
PHYLOGENETIC AND
BIOGEOGRAPHIC
DIVERSIFICATION IN
OSMORHIZA (APIACEAE)¹

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ABSTRACT

Osmorhiza Raf. (Apiaceae) consists of 10 species disjunctly distributed in temperate Asia (1 sp.) and the Americas (9 spp.). *Osmorhiza berteroi* and *O. depauperata* show an American antitropical disjunction. Within North America, these two species are also disjunctly distributed in eastern and western North America and the Great Lakes regions. A phylogenetic analysis was conducted to clarify inter- and intraspecific relationships based on sequences of the ITS and 5.8S regions of nrDNA. With *Anthriscus*, *Geocaryum*, and *Myrrhis* as outgroups, the monophyly of *Osmorhiza* is strongly supported. The ITS phylogeny suggests the basal position of the Asiatic *O. aristata* and the monophyly of the nine New World species. The ITS sequence of *Osmorhiza aristata* is relatively divergent from those of all other species even though it is morphologically similar to the eastern North American *O. claytonii* and *O. longistylis* (which form a clade), suggesting early divergence followed by morphological stasis. *Osmorhiza berteroi*, *O. brachypoda*, *O. depauperata*, *O. mexicana*, *O. occidentalis*, and *O. purpurea* constitute a monophyletic group (= western North American clade). The morphologically distinct *O. glabrata* from the central Andes forms a trichotomy with the eastern North American clade (*O. claytonii* and *O. longistylis*) and the western North American clade in parsimony and maximum likelihood analyses. The 11 populations studied of the widespread *O. berteroi* form a clade, and showed little sequence divergence, suggesting recent establishment of the widely disjunct populations following long-distance dispersal. Disjunct populations of *O. depauperata* from the Rocky Mountains and eastern North America have an identical ITS profile. *Osmorhiza occidentalis*, however, shows a high level of infraspecific sequence divergence. The ITS phylogeny and the low sequence divergence values suggest rapid diversification of *Osmorhiza* in western North America.

Key words: Apiaceae, biogeography, disjunction, morphological stasis, *Osmorhiza*.

Osmorhiza Raf. (Apiaceae) comprises ten species, with one species in Asia, eight species in North America (three of which also occur in South America), and one species restricted to the central Andes. The distribution of species within the genus provides an ideal model for studying the evolution of both intra- and intercontinental disjunctions, with three distinct patterns represented among its members (Fig. 1; cf. also Lowry & Jones, 1984). According to the most recent classification of the

genus (Lowry & Jones, 1984; see also Kartesz & Ghandi, 1993), the sole Asian species, *O. aristata*, is closely related to two species found in eastern North America (*O. claytonii* and *O. longistylis*). The second distributional pattern is seen in *O. berteroi* and *O. depauperata*, both of which exhibit an antitropical (often inappropriately referred to as “amphitropical,” see Cox, 1990) disjunction between temperate North and South America. Also, the more distantly related *O. mexicana* subsp. *mexicana* has

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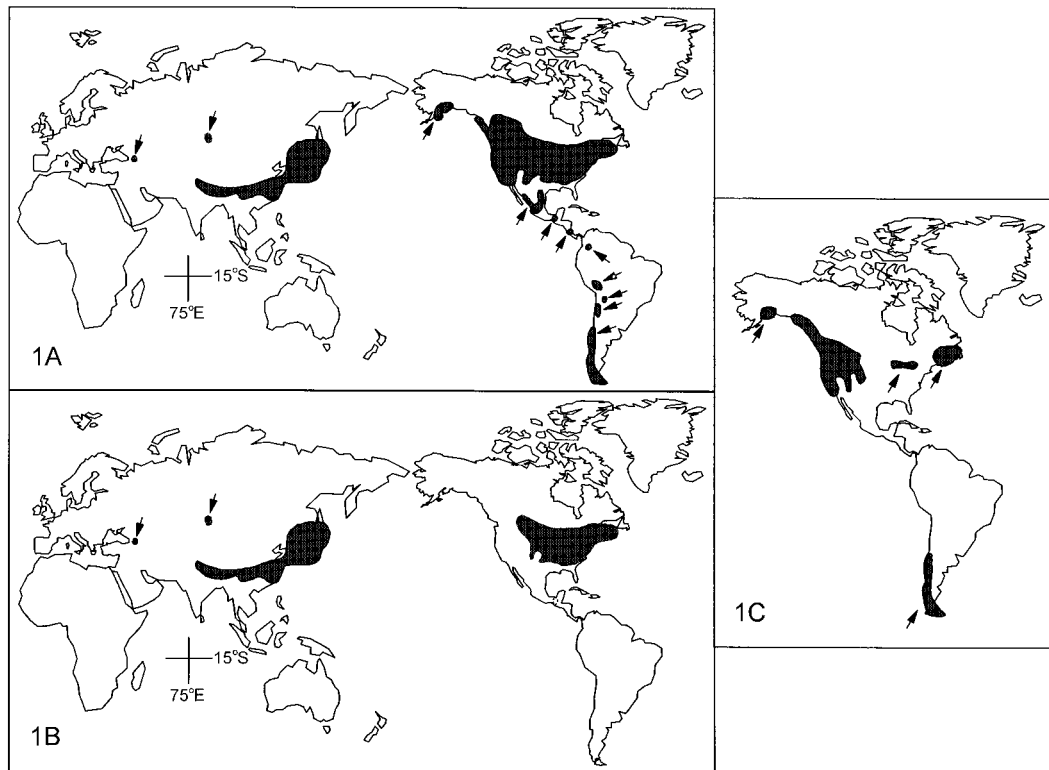


Figure 1.—A. Distribution of *Osmorhiza* (Apiaceae). —B. Distribution of *Osmorhiza* sect. *Osmorhiza*, showing Asian and eastern North American disjunction. —C. Distribution of *O. berteroi* showing American antitropical, and eastern-western North American disjunctions. Arrows indicate isolated areas of distributions.

a range that spans the American cordillera, with isolated populations extending from northern Mexico to northern Argentina. Finally, a third disjunction is found in *O. berteroi* and *O. depauperata*, in which populations occur in western North America, the Great Lakes region, and northeastern North America.

The disjunct distribution of plants between eastern Asia and eastern North America is a classic topic in biogeography (Gray, 1840, 1878; Li, 1952; Graham, 1972; Thorne, 1972; Boufford & Spongberg, 1983; Wu, 1983; Wen, 1998, 1999, 2001), and most genera exhibiting this pattern have now been well documented. Recent phylogenetic studies have confirmed the close affinity of the intercontinental disjuncts in many groups (reviewed in Wen, 1999), but most presumed species pairs studied so far between the two continents do not show a direct sister-species relationship. Morphological stasis (i.e., the lack of significant morphological change in a species over long periods of time) has been suggested as a common phenomenon among these disjuncts (Parks & Wendel, 1990; Qiu et al., 1995a, 1995b) and may at least partially explain

the reported findings (Wen, 1999, 2001). To test this hypothesis, Wen (1998) suggested the examination of eastern Asian–eastern North American disjunct taxa within the context of biogeographic studies covering the Northern Hemisphere or even at a global scale. In-depth comparative studies of disjunct groups that also have apparent close relatives in western North America may provide insight into the relative morphological, molecular, physiological, and cladogenetic rates of evolution of taxa in all three areas.

Antitropical disjunctions between western North America and southern South America were recognized as early as 1880 by Gray and Hooker, and have been discussed by many authors since (e.g., Engler, 1882; DuReitz, 1940; Campbell, 1944; Constance, 1963; Raven, 1963; Cruden, 1966; Moore, 1972; Thorne, 1972, 1993; Cox, 1990; Peterson & Morrone, 1997; Peterson & Ortiz-Diaz, 1998). Constance (1963) pointed out that the species exhibiting this pattern comprise a highly unrepresentative sample of the floras in the two disjunct areas, and that many of them are self-compatible and often autogamous. Based on

these and other considerations, Raven (1963) concluded that the most plausible explanation for the observed antitropical disjunctions is relatively recent long-distance dispersal, although Cruden (1966) presented arguments in favor of “mountain hopping” that involves shorter distance dispersal along the western cordillera.

Fernald (1924, 1925, 1935) was the first to draw attention to disjunctions between western North America, the Great Lakes region, and northeastern North America, which he thought involved primarily arctic and western taxa that were able to survive during the Pleistocene in unglaciated areas (“nunataks”) around Lake Superior, on the Gaspé Peninsula, and in Newfoundland and Labrador, but could not expand their range following the glaciations because of their antiquity. However, all of Fernald’s nunataks were in fact glaciated, and the notion of senescent species is now widely rejected (Wood, 1972). A modified version of this hypothesis was proposed by Schofield (1969), who regarded the eastern populations as remnants of a previously widespread flora whose members were able to survive only in some areas (probably south of the glacial boundaries, but perhaps also in nunataks) and then move to their present sites following the Pleistocene, while being eliminated from the refugia. Lowry and Jones (1984) viewed this as a plausible explanation for the western North American–eastern North American disjunctions in *O. berteroi* and *O. depauperata*, and further suggested that taxa occurring in the Great Lakes area and the northeast are most likely now restricted to sites with less competition from eastern boreal taxa and where climatic conditions (especially snowfall and moisture availability in spring) are similar to those in western parts of the continent.

Another fascinating aspect of *Osmorhiza* is that the type of seed dormancy differs among the species, and in particular between the putative close relatives in eastern Asia and eastern North America. Very little comparative research has been done on physiological traits of species that exhibit this classical disjunct pattern (Terui & Okagami, 1993; Wen et al., 1996), with only one investigation conducted within a phylogenetic framework (Wen et al., 1996). Studies on seeds of five *Osmorhiza* species found that they have morphophysiological dormancy (MPD) (Baskin & Baskin, 1984, 1991; Baskin et al., 1995; Walck et al., 2002). Seeds with MPD have embryos that are very small relative to the size of the seed, and the embryo must grow to the full length of the seed to germinate. In addition to being small, the embryos also have physiological dormancy that must be broken before seeds can

germinate (Baskin & Baskin, 1998). Seeds of *O. claytonii* and *O. longistylis* have nondeep complex MPD, i.e., they require warm followed by cold stratification to germinate, and gibberellic acid (GA_3) substitutes for warm stratification (Baskin & Baskin, 1984, 1991). In contrast, seeds of *O. aristata* and those from western North American populations of *O. berteroi* and *O. occidentalis* have deep complex MPD, requiring only cold stratification to overcome dormancy, and GA_3 does not substitute for stratification (Baskin et al., 1995; Walck et al., 2002). Thus, mapping the types of MPD on a phylogenetic tree can provide an opportunity to evaluate the evolution and adaptive significance of this physiological trait.

Constance and Shan (1948) proposed an infrageneric classification for *Osmorhiza* that was largely adopted by Lowry and Jones (1984) with a few modifications (Table 1). Several hypotheses of relationships are implied in this classification. Two subgenera are recognized, one of which (subg. *Glycosma*) comprises a single western North American species, *O. occidentalis*, which would thus represent the sister species to a clade that includes the remaining nine species. Within the typical subgenus, three sections are defined (each with three species), which are likewise assumed to comprise monophyletic groups. This classification thus offers a framework against which to test alternative hypotheses of relationships using molecular sequence data that were not available previously.

The objectives of this study are to: (1) reconstruct the phylogeny of *Osmorhiza* using sequences of the internal transcribed spacer (ITS) and 5.8S regions of nuclear ribosomal DNA; (2) examine the biogeographic diversification within the group; (3) re-evaluate the earlier hypotheses regarding the possible origins of the observed disjunct distributional patterns; and (4) examine the evolution of seed dormancy in a phylogenetic framework for disjunct taxa of *Osmorhiza* and determine which type(s) of MPD are plesiomorphic versus derived. Sequences of ITS and 5.8S regions were employed because they have been shown to be appropriate to assess evolutionary relationships within other groups exhibiting disjunct distributions between Asia and North America (Fritsch, 1996; Lee et al., 1996; Wen & Zimmer, 1996; Wen et al., 1998) as well as interspecific relationships in Apiaceae (Downie et al., 1998) and evolution within many other north temperate plants (e.g., Xiang et al., 1998; Lee & Wen, 2001).

MATERIALS AND METHODS

Forty-eight populations representing all ten species of *Osmorhiza* and three outgroup taxa were

Table 1. Taxa of *Osmorhiza* Raf. and their distributions following the classification scheme of Lowry and Jones (1984), as modified from Constance and Shan (1948).

Classification	Taxon	Distribution
Subgenus <i>Glycosma</i> (Nutt.) Drude	<i>O. occidentalis</i> (Nutt.) Torr.	W North America
Subgenus <i>Osmorhiza</i>		
Section <i>Mexicanae</i> Constance & Shan ex Lowry & A. G. Jones	<i>O. brachypoda</i> Torr. <i>O. glabrata</i> Phil. <i>O. mexicana</i> Griseb. subsp. <i>bipatriata</i> (Constance & Shan) Lowry & A. G. Jones subsp. <i>mexicana</i>	California, Nevada, and Arizona central Andes SW Texas and N Mexico N Mexico to N Argentina
Section <i>Nudae</i> Constance & Shan ex Lowry & A. G. Jones	<i>O. berteroi</i> DC. <i>O. depauperata</i> Phil. <i>O. purpurea</i> (J. M Coult. & Rose) Suksd.	W North America, Great Lakes area, NE North America, and South America W North America, Great Lakes area, NE North America, and South America NW North America
Section <i>Osmorhiza</i>	<i>O. aristata</i> (Thunb.) Rydb. <i>O. claytonii</i> (Michx.) C. B. Clarke <i>O. longistylis</i> (Torr.) DC.	temperate Asia E North America E North America

sampled in this study (Table 2). Populations of widespread disjunct taxa such as *O. berteroi* and *O. depauperata* were examined from throughout much of their distributional ranges. *Anthriscus caucalis* M. Bieb., *Geocaryum macrocarpum* (Boiss. & Spruner) Engstrand, and *Myrrhis odorata* (L.) Scop. were chosen as the outgroups because of their close relationship to *Osmorhiza* (Lowry & Jones, 1984; Downie et al., 2000).

Total DNA was extracted with the CTAB method of Doyle and Doyle (1987) and purified over CsCl/ethidium bromide gradients. DNA amplifications were performed in 100- μ L reactions following Wen and Zimmer (1996) using the primers C26A and Nnc18S10 (see Wen & Zimmer for sequences of primers). The entire ITS and 5.8S regions were sequenced manually from both directions following Wen et al. (1998) using four primers: C5.8S, C26A, ITS4, and N18L18 (see Wen & Zimmer for sequences of primers).

The DNA sequences obtained were assembled, and the boundaries between the coding and spacer regions were determined by comparing them with the sequences of *Daucus carota* L. (Yokota et al., 1989). The sequences were then exported to PAUP* (vers. 4.0, Swofford, 1999). Most mutations were base substitutions, thus allowing manual alignment. All the sequences have been deposited at GenBank (see Table 2 for accession numbers).

Phylogenetic analyses were performed with PAUP* using maximum parsimony (Swofford et al.,

1996), maximum likelihood (Felsenstein, 1981), and neighbor-joining (Saitou & Nei, 1987) methods. Parsimony analysis was performed using a branch-and-bound search with MULPARS and furthest addition sequence options. The amount of support for monophyletic groups revealed in the maximally parsimonious tree(s) (MPTs) was examined with 1000 bootstrap replicates (Felsenstein, 1985) with random addition and heuristic search options. The maximum likelihood analysis was performed with the input order of sequences randomized and the transition/transversion ratio set at 1.42 based on the observed frequencies in the MPTs of the parsimony analysis. A neighbor-joining tree was constructed using Kimura two-parameter distance (Kimura, 1980).

Relative rate tests were performed with the method of Wu and Li (1985) to detect any rate asymmetries of the ITS and 5.8S regions among taxa in *Osmorhiza*. The proportions of site differences were estimated using the Kimura two-parameter distance (Kimura, 1980).

The optimal area cladogram was constructed from taxon cladograms using the optimality method in COMPONENT (vers. 2.0, Page, 1993). The following options were used: nearest-neighbor interchanges and minimizing the number of leaves added. Four areas of endemism were defined for *Osmorhiza* based on the distribution of taxa and previous biogeographic studies of the North Temperate zone (e.g., Wood, 1970; Patterson, 1981;

Table 2. Accessions of *Osmorhiza* species and the outgroup sampled. Collections marked with “*” were used in the relative rate tests (see Table 3).

Taxon	Voucher	Source	GenBank accession
<i>O. aristata</i>	*S. Zhou s.n. (F) K. Yonekura s.n. (F) J. H. Lee & B. W. Han s.n. (F)	China, Zhejiang Prov. Japan, Aomori Pref. Korea, Odaesan	AF453952 AF453953 AF453954
<i>O. berteroi</i>	*M. Baeza & G. Kottirsch 675 (F) M. Baeza & G. Kottirsch 676 (F) T. Stuessy et al. 15556 (F) M. Baeza & G. Kottirsch 1172 (F) L. P. Janeway & D. Isle 5710 (CS) V. H. Oswald 9248 (CS) J. Wen 4707 (F) J. Wen 4726 (F) J. Wen 4732 (F) P. P. Lowry II et al. 4949 (MO) P. P. Lowry II et al. 4950 (MO) V. H. Oswald 9253 (CS) *J. Wen 1069 (A) J. Wen 1089 (A) J. Wen 4716 (F) J. Wen 812 (A) J. L. Walck & S. N. Hidayati 565 (F) J. L. Walck & S. N. Hidayati 567 (F) *J. Wen 3516 (F) J. Wen 4742 (F) M. DeVore 1118 (OS) *M. DeVore 1219 (OS) S. Teillier et al. 2419 (MO) J. Wen 827 (A) J. Wen 861 (A) J. Wen 4715 (F) J. L. Walck & S. N. Hidayati 564 (F) *H. Vibrans s.n. (10-VII-98) (CS) F. Chiang 1276 (CS) P. P. Lowry II & M. J. Warnock 3182 (F)	Chile, Prov. Ñuble Chile, Prov. Ñuble Chile, Valle Hermoso Chile, Prov. Malleco U.S.A., California, Colusa Co. U.S.A., California, Butte Co. U.S.A., Michigan, Luce Co. Canada, Quebec, Rimouski Co. Canada, Quebec, Gaspé Co. U.S.A., Oregon, Clackamas Co. U.S.A., Oregon, Marion Co. U.S.A., California, Butte Co. U.S.A., Kentucky, Menifee Co. U.S.A., Pennsylvania, Blair Co. U.S.A., Michigan, Baraga Co. U.S.A., Maryland, Baltimore Co. U.S.A., Kentucky, Fayette Co. U.S.A., West Virginia, Pocahontas Co. U.S.A., Colorado, Larimer Co. Canada, Quebec, Rimouski Co. Chile, Icalma Valley Chile, Prov. de Talca Chile, Frente a Santiago, PN El Morado U.S.A., Virginia, Giles Co. U.S.A., North Carolina, Watauga Co. U.S.A., Michigan, Baraga Co. U.S.A., Kentucky, Fayette Co. Mexico, Edo Mexico, Mexico D.F. Mexico, Nuevo León	AF453955 AF453956 AF453965 AF453957 AF453963 AF453964 AF453958 AF453959 AF453960 AF453961 AF453962 AF453966 AF453970 AF453971 AF453972 AF453969 AF453967 AF453968 AF453974 AF453975 AF453973 AF453976 AF453977 AF453979 AF453980 AF453981 AF453978 AF453983 AF453982 AF453984
<i>O. brachypoda</i>			
<i>O. claytonii</i>			
<i>O. depauperata</i>			
<i>O. glabrata</i>			
<i>O. longistylis</i>			
<i>O. mexicana</i> subsp. <i>mexicana</i>			
<i>O. mexicana</i> subsp. <i>bipatriata</i>			

Table 2. Continued.

Taxon	Voucher	Source	GenBank accession
<i>O. occidentalis</i>	*B. Painter et al. 18 (CS)	U.S.A., Colorado, Garfield Co.	AF453985
	J. Wen 3860 (F)	U.S.A., Colorado, Routt Co.	AF453986
	L. M. Moore 6672 (CS)	U.S.A., Colorado, Dolores Co.	AF453991
	V. H. Oswald & L. Ahart 9493 (CS)	U.S.A., California, Plumas Co.	AF453992
	V. H. Oswald & L. Ahart 9532 (CS)	U.S.A., California, Nevada Co.	AF453993
	P. P. Lowry II 4633 (MO)	U.S.A., Nevada, Elko Co.	AF453987
	P. P. Lowry II 5155 (MO)	U.S.A., Nevada, Lander Co.	AF453988
	R. R. Halse 5561 (CS)	U.S.A., Oregon, Benton Co.	AF453989
	R. R. Halse 5565 (CS)	U.S.A., Oregon, Benton Co.	AF453990
<i>O. purpurea</i>	P. P. Lowry II 4963 (MO)	U.S.A., Oregon, Tillamook Co.	AF453994
	P. P. Lowry II 5069 (MO)	U.S.A., Oregon, Linn Co.	AF453995
	P. P. Lowry II 5070 (MO)	U.S.A., Oregon, Linn Co.	AF453996
<i>Anthriscus caucalis</i>		Downie et al. (1998)	U79601, U79602
<i>Geocaryum macrocarpum</i>		Downie et al. (2000)	AF073605, AF073606
<i>Myrrhis odorata</i>		Vallejo-Roman et al. (1998)	AF077901

Hoey & Parks, 1991), work on *Osmorhiza* (Lowry & Jones, 1984), and results of this phylogenetic analysis. These areas are western North America, eastern North America, central Andes, and Asia. South America was not chosen as one of the areas of endemism because it is linked to western North America via disjunction and shares the same species, suggesting that it was recently colonized (also see Discussion).

RESULTS

CHARACTERISTICS OF ITS SEQUENCES

The combined length of the ITS1, 5.8S, and ITS2 regions in *Osmorhiza* species is 605 bases, with an ITS1 of 217–220 bases, a 5.8S of 164 bases, and an ITS2 of 224–225 bases. Two insertions in ITS1 were required to align the sequences within the genus. One occurs in the three accessions of Asian *O. aristata* (1 bp), and the other is a 3-bp insertion in *O. mexicana* subsp. *bipatriata*. Two additional insertions are in ITS2, each 1-bp in length. The alignment of sequences of *Osmorhiza* with those of the outgroup species required five additional 1-bp and one 2-bp indels, and sequences of *Osmorhiza* and the outgroups could be aligned manually without difficulty. The sequence divergence of *Osmorhiza* taxa and the outgroups ranges from 4.679 (between *Myrrhis odorata* and *O. berteroi*) to 14.05% (between *Anthriscus caucalis* and *O. mexicana* subsp. *mexicana*).

PHYLOGENETIC ANALYSES

Of the 693 aligned positions, 116 sites were variable; of these, 62 were phylogenetically informative. Treating gaps as new characters, the parsimony analysis generated a single most parsimonious tree (MPT) with a total length of 157 steps, a consistency index (CI) of 0.860 (0.763 excluding uninformative characters), a retention index (RI) of 0.912, and a rescaled consistency index (RC) of 0.785 (Fig. 2). Treating gaps as missing data, the parsimony analysis produced a single MPT with an identical topology to Figure 2 with a length of 141 steps, a CI = 0.851 (0.756 when uninformative characters are excluded), an RI = 0.974, and an RC = 0.916. The tree from the weighted parsimony analysis (weighting transversions over transitions 1.42 times) also had an identical topology to the MPT shown in Figure 2. Several relationships are suggested by the parsimony analyses: (1) the three populations of *O. aristata* from Asia form a monophyletic group; (2) *O. clay-*

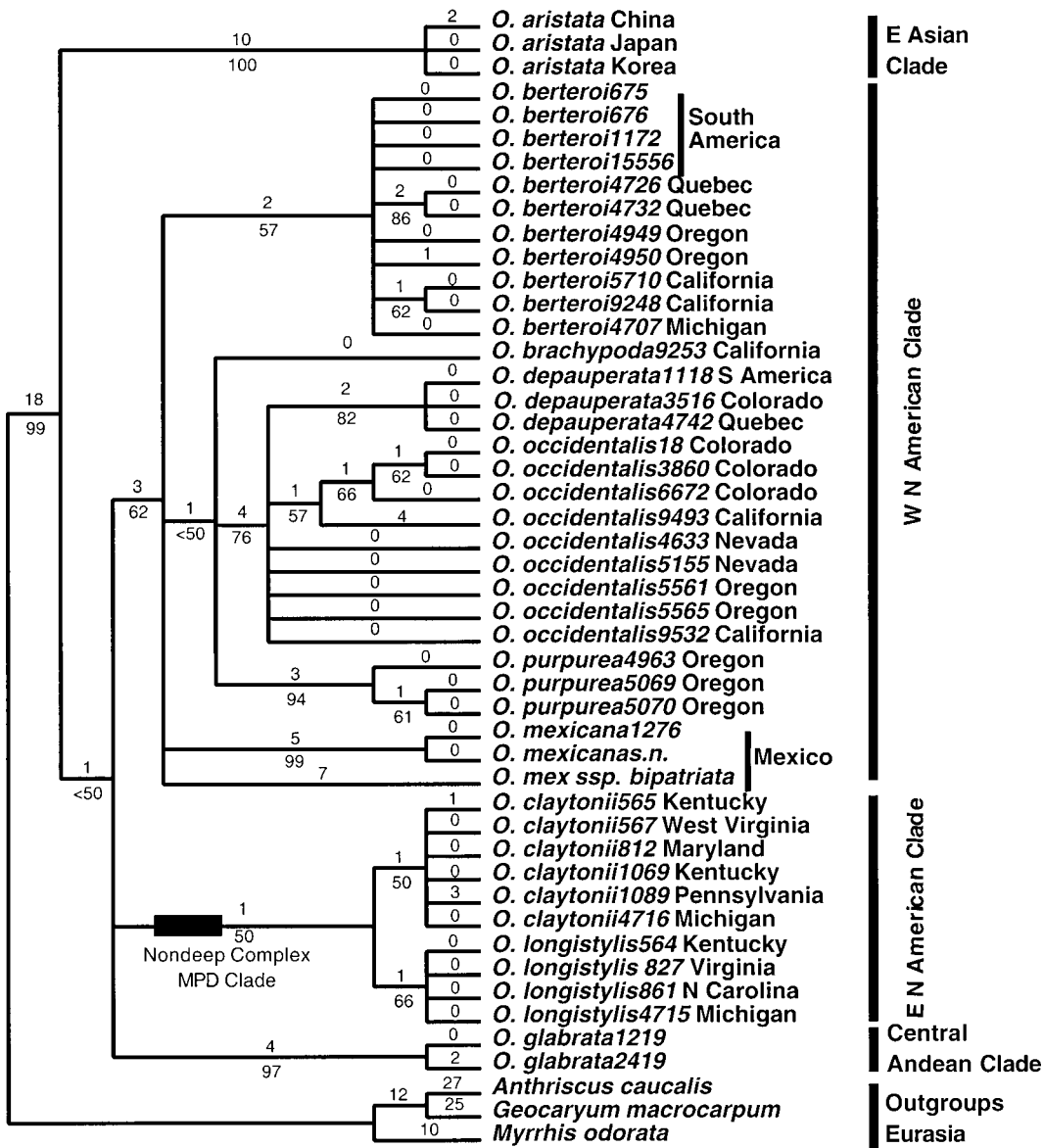


Figure 2. The single most parsimonious tree of *Osmorhiza* with a total length of 157 steps, a CI of 0.860 (0.763 excluding uninformative characters), an RI of 0.912, and an RC of 0.785, treating gaps as new characters. Numbers above lines are branch lengths and those below are bootstrap values in 1000 replicates. Numbers following the taxon names are accession numbers as in Table 2 to help identify the source of study material. The solid black bar at the node of the *O. claytonii*-*O. longistylis* clade indicates the most parsimonious explanation of the evolution of the nondeep complex morphophysiological dormancy (MPD).

tonii and *O. longistylis* from eastern North America form a clade (the E N American clade); (3) the multiple populations examined of the widespread *O. depauperata* form a monophyletic group; (4) *O. depauperata* and *O. occidentalis* form a clade; (5) *O. brachypoda* and *O. purpurea* are allied with the *O. depauperata*-*O. occidentalis* clade; (6) populations of the widespread *O. berteroi* form a mono-

phyletic group; (7) several species (*O. berteroi*, *O. brachypoda*, *O. depauperata*, *O. mexicana*, *O. occidentalis*, and *O. purpurea*) form a largely western North American clade with *O. berteroi* and *O. depauperata* extending to eastern North America and South America, and *O. mexicana* ranging from Texas to South America (the W N American clade); and (8) *O. glabrata* from the central Andes forms

Table 3. Relative rate tests to detect rate asymmetry. *Myrrhis odorata* was used as the reference taxon. (* Significant at the 5% level.)

Species 1	Species 2	K ₁₃	K ₂₃	K ₁₃ -K ₂₃ ± SE
<i>O. aristata</i>	<i>O. berteroi</i>	0.06582	0.05165	0.01417 ± 0.00611*
<i>O. aristata</i>	<i>O. claytonii</i>	0.06582	0.06015	0.00567 ± 0.00619
<i>O. aristata</i>	<i>O. depauperata</i>	0.06582	0.06759	-0.00177 ± 0.00701
<i>O. aristata</i>	<i>O. glabrata</i>	0.06582	0.05347	0.01235 ± 0.00545*
<i>O. aristata</i>	<i>O. mexicana</i>	0.06582	0.06442	0.00140 ± 0.00669
<i>O. aristata</i>	<i>O. occidentalis</i>	0.06582	0.06993	-0.00411 ± 0.00752
<i>O. berteroi</i>	<i>O. claytonii</i>	0.05165	0.06015	-0.00850 ± 0.00499
<i>O. berteroi</i>	<i>O. depauperata</i>	0.05165	0.06759	-0.01594 ± 0.00450*
<i>O. berteroi</i>	<i>O. glabrata</i>	0.05165	0.05347	-0.00182 ± 0.00397
<i>O. berteroi</i>	<i>O. mexicana</i>	0.05165	0.06442	-0.01277 ± 0.00398*
<i>O. berteroi</i>	<i>O. occidentalis</i>	0.05165	0.06993	-0.01828 ± 0.00493*
<i>O. claytonii</i>	<i>O. depauperata</i>	0.06015	0.06759	-0.00744 ± 0.00619
<i>O. claytonii</i>	<i>O. glabrata</i>	0.06015	0.05347	0.00668 ± 0.00415
<i>O. claytonii</i>	<i>O. mexicana</i>	0.06015	0.06442	-0.00427 ± 0.00574
<i>O. claytonii</i>	<i>O. occidentalis</i>	0.06015	0.06993	-0.00978 ± 0.00641
<i>O. depauperata</i>	<i>O. glabrata</i>	0.06759	0.05347	0.01412 ± 0.00520*
<i>O. depauperata</i>	<i>O. mexicana</i>	0.06759	0.06442	0.00317 ± 0.00481
<i>O. depauperata</i>	<i>O. occidentalis</i>	0.06759	0.06993	-0.00234 ± 0.00347
<i>O. glabrata</i>	<i>O. mexicana</i>	0.05347	0.06442	-0.01095 ± 0.00477*
<i>O. glabrata</i>	<i>O. occidentalis</i>	0.05347	0.06993	-0.01646 ± 0.00550*
<i>O. mexicana</i>	<i>O. occidentalis</i>	0.06442	0.06993	-0.00551 ± 0.00568

a trichotomy with the E N American clade and the W N American clade.

The maximum likelihood tree (MLT, not shown, with a log likelihood of -1512.26) has an identical topology to the MPT. The neighbor-joining tree (NJT) is similar to the MPT and the MLT, but differs in that (1) *O. glabrata* is placed sister to the *O. claytonii*-*O. longistylis* clade in the NJT; and (2) the *O. claytonii*-*O. longistylis*-*O. glabrata* clade is sister to the Asian *O. aristata*. The new clades are weakly supported with bootstrap values less than 50%.

SEQUENCE DIVERGENCE

Treating gaps as missing data, the Kimura two-parameter distance among species of *Osmorhiza* was estimated to be 0.299-3.784%. These divergence values are comparable to those in *Aralia* sect. *Aralia*, which shows a similar disjunct pattern of distribution, with representatives in eastern Asia and in eastern and western North America (Wen et al., 1998). The highest divergence occurs between *O. occidentalis* from western North America and *O. aristata* from eastern China, and the lowest between *O. claytonii* and *O. longistylis* from eastern North America, which are sympatric throughout much of their ranges. Overall, divergence values between *O. aristata* and other members of the genus are comparatively high, ranging from 1.627% (with *O. clay-*

tonii) to 3.784% (with *O. occidentalis*). Among the New World species, sequence divergence of *O. glabrata* from the central Andes ranges from 0.601% (with *O. longistylis*) to 2.552% (with *O. occidentalis*). The western North American *O. occidentalis* has a relatively high level of sequence divergence, ranging from 0.319% (with *O. depauperata*) to 2.552% (with *O. glabrata*). Intraspecific variation was detected in six species for which multiple samples were available, *O. aristata* (0-0.302%), *O. berteroi* (0-0.473%), *O. claytonii* (0-0.517%), *O. mexicana* (0-1.415%), *O. occidentalis* (0-1.013%), and *O. purpurea* (0-0.148%), whereas multiple populations sampled of *O. depauperata* and *O. longistylis* showed no variation in their ITS profiles, despite the fact that these species have wide distributions. The absence or relatively low level of sequence variation within these taxa supports the species level circumscriptions proposed by Lowry and Jones (1984). On the other hand, intraspecific variation within *O. occidentalis* was 1.013% between populations from the Rocky Mountains and those from California.

RELATIVE RATE TESTS

Twenty-one relative rate tests (Wu & Li, 1985) were conducted to detect rate asymmetry (Table 3). *Myrrhis odorata* was used as the reference taxon. Rate differences of most pairs of species were not

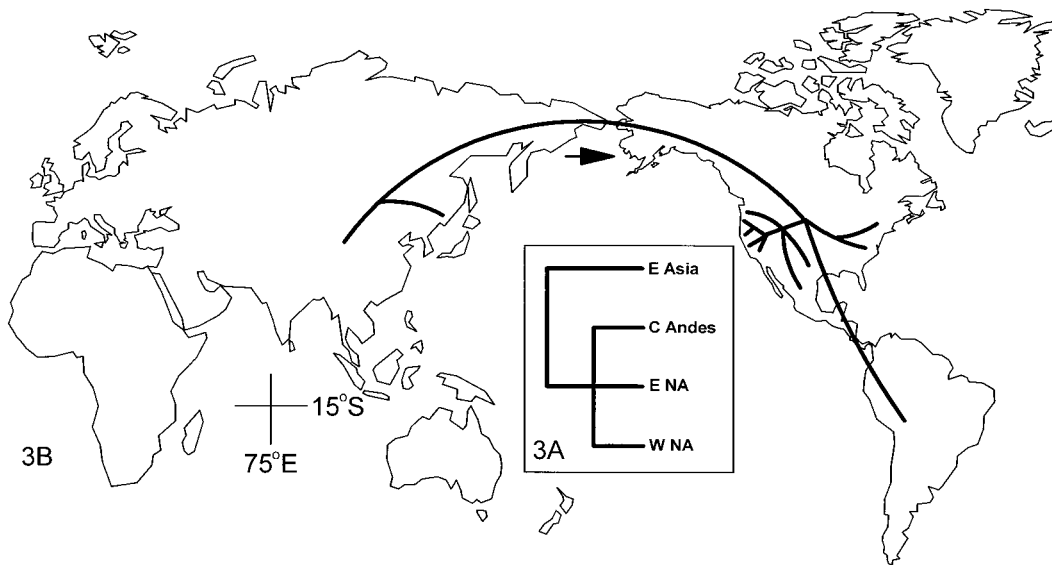


Figure 3. Biogeography of *Osmorhiza*.—A. Area cladogram of *Osmorhiza* based on the nuclear ribosomal ITS phylogeny. —B. Model of diversification in *Osmorhiza*.

statistically significant at the 5% level, whereas eight species pairs showed significant differences. These tests suggest that *O. berteroi* had a slower rate of nucleotide substitutions in the ITS regions than *O. aristata*, *O. depauperata*, *O. mexicana*, and *O. occidentalis*. They also show that *O. glabrata* had a slower rate of nucleotide substitution than *O. aristata*, *O. depauperata*, *O. mexicana*, and *O. occidentalis*. The molecular clock hypothesis for the ITS sequences in *Osmorhiza* was therefore rejected.

AREA CLADOGRAM CONSTRUCTION

The area cladogram (Fig. 3A) was constructed with the MPT and MLT using the optimality method in COMPONENT (vers. 2.0, Page, 1993). Among the four areas of endemism, Asia is basal and eastern North America, western North America, and central Andes form a trichotomy.

DISCUSSION

PHYLOGENY AND PATTERNS OF DIFFERENTIATION

Parsimony and maximum likelihood analyses support the basal position of the Asian *O. aristata* within the genus and the monophyly of the New World taxa; these relationships were previously suggested in Downie et al. (2000) in a broader analysis of the tribe Scandiceae of Apiaceae. Within the New World species, three major subclades are suggested: (1) the eastern North American *O. claytonii* and *O. longistylis*; (2) a largely western North

American clade comprising *O. berteroi*, *O. brachypoda*, *O. depauperata*, *O. mexicana*, *O. occidentalis*, and *O. purpurea*; and (3) the central Andean endemic *O. glabrata*. The neighbor-joining tree, however, groups the central Andean endemic *O. glabrata* with the eastern North American *O. claytonii* and *O. longistylis*. The *O. claytonii*–*O. longistylis*–*O. glabrata* group is then sister to *O. aristata* from Asia. As in the MPT and the MLT, the NJT also suggests a largely western North American group comprising *O. berteroi*, *O. brachypoda*, *O. depauperata*, *O. mexicana*, *O. occidentalis*, and *O. purpurea*.

The ITS phylogeny conflicts in several aspects with the phylogenetic hypothesis implied in the infrageneric classification of Lowry and Jones (1984) based on morphology (cf. Table 1). *Osmorhiza occidentalis* is a morphologically distinct species and was thus originally described in the monotypic genus *Glycosma* by Nuttall (in Torrey & Gray, 1840). Recent treatments have included this species within *Osmorhiza*, but placed it in its own section (Constance & Shan, 1948) or subgenus (Lowry & Jones, 1984) because of the numerous features that distinguish it from other members of the genus, including glabrous fruits lacking a caudate appendage, numerous staminate umbellules and flowers, bipinnate leaves, and yellow to greenish yellow flowers. The inclusion of *O. occidentalis* within a well-supported western North American clade in the ITS phylogeny suggests, however, that its dis-

tinctive morphological characters are most likely attributable to rapid evolution within the species, resulting in many autapomorphies.

Another discrepancy between the current infrageneric classification and the ITS phylogeny involves the three species currently included in *Osmorhiza* sect. *Osmorhiza*: the Asian *O. aristata* and the eastern North American *O. claytonii* and *O. longistylis*. These taxa form a morphologically coherent group and were even treated as a single species by some authors (e.g., Gray, 1859; Clarke, 1879; Kuntze, 1891; Boivin, 1968). They share several diagnostic features, including an involucre composed of (1–)2–3(–5) conspicuous, foliaceous bractlets, and styles (including the high-conic stylopodium) that are 1–3.6 mm long. However, based on ITS data, *O. aristata* is not only basal within the genus, but also shows a high level of sequence divergence from its congeners (1.627–3.784%), suggesting its early divergence within the group. The relative antiquity of *O. aristata* and the comparatively high level of morphological similarity between it and the eastern North American species are consistent with the hypothesis of morphological stasis among eastern Asian–eastern North American disjuncts, as proposed by Parks and Wendel (1990). Taxa of the eastern North American clade are sister to the western North American clade and not to *O. aristata*. The morphological similarity between *O. aristata*, *O. claytonii*, and *O. longistylis* could, however, be explained by their having experienced morphological stasis while concurrently the largely western North American clade diversified more rapidly.

Morphological stasis was first proposed by Parks and Wendel (1990) as a possible explanation for the presence of morphological similarities among eastern Asian–eastern North American disjunct taxa that are not closely related or do not represent sister groups. They found that two morphologically similar disjunct species of *Liriodendron* (*L. chinense* (Hemsley) Sargent and *L. tulipifera* L.) show a high level of allozyme and cpDNA divergence. Molecular and fossil data suggest the divergence time of the two species to be 10–16 million years ago (middle Miocene). Morphological stasis has also been suggested to explain similarities between the Asian and North American species of *Aralia* sect. *Dimorphanthus* (Wen, 2000), *Liquidambar* (Hoey & Parks, 1991; Shi et al., 1998), and *Magnolia* sect. *Rytidospermum* (Qiu et al., 1995a, 1995b).

A third conflict between the current infrageneric classification of *Osmorhiza* and the ITS data involves the central Andean endemic *O. glabrata*. The relationships and taxonomic placement of this

species have been somewhat ambiguous. Constance and Shan (1948) included *O. glabrata* in their section “*Glycosmae*” along with *O. occidentalis*, largely based on apparent similarities in their fruits. By contrast, Lowry and Jones (1984) placed the Andean endemic in section *Mexicanae*, which also comprises *O. brachypoda* and *O. mexicana*. Shared characters among these species include short styles (0.5–1.2 mm long), a low-conic stylopodium, a conspicuous involucre of 1–6 bractlets, and an absent or poorly developed involucre subtending the umbellules. By contrast, the maximum parsimony and the maximum likelihood analyses suggest that *O. glabrata* forms a trichotomy with the eastern North American clade (*O. claytonii* and *O. longistylis*) and the largely western North American clade. The neighbor-joining tree, however, places *O. glabrata* sister to the eastern North American *O. claytonii*–*O. longistylis* group. The ITS phylogeny (Fig. 2) thus suggests the relative antiquity of *O. glabrata* among the New World species. It seems most plausible that *O. glabrata* was derived from a North American ancestor, perhaps shared with *O. claytonii* and *O. longistylis* (as in the NJT) or with taxa of the eastern North American clade and the western North American clade (Fig. 2). The present narrow distribution of *O. glabrata* in the central Andes may be due to local survival following the Pleistocene glaciations, which have been considered important in the evolution of the Andean flora (Vuilleumier, 1971; Simpson, 1975, 1983; Prance, 1982). Additional data are, however, needed to ascertain the exact phylogenetic position of *O. glabrata*.

INFRASPECIFIC VARIATION

Differentiation within O. occidentalis. *Osmorhiza occidentalis* showed a relatively high level of infraspecific sequence divergence, with a Kimura two-parameter distance of 1.013% between populations from the Rocky Mountains and California. Morphologically, *O. occidentalis* is somewhat variable, and several segregate species have been recognized in the past based primarily on differences in inflorescence structure, leaf pubescence, and fruit size (see Lowry & Jones, 1984). These differences, however, were regarded as minor by Lowry and Jones (1984), who provided data showing that a broadly defined *O. occidentalis* comprises a morphologically coherent group despite the presence of a few rather atypical collections (some of which served as the basis for the segregate species). Because *O. occidentalis* is so distinct from its congeners, it may be that infraspecific morphological variation has been over-

looked by previous workers. Additional studies should be undertaken to re-assess variability within *O. occidentalis* and to evaluate whether the molecular divergence is correlated with morphology and/or geographic distribution.

Differentiation between O. mexicana subsp. mexicana and subsp. bipatriata. The two subspecies of *O. mexicana* show a relatively high level of ITS sequence divergence (1.415%). Moreover, they did not form a monophyletic group in the phylogenetic analysis (Fig. 2). Initially, Constance and Shan (1948) recognized these taxa as distinct species, but Lowry and Jones (1984) treated them as subspecies because of the presence of occasional morphological intermediates at several localities in northern Mexico, including one area (Cerro Poposí, Nuevo León) where they co-occur with typical material of both subspecies. The biphyly of *O. mexicana*, as currently circumscribed, suggests the need to reexamine the status of subspecies *bipatriata* and the possible causes of the morphological intermediates. Although no interspecific hybridization has been reported for *Osmorhiza*, occasional morphological intermediates have been observed in sympatric populations of *O. berteroi* and *O. occidentalis* in western Oregon (R. Halse, pers. comm.).

BIOGEOGRAPHY

Diversification in major geographic areas. The ITS phylogeny (Fig. 2) shows that rates of cladogenesis vary among the four major areas occupied by *Osmorhiza*: eastern Asia, eastern North America, western North America, and the central Andes. Cladogenesis clearly appears to have been more rapid in the New World than in Asia, and the western North American clade shows a particularly high level of species diversity (Fig. 3B). It is unusual that Asia is species-depauperate considering that most other Asian–North American disjunct genera show a higher species diversity there (Tiffney, 1985; Wen, 1999). The number of *Osmorhiza* species occurring in each area may in part be the result of differential rates of extinction, especially during the Quaternary glaciations. However, it is generally accepted that North America was more severely affected by glaciation than eastern Asia due to its more complex topography and the north–south rather than primarily east–west orientation of its mountain ranges (Axelrod et al., 1998; Wen, 1999).

Western North America is the center of species diversity in *Osmorhiza*, with six of the ten species occurring in this region. The monophyly of the western North American clade is well supported in

all three analyses presented here. Morphologically, however, the clade is rather diverse, with members from both subgenera and two of the three sections recognized by Lowry and Jones (1984). The ITS phylogeny suggests two successive diversifications within *Osmorhiza* in western North America. First, the common ancestor of the western North American clade may have diversified into *O. berteroi*, *O. mexicana*, and the ancestor of the *O. brachypoda*–*O. depauperata*–*O. occidentalis*–*O. purpurea* subclade. The latter subclade then appears to have differentiated further. This diversification among western North American *Osmorhiza* may have been highly influenced by the availability of a range of habitats associated with the uplifting of the Rocky Mountains and the western cordillera during the Tertiary (Barbour & Christensen, 1993; Graham, 1993).

The Andean endemic *O. glabrata* most likely represents an isolated relict. Its phylogenetic position is not well-resolved in the ITS phylogeny. It may have diverged rapidly early in the evolutionary history of the North American *Osmorhiza*, persisting in a relatively small portion of the central Andes. The fruits of *O. glabrata* are glabrous to sparsely hispid, which may afford them limited opportunities for long-distance dispersal by animals. It should be noted, however, that *O. occidentalis* almost always has completely glabrous fruits but nevertheless extends throughout a much larger area of western North America (Lowry & Jones, 1984).

Eastern Asian–eastern North American disjunction. The relative antiquity of the eastern Asian–eastern North American disjunction in *Osmorhiza* is suggested by the phylogenetically basal position of the Asiatic *O. aristata*, the monophyly of the diverse New World species (Fig. 2), and the relatively high ITS sequence divergence between *O. aristata* and its congeners. The most commonly accepted interpretation of the origin of eastern Asian–eastern North American disjunctions is that this pattern reflects an initial widespread distribution of temperate forest elements in the Northern Hemisphere during the mid Tertiary followed by subsequent extirpations in western North America and western Europe as a result of late Tertiary and Quaternary climatic cooling (Graham, 1993; Manchester, 1999; Wen, 1999). No fossils of *Osmorhiza* have been reported that could help to date the biogeographic disjunction. Also, the rejection of the molecular clock hypothesis for the ITS sequences in *Osmorhiza* (see Results) makes it questionable to attempt an indirect estimate of the times of divergence between the eastern Asian and the North American members of the genus.

The apparent close relatives of *Osmorhiza*, the genera *Myrrhis* and *Geocaryum* (Downie et al., 2000), are restricted to the Old World, which is consistent with a hypothesized Old World origin of *Osmorhiza* (Downie et al., 2000). The basal position of the Asian *O. aristata* and the Old World distribution of *Myrrhis* and *Geocaryum* are consistent with the idea that the ancestor of the North American *Osmorhiza* migrated from Asia.

American antitropical disjunction. *Osmorhiza berteroi* and *O. depauperata* show a similar pattern of antitropical disjunction between western North America and temperate South America. Constance (1963) suggested that these species may have migrated south in a step-wise manner along the western American cordillera throughout the Tertiary, with subsequent elimination of populations from intervening tropical areas. The present-day distribution of *O. mexicana* subsp. *mexicana*, which comprises isolated populations that bridge the areas currently occupied by *O. berteroi* and *O. depauperata*, appears to support the idea that the now-disjunct species could also have had a more continuous distribution in the past. However, the absence of ITS sequence divergence between the western North American and South American populations of both species suggests a recent origin of the observed antitropical disjunctions. Species of *Osmorhiza* are facultatively autogamous, which would enable establishment of a new population from a single propagule, and their armed fruits with caudate appendages and numerous retrorse bristles appear to be well adapted for epizoochorous dispersal (Lowry & Jones, 1984). As indicated by Raven (1963), the pattern of disjunction of temperate taxa between western North America and southern South America corresponds closely to the migration routes of many bird species, and this probably accounts for the disjunctions seen in *O. berteroi* and *O. depauperata* (Lowry & Jones, 1984). Thus, the lack of ITS sequence divergence, the monophyly of both species, and their fruit morphology are all consistent with an origin of the antitropical disjunction due to relatively recent long-distance dispersal via birds from western North America to South America. A similar explanation has been used for several disjunct taxa of grasses (Peterson & Morrone, 1997; Peterson & Ortiz-Diaz, 1998).

Eastern-western North American disjunction. The phytogeographic disjunction between western North America, the Great Lakes region, and northeastern North America has been discussed repeatedly since it was first reported by Fernald in 1925 (Fernald, 1925, 1935; see also Stebbins, 1935; Rousseau, 1953; Drury, 1969;

Schofield, 1969; Morisset, 1971; Voss, 1972; Wood, 1972; Miller & Thompson, 1979). It is now generally regarded that the disjunct populations of species showing this pattern are remnants of a previously widespread flora that probably survived south of the glacial boundaries, but perhaps also in nunataks (cf. Fernald, 1925), and then migrated to their present sites following the Pleistocene, while being eliminated from the refugia in the south (Schofield, 1969; Miller & Thompson, 1979). In explaining the western North American-eastern North American disjunctions in *O. berteroi* and *O. depauperata*, Lowry and Jones (1984) also emphasized that taxa occurring in the Great Lakes area and the northeast are most likely now restricted to sites with less competition from eastern boreal taxa and where climatic conditions are similar to those in western parts of the continent. In the present study, the western cordilleran populations of *O. berteroi* showed an identical ITS profile to those of the Great Lakes region, while the eastern populations had just a single nucleotide substitution. The fact that there is little or no sequence divergence within each of these species is consistent with a relatively recent origin of the disjunct pattern during the glacial or postglacial periods.

PHYSIOLOGICAL ADAPTATION—SEED DORMANCY

Disjunct species of herbaceous plants with relict distributions in both eastern North America and Asia appear to exhibit stasis in ecological traits (Ricklefs & Latham, 1992). Similarly, closely related species groups with members occurring in one or the other area may also show stasis with regard to various traits, including the type of morphophysiological dormancy (MPD) they exhibit. This is the case for seeds of the eastern North American-Asian disjunct species pairs *Jeffersonia diphylla* (L.) Pers.—*J. dubia* (Maxim.) Benth. & Hook. f. (Berberidaceae) and *Panax quinquefolius* L.—*P. ginseng* C. A. Meyer (Araliaceae), all of which have deep simple MPD (cf. Baskin & Baskin, 1998). In *Osmorhiza*, however, seeds of *O. aristata* from Asia and *O. berteroi* and *O. occidentalis* from western North America share the same type of MPD (deep complex MPD), whereas those of the eastern North American *O. claytonii* and *O. longistylis* exhibit a different type of dormancy (nondeep complex MPD). Wake et al. (1983) suggested that changes in some traits, including physiological ones, might help an organism to compensate for maintaining a stable morphology over long periods of time. This would appear to be the case in members of *Osmorhiza*.

Although much additional work must be completed before we have a robust understanding of the evolution of various types of MPD, some insights into their evolution may be drawn from the phylogeny of *Osmorhiza*. The plesiomorphic condition in the genus appears to be deep complex MPD, whereas nondeep complex MPD is derived. This is further supported by studies that show seeds of the outgroup *Myrrhis odorata* also have deep complex MPD (Lhotska, 1977; Deno, 1994). The suggestion of Baskin et al. (1995) that deep complex MPD may have been derived from nondeep complex MPD is not upheld according to the ITS phylogeny of *Osmorhiza*.

Seeds of most (and probably all) *Osmorhiza* species are dispersed during summer/autumn, with germination occurring in spring (Baskin & Baskin, 1984, 1991; Baskin et al., 1995; Walck et al., 2002). A cold stratification period would thus ensure that germination does not occur until the following spring, since dormancy is broken with cold (winter) stratification. The selective forces responsible for the evolution of the requirement for a warm stratification pretreatment prior to cold stratification observed in the eastern North American *O. claytonii* and *O. longistylis* are unclear. The most parsimonious explanation based on the ITS phylogeny is that the evolution of this trait occurred in the common ancestor of these closely related sister species. It seems reasonable, however, to assume that a common environmental selection pressure (or a set of pressures) was responsible for the acquisition of the warm + cold stratification requirement. Interestingly, species of *Erythronium* have a similar geographic pattern of MPD types, with seeds from the western North American *E. grandiflorum* Pursh exhibiting deep complex MPD and those from the eastern North American *E. albidum* Nutt. and *E. americanum* Ker Gawler showing nondeep complex MPD (Baskin et al., 1995).

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