximum likelihood methods. We constructed a data set and included all of the aligned base pairs with indels coded as missing (we then considered the phylogenetic distribution of each indel by mapping its occurrence on trees derived from analysis of base substitutions alone).

This data matrix was first analyzed using parsimony with PAUP (Phylogenetic Analysis Using Parsimony) version 4.0\* (Swofford, 1999) using the heuristic search option, saving all minimal length trees (MULPARS on), tree

bisection-reconnection (TBR) branch-swapping, and 100 replicates with random taxon addition; characters were equally weighted, and character states were specified as unordered. Bootstrap (Felsenstein, 1985) and decay analyses (Bremer, 1988; Donoghue et al., 1992) were then conducted to obtain estimates of reliability for monophyletic groups. For the bootstrap analysis (1000 replicates), simple taxon addition and TBR branch swapping, with characters equally weighted, were used. The decay analysis was conducted following the method of Eernisse and Kluge (1993; e.g., Xiang, Soltis, and Soltis, 1998; Xiang et al., 1998). The method involves examining each node of interest in turn using a constraint statement that specifies only the node of interest being monophyletic and saving the shortest trees that do not satisfy this criterion. The difference between the length of these trees and the shortest trees is used as the decay value for that node.

We conducted two maximum likelihood (MLE) analyses with PAUP\* version 4.0 using heuristic search, TBR branch-swapping, and the Hasegawa-Kishino-Yano model (1985) with different settings for ratio of transitions (ti)/transversions (tv), base frequencies, and rate heterogeneity among sites. The first analysis used a ti/tv ratio of 2.0 and empirical base frequencies and assumed equal rate of evolution for all sites. Trees resulting from this analysis were then used to calculate base frequencies, ti/tv ratio, and the shape parameter of the gamma distribution to estimate rate heterogeneity among sites. A second analysis with the latter values was then conducted.

**Character evolution**—The phylogenetic distribution of chromosome number, ovary position, and geographic distribution was investigated. Chromosome numbers were obtained from Fedorov (1969); ovary positions were based on Hara (1957) and personal observations. Using MacClade 3.05 (Maddison and Maddison, 1992), data were traced onto the strict consensus of shortest trees obtained (see below). To gain insights into the geographic history of *Chrysosplenium* from a phylogenetic perspective, we inferred the ancestral areas of clades recognized in the *matK* tree using MacClade following the general approach of Xiang, Soltis, and Soltis (1998) and Xiang et al. (1998). As noted, *Peltoboykinia* is the sister group of *Chrysosplenium* and was therefore used as the outgroup. However, subsequent sister taxa to *Chrysosplenium/Peltoboykinia* are uncertain (Soltis et al., 1993, 1996). This limits our ability to reconstruct unambiguously the ancestral state for characters in *Chrysosplenium* (Maddison, Donoghue, and Maddison, 1994).

## RESULTS

**Phylogenetic relationships**—Parsimony analysis (indels scored as missing) produced 18 shortest trees, all in one island (Maddison, 1991), each of 371 steps, CI (consistency index) = 0.6852 excluding uninformative characters, RI (retention index) = 0.8509. One of these 18 shortest trees, picked at random, is depicted in Fig. 1 with the nodes that collapse in the strict consensus indicated. As noted in more detail below, the distribution of indels lends further support to relationships suggested by base substitutions.

The two MLE analyses yielded identical trees that are nearly identical to those retrieved with parsimony. For example, the best MLE tree ( $-\ln = 3593.48$ ) differs from the shortest parsimony trees only in the relationships depicted within the *Macrostemon* subclade. The MLE tree (not shown) placed *C. echinus*, *C. fauriae* (Wakabayashi & Nakazawa collection), and *C. macrostemon* in a trichotomy, as sister to *C. kiotense/C. nagei*. In contrast, all of the shortest parsimony trees indicate that *C. fauriae* (Wakabayashi & Nakazawa collection) is sister to *C. echinus*, which in turn is the sister to *C. macrostemon*; *C. macrostemon* is sister to *C. kiotense/C. nagei*. Because of the high degree of overall similarity between MP and MLE trees, only one of the shortest parsimony trees is shown (Fig. 1) and discussed in more detail below.

All shortest trees recognize two large sister clades corresponding to the two groups of Franchet (1890): sect. *Oppos-*

TABLE 1. Species of *Chrysosplenium* sequenced for *matK*. Included are the series (Hara, 1957) to which each species was assigned. Also provided are voucher and GenBank information.

Species	Series	Locality	Collection data	GenBank accession no. <sup>a</sup>
<i>Chrysosplenium</i>				
<i>C. valdivicum</i> Hook.	<i>Valdivica</i>	enroute to Antillanca, Valdivia, Chile	<i>Ono &amp; Nakazawa 942100</i>	GBAN-AB004835
<i>C. americanum</i> Schwein. ex Hook.	<i>Oppositifolia</i>	Virginia, USA	<i>Wieboldt, s.n.</i>	**
<i>C. ramosum</i> Maxim.	<i>Oppositifolia</i>	near Kadoma, Kawai-mura, Iwate Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943129</i>	GBAN-AB003057
<i>C. delavayi</i> Franch.	<i>Delavayi</i>	Sapa, Hoang Lien Son Prov., Viet Nam	<i>Wakabayashi &amp; Nakazawa 943181</i>	GBAN-AB004834
<i>C. glehomaefolium</i> Nutt. ex Torr. et A. Gray	<i>Nepalensis</i>	Humboldt, California, USA	<i>Hardig s.n.</i>	GBAN-L34123
<i>C. grayanum</i> Maxim.	<i>Nepalensis</i>	Shiruchi-machi, Tehigi Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943099</i>	GBAN-AB003048
<i>C. pseudofauriei</i> H. Lev.	<i>Sinica</i>	Iiyama-mura, Tokushima Pref., Japan	<i>Wakabayashi 93045</i>	GBAN-AB003056
<i>C. kamischaticum</i> Fisch. ex Seringe	<i>Kamischatica</i>	Kadoma, Kawai-mura, Iwate Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943117</i>	GBAN-AB003050
<i>C. echinus</i> Maxim.	<i>Macrostemon</i>	Jakkono-taki, Nikko-shi, Tochigi Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943091</i>	GBAN-AB003045
<i>C. fauriae</i> Franch.	<i>Macrostemon</i>	Maeharatani-kawa, Maehara-machi, Takayama-shi, Gifu Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943029</i>	GBAN-AB003046
<i>C. fauriae</i> Franch.	<i>Macrostemon</i>	Ohiwa, Kamichii-machi, Toyama Pref., Japan	<i>Soltis J2503</i>	**
<i>C. kiotense</i> Ohwi.	<i>Macrostemon</i>	Morito-kawa, Futago-yama, Zushi-shi, Kanagawa Pref., Japan	<i>Nakazawa 942009</i>	GBAN-AB003052
<i>C. macrostemon</i> Maxim. ex Franch. et Sav.	<i>Macrostemon</i>	Kakino-kawa, Nishihora, Miyama-machi, Gifu Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943059</i>	GBAN-AB003051
<i>C. nagasei</i> Wakab. et H. Ohba.	<i>Macrostemon</i>	Ohara, Kiyomi-mura, Ono-gun, Gifu Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943024</i>	GBAN-AB003054
<i>C. maximowiczii</i> Franch. et Sav.	<i>Pilosa</i>	Morito-kawa, Futago-yama, Zushi-shi, Kanagawa Pref., Japan	<i>Nakazawa 942014</i>	GBAN-AB003053
<i>C. rhabdospermum</i> Maxim.	<i>Pilosa</i>	Tara-dake, Omura-shi, Nagasaki Pref., Japan	<i>Wakabayashi 90034</i>	GBAN-AB003058
<i>C. pilosum</i> var. <i>sibiricum</i> (Maxim.) Hara	<i>Pilosa</i>	Shichiso-cho, Gifu Pref., Japan	<i>Wakabayashi 93039</i>	GBAN-AB003055
<i>C. pilosum</i> var. <i>fulvum</i> (Terracc.) Hara	<i>Pilosa</i>	Jakko Falls, Tochigi Pref., Japan	<i>Soltis J2535</i>	**
<i>C. album</i> Maxim.	<i>Pilosa</i>	Kamichi, Souryo-cho, Koya-gun, Hiroshima Pref., Japan	<i>Wakabayashi 89034</i>	GBAN-AB003044
<i>C. album</i> Maxim.	<i>Pilosa</i>	Jakko Falls, Tochigi Pref., Japan	<i>Soltis J2534</i>	**
<i>C. carnosum</i> J. D. Hook. et Th. Thomson	<i>Ovalifolia</i>	Sagarmatha zone, Solukhumba Distr., around Duhkund, Nepal	<i>Wakabayashi et al. 9710339</i>	GBAN-AB004833
<i>C. alternifolium</i> L.	<i>Alternifolia</i>	Germany	<i>Hibsch-Jetter s.n.</i>	**
<i>C. alternifolium</i> var. <i>sibiricum</i> Seringe ex DC.	<i>Alternifolia</i>	Iowa, USA	<i>Wendel s.n.</i>	GBAN-L34120
<i>C. alternifolium</i> var. <i>sibiricum</i> Seringe ex DC.	<i>Alternifolia</i>	Alberta, Canada	<i>Packer s.n.</i>	**
<i>C. griffithii</i> J. D. Hook. et Th. Thomson	<i>Alternifolia</i>	Sichuan, Daocheng, Gongga Shan, China	<i>Wu et al. 96-726</i>	**
<i>C. japonicum</i> (Maxim.) Makino	<i>Alternifolia</i>	Taihaku-san, Sendai-shi, Miyagi Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943113</i>	GBAN-AB003049
<i>C. tetrandrum</i> (N. Lund) Th. Fries	<i>Alternifolia</i>	Pink Mt., British Columbia, Canada	<i>Staley 6250, UBC</i>	**
<i>C. tosaense</i> Makino	<i>Alternifolia</i>	Yugate, Torahide, Hanno-shi, Saitama Pref., Japan	<i>Nakazawa 942008</i>	GBAN-AB003059
<i>C. flagelliferum</i> Fr. Schmidt	<i>Flagellifera</i>	Anedaki, Shiraiishi-shi, Gifu Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943108</i>	GBAN-AB003047
<i>C. flagelliferum</i> Fr. Schmidt	<i>Lanuginosa</i>	Sichuan, Mt. Omei, China; cultivated in Nikko Botanical Garden, Japan	<i>W-96002</i>	**
<i>C. davidianum</i> Decne. ex Maxim	<i>Petaloides</i>	Yunnan, Lijiang, China	<i>W-97011</i>	**
Outgroup				
<i>Peltoboykinia tellimoides</i> (Maxim.) Hara		Mito-san, Hinohara-mura, Tokyo Pref., Japan	<i>Wakabayashi &amp; Nakazawa 943165</i>	GBAN-AB003060

<sup>a</sup> The prefix GBAN- has been added to link the online version of *American Journal of Botany* to GenBank, but is not part of the actual accession number.

TABLE 2. Insertions (I-1 to I-9) and deletions (D-1 to D-5) in the *matK* sequences of *Chrysosplenium*, inferred using the sequence of *Peltoboykinia* as the reference. The reference nucleotide (RN) is the position of the nucleotide preceding the indel in the sequence of the reference taxon—this nucleotide is indicated in boldface type in the sequence region. Starting site of *matK* for the reference sequence corresponds to that of the published sequences of mustard and tobacco (Neuhaus and Link, 1987). Dashes represent missing bases associated with indels.

Indel	Taxa involved	RN	Sequence region
I-1	<i>Peltoboykinia</i> <i>C. glechomaefolium</i>	206	ATCAT-----TTG ATCAATTGGTTATTCTGCTTTG
I-2	<i>Peltoboykinia</i> <i>C. americanum</i>	252	GGGG-----CATA GGGGCATAATCATA
I-3	<i>Peltoboykinia</i> <i>C. glechomaefolium</i> <i>C. oppositifolium</i>	264	AAT-----TTATATTA AATAATAATTATATTA AATAATAA?TTATATTA
I-4	<i>Peltoboykinia</i> <i>C. album</i> <i>C. rhabdospermum</i>	303	GTCATT-----GTGGA GTCATTTCAGTCATTGTGGA GTCATTTCAGTCATTGCGGA
I-5	<i>Peltoboykinia</i> <i>C. alternifolium</i> <i>C. tetrandrum</i> <i>C. japonicum</i> <i>C. henryi</i> <i>C. carnosum</i> <i>C. davidianum</i> <i>C. griffithii</i>	549	AGTAT-----TACAA AGTATTATATTACAA AGTATTATATTACAA AGTATTATATTATAA AGTATTACAATTTAAA AGTATTACAATTTAAA AGTATTACAATTTAAA AGTATTACAATTTAAA
I-6	<i>Peltoboykinia</i> <i>C. tosaense</i>	577	CTCCAA-----CTCCAA CCCCAATCCCAACTCCAA
I-7	<i>Peltoboykinia</i> <i>C. grayanum</i> <i>C. kiotense</i> <i>C. macrostemon</i>	607	CCATTG-----TTTCA CCA---CTTCCACTGTTCA CCATTGATTCAAATGATTCA
I-8	<i>Peltoboykinia</i> <i>C. flagelliferum-S</i>	774	TGTA-----GAAGT TGTAATAAATCTTGTAGAAGT
I-9	<i>Peltoboykinia</i> <i>C. album</i> <i>C. rhabdospermum</i>	774	TGTA-----GAAGT TGTAATCTGTAGAAGT TGTAATCTGTAGAAGT
D-1	<i>Peltoboykinia</i> <i>C. fauriae-S</i>	537	TTTCTCTATGAGTAT TTT-----CAGTAT
D-2	<i>Peltoboykinia</i> <i>C. echinus</i> <i>C. fauriae-W &amp; N</i> <i>C. nagasei</i> <i>C. kiotense</i> <i>C. macrostemon</i>	546	TGAGTATTACAATTTAAGTAGTC TTAC-----AGTT TTAC-----AGTT TTAC-----AGTT TTAC-----AGTT TTAC-----AGTT
D-3	<i>Peltoboykinia</i> <i>C. fauriae-S</i>	549	GTATTACAATTTAAGTA GTATTACA-----
D-4	<i>Peltoboykinia</i> <i>C. henryi</i> <i>C. carnosum</i> <i>C. griffithii</i> <i>C. davidianum</i>	577	CTCCAACTCCAAAGAAAT CCCCAA-----ATAAAT CTCCAA-----ATAAAT TCCAAA-----ATAAAT CCCCAA-----ATAAAT
D-5	<i>Peltoboykinia</i> <i>C. grayanum</i>	607	CCATTG-----TTTCA CCA---CTTCCACTGTTCA

*itifolia*, with bootstrap and decay values of 92% and 5, respectively, and a poorly supported sect. *Alternifolia* (44%, 2). Within the *Oppositifolia* clade, *C. pseudofauriei*, *C. grayanum* (both from eastern Asia), *C. oppositifolia* (from Europe), *C. americanum* (eastern North American), and *C. glechomaefolium* (western North America) form a weakly supported clade (62%, 2). A second clade (72%, 3) within *Oppositifolia* contains *C. delavayi* (China and Taiwan) as sister to *C. valdivicum* (Chile) and *C. ramosum* (northeastern China). The remaining members of the *Oppositifolia* clade, all from eastern Asia, form a moderately supported (78%, 3) subclade, within which *C. kantschaticum* is sister to the remaining members; they, in

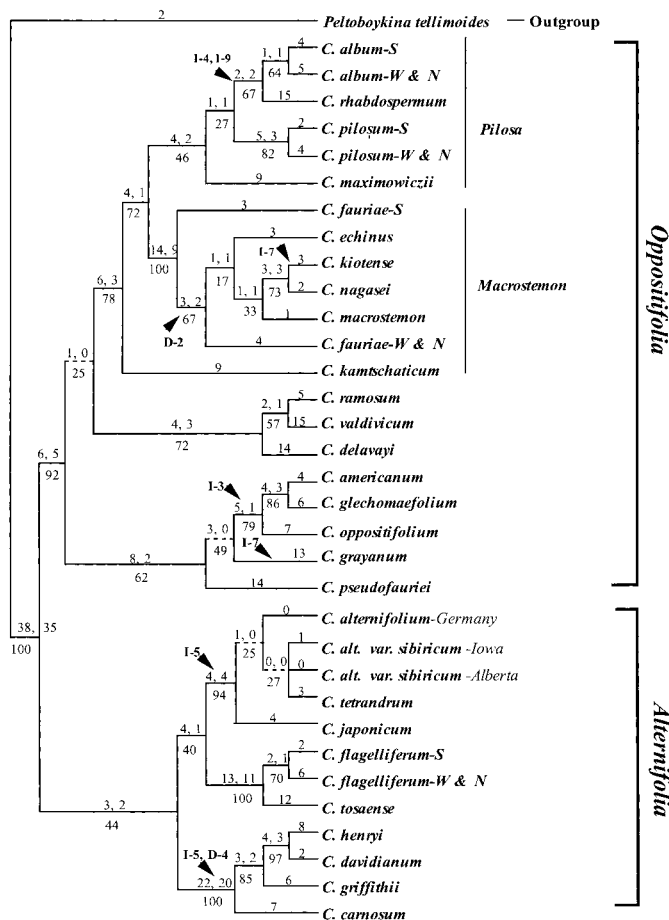


Fig. 1. One of the 18 most parsimonious trees resulting from parsimony analysis of *matK* sequences with indels coded as missing data (371 steps, CI = 0.6852 excluding uninformative characters, RI = 0.8509). Numbers above branches represent numbers of nucleotide changes and Bremer (1988) support, respectively, and numbers below branches are bootstrap values in percentages. Dashed lines indicate the nodes not found in all shortest trees. For those species for which more than one population was sampled, population designations follow the name of the species (W & N = collection of Wakabayashi & Nakazawa; S = collection of Soltis).

turn, form two subclades. The first is a well-supported (100%, 9) *Macrostemon* clade of *C. fauriae*, *C. echinus*, *C. kiotense*, *C. nagasei*, and *C. macrostemon*, all of series *Macrostemon* and occurring in Japan, and the second is a *Pilosa* clade (without support >50%) of *C. maximowiczii*, *C. pilosum*, *C. rhabdospermum*, and *C. album*, all of series *Pilosa* and occurring primarily in Japan.

The *Alternifolia* clade comprises two subclades, one of which is strongly supported (100%, 20) and contains *C. carnosum*, *C. griffithii*, *C. davidianum*, and *C. henryi*, all from eastern Asia. The second major subclade within *Alternifolia* does not receive bootstrap support >50%. It comprises *C. flagelliferum* and *C. tosaense* from eastern Asia as the well-supported (100%, 11) sister group to a clade (94%, 4) that includes *C. japonicum* (eastern Asia) as the sister to a clade of *C. alternifolium* and *C. tetrandrum* (both circumboreal).

Multiple populations of five species were sampled. In *C. album*, *C. pilosum*, and *C. flagelliferum*, the two populations sampled are sisters. However, the two populations sampled of *C. fauriae* are not immediate sisters, but rather successive,

basal branches of the *Macrostemon* clade. Similarly, the populations of *C. alternifolium* sampled form a clade only with the inclusion of *C. tetrandrum*.

Some of the indels detected provide additional support for clades. Indels I-1, I-2, I-6, I-8, D-1, D-3, and D-5 are unique to individual taxa and will not be discussed further (Table 2). Other indels further support relationships indicated by base substitutions; these findings for *Chrysosplenium* further support a trend noted in other analyses employing *matK* sequence data—indels in *matK* are often phylogenetically informative (reviewed in Soltis and Soltis, 1998). Two insertions, I-4 and I-9, both unite *C. album* with *C. rhabdospermum*. The deletion D-2 is an additional synapomorphy for all members of the *Macrostemon* subclade, with the exception of the first-branching member, *C. fauriae* (Soltis collection); thus, D-2 unites the subclade consisting of *C. echinus*, *C. fauriae* (Wakabayashi & Nakazawa collection), *C. nagasei*, *C. kiotense*, and *C. macrostemon* (Table 2; Fig. 1). This same deletion provides additional evidence for the distinctness of the two populations of *C. fauriae* sampled. More populations of *C. fauriae* should be carefully studied; these two populations should perhaps ultimately be treated as distinct species. Insertion I-5 appears to have arisen twice independently, with each occurrence representing a synapomorphy for a distinct clade: (1) *C. alternifolia*, *C. tetrandrum*, and *C. japonicum*; (2) *C. henryi*, *C. davidianum*, *C. griffithii*, and *C. carnosum* (Table 2; Fig. 1). Insertion I-3 is present in *C. oppositifolium* and *C. glechomaefolium*, but not *C. americanum* (the sister of *C. glechomaefolium*). Hence, this insertion either arose independently in *C. oppositifolium* and *C. glechomaefolium* or arose in the ancestor of this clade of three species with a subsequent loss in *C. americanum*. Insertion I-7 occurs in two distantly related members of the *Oppositifolia* clade (*C. grayanum* and *C. kiotense*) and thus appears to be homoplasious. It is noteworthy that three of the nine bases involved in the insertion differ between the two species, possibly reflecting the independent nature of this insertion in these taxa. In addition, a deletion (D-5) occurs at the beginning of this same region in *C. kiotense*, suggesting that the history of insertion and deletion for this area may be more complex in this species than in *C. grayanum*.

## DISCUSSION

**Phylogenetic relationships**—*Chrysosplenium* has been historically divided into two major groups, section *Oppositifolia* with opposite leaves and section *Alternifolia* with alternate leaves (Franchet, 1890). Phylogenetic analyses of *matK* sequence data for 33 collections representing much of the diversity of *Chrysosplenium* support the division of the genus into two major clades, one with opposite leaves and one with alternate leaves. Thus, sections *Oppositifolia* and *Alternifolia* reflect phylogenetic relationships, and leaf phyllotaxy is a diagnostic morphological character.

Hara also recognized 17 series in *Chrysosplenium*, many of which contain only one or a few species; two of the series he recognized appear monophyletic in our analyses. Series *Macrostemon*, consisting of *C. echinus*, *C. fauriae*, *C. kiotense*, *C. macrostemon*, and *C. nagasei*, all from Japan, receives strong support (100%, 9) as a monophyletic group. Series *Pilosa*, represented in our study by *C. maximowiczii*, *C. rhabdospermum*, *C. pilosum*, and *C. album* also is monophyletic in all phylogenetic analyses, albeit without support >50%. The *Pilosa* subclade is also primarily centered in Japan, with

*C. pilosum* also known from Korea. However, in contrast to our findings for series *Macrostemon* and *Pilosa*, *matK* sequence data do not provide support for the monophyly of Hara's series *Oppositifolia*, *Nepalensia*, or *Alternifolia*. Of the three species sampled of series *Oppositifolia* (Table 1), only *C. americanum* and *C. oppositifolium* are closely related; *C. ramosum* appears as a member of a distinct clade. *Chrysosplenium glechomaefolium* and *C. grayanum* of series *Nepalensia* are both members of the same subclade, but are not sister taxa. Instead, *C. glechomaefolium*, from western North America, is sister (with strong support) to *C. americanum* (series *Oppositifolia*) from eastern North America. Series *Alternifolia* appears grossly polyphyletic with species spread among the three subclades present within the *Alternifolia* clade (Fig. 1).

**Chromosome evolution**—The most frequently reported haploid chromosome numbers for *Chrysosplenium* are  $n = 11$  and  $n = 12$ , but several species have  $n = 4$ ,  $n = 8$ ,  $n = 9$ , and  $n = 21$  (Fedorov, 1969; Spongberg, 1972). For example, *C. delavayi* has  $n = 4$ ; *C. henryi* has  $n = 8$ ; *C. glechomaefolium*, *C. griffithii*, and *C. davidianum* have  $n = 9$ ; and *C. oppositifolium* has  $n = 21$ . These are unusual numbers for Saxifragaceae, a family in which most genera have  $n = 7$ . For the two most common numbers in *Chrysosplenium* ( $n = 11$ , 12), these numbers are known elsewhere only in some species of *Saxifraga* (Webb and Gornall, 1989), with  $n = 11$  also reported for *Peltoboykinia*, the sister group of *Chrysosplenium*. Given that  $n = 11$  in *Peltoboykinia*, it is likely that the original base chromosome number for *Chrysosplenium* was also  $n = 11$  (Fig. 2). Reconstructions of the evolution of chromosome numbers in *Chrysosplenium* (using MacClade version 3.05; Maddison and Maddison, 1992) suggest that  $n = 12$  arose independently several times. For example, if  $n = 11$  is ancestral, considering first the large *Oppositifolia* clade,  $n = 12$  has evolved independently in the *Pilosa* subclade, *C. ramosum* or the ancestor of the small clade to which it belongs, and either in the ancestor of the *C. americanum/C. glechomaefolium/C. oppositifolium/C. pseudofauriei* clade (with subsequent chromosomal evolution in *C. oppositifolium* and *C. glechomaefolium*), or perhaps multiple times just within this clade (Fig. 2). For the large *Alternifolia* clade,  $n = 12$  characterizes the large subclade containing *C. tosaense*, *C. flagelliferum*, *C. japonicum*, *C. tetrandrum*, and *C. alternifolium*.

Our data also indicate several examples of aneuploid decrease in *Chrysosplenium*. For example, in the small clade composed of *C. davidianum*, *C. henryi*, *C. griffithii*, and *C. carnosum*, *C. carnosum* is sister to the remainder of the clade. Beginning with an ancestral number of  $n = 11$ , a reduction to  $n = 9$  subsequently occurred in the ancestor of *C. davidianum*, *C. henryi*, *C. griffithii*; this was followed by a reduction to  $n = 8$  in *C. henryi*. A separate, independent reduction to  $n = 9$  also occurred in *C. glechomaefolium*. An additional instance of aneuploid decrease is also evident in *C. delavayi*, which has  $n = 4$ . Multiple episodes of aneuploidy have similarly been documented across Saxifragaceae, having occurred on a massive scale independently in both *Micranthes* and *Saxifraga* (Soltis et al., 1996).

An example of chromosome increase, probably involving polyploidy, is observed in *C. oppositifolium*, with  $n = 21$ . The first two branches of the clade to which *C. oppositifolium* belongs are *C. grayanum* and *C. pseudofauriei*, with  $n = 11$  and  $n = 12$ , respectively. A likely scenario is a polyploid event, perhaps involving ancestor(s) with  $n = 11$  leading to a poly-

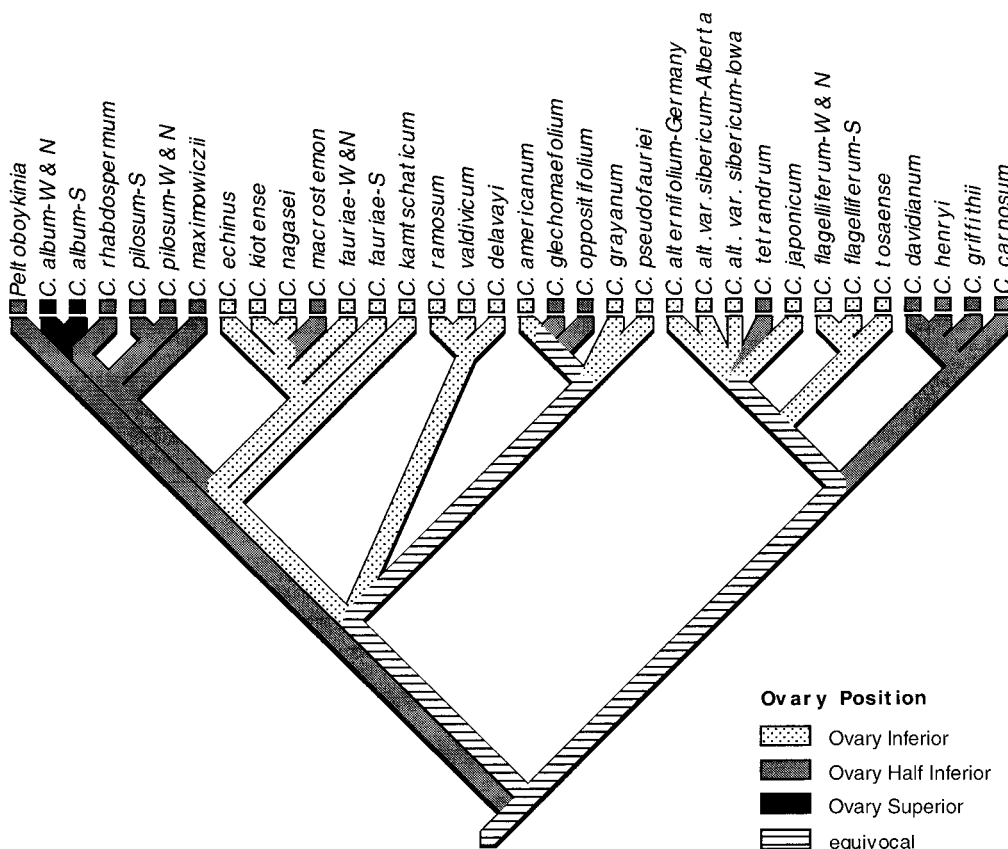


Fig. 2. Strict consensus of the shortest trees obtained for *Chrysosplenium* using *matK* sequence data, onto which chromosome numbers have been mapped using MacClade (Maddison and Maddison, 1992). Chromosome numbers are from Fedorov (19690). For those species for which more than one population was sampled, population designations follow the name of the species (W & N = collection of Wakabayashi & Nakazawa; S = collection of Soltis).

ploid with  $n = 22$ ; this would be followed by aneuploid decrease to  $n = 21$ .

**Ovary position**—Ovary position has been proposed to evolve in a unidirectional manner throughout the angiosperms from superior to greater inferiority, generally via congenital fusion of the hypanthium to the ovary wall (e.g., Eames, 1931, 1961; Langdon, 1939; Douglas, 1944, 1957; Gauthier, 1950; reviewed in Kuzoff, Hufford, and Soltis, 2001). This model of unidirectional ovary evolution has been invoked to account for the remarkable range of ovary positions seen in some genera of Saxifragaceae, for example *Saxifraga* (Stebbins, 1974) and *Lithophragma* (Taylor, 1965). As is the case for *Lithophragma*, *Saxifraga*, and other genera and small clades within Saxifragaceae, the ovary position reported for species of *Chrysosplenium* varies widely. Ovary position has been reported as superior for *C. album* (a superior ovary has also been reported for *C. ludlowii*, but this species is known only from the type collection), with half-inferior and inferior ovaries present in the remaining taxa we sampled (Fig. 3). *Peltoboykinia*, the sister of *Chrysosplenium*, has an ovary that is half-inferior. The ancestor of *Chrysosplenium* is reconstructed as having an ovary that is either half-inferior or completely inferior. Considering first the opposite-leaved clade, the ancestor of this clade also had an ovary position that was either half or fully inferior. The common ancestor of the *Pilosa/Macrostemon/C. kamtschaticum* clade is reconstructed as having an inferior ovary. An inferior ovary characterizes the *Macrostemon* clade, with

the evolution of a half-inferior ovary occurring in one member, *C. macrostemon*. Both inferior and half-inferior ovaries also evolved within the *C. americanum/C. glechomaefolium/C. oppositifolium/C. grayanum/C. pseudofauriei* clade, although the lack of resolution within this clade precludes an accurate interpretation of the transitions that occurred. A half-inferior ovary characterizes the *Pilosa* clade. It is noteworthy that *C. album*, with what has been termed a superior ovary, is derived within this clade from an ancestor with a half-inferior ovary.

The ovary position of the ancestor of the alternate-leaved clade was either inferior or half-inferior. A half-inferior ovary is found in all members of the *C. davidianum/C. henryi/C. griffithii/C. carnosum* clade. The remaining alternate-leaved taxa have inferior ovaries, with a half-inferior ovary present in *C. tetrandrum*. Thus, regardless of the ovary position of the ancestor of this clade, our reconstruction suggests a dynamic course of ovary diversification. That is, if the ancestor was inferior, a half-inferior ovary evolved independently in *C. tetrandrum* and again in the *C. davidianum*, *C. henryi*, *C. griffithii*, and *C. carnosum* clade. Alternatively, if the ancestral condition was a half-inferior ovary, an inferior ovary evolved in the *C. alternifolium/C. tetrandrum/C. flagelliferum/C. tosaense* clade, with a subsequent reversal in *C. tetrandrum*.

Thus, MacClade reconstructions of character states indicate that the evolution of ovary position in *Chrysosplenium* has been dynamic, with several shifts between completely inferior and half-inferior ovaries (Fig. 3). It is particularly noteworthy that the superior ovary of *C. album* is derived from a half-

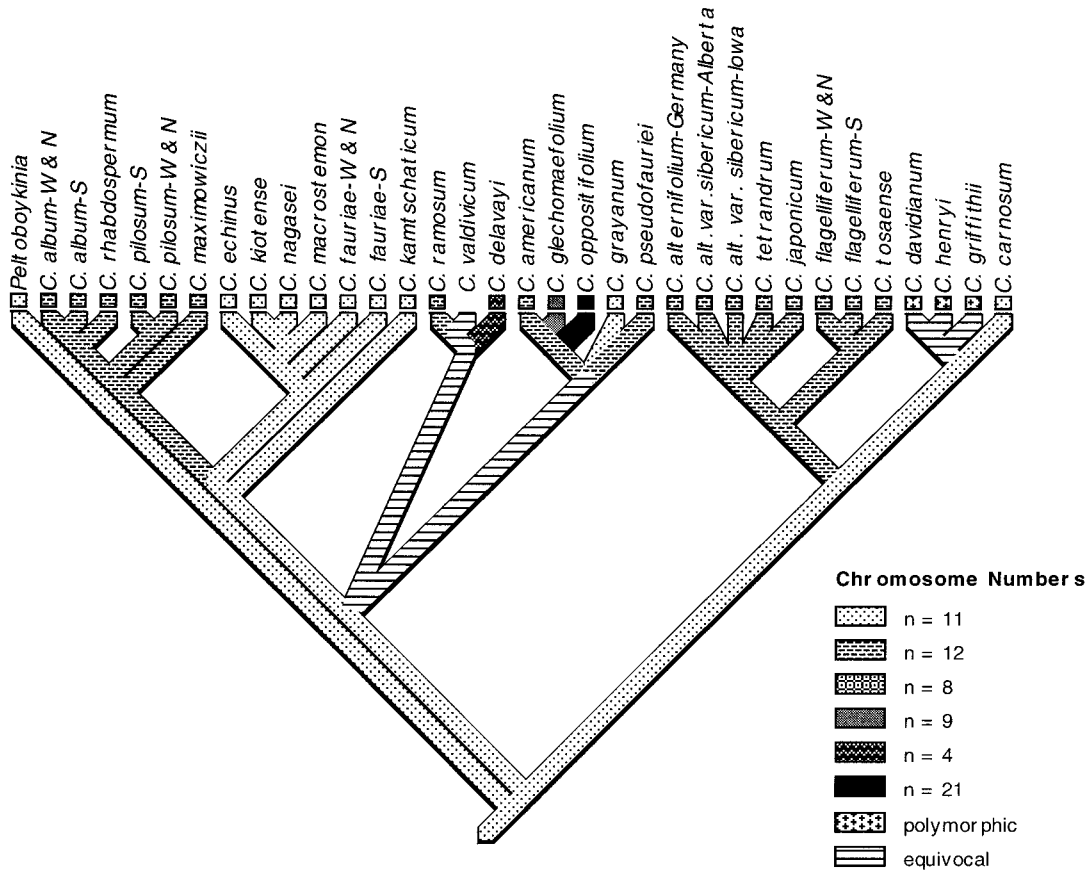


Fig. 3. Strict consensus of the shortest trees obtained for *Chrysosplenium* using *matK* sequence data, onto which ovary position has been mapped using MacClade (Maddison and Maddison, 1992). Ovary positions are based on the descriptions provided by Hara (1957). For those species for which more than one population was sampled, population designations follow the name of the species (W & N = collection of Wakabayashi & Nakazawa; S = collection of Soltis).

inferior ancestor. The labile nature of gynoecial diversification in *Chrysosplenium* is in agreement with similar results based on analyses of other well-defined genera within Saxifragaceae in which substantial variation in ovary position has been observed (e.g., Kuzoff et al., 1999; Mort and Soltis, 1999). For example, ovary position in *Lithophragma* ranges from inferior to what appears to be completely superior, with a wide range of intermediate conditions. Kuzoff et al. (1999) demonstrated that: (1) the ancestor of *Lithophragma* had an ovary that was half-inferior; (2) ovary position has evolved toward greater inferiority in some species (e.g., *L. affine*, *L. parviflorum*, and *L. trifoliatum*) and greater superiority in others (e.g., *L. glabrum*, *L. heterophyllum*, and *L. campanulatum*); and (3) variation in ovary position in *Lithophragma* is not the result of a unidirectional trend. Very similar conclusions can now be drawn for *Chrysosplenium*. That is, the ancestor of *Chrysosplenium* had an ovary that was half-inferior, and there has been evolution to increasing inferiority in some species and increasing superiority in others. Ontogenetic analyses of the early gynoecial development in the Saxifragaceae indicate that there are no truly superior ovaries in the family (Kuzoff, Hufford, and Soltis, 2001; Soltis et al., unpublished data). That is, all ovaries appear to develop from what is termed an appendicular epigynous ground plan and hence are technically “inferior.” Truly superior ovaries, in contrast, develop from a hypogynous ground plan, and this ground plan has not been observed in the family. Subtle allometric shifts in early ontogeny in the

superior vs. inferior region of the ovary appear to be responsible for the diverse array of ovary positions present in Saxifragaceae (Kuzoff, Hufford, and Soltis, in press). Thus, in Saxifragaceae, ovaries that appear superior are not homologous to truly superior ovaries but are best referred to as “superior mimics” (Kuzoff, Hufford, and Soltis, in press).

**Biogeography**—*Chrysosplenium* exhibits a wide distribution in the arctic and north temperate zones, with two species disjunctly distributed in the Southern Hemisphere. Most species of the genus occur in eastern Asia, a few in circumpolar regions, two in eastern North America, and four in western North America.

Hara (1957) considered the two species from South America, *C. valdivicum* and *C. macranthum*, to be ancestral in the genus because they seem to retain features that Hara considered “primitive,” such as glabrous and isophyllous stems, opposite leaves, simple inflorescences, short stamens and styles, smooth seeds, and an inferior ovary. The two species from South America are similar morphologically, differing slightly in leaf morphology. We obtained material of *C. valdivicum*, and our phylogenetic trees indicate clearly that this species is not the first-branching member of *Chrysosplenium*, but instead occurs as a derived member of the genus in a small clade with *C. delavayi* and *C. ramosum*, both of which occur in eastern Asia; *C. delavayi* appears as the sister to *C. valdivicum/C. ramosum*. Rather than a South American origin of *Chryso-*

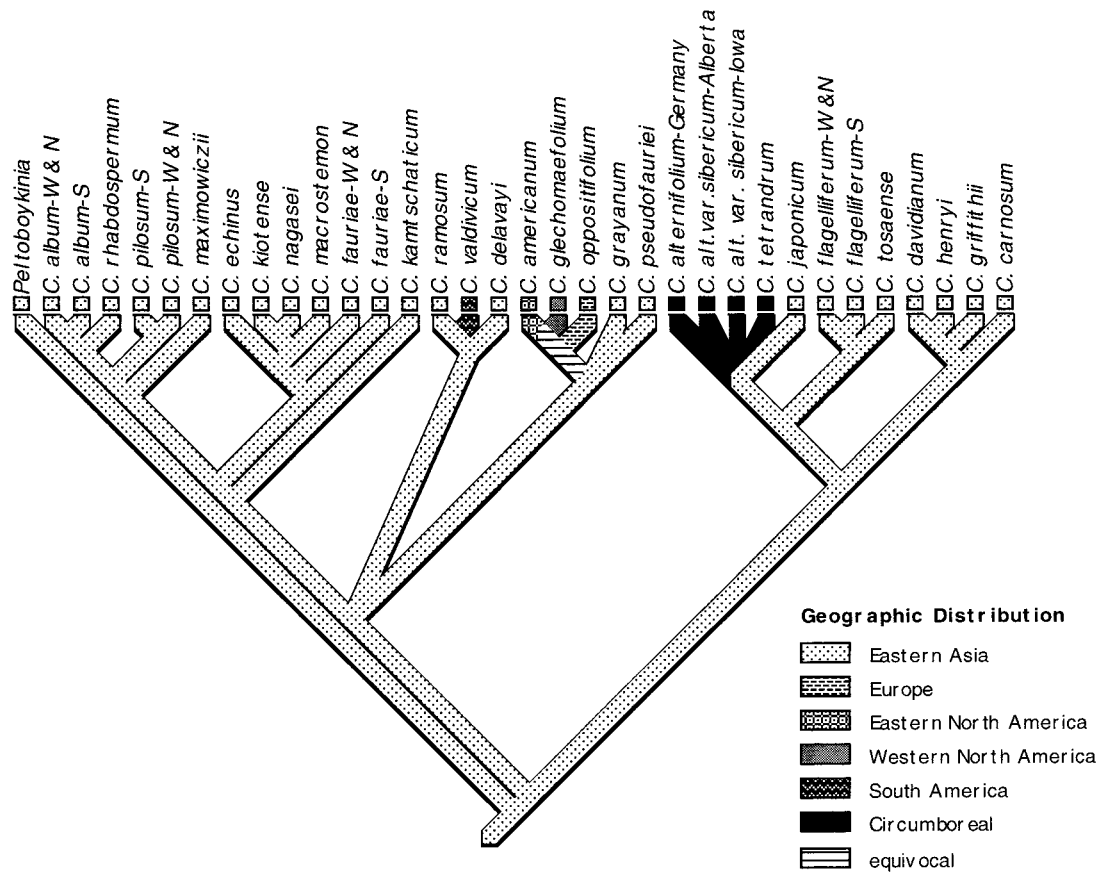


Fig. 4. Strict consensus of the shortest trees obtained for *Chrysosplenium* using *matK* sequence data, onto which geographic area has been mapped using MacClade (Maddison and Maddison, 1992). Geographic distributions are from Hara (1957) and Spongberg (1972). For those species for which more than one population was sampled, population designations follow the name of the species (*W* & *N* = collection of *Wakabayashi* & *Nakazawa*; *S* = collection of *Soltis*).

*splenium*, our phylogenetic reconstructions suggest an Asian origin of the genus, with at least three separate instances of migration to the New World.

Phylogenetic analyses of *Chrysosplenium* demonstrate that the eastern Asian species are distributed in both major clades (*Alternifolia* and *Oppositifolia*), as well as among all of the larger subclades. Our results indicate that the ancestral area of the genus is eastern Asia, suggesting that *Chrysosplenium* originated in eastern Asia, with subsequent movement to Europe and North and South America (Fig. 4). Our data also suggest that the genus had undergone diversification into the alternate- and opposite-leaved groups in eastern Asia before it spread into other parts of the world. This scenario of an Asian origin is consistent with the hypothesis of Savile (1975). Based on relationships among rust parasites (genus *Puccinia*) that use members of Saxifragaceae as hosts, Savile (1975) proposed that several genera of Saxifragaceae having disjunct distributions in eastern North America, eastern Asia, and western North America actually originated in eastern Asia with subsequent migration from that area of origin to other regions of the Northern Hemisphere.

As noted, the most parsimonious trees indicate that *Chrysosplenium* early diverged into two lineages that correspond to the two recognized sections *Alternifolia* and *Oppositifolia*. The alternate-leaved lineage subsequently diverged into two subgroups. One subgroup of the *Alternifolia* clade consists of a few species (*C. henryi*, *C. davidianum*, *C. griffithii*, and *C.*

*carnosum*) occurring in the Himalayan region; the other subgroup is composed of three Japanese species (*C. japonicum*, *C. flagelliferum*, and *C. tosaense*) and two circumpolar species (*C. alternifolium* and *C. tetrandrum*). Thus, our results suggest at least one migration event out of eastern Asia occurred to establish the circumboreal distributions of the latter two species.

Two additional migration events out of eastern Asia are also apparent in the *Oppositifolia* clade. The opposite-leaved lineage diverged into three subclades, one of which is exclusively Asian in distribution (the *Pilosa/Macrostemon* clade); a second small clade of three species also occurs in Asia, with one species (*C. valdivicum*) in Chile. The third subclade consists of several species displaying a disjunct distribution in eastern Asia, eastern North America, western North America, and Europe (i.e., *C. grayanum* and *C. pseudofauriei* from eastern Asia, *C. oppositifolium* from Europe, *C. americanum* from eastern North America, and *C. glechomaefolium* from western North America) (Fig. 4). Within this clade, the two North American species (*C. americanum*/*C. glechomaefolium*) are sisters; their immediate sister is the European species, *C. oppositifolium*, followed by the two Asian species, *C. grayanum* and *C. pseudofauriei*. Thus, the data indicate an additional migration event out of eastern Asia in the *Oppositifolia* clade. Furthermore, within this subclade the Old World species are the sister taxa to the New World species, with the eastern and western North American taxa appearing as sisters. The phy-

logenetic pattern within this clade is identical to that found in *Aesculus* (Hippocastanaceae), which exhibits the same disjunct distribution in eastern Asia, eastern North America, western North America, and Europe (Xiang et al., 1998). This phylogenetic pattern is also similar to that found for several other genera (e.g., *Tiarella*, *Boykinia*, *Trautvetteria*, *Calycanthus*, *Aralia* sect. *Aralia*, big-bracted species of *Cornus*) displaying disjunct distributions in eastern Asia, western North America, and eastern North America (Xiang, Soltis, and Soltis, 1998). In all of these taxa, the North American species form a monophyletic group sister to the Asian species. This pattern suggests a closer relationship between the floras of eastern and western North America than between eastern Asia and eastern North America. This relationship is in contrast to the long-standing view (first proposed by Gray, 1846) that a closer relationship exists between eastern Asian and eastern North American floras.

The second subclade in the *Oppositifolia* clade consists of three species showing a disjunct distribution in eastern Asia and the Southern Hemisphere (i.e., *C. valdivicum* from Chile, *C. ramosum* from northeastern China, and *C. delavayi* from eastern Asia; with the first two species being sisters) (Figs. 1, 4). Thus, the data imply still another migration event from Asia to the New World. The occurrence of *Chrysosplenium* in the southernmost portion of South America represents an intriguing disjunction. This disjunction pattern is rare and has been considered difficult to explain; long-distance dispersal or a relictual pattern resulting from vicariance have both been proposed to explain the disjunction (see Thorne, 1972, Schnabel and Wendel, 1998). For example, Qin (1997) proposed that the disjunct distribution of Lardizabalaceae in eastern Asia and the Southern Hemisphere resulted from the fragmentation of a once worldwide distribution of the family that formed before the separation of Laurasia and Gondwana. In contrast, Thorne (1972) proposed that *Coriaria* (Coriariaceae) obtained its distribution in the two hemispheres via long-distance dispersal by birds. Schnabel and Wendel (1998) argue against vicariance and also favor long-distance dispersal to explain the distribution of *Gleditsia* (Fabaceae), a genus of 13 species found disjunctly in eastern North America and eastern Asia with a single species from South America. As in *Chrysosplenium*, a molecular-based phylogenetic tree demonstrated that the South American species of *Gleditsia* forms a clade with two species from eastern Asia. Sequence divergence between the single South American species and its close relatives in Asia suggested long-distance dispersal (within the last 8 million years) for this disjunction in *Gleditsia* (Schnabel and Wendel, 1998).

Thus, long-distance dispersal is also one possible explanation for the disjunction in *Chrysosplenium*. As argued by Schnabel and Wendel (1998) for *Gleditsia*, there is no evidence to suggest that this particular clade (*C. valdivicum*/*C. ramosum*/*C. delavayi*) within the opposite-leaved lineage of *Chrysosplenium* (a derived lineage within Saxifragaceae; Soltis et al., 1993, 1996) is an ancient lineage that evolved before the separation of Laurasia and Gondwana. We cannot provide an estimate of divergence times for species of *Chrysosplenium* occurring in Asia and South America because no absolute substitution rate (or clock) has been estimated for *matK* and no suitable fossil record exists in Saxifragaceae, or a close relative, for calibration. A comparison of sequence divergence values suggests, however, that the disjunction is probably not of recent origin. The following sequence divergence values were estimated using total substitutions calculated using Jukes-Can-

tor distance: 0.025 between *C. ramosum* (eastern Asia) and *C. delavayi* (southwestern China), 0.025 between *C. ramosum* and *C. valdivicum* (Chile), and 0.034 between *C. valdivicum* and *C. delavayi*. In the sister clade, sequence divergence is 0.012 between *C. americanum* (eastern North America) and *C. glechomaefolium* (western North America), and 0.020 between *C. oppositifolium* (Europe) and the clade of *C. americanum*/*C. glechomaefolium*. Assuming that *matK* is evolving in a roughly clock-like manner in these two clades, the data suggest that the species in eastern Asia and Chile diverged before the isolation of the North American and European species. Although the data seem to preclude relatively recent long-distance dispersal as a likely explanation for this disjunction, ancient long-distance dispersal remains a likely explanation. The seeds of *Chrysosplenium* are small, as in other Saxifragaceae, so long-distance dispersal of seeds from Asia to South America is a possibility. However, these plants occur on forest floors, typically in very wet areas, under dense forest canopies, all of which would make long-distance dispersal on such a large geographic scale more difficult.

Alternatively, this disjunct distribution could represent remnants of a once more continuous geographic distribution of this particular subclade of the genus *Chrysosplenium*, involving migration from Asia down the western Cordillera of North America and South America with subsequent extinction in much of the intervening area. Interrupted migration of plants between North and South America was possible via the West Indies as early as the Oligocene, and direct migration between North and South America became feasible 5.7 million years ago when the union of North and South America occurred (Raven and Axelrod, 1974). One difficulty with this hypothesis is the absence of members of this *C. valdivicum*/*C. ramosum*/*C. delavayi* alliance in the area between Asia and South America. Suitable habitats seem to be present given that other species of *Chrysosplenium* (from other subclades) are found throughout the Pacific Northwest of North America, extending as far south as California. Thus, these data would also seem to favor ancient long-distance dispersal.

*Chrysosplenium* represents one of several genera in Saxifragaceae having a largely Northern Hemisphere distribution with highly disjunct members in South America. For example, the monotypic *Hieronymusia* is known only from a small area of northern Argentina and southern Bolivia; its putatively closest relatives (species of *Suksdorfia*) occur in the Pacific Northwest of North America (Gornall and Bohm, 1985). Species of *Saxifraga* (in the strict sense; see Soltis et al., 1996) are similarly widely distributed in North America, Europe, and Asia, with a few disjunct species (typically considered to represent distinct genera, *Saxifragoides* and *Saxifragella*; see Engler, 1930) known from the southern tip of South America. In contrast to our results for *Chrysosplenium*, however, phylogenetic analyses of *matK* sequences suggest that *Saxifragella*, endemic to Tierra del Fuego, actually represents an early branch of the *Saxifraga* lineage (Soltis et al., unpublished data), with the other early-branching species occurring in Asia and the Pacific Northwest of North America (the relationships of *Saxifragoides* are less clear). Thus, South American disjuncts in different genera of Saxifragaceae may have different relationships with congeners, and perhaps also different origins. At this point, however, either ancient long-distance dispersal or migration from Asia down the western Cordillera of North America and South America with subsequent extinction in much of the intervening area could explain the disjunctions in *Chrysosplen-*

*ium* and *Saxifraga*. More examples of these disjunctions involving North America and South America and eastern Asia and South America should be critically examined.

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