American Journal of Botany 92(3): 477–486. 2005.

MARKED GENETIC DIVERGENCE AMONG SKY ISLAND POPULATIONS OF *SEDUM LANCEOLATUM* (CRASSULACEAE) IN THE ROCKY MOUNTAINS[1]

ERIC G. DECHAINE[2,4] AND ANDREW P. MARTIN[3]

[2]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138 USA; and
[3]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado 80309 USA

Climate change during the Quaternary played an important role in the differentiation and evolution of plants. A prevailing hypothesis is that alpine and arctic species survived glacial periods in refugia at the periphery of glaciers. Though the Rocky Mountains, south of the southernmost extent of continental ice, served as an important glacial refuge, little is known about how climate cycles influenced populations within this region. We inferred the phylogeography of *Sedum lanceolatum* (Crassulaceae) within the Rocky Mountain refugium to assess how this high-elevation plant responded to glacial cycles. We sequenced 884 base pairs (bp) of cpDNA intergenic spacers (tRNA-L to tRNA-F and tRNA-S to tRNA-G) for 333 individuals from 18 alpine populations. Our highly variable markers allowed us to infer that populations persisted across the latitudinal range throughout the climate cycles, exhibited significant genetic structure, and experienced cycles of range expansion and fragmentation. Genetic differentiation in *S. lanceolatum* was most likely a product of short-distance elevational migration in response to climate change, low seed dispersal, and vegetative reproduction. To the extent that *Sedum* is a good model system, paleoclimatic cycles were probably a major factor preserving genetic variation and promoting divergence in high-elevation flora of the Rocky Mountains.

Key words: alpine; climate cycles; cpDNA; Crassulaceae; genetic diversity; historical biogeography; Quaternary; Rocky Mountain refuge; *Sedum lanceolatum*.

During the long glacial episodes of the Quaternary (from two million years ago to the present) many taxa were restricted to one or a few regional refugia (Webb and Bartlein, 1992; Hewitt, 1996). Evidence from studies on European flora (reviewed in Taberlet et al., 1998; Stehlik, 2000) suggests that glacial refugia were located at the periphery of ice sheets (Tollefsrud et al., 1998; Despres et al., 2002; Holderegger et al., 2002; Schönswetter et al., 2002) and that some populations persisted on ice-free nunataks protruding above the glaciers (Stehlik, 2002; Stehlik et al., 2002b; Tribsch et al., 2002; Schönswetter et al., 2003). Though less information is available for North American taxa, several important Ice Age refugia have been proposed and supported empirically, including Beringia, the Rocky Mountains south of the southernmost extent of sheet ice, and coastal regions to the southeast and west of the ice sheets (Hultén, 1937; Mooney and Billings, 1961; Steinhoff et al., 1983; Cwynar and MacDonald, 1987; Sewel et al., 1996; Soltis et al., 1997; Comes and Kadereit, 1998; Tremblay and Schoen, 1999; Abbott et al., 2000; Abbott and Brochmann, 2003; Abbott and Comes, 2003; Dobes et al., 2004). Populations inhabiting the area of the Rocky Mountain glacial refugium harbor high levels of genetic variation, presumably because Pleistocene conditions promoted the persistence and differentiation of populations in this region (Golden and Bain, 2000; Dobes et al., 2004). These molecular studies corroborated previous findings from the pollen and plant macrofossil records, which revealed that as the climate warmed and ice receded, low-elevation plants expanded their ranges northward, while high-elevation taxa retreated upslope and that the response to climate change varied predictably between the Northern and Southern Rockies (Fall, 1997; Vierling, 1998).

The Rocky Mountains, south of the Laurentide and Cordilleran ice sheets, served as one of the most important glacial refuges for high-latitude and high-elevation flora in North America. Within this ice-free area, the Rocky Mountains are divided into three physiographic regions (Brouillet and Whetstone, 1993): the Northern Rockies of Montana, the Central Rockies from Montana to the Wyoming Basin, and the Southern Rockies that extend from the Wyoming Basin south into New Mexico (Fig. 1). The Rocky Mountains comprise a topographically and climatologically heterogeneous cordillera along the Continental Divide with elevations ranging roughly from 1000 to 4000 m. Climate is highly variable due to topography, but in general the Northern Rockies receive moisture during winter from the northern Pacific while the Southern Rockies are more affected by subtropical summer monsoons (Thompson and Anderson, 2000; Kittel et al., 2002). At the time of the Last Glacial Maximum (about 18 000 yr ago), the Cordilleran and Laurentide Ice Sheets extended south into northern Montana, but the highest peaks rose above the ice as nunataks (Carrara, 1989). Most of the central Rockies was covered by a 1000 m thick layer of ice (Pierce, 1979) whereas in the south, mountain glaciers were more localized and not as continuous (Elias, 1996).

Not only were high-elevation plants within the Rocky Mountain refugium restricted in latitudinal range due to cold glacial periods, but they probably became fragmented during

---

[1] Manuscript received 21 May 2004; revision accepted 24 November 2004.

The authors thank Glacier National Park, Yellowstone National Park, Grand Teton National Park, and Rocky Mountain National Park for permission to collect plants. The work was funded by an NSF Doctoral Dissertation Improvement Grant, the University of Colorado, EPOB Graduate Student Research Grants and Fellowships, the Beverly Sears Graduate Student Grants, the John W. Marr Ecology Fund, the Indian Peaks Wilderness Association, Canon-National Parks Scholarships, the Edna Bailey Sussman Fellowship, and the Colorado Mountain Club Academic Fellowship, with additional support from the Southern Rockies Ecosystem Project. For help collecting specimens, we thank Gerald DeChaine, Mathew Burt, and Thomas Walla. We also thank Deane Bowers, William Bowman, Yan Linhart, Tom Veblen, and two anonymous reviewers for valuable discussions and feedback.

[4] Author for correspondence (e-mail: dechaine@fas.harvard.edu).

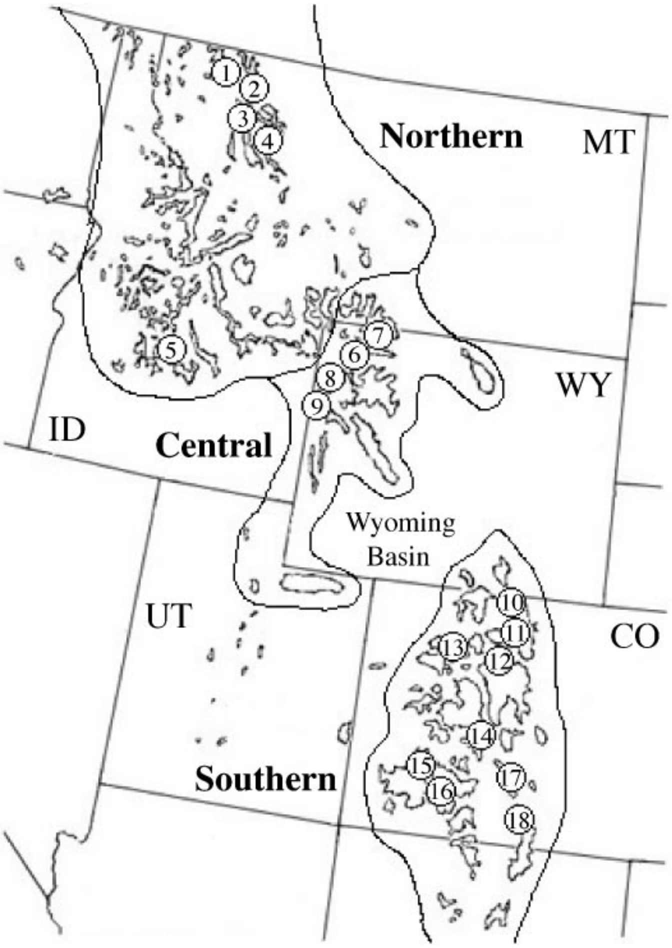

Fig. 1. Rocky Mountain biogeographic provinces and collection sites for *Sedum lanceolatum*. The biogeographic provinces included within this study are outlined: the Northern Rockies, the Central Rockies, and the Southern Rockies (Brouillet and Whetstone, 1993). The current, fragmented distribution of alpine and subalpine habitat within the region (Küchler, 1985) is illustrated. In addition, the general location of the Wyoming Basin is shown. Sampling locations are represented by circles and population numbers correspond to those in Table 1. States are abbreviated as follows: Colorado (CO), Idaho (ID), Montana (MT), Utah (UT), and Wyoming (WY).

warm interglacials due to the upward migration of habitat (Hewitt, 2000). During glacial periods, the alpine habitat was broadly distributed and interconnected, except for a discontinuity associated with the Wyoming Basin (Fig. 2A; Pewe, 1983; Elias, 1996). Interconnection during the glacial periods might have facilitated genetic and demographic cohesion across a broad geographic range. By contrast, contemporary surveys of the distribution of alpine habitat in the Rocky Mountains shows a highly dissected archipelago of habitat islands confined to high elevations (Fig. 2B; Küchler, 1985). In the current interglacial, many populations of alpine species are isolated and probably exhibit different demographic characteristics.

The objective of this study was to infer how high-elevation plants of the Rocky Mountain region south of the Cordilleran glacier responded to repeated cycles of climate change during the Quaternary. Though alpine plants are incredibly diverse in the Rocky Mountains, with more than 600 species in the Central Rockies alone (Scott, 1995), little is known about how plant populations responded to the dynamic climatic history of the Pleistocene. Given the expanding/contracting response of the alpine tundra to climate change, as outlined above, we expected populations to have mixed during glacial periods and become isolated during interglacials. Overall, we predicted that these cycles would yield a high degree of genetic diversity within the region as found in other glacial refugia (Comes and Kadereit, 1998), no loss of genetic diversity associated with increasing latitude because populations could have persisted across the range throughout the climate cycles, and high levels of genetic differentiation among populations due to isolation on sky islands during interglacials. Herein we adopted population genetic and phylogenetic approaches to evaluate the history of 18 alpine populations, ranging from northern Montana that was blanketed in sheet ice to southern Colorado where ice-free habitat existed throughout the climate cycles.

MATERIALS AND METHODS

Study organism—After its initial discovery by the Lewis and Clark expedition, *Sedum lanceolatum* Torr. (Crassulaceae), the yellow stonecrop, was found to be widespread throughout the western cordillera of North America, ranging from New Mexico to Alaska (Clausen, 1975). Plants are patchily distributed over a broad range of elevations, from 1700 m in the Great Plains to 4048 m in the alpine tundra of the Rocky Mountains. *Sedum lanceolatum* is a tufted, slow-growing, perennial herb that inhabits sandy soils and rocky outcrops of limestone, sandstone, marble, andesite, basalt, granodiorite, and granite (Clausen, 1975). Yellow, pentamerous flowers, occurring in terminal cymes, are pollinated by a variety of insects, including Diptera, Hymenoptera, and Lepidoptera (Scott, 1973; Clausen, 1975), but self-pollination is possible (Clausen, 1975). Although the small seeds (1.0 mm × 0.4 mm) generally drop to the ground and germinate, they are lightweight, and long-distance dispersal may occur (Clausen, 1975). Vegetative reproduction occurs readily in *S. lanceolatum* when leafy rosettes separate from primary stems and take root (Clausen, 1975). Within *Sedum*, hybridization is common. Diploid, tetraploid, and hexaploid populations of *S. lanceolatum* exist, but can be differentiated morphologically (Clausen, 1975). Finally, though populations are generally large (Jolls, 1980), population density, ratio of seedlings to adults, average number of leaves per rosette, height of inflorescence, and number of flowers per plant decrease with increasing elevation (Jolls, 1980).

Sampling—*Sedum lanceolatum* was collected from 18 alpine sites throughout the Rocky Mountains, from southern Colorado, which was greatly impacted by mountain glaciers, to northern Montana where continental ice sheets buried the landscape but populations could have persisted on nunataks (Fig. 1, Table 1). All specimens were morphologically similar and we used this criterion to limit comparison to individuals with the same ploidy. At each location, leaves from approximately 20 spatially separated individuals were taken, for a total of 333 samples (Table 1). Seven of the collection sites were within national parks: Glacier National Park (permit # GLAC-2001-SCI-0020), Yellowstone National Park (permit # YELL-2001-SCI-0212), Grand Teton National Park (permit # GRTE-2001-SCI-0009), and Rocky Mountain National Park (permit # ROMO-2001-SCI-0037). Specimens were transported on dry ice and stored at −80°C at the University of Colorado, Boulder, Colorado, USA.

cpDNA extraction and sequencing—DNA was extracted from plant material with DNeasy Plant Extraction kits (Qiagen, Valencia, California, USA). The chloroplast genome is the most widely used marker for population level studies in plants (Gielly and Taberlet, 1994) because in angiosperms, cpDNA is nonrecombinant, maternally inherited, dispersed via seed, permitting history to be separated from more recent gene flow due to pollen (McCauley, 1995), and evolves at a rate that retains intraspecific patterns and processes (Palmer, 1987; Comes and Kadereit, 1998). We sequenced two intergenic spacers from the chloroplast genome: the noncoding regions between tRNA-L and tRNA-

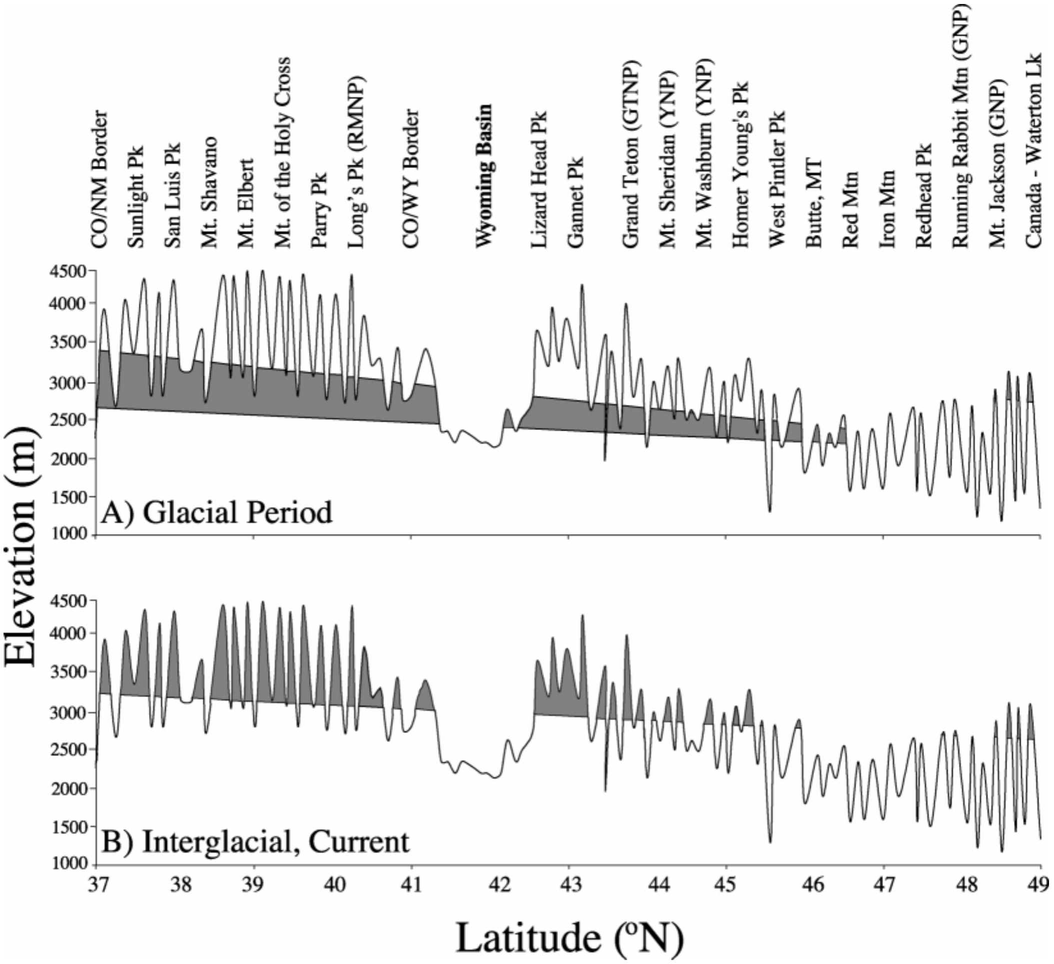

Fig. 2. Elevation profile of the Rocky Mountains showing hypothetical biogeographic response of *Sedum lanceolatum* to paleoclimatic cycles of the Quaternary. In general the north–south transect follows the Continental Divide from the San Juan Range in southern Colorado to Glacier National Park in Montana, but incorporates study sites and other locations that emphasize differences in elevation. The gray regions represent the distribution of alpine habitat during (A) a glacial period, given a 1000-m drop in elevation (Pewe, 1983) and (B) an interglacial, using the current distribution of alpine habitat (from Küchler, 1985). Latitude and elevation data were obtained from topographic atlases of the region (DeLorme, 1998a, b, c, d).

Table 1. Collection sites, within-population genetic diversity, and distribution of private alleles.

Site	Population	*n*	Latitude (N)	Longitude (W)	π	SD π	θ_W	SD θ_W	Private Alleles	% Private
1	GNP Numa Peak	20	48.8963	114.1691	0.0027	0.0017	0.8456	0.5403	G1,G6,G13	70
2	GNP Gunsight Pass	19	48.6111	113.7333	0.0024	0.0016	0.8583	0.5497	G7,G11	68
3	GNP Triple-Divide Pass	19	48.5694	113.5139	0.0038	0.0023	0.8583	0.5497	G4,G8,G9,G10	84
4	GNP Dawson Pass	19	48.4833	113.4667	0.0016	0.0011	0.2861	0.2861	G3,G5,G12	100
5	Idaho Hyndman Peak	17	43.7381	114.1394	0.0023	0.0015	0.5916	0.4430	I1,I2,I3,I4	94
6	YNP Mt. Washburn	20	44.7999	110.4256	0.0000	0.0000	0.0000	0.0000	Y2	100
7	YNP Amethyst Mtn.	17	44.8194	110.2306	0.0005	0.0005	0.2958	0.2958	Y4	6
8	GTNP Moose Pass	21	43.9528	110.8444	0.0019	0.0013	0.2780	0.2780	Y3	52
9	GTNP Static Peak	20	43.6797	110.8178	0.0005	0.0005	0.2819	0.2819	—	0
10	RMNP Sundance Mtn.	15	40.4041	105.7019	0.0036	0.0022	0.9226	0.5978	R1,R2,R3,R4,R9,R10	100
11	RMNP Long's Peak	19	40.2583	105.6167	0.0022	0.0014	1.1445	0.6638	R5,R6,R7,R8	100
12	Quandary Peak	20	39.3947	106.0981	0.0034	0.0021	0.8456	0.5403	C5,C6	30
13	Maroon Bells	18	39.0417	106.9878	0.0015	0.0011	0.8722	0.5600	C1,C4	100
14	Mt. Shavano	18	38.6167	106.2417	0.0052	0.0030	1.1629	0.6769	C12	11
15	American Basin	17	37.9139	107.5139	0.0000	0.0000	0.0000	0.0000	C3	100
16	San Luis Peak	19	37.9875	106.9292	0.0010	0.0008	0.8583	0.5497	C7	16
17	Humboldt Peak	17	37.9750	105.5611	0.0023	0.0015	0.2958	0.2958	C10	59
18	Iron Nipple	18	37.5917	105.4542	0.0020	0.0013	0.5815	0.4348	C8,C9	100

Notes: Site numbers correspond to those in Fig. 1. Abbreviations are as follows: GNP (Glacier National Park), YNP (Yellowstone National Park), GTNP (Grand Teton National Park), RMNP (Rocky Mountain National Park), ID (Idaho), and CO (Colorado). Private alleles only occur within one population, by definition. % Private is defined as the percentage of all individuals within a population that exhibit private alleles.

F (304 base pairs [bp]) and between tRNA-S and tRNA-G (580 bp), for a total of 884 bp. Amplification of the desired markers was accomplished by using specific primers for those regions: tRNA-L: (5′-ATTTGAACTGGTGACACGAG-3′) and tRNA-F: (5′-GGTTCAAGTCCCTCTATCCC-3′) (Fujii et al., 1999) and tRNA-S: (5′-GCCGCTTTAGTCCACTCAGC-3′) and tRNA-G: (5′-GAACGAATCACACTTTTACCAC-3′) (Hamilton, 1999). Each 50 μL reaction volume contained 2.25 mmol/L $MgCl_2$, 0.02 mmol/L dNTPs, 0.05 mmol/L of each primer, 2.5 units of Promega (Madison, Wisconsin, USA) B *Taq* polymerase, Promega 10× reaction buffer, and approximately 100 ng of genomic DNA. The thermal cycler profile was one cycle at 96°C for 5 min, 40 cycles at 96°C for 45 s, 52°C for 1 min, 72°C for 1 min 30 s, and one extension cycle at 72°C for 7 min. Polymerase chain reaction (PCR) products were cleaned with ExoI-SAP (0.5 μL exonuclease I, 0.5 μL shrimp alkaline phosphatase; 37 for 15°C min and 85°C for 15 min). Forward and reverse strands were cycle sequenced with the same PCR primers, using BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems, Hayward, California, USA). For the cycle sequencing reaction, the thermal cycler profile was 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Cycle sequence products were cleaned with Performa DTR 96-well Standard Plate Kit (Edge BioSystems, Gaithersburg, Maryland, USA). Sequencing was performed by the DNA Sequencing and Synthesis Facility at Iowa State University, Ames, Iowa, USA. Sequences were edited on Sequencher version 4.1.2 software (Gene Codes, Ann Arbor, Michigan, USA) and aligned with Clustal X (Thompson et al., 1997). Because recombination is limited in the chloroplast genome (McCauley, 1995), sequences from the two intergenic spacers were combined in all analyses. For all analyses, indels were considered to be a single polymorphic position (Widmer and Baltisberger, 1999; Stehlik et al., 2002a), unless sites within indels varied between haplotypes. In these cases, indels were reduced in length to the minimum number of polymorphic sites needed to represent the variation within the indel.

Analyses of genetic variation and population differentiation—Measures of within-population genetic variation and among-population genetic differentiation were estimated to test the predictions of the expansion hypotheses. Within population genetic diversity was estimated as the average pairwise nucleotide diversity (π) and based on the relationship between the number of segregating sites and the number of alleles sampled (θ_W). Linear regressions were performed using both estimators of θ to evaluate the relationship between latitude and genetic diversity (θ). In addition, regions with high levels of genetic variation were used to implicate the location of refugia (Comes and Kadereit, 1998; Hewitt, 1999). To test whether or not populations had been isolated on sky islands and to examine hierarchical population structure, we estimated the degree of differentiation among populations (F_{ST}) and performed an analysis of molecular variation (AMOVA). For the AMOVA, variation was partitioned at three levels: among northern, central, and southern biogeographic regions (Brouillet and Whetstone, 1993); among populations within regions; and within populations. We also tested for isolation-by-distance following Rousset (1997) using linear regression of standardized pairwise population F_{ST} $[-F_{ST}/(1-F_{ST})]$ on the logarithm of geographic distance. All calculations were performed on Arlequin version 2.0 (Schneider et al., 1997).

Phylogenetic and nested clade analyses—Two approaches were taken to infer phylogenetic relationships among haplotypes. First, the intraspecific phylogenetic relationships among haplotypes was inferred using Bayesian posterior probabilities implemented with MRBAYES version 2.0 (Huelsenbeck and Ronquist, 2001) using four-chain Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analysis. Base frequencies were determined empirically and substitution rates and the gamma distribution were estimated. The first 400 000 generations were discarded as the ‘‘burn-in.’’ Chains were sampled every 100 generations and inferences were based on a total of 5000 sampled trees. A consensus tree with posterior probabilites was generated in PAUP version 4.10b (Swofford, 2001) from the base frequency, substitution rate, gamma distribution, and 5000 Bayesian trees.

We also used statistical parsimony (Templeton et al., 1992), implemented on TCS 1.13 (Clement et al., 2000), for estimating the relationships among haplotypes. The resulting genealogy was tested for geographic structure using nested clade analysis (NCA; Templeton et al., 1995) implemented on GeoDis (Posada et al., 2000). While NCA permits the examination of potential population processes underlying the geographic distribution of genetic variation (Templeton, 1998), it is unable to statistically examine alternative inferences (Knowles and Maddison, 2002), and thus caution is warranted when interpreting these analyses. The few studies of plant phylogeography that incorporated NCA support its utility in elucidating processes that have shaped genetic patterns when compared with more traditional approaches such as AMOVA (see Stehlik, 2002; Dobes et al., 2004).

RESULTS

Chloroplast sequence data—Approximately 304 bp of intergenic spacer between tRNA-L and tRNA-F and 580 bp between tRNA-S and tRNA-G were sequenced from 333 individual plants for a total of 884 bp of chloroplast intergenic spacer per sample (Genbank accession numbers: AY704273–AY704317 for tRNA-L to tRNA-F and AY704318–AY704362 for tRNA-S to tRNA-G). In surprising contrast to other studies that showed very low levels of sequence variation for cpDNA (e.g., Stehlik et al., 2002a; Holderegger and Abbot, 2003), 45 haplotypes were identified in *S. lanceolatum*. The sequence data revealed three indels, which varied in length from 1 to 7 bp, four regions of single nucleotide repeat length polymorphisms (strings of 6–7 A’s, 13–18 A’s, 8–10 T’s, 7–10 T’s, and 7–8 T’s), and an additional seven variable sites. After converting the indels to one base substitution each, we described 20 variable sites for the 45 haplotypes, of which 16 sites were parsimony informative.

Population genetic analysis—The average pairwise nucleotide diversity within populations, π, ranged from 0.0 to 4.6 (Table 1). We found no relation between genetic diversity, as measured by π or θ_W, and latitude (Table 1; for π, $r = 0.01$, $P = 0.96$; for θ_W, $r = 0.01$, $P = 0.67$). Moreover, estimates of genetic diversity were highly variable among sites at similar latitudes, but generally lower in the mid-latitudes around the Greater Yellowstone Ecosystem. The high levels of genetic variation associated with northern and southern regions suggest the presence of two refugia (Comes and Kadereit, 1998). These findings on the distribution of genetic variation suggest that, generally, populations persisted across the latitudinal range sampled for this species throughout the climate cycles.

Several lines of evidence indicated that *S. lanceolatum* exhibited strong geographic structure. First, 39 out of 45 haplotypes (87%) were restricted to only a single locality (we refer to these haplotypes as private alleles) (Table 1). Second, nearly all pairwise population comparisons of F_{ST} were significant (not shown). Third, regression of $F_{ST}/(1 - F_{ST})$ against the logarithm of geographic distance showed a strong signal of isolation-by-distance ($r = 0.21$, $P < 0.01$). Finally, the results of the AMOVA (Table 2) were consistent with our other findings and showed significant genetic structure at all hierarchical levels. The majority of variation (52%) was explained by differences among populations within regions. Differences among northern, central, and southern regions explained 38% of the variation. Only 18% of the variation occurred within populations. Overall, the genetic structure and high degree of differentiation between populations suggests extremely low rates of gene flow among biogeographic provinces or among populations within regions and long-term isolation of high-elevation *S. lanceolatum* populations on multiple sky islands.

TABLE 2. Tests of genetic subdivision for populations of *Sedum lanceolatum.*

Source of variation	df	Sum of squares	Variance Components	Percentage of Variation
Among regions	2	185.0	0.671***	30.5
Among populations within regions	15	320.2	1.137***	51.6
Within populations	315	123.7	0.393***	17.9
Total	332	629.0	2.201***	

Notes: The AMOVA was partitioned at three levels: among northern, central, and southern biogeographic regions, among populations within regions, and within populations. Significant genetic structure was evident at all hierarchical levels (*** $P < 0.001$).

Intraspecific phylogeny and network—The consensus tree from Bayesian analysis revealed that the majority of haplotypes in Colorado, south of the Wyoming Basin, fall within one clade, while evolutionary relationships among the northern haplotypes and the few remaining Colorado haplotypes could not be resolved. Lower resolution of northern haplotypes, in comparison to those in the south, implies longer isolation of southern populations and a greater degree of haplotype mixing among northern populations.

The genealogy of *S. lanceolatum* haplotypes inferred using statistical parsimony (Fig. 3) included 28 unsampled, hypothetical ancestors in addition to the 45 sampled haplotypes. Only one haplotype (Y5) was broadly distributed. The deepest divergence between clades (clades 4-1 and 4-2) corresponded to populations north and south of the Wyoming Basin. The internal position of the geographically widespread haplotype implies that it is probably of ancestral origin, but we did not determine an ancestral root for the cladogram. Again, this disjunct distribution of haplotypes corroborates our AMOVA findings and implies long-term separation of northern and southern populations in two region-scale refugia and little gene flow among any sites.

Nested clade analysis—Nested clade analysis (NCA) grouped the 73 haplotypes (which includes the 28 hypothetical unsampled ancestors) into 31 first level clades, 13 second level clades, five third level clades, and two fourth level clades, within the entire clade (Fig. 3). The NCA identified 16 clades that exhibited significant geographic structure, using $\alpha = 0.05$ (boxes in Fig. 3 and all those shown in Fig. 4). Using Templeton et al. (1995) inference key, we inferred that the history of *S. lanceolatum* included several cycles of fragmentation and range expansion (Table 3). In the north, range expansion (clade 4-1) was followed by restricted gene flow (clades 3-2) then range expansion again (clade 3-1, 2-4, 2-1) and finally more restricted gene flow (clade 2-2) and fragmentation (clades 1-1, 1-3). The southern clade was more influenced by restricted gene flow and fragmentation events (clades 4-2, 3-3, 2-9, 2-7), but did exhibit range expansion (clade 2-6) followed by a recent fragmentation event (clade 1-8). Overall, the north experienced oscillation between expansion and fragmentation or isolation of populations whereas the predominant inference in the south was one of fragmentation and isolation.

DISCUSSION

Because of the large amount of variation observed in the cpDNA intergenic spacers of *S. lanceolatum*, we were able to discern a high level of geographic structure in this species. The chloroplast genome has historically been considered to exhibit low variation (Schaal et al., 1998; reviewed in Widmer and Baltisberger, 1999). Recently, however, several studies have found high levels of variation within chloroplast regions and that the degree of variation differs among taxa (Soltis et al., 1991; Dobes et al., 2004) and between markers within the genome (Gaut et al., 1993; Gielly and Taberlet, 1994; Widmer and Baltisberger, 1999). Most of the variation occurs in large single-copy regions (Schaal et al., 1998), as in our study. The few studies that used cpDNA sequence data from intergenic spacers (e.g., Fujii et al., 1999; Dobes et al., 2004) were highly successful at inferring the historic biogeography of a species in response to Quaternary climate change. Our cpDNA intergenic markers exhibited an unusually high level of sequence variation (2.26%) in comparison to other studies and permitted well-resolved inferences of the *S. lanceolatum* genealogy and genetic structure. Undoubtedly, *S. lanceolatum* is not alone in its rate of sequence evolution for cpDNA intergenic spacer regions, and increased sampling will most likely reveal that other species harbor extensive variation in cpDNA.

The Rocky Mountains south of the contiguous ice sheets is topographically and climatologically heterogeneous, with abrupt changes in elevation, resources, climate, and abundance of alpine habitat. The disjunct geography and topography of the Rocky Mountains suggest isolation between the northern and southern Rockies across the Wyoming Basin. Both climatic (low precipitation) and geographic (low elevation) barriers meet at approximately 42° N latitude on the Continental Divide. The Wyoming Basin has acted as a formidable barrier to gene flow between the north and south for high-elevation organisms (Noonan, 2001; DeChaine and Martin, 2004).

The genetic structure in *S. lanceolatum* implies that both climate and geography within the Rocky Mountains played major roles in shaping the distribution of genetic variation and historic biogeography of this species. A genetic split across the Wyoming Basin is immediately apparent from the data. The distribution of genetic diversity (Table 1), as measured by π and θ_W showed no relationship with latitude in *S. lanceolatum*, but rather revealed two centers of diversity within the region, demonstrating that populations persisted across the latitudinal range of the Rocky Mountain refugium throughout the paleoclimatic cycles. We also found that populations were highly differentiated, implying a low frequency of gene flow and long distance colonization, as expected (Van der Velde and Bijlsma, 2003). Moreover, genetic diversity was structured at all hierarchical levels. While genetic divergence was strong among the northern, central, and southern regions, the greatest degree of differentiation occurred among populations within regions (Table 2). The signal of isolation-by-distance also implied limited gene flow among populations up and down the cordillera. The significant geographic structure associated with the intraspecific phylogeny for *S. lanceolatum* further illustrated northern and southern centers of diversity and long-term isolation of populations. Moreover, some of the highest levels of within-population genetic variation and an assortment of private alleles were observed for the most northern populations in Glacier National Park (Table 1), indicating that populations either persisted on the highest peaks, or nunataks, that protruded above glacial ice or that other local, peripheral refugia existed during the last glacial period.

Though high elevation species are expected to exhibit low levels of differentiation among populations due to population mixing during relatively long glacial periods (Winograd et al.,

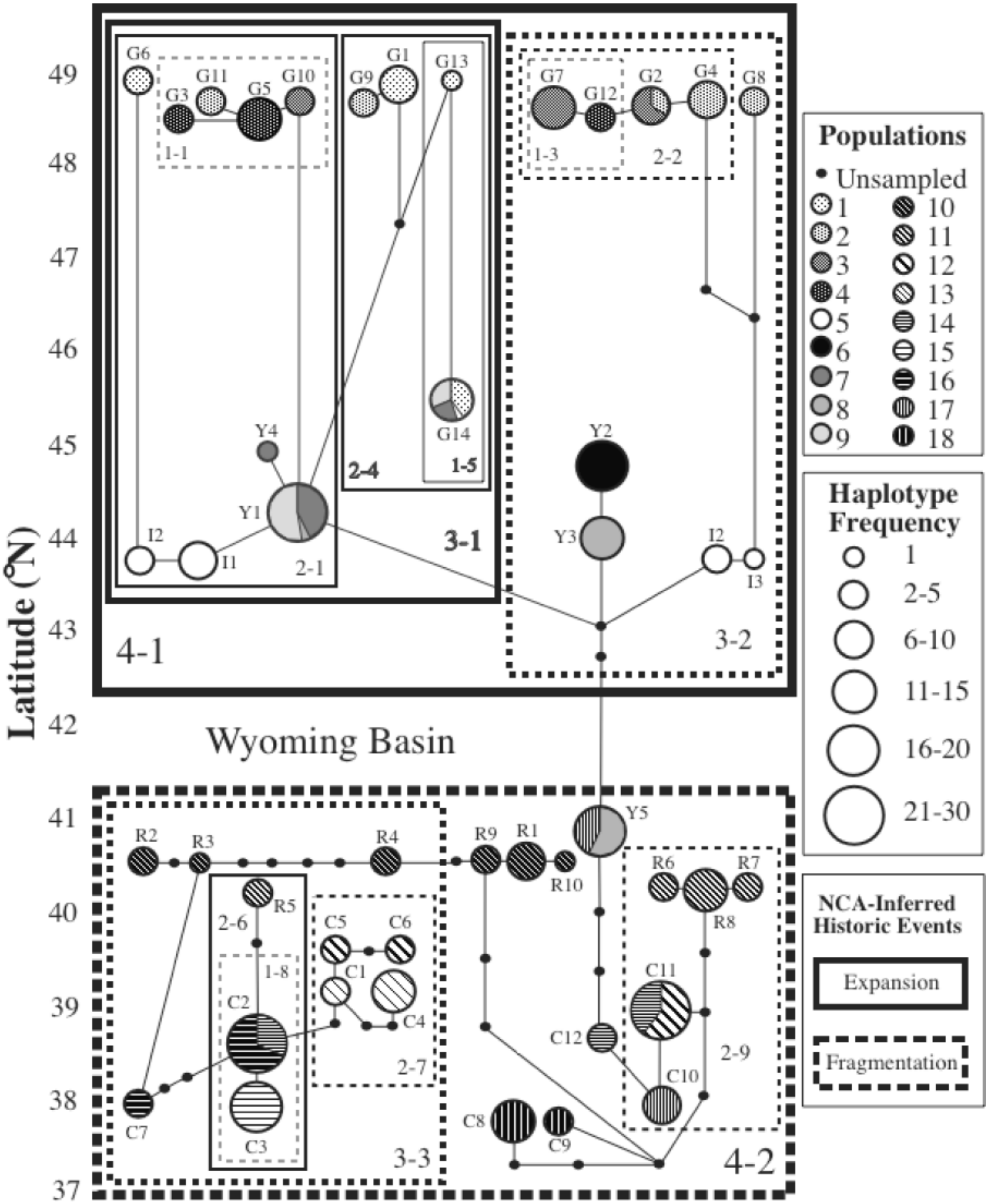

Fig. 3. Intraspecific genealogy and nested clade analysis-inferred geographic structure for *Sedum lanceolatum* across the latitudinal gradient of the Rocky Mountains. This figure combines the intraspecific phylogeny of *S. lanceolatum* with the frequency of haplotypes and the geographic location of the haplotype (latitude [°N] is given along the *y*-axis). The Wyoming Basin is shown for geographic reference. Hypothetical, unsampled haplotypes inferred through TCS 1.13 (Clements et al., 2000) are shown as black dots. Haplotypes are shown as labeled circles. The size of the circle represents the frequency of that haplotype. The shading of a circle shows the population(s) at which the haplotype was found. Private alleles only occur at one site by definition. For haplotypes shared among sites, the sites and the frequency that the haplotype was sampled for each site are shown as a pie chart. In addition, shared haplotypes are plotted by the average latitude for that haplotype. Each line separating two haplotypes corresponds to one mutational difference between them. Furthermore, boxes delineate clades (labeled) with significant geographic structure used for nested clade analysis (NCA) inferences. The thickness of the box lines corresponds to clade depth (from 1 to 4), with thicker boxes representing deeper clades. Solid boxes denote inferred range expansion, while dashed boxes indicate clades that show haplotype distributions consistent with fragmentation or restricted gene flow.

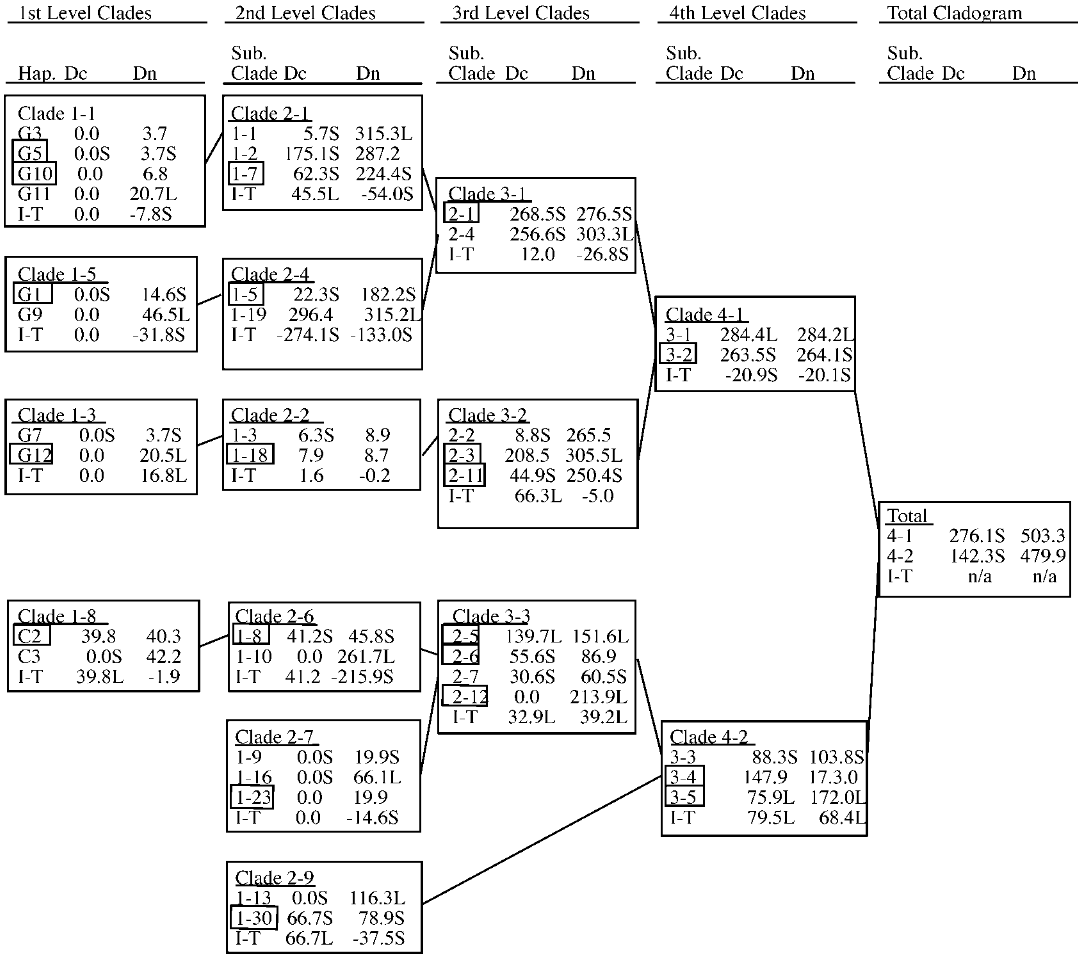

Fig. 4. Nested clade analysis (NCA) results for clades with significant geographic structure. Nesting hierarchy proceeds from the presumed most recent first-level clades on the left to the deeper clades on right. Within each clade (boxed), columns indicate haplotypes/subclades (from Fig. 3) or interior-tip (I-T) contrasts, clade distance (Dc), and nested distance (Dn). Individual haplotypes/clades that are boxed are interior, while tip clades are left unboxed. Significantly small (S) and large (L) statistics used for the NCA inference key (Templeton et al., 1995) in Table 3 are shown.

1997), *S. lanceolatum* exhibited high levels of genetic differentiation. High-elevation populations could have remained isolated throughout the paleoclimatic cycles by migrating up- and downslope with little opportunity for interpopulation gene flow. Isolation of populations, whether on a chain of interglacial sky-islands or glacial nunataks within the confines of Cordilleran ice, could have led to genetic differentiation among populations. While downslope migration, expansion, and connection of previously fragmented habitat during glacial periods (or during interglacials for northern populations) might have

TABLE 3. Inferences of historical processes.

Clade total	Steps in inference key	Inferred event
4–1	1,2,11,12,no	contiguous range expansion
4–2	1,2,3,4,no	restricted gene flow with isolation by distance
3–1	1,2,11,12,13,14,no	contiguous range expansion or long-distance colonization
3–2	1,2,3,4,no	restricted gene flow with isolation by distance
3–3	1,2,3,4,no	restricted gene flow with isolation by distance
2–1	1,2,11,12,13,14,no	contiguous range expansion or long-distance colonization
2–2	1,2,3,4,no	restricted gene flow with isolation by distance
2–4	1,2,11,12,no	contiguous range expansion
2–6	1,2,11,12,no	contiguous range expansion
2–7	1,2,3,5,15,no	past fragmentation
2–9	1,2,3,5,15,no	past fragmentation
1–1	1,2,3,5,15,no	past fragmentation
1–3	1,2,4,9,no	past fragmentation
1–5	1,2,11,12,no	contiguous range expansion
1–8	1,2,3,4,9,no	past fragmentation
1–22	1,2,11,12,13,yes	long-distance colonization

promoted population admixture, which could lead to increased genetic diversity (Walter and Epperson, 2001), population mixing could have been inhibited by the persistent presence of individuals at a site or within a region throughout the glacial cycles (Soltis et al., 1997). This could account for the observed high frequency of private alleles and geographic structure in the haplotypes of *S. lanceolatum*. When populations receded upslope, and experienced another period of isolation, they likely became further differentiated, while preserving genetic variation across the species' distribution.

Historic events inferred through NCA revealed that northern clades were more affected by expansion events, while those in the south responded to fragmentation of habitat (Table 3, Fig. 3), which was probably due to differences in topography and the extent of ice. The south is more topographically heterogeneous and was not impacted by sheet ice, while the north experienced a higher degree of connectivity during glacial periods with more of an opportunity for gene flow and colonization of novel habitat. For the alpine tundra, population processes were probably uncoupled across the latitudinal range due to the varying effects of ice. During glacial periods, northern populations were probably isolated on nunataks or in peripheral refugia, while in the south populations broadened their ranges and became connected with downslope migration of alpine habitat. In contrast, glacial retreat probably served to connect the disparate northern populations, while interglacial warming marooned more southerly populations on fragmented sky islands.

Unfortunately, fossil pollen of alpine plant species is scarce and *Sedum* pollen has not been identified from glacial deposits or regions south of the ice sheets, so direct comparison with the palynological record is not possible. Nonetheless, our findings match palynological records for other high-elevation taxa in the Rocky Mountains, including elevational migration of the alpine tundra and treeline and a differential response of flora north and south of the Wyoming Basin (Whitlock, 1993; Fall, 1997; Vierling, 1998).

The phenomenon of ''chloroplast capture'' cannot be ruled out as a factor affecting the geographic structure of haplotypes in our study. In the absence of reproductive barriers, interspecific gene flow can cause the introgression of one species' chloroplast into a population of another species with reticulation of chloroplast lineages but permanence of morphological traits (reviewed in Schaal et al., 1998). Several examples have been documented, including sunflowers (Rieseberg et al., 1990), poplars (Smith and Sytsma, 1990), oaks (Whittemore and Schaal, 1991), and the sister family to Crassulaceae, the Saxifragaceae (Soltis et al., 1991). Members of the genus *Sedum* frequently hybridize (Clausen, 1975) and thus chloroplast introgression is likely. For nearly all of our collecting sites *S. lanceolatum* was the only species of *Sedum* present. Nonetheless, comparisons with other co-distributed species in the genus are warranted.

Biogeographic responses of flora to recurrent cycles of climate change greatly impact the evolution of a species (Stebbins, 1984) and that response is species dependent (Jackson and Overpeck, 2000), based on seed-dispersal abilities, pollination strategies, life span, and whether the species is asexual, a self-pollinator, or outcrossing. Several life history traits in *S. lanceolatum* (Clausen, 1975), including a long-lived perennial habit, slow growth, local outcrossing through insect pollination, vegetative reproduction, and limited or short-distance seed dispersal, undoubtedly influenced the high degree of isolation and spatial stability of this species. High levels of differentiation among regions without the loss of genetic variation is presumably due in part to long life span, vegetative propagation, and local selection (Hamrick and Godt, 1990; Reisch et al., 2003), in contrast to outcrossing plants (Hamrick et al., 1991). The wide range of altitudes and soil types inhabited by *S. lanceolatum* suggests that localized adaptation may be contributing to the high genetic differentiation among sky island populations in this species.

Summary of conclusions—The expanse of the Rocky Mountain cordillera running south of the Pleistocene continental ice sheets has been a major influence on the evolution of plants in North America. Our inferences from the high-resolution cpDNA markers of *S. lanceolatum* suggest that high-elevation populations underwent extensive genetic divergence in response to habitat fragmentation associated with the paleoclimatic cycles. Not only was this region an important glacial refugium that preserved genetic diversity of arctic and alpine taxa throughout glacial periods, but its varied topography also promoted genetic differentiation of populations during warm interglacials.

LITERATURE CITED

ABBOTT, R. J., AND C. BROCHMANN. 2003. History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology* 12: 299–313.

ABBOTT, R. J., AND H. P. COMES. 2003. Evolution in the Arctic: a phylogeographic analysis of the circumarctic plant, *Saxifraga oppositifolia* (Purple saxifrage). *New Phytologist* 161: 211–224.

ABBOTT, R. J., L. C. SMITH, R. I. MILNE, R. M. M. CRAWFORD, K. WOLFF, AND J. BALFOUR. 2000. Molecular analysis of plant migration and refugia in the Arctic. *Science* 289: 1343–1346.

BROUILLET, L., AND R. D. WHETSTONE. 1993. Climate and physiography. *In* Flora of North America North of Mexico Editorial Committee [eds.], Flora of North America, 15–46. Oxford University Press, New York, New York, USA.

CARRARA, P. E. 1989. Late Quaternary glacial and vegetative history of the Glacier National Park Region, Montana. U.S. Ecological Survey Bulletin No. 1902.

CLAUSEN, R. T. 1975. *Sedum* of North America north of the Mexican Plateau. Cornell University Press, Ithaca, New York, USA.

CLEMENT, M., D. POSADA, AND K. A. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1659.

COMES, H. P., AND J. W. KADEREIT. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science* 3: 432–438.

CWYNAR, L. C., AND G. M. MACDONALD. 1987. Geographical variation of lodgepole pine in relation to population history. *American Naturalist* 129: 463–469.

DECHAINE, E. G., AND A. P. MARTIN. 2004. Historic cycles of fragmentation and expansion in *Parnassius smintheus* (Papilionidae) inferred using mitochondrial DNA. *Evolution* 58: 113–127.

DELORME. 1998a. Colorado atlas and gazetteer. DeLorme, Yarmouth, Massachusetts, USA.

DELORME. 1998b. Idaho atlas and gazetteer. DeLorme, Yarmouth, Massachusetts, USA.

DELORME. 1998c. Montana atlas and gazetteer. DeLorme, Yarmouth, Massachusetts, USA.

DELORME. 1998d. Wyoming atlas and gazetteer. DeLorme, Yarmouth, Massachusetts, USA.

DESPRES, L., S. LORIOT, AND M. GAUDEUL. 2002. Geographic pattern of genetic variation in the European globeflower *Trollius europaeus* L. (Ranunculaceae) inferred from amplified fragment length polymorphism markers. *Molecular Ecology* 11: 2337–2347.

DOBES, C. H., T. MITCHELL-OLDS, AND M. A. KOCH. 2004. Extensive chloroplast haplotype variation indicates Pleistocene hybridization and radiation of North American *Arabis drummondii*, *A.* × *divaricarpa*, and *A. holboellii* (Brassicaceae). *Molecular Ecology* 13: 349–370.

Elias, S. A. 1996. The Ice Age history of national parks in the Rocky Mountains. Smithsonian Institution Press, Washington, D.C., USA.

Fall, P. L. 1997. Timberline fluctuations and late Quaternary paleoclimates in the southern Rocky Mountains, Colorado. *Geological Society of America Bulletin* 109: 1306–1320.

Fujii, N., K. Ueda, Y. Watano, and T. Shimizu. 1999. Further analysis of intraspecific sequence variation of chloroplast DNA in *Primula cuneifolia* Ledeb. (Primulaceae): implications for biogeography of the Japanese alpine flora. *Journal of Plant Research* 112: 87–95.

Gaut, B. S., S. V. Muse, and M. T. Clegg. 1993. Relative rates of nucleotide substitution in the chloroplast genome. *Molecular Phylogeny and Evolution* 2: 89–96.

Gielly, L., and P. Taberlet. 1994. The use of chloroplast DNA to resolve plant phylogenies: non-coding vs. rbcL sequences. *Molecular Biology and Evolution* 11: 769–777.

Golden, J. L., and J. F. Bain. 2000. Phylogeographic patterns and high levels of chloroplast DNA diversity in four *Packera* (Asteraceae) species in southwestern Alberta. *Evolution* 54: 1566–1579.

Hamilton, M. B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* 8: 513–525.

Hamrick, J. L., and M. J. W. Godt. 1990. Allozyme diversity in plant species. *In* A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir [eds.], Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, Massachusetts, USA.

Hamrick, J., M. J. W. Godt, D. A. Murawski, and M. D. Loveless. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. *In* D. Falk and K. E. Holsinger [eds.], Genetics and conservation of rare plants, 75–86. Oxford University Press, New York, New York, USA.

Hewitt, G. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* 58: 247–276.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405: 907–913.

Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* 68: 87–112.

Holderegger, R., and R. J. Abbott. 2003. Phylogeography of the arctic-alpine *Saxifraga oppositifolia* (Saxifragaceae) and some related taxa based on cpDNA and ITS sequence variation. *American Journal of Botany* 90: 931–936.

Holderegger, R., I. Stehlik, and R. J. Abbott. 2002. Molecular analysis of Pleistocene history of *Saxifraga oppositifolia* in the Alps. *Molecular Ecology* 11: 1409–1418.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Hultén, E. 1937. Outline of the history of arctic and boreal biota during the Quaternary Period. Lehre J. Cramer, New York, New York, USA.

Jackson, S. T., and J. T. Overpeck. 2000. Responses of plant populations and communities to environmental changes of the late Quaternary. *Paleobiology* 26: 194–220.

Jolls, C. L. 1980. Variation in the reproductive biology of *Sedum lanceolatum* Torr. (Crassulaceae) along an elevational gradient in the front range, Colorado. Ph.D. dissertation, University of Colorado, Boulder, Colorado, USA.

Kittel, T. G. F., P. E. Thornton, J. A. Royle, and T. N. Chase. 2002. Climates of the Rocky Mountains: historical and future patterns. *In* J. S. Baron [ed.], Rocky Mountain Futures, 59–82. Island Press, Washington, D.C., USA.

Knowles, L. L., and W. P. Maddison. 2002. Statistical phylogeography. *Molecular Ecology* 11: 2623–2635.

Küchler, A. W. 1985. National atlas potential natural vegetation. *In* National atlas of the United States of America. Department of the Interior, U.S. Geological Survey, Reston, Virginia, USA.

McCauley, D. E. 1995. The use of chloroplast DNA polymorphism in studies of gene flow in plants. *Trends in Ecology and Evolution* 10: 198–202.

Mooney, H. A., and W. D. Billings. 1961. Comparative physiological ecology of arctic and alpine populations of *Oxyria digyna*. *Ecological Monographs* 31: 1–29.

Noonan, G. R. 2001. Systematics and cladistics of the North American subgenus *Anadaptus* Casey (Genus *Anisodactylus* Dejean) and a geographic information system analysis of the biogeography of included species. *Annals of the Entomological Society of America* 94: 301–332.

Palmer, J. D. 1987. Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. *American Naturalist* 130: S6–S29.

Pewe, T. L. 1983. The periglacial environment in North America during Wisconsin time. *In* S. C. Porter [ed.], Late Quaternary environments of the United States, 157–189. University of Minnesota Press, Minneapolis, Minnesota, USA.

Pierce, K. L. 1979. History and dynamics of glaciation in the northern Yellowstone National Park area. U.S. Geological Survey Professional Paper 729-F.

Posada, D., K. A. Crandall, and A. R. Templeton. 2000. GeoDis: a program for the cladistic nested clade analysis of the geographic distribution of genetic haplotypes. *Molecular Ecology* 9: 487–488.

Reisch, C., P. Poschlod, and R. Wingender. 2003. Genetic variation of *Saxifraga paniculata* Mill. (Saxifragaceae): molecular evidence for glacial relict endemism in central Europe. *Biological Journal of the Linnean Society* 80: 11–21.

Reiseberg, L. H., S. Beckstrom-Sternberb, and K. Doan. 1990. *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius*. *Proceedings of the National Academy of Sciences, USA* 87: 593–597.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219–1228.

Schaal, B. A., D. A. Hayworth, K. M. Olsen, J. T. Rauscher, and W. A. Smith. 1998. Phylogeographic studies in plants: problems and prospects. *Molecular Ecology* 7: 465–474.

Schneider, S., J. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin version 1.l: a software for population genetic data analysis. Genetics and Biometry Laboratory. University of Geneva, Geneva, Switzerland.

Schönswetter, P., A. Tribsch, G. M. Schneeweiss, and H. Niklfeld. 2003. Disjunctions in relict alpine plants: phylogeography of *Androsace brevis* and *A. wulfeniana* (Primulaceae). *Biological Journal of the Linnean Society* 141: 437–446.

Schönswetter, P., A. Tribsch, G. M. Barfuss, and H. Niklfeld. 2002. Several Pleistocene refugia detected in the high alpine plant *Phyteuma globulariifolium* Sternb. & Hoppe (Campanulaceae) in the European Alps. *Molecular Ecology* 11: 2637–2647.

Scott, J. A. 1973. Population biology and adult behavior of the circumpolar butterfly *Parnassius phoebus* (Papilionidae). *Entomologia Scandinavia* 4: 161–168.

Scott, R. W. 1995. The alpine flora of the Rocky Mountains, vol. 1, The Middle Rockies. University of Utah Press, Salt Lake City, Utah, USA.

Sewell, M. M., C. R. Parks, and M. W. Chase. 1996. Intraspecific chloroplast DNA variation and biogeography of North American *Liriodendron* L. (Magnoliaceae). *Evolution* 50: 1147–1154.

Smith, S. L., and K. J. Sytsma. 1990. Evolution of *Populus nigra* (sect. *Aigeiros*): introgressive hybridization and the chloroplast contribution of *Populus alba* (sect. *Populus*). *American Journal of Botany* 77: 1176–1187.

Soltis, D. E., M. A. Gitzendanner, D. D. Strenge, and P. S. Soltis. 1997. Chloroplast phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution* 206: 353–373.

Soltis, D. E., P. S. Soltis, T. G. Collier, and M. L. Edgerton. 1991. Chloroplast DNA variation within and among genera of the *Heuchera* group (Saxifragaceae): evidence for chloroplast transfer and paraphyly. *American Journal of Botany* 78: 1091–1112.

Stebbins, G. L. 1984. Polyploidy and the distribution of the arctic-alpine flora: new evidence and a new approach. *Botanica Helvetica* 94: 1–13.

Stehlik, I. 2002. Glacial history of the alpine herb *Rumex nivalis* (Polygonaceae): a comparison of common phylogeographic methods with nested clade analysis. *American Journal of Botany* 89: 2007–2016.

Stehlik, I. 2000. Nunataks and peripheral refugia for alpine plants during quaternary glaciation in the middle parts of the Alps. *Botanica Helvetica* 110: 25–30.

Stehlik, I., F. R. Blattner, R. Holderegger, and K. Bachmann. 2002a. Nunatak survival of the high alpine plant *Eritrichium nanum* (L.) Gaudin in the central Alps during the ice ages. *Molecular Ecology* 11: 2027–2036.

Stehlik, I., J. J. Schneller, and K. Bachmann. 2002b. Immigration and in situ glacial survival of the low-alpine *Erinus alpinus* (Scrophulariaceae). *Biological Journal of the Linnean Society* 77: 87–103.

STEINHOFF, R. J., D. G. JOYCE, AND L. FINS. 1983. Isozyme variation in *Pinus monticola*. *Canadian Journal of Forestry Research* 13: 1122–1132.

SWOFFORD, D. L. 2001. PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0b8a. Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. FUMAGALLI, A.-G. WUST-SAUCY, AND J.-F. COSSON. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453–464.

TEMPLETON, A. R. 1998. Nested clade analysis of phylogeographic data: testing hypothesis about gene flow and population history. *Molecular Ecology* 7: 381–397.

TEMPLETON, A. R., K. A. CRANDALL, AND C. F. SING. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132: 619–633.

TEMPLETON, A. R., E. ROUTMAN, AND C. A. PHILLIPS. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics* 140: 767–782.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876–4882.

THOMPSON, R. S., AND K. H. ANDERSON. 2000. Biomes of western North America at 18,000, 6000 and 0 ^{14}C yr BP reconstructed from pollen and packrat midden data. *Journal of Biogeography* 27: 555–584.

TOLLEFSRUD, M. M., K. BACHMANN, K. S. JAKOBSEN, AND C. BROCHMANN. 1998. Glacial survival does not matter. II. RAPD phylogeography of Nordic *Saxifraga cespitosa*. *Molecular Ecology* 7: 1217–1232.

TREMBLAY, N. O., AND D. J. SCHOEN. 1999. Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. *Molecular Ecology* 8: 1187–1198.

TRIBSCH, A., P. SCHÖNSWETTER, AND T. F. STUESSY. 2002. *Saponaria pumila* (Caryophyllaceae) and the Ice Age in the European Alps. *American Journal of Botany* 89: 2024–2033.

VAN DER VELDE, M., AND R. BIJLSMA. 2003. Phylogeography of five *Polytrichum* species within Europe. *Biological Journal of the Linnean Society* 78: 203–213.

VIERLING, L. A. 1998. Palynological evidence for late- and postglacial environmental change in central Colorado. *Quaternary Research* 49: 222–232.

WALTER, R., AND B. K. EPPERSON. 2001. Geographic pattern of genetic variation in *Pinus resinosa*: area of greatest diversity is not the origin of postglacial populations. *Molecular Ecology* 10: 103–111.

WEBB III, T., AND P. J. BARTLEIN. 1992. Global changes during the last 3 million years: climatic controls and biotic responses. *Annual Review of Ecology and Systematics* 23: 141–173.

WHITLOCK, C. 1993. Postglacial vegetation and climate of Grand Teton and southern Yellowstone National Parks. *Ecological Monographs* 63: 173–198.

WHITTEMORE, A. T., AND B. A. SCHAAL. 1991. Interspecific gene flow in oaks. *Proceedings of the National Academy of Sciences, USA* 88: 2540–2544.

WIDMER, A., AND M. BALTISBERGER. 1999. Extensive intraspecific chloroplast DNA (cpDNA) variation in the alpine *Draba aizoides* L. (Brassicaceae): haplotype relationships and population structure. *Molecular Ecology* 8: 1405–1415.

WINOGRAD, I. J., J. M. LANDWEHR, K. R. LUDWIG, T. B. COPLEN, AND A. C. RIGGS. 1997. Duration and structure of the past four interglaciations. *Quaternary Research* 48: 141–154.