Systematic Botany (1995), 20(2): pp. 179–190

Allozyme Variation in the Athabasca Sand Dune Endemic, *Salix silicicola*, and the Closely Related Widespread Species, *S. alaxensis*

BRETT G. PURDY AND RANDALL J. BAYER

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

Communicating editor: Peter K. Bretting

ABSTRACT. Genetic variation was estimated by starch gel electrophoretic resolution of 17 putative isozyme loci in eight populations of the Athabasca sand dune endemic *Salix silicicola* from northern Saskatchewan, Canada, and five populations of its putative progenitor species, *Salix alaxensis*, from northern British Columbia, Canada. Thirty-seven alleles were detected at 10 polymorphic loci. Six loci were polymorphic in the endemic species and 10 loci were polymorphic in the presumed progenitor species. *Salix silicicola* had one unique allele, whereas *Salix alaxensis* had 17 alleles not found in the former species. Populations of the endemic contained fewer alleles per locus (1.30 vs. 1.85), fewer alleles per polymorphic locus (2.05 vs. 2.85), lower percent polymorphic loci (28.7 vs. 45.9) and lower genetic diversity (0.073 vs. 0.195) than did populations of the putative progenitor species. Genetic identities within species averaged 0.981 for *Salix silicicola* and 0.973 for *Salix alaxensis* and between species identities ranged from 0.902 to 0.963 with a mean of 0.932. At polymorphic loci, total gene diversity was relatively high in *Salix silicicola* (H_T = 0.305) and *Salix alaxensis* (H_T = 0.384). Population differentiation was relatively low in both species (*Salix silicicola*, G_{ST} = 0.159; *Salix alaxensis*, G_{ST} = 0.097) whereas estimates of gene flow based on G_{ST} values were moderate (*Salix silicicola*, Nm_W = 1.32; *Salix alaxensis*, Nm_W = 2.33), consistent with these trees' dioecious breeding system and wind-dispersed seeds.

The amount and pattern of genetic variation within and among populations and closely related species can be influenced by a wide range of factors, including mating system, natural selection, geographic distribution, and historical events (Loveless and Hamrick 1988). Plant species with limited geographic ranges typically have lower levels of genetic diversity than do widespread taxa (Karron 1987; Hamrick and Godt 1989). In general, endemic species contain fewer polymorphic loci, fewer alleles per polymorphic locus, and less heterozygosity than do more widespread species (Hamrick and Godt 1989). This paucity of genetic diversity may be due to stochastic processes, such as genetic drift and founder effects, and/or strong directional selection toward genetic uniformity (Karron 1987). Recently-evolved species are often classified as narrow endemics because of their restricted geographic ranges.

Although the number of plant allozyme studies of genetic diversity in rare and endemic taxa has increased in recent years (Hamrick and Godt 1989), there are few studies of endemic and widespread taxa that are closely related, especially species pairs that are progenitors-derivatives as well (Edwards and Wyatt 1994). Population genetic studies have revealed that derivative species generally harbor less genetic diversity than the progenitor (Gottlieb 1973, 1974; Crawford and Smith 1982a, 1982b; Crawford et al. 1985; Loveless and Hamrick 1988; Pleasants and Wendel 1989), though not always (Gottlieb et al. 1985; Linhart and Premoli 1993). Information on the genetic diversity of rare and endangered species is an important factor for determining approaches to conservation (Soulé and Kohm 1989).

The flora of boreal and arctic regions typically contains few endemic taxa because of the short time period available, since the Pleistocene, for speciation to occur (Kruckeberg and Rabinowitz 1985). Yet, the Athabasca sand dunes in northern Saskatchewan, Canada, contain a number of rare plant species, including ten endemic taxa (Raup 1936; Raup and Argus 1982). Previous investigations on the population genetics of Athabasca sand dune endemics *Stellaria arenicola* Raup (Purdy et al. 1994) and *Deschampsia mackenzieana* Raup (Purdy and Bayer 1995) focused on species that evolved while sympatric with their progenitor taxon. The tetraploid nature of *D. mackenzieana* isolated it from the diploid *D. cespitosa* (L.) Beauv., with genetic diver-

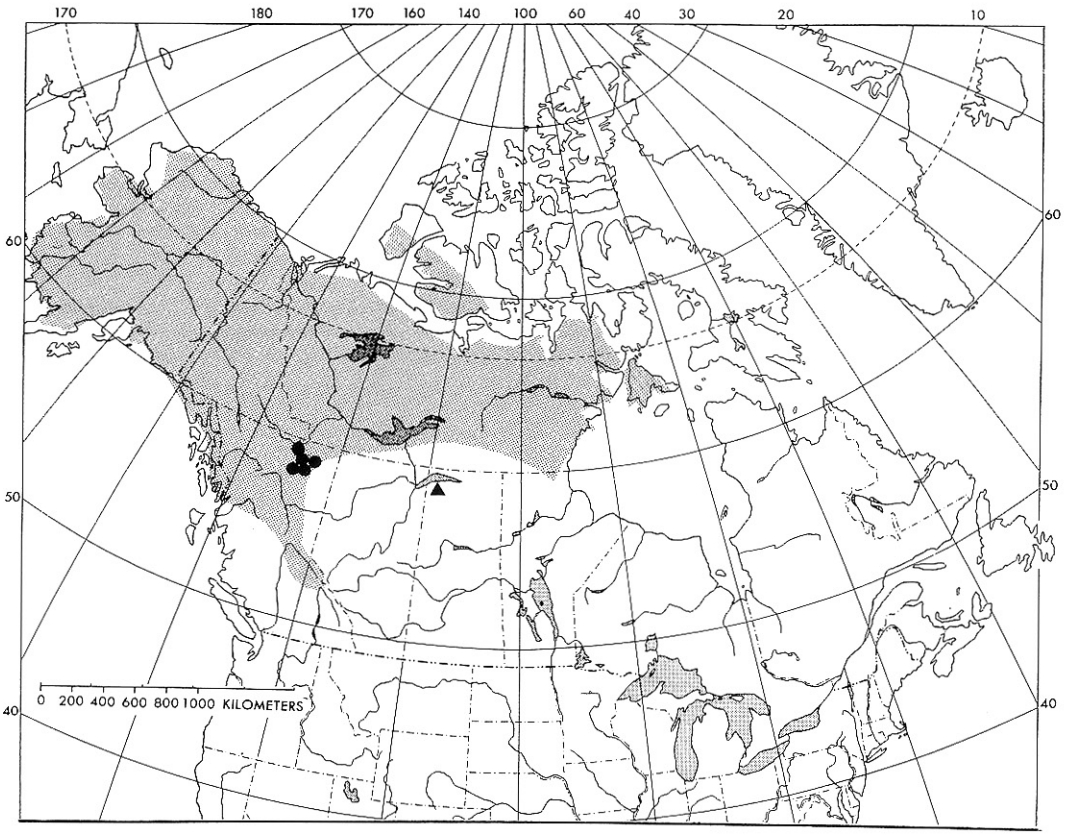

FIG. 1. Location of populations of the widespread *Salix alaxensis* (circles) sampled in this study. The shaded area represents the North American distribution for *S. alaxensis*. The triangle indicates the location of the Athabasca sand dunes.

sity in the tetraploid being considerably lower than in its progenitor (Purdy and Bayer 1995). *Stellaria arenicola* had lower genetic diversity than did *S. longipes* Goldie, the species from which it evolved, but gene flow between species may have been important in maintaining higher levels of genetic variation in this endemic (Purdy et al. 1994).

Salix silicicola Raup, one of four endemic willows of the Athabasca sand dunes, is restricted to the active sand dunes, gravel pavements, and beach ridges on the south shore of Lake Athabasca (Raup 1936, 1959). A conspicuous willow, it is thought to be derived from *S. alaxensis* (Anders.) Cov., from which it differs by its short stipules (up to 7 mm long, compared to 22 mm) that are broadly ovate to lanceolate rather than linear-lanceolate to filiform, and by its persistent, heavy, hairy-tomentose vestiture on both sides of the laminae instead of being glabrous or glabrate and bright green on the upper surface (Raup 1959). *Salix alaxensis*, a common willow in Alaska, Yukon, Northwest Territories, and the northern Rocky Mountains in British Columbia (Raup 1959), occurs with other pioneer vegetation on river alluvia, glacial moraines, immature forests, and alpine tundra (Argus 1973). Populations of *S. silicicola* are allopatric with populations of *S. alaxensis* at present (Argus and Steele 1979; Porsild and Cody 1980).

This study was conducted to compare the amount and pattern of genetic diversity of *Salix silicicola*, a recently evolved narrow endemic, with *S. alaxensis*, its presumed widespread progenitor. This is the first study of an endemic from the Athabasca sand dunes that has an allopatric distribution with its presumed progenitor. We compared our results with studies of

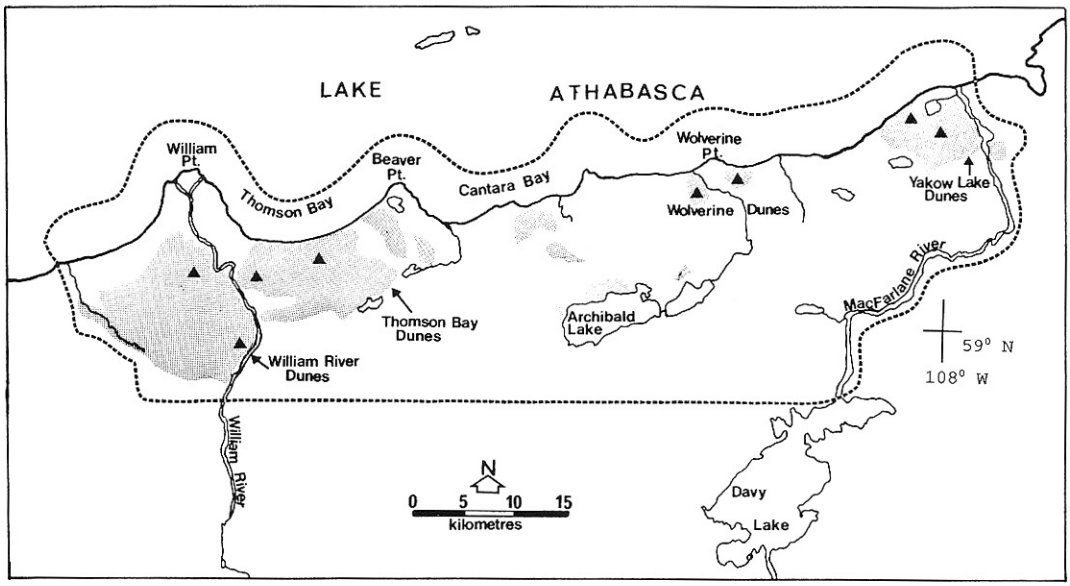

FIG. 2. Location of populations of the endemic *Salix silicicola* (triangles) sampled in this study from the Athabasca sand dunes. Active sand dunes are shaded on the map. The dotted line indicates the boundaries of the Athabasca Sand Dunes Wilderness Provincial Park.

other endemic species from the Athabasca sand dunes that have evolved in sympatry with their progenitor taxon. We also wanted to confirm the close genetic relationship of the two taxa as well as to establish population genetic information for *S. silicicola*, a candidate for the endangered species list in Canada.

MATERIALS AND METHODS

Sample Collection. Summer cuttings from two or three year old stems were collected from five populations of *S. alaxensis* from northern British Columbia (Fig. 1; Table 1) and eight populations of *Salix silicicola* on the Athabasca sand dunes in Saskatchewan (Fig. 2; Table 1). The cuttings were wrapped in live *Sphagnum* and kept moist and cool until they were transported to the University of Alberta greenhouse. After being planted in soil in Spencer-Lemaire containers, the cuttings were placed on a mist bench until leaves began to emerge. At least 50 stems were collected from each population, although a reduced number of cuttings actually grew new leaves (Table 3).

Electrophoresis. Standard methods for starch gel electrophoresis were employed in this study (Soltis et al. 1983). Fresh pieces of actively growing leaf tissue were ground in cold Tris-HCl extraction buffer (Purdy et al. 1994). The supernatant was absorbed into filter paper wicks, frozen at −20°C overnight, and electrophoresed the following morning. The filter paper wicks were loaded onto 12% electrolyzed potato starch gels (Sigma Chemical, St. Louis, Missouri).

Three buffer systems were used to resolve 11 enzyme systems in this study. Glutamate oxalate-transaminase (GOT) and glutamate dehydrogenase (GDH) were resolved on buffer system VI of Soltis et al. (1983). Acid phosphatase (ACP), glucose-6-phosphate dehydrogenase (G6PDH), leucine aminopeptidase (LAP), and phosphoglucose isomerase (PGI) were resolved on buffer system VII of Soltis et al. (1983). 6-phosphogluconate dehydrogenase (PGD), glyceraldehyde-3-phosphate dehydrogenase (G3PDH), isocitrate dehydrogenase (IDH), menadione reductase (MNR), and shikimic acid dehydrogenase (SKD) were resolved on a histidine-citric acid buffer system (Cardy et al. 1983). The first two buffer systems were run at 50 mA for 30 min, then at 60mA for 4–5 hr, the third buffer system was run at 30 mA for 4–5 hr. Electrophoresis was performed at 4°C.

Enzymatic assays followed Soltis et al. (1983), except for MNR (Wendel and Weeden 1989).

TABLE 1. Population designation, collection location, and habitat for eight populations of *Salix silicicola* and five populations of *S. alaxensis* used in this study.

Population	Population location and habitat
	S. silicicola
WRD1	Central William River dune field. Lat. 59° 03′ N, Long. 109° 15′ W, elev. 244 m. Gravel plains.
WRD2	Southern William River dune field. Lat. 58° 55′ N, Long. 109° 12′ W, elev. 275 m. Active sand dunes.
TBD1	Western Thomson Bay dune field. Lat. 59° 05′ N, Long. 109° 11′ W, elev. 244 m. Active sand dunes.
TBD2	Central Thomson Bay dune field. Lat. 59° 03′ N, Long. 109° 05′ W, elev. 270 m. Active sand dunes.
CAD1	East Wolverine dune field. Lat. 59° 07′ N, Long. 108° 20′ W, elev. 215 m. Beach ridges.
CAD2	West Wolverine dune field. Lat. 59° 08′ N, Long. 108° 25′ W, elev. 245 m. Active sand dunes.
YLD1	Northern Yakow dune field. Lat. 59° 11′ N, Long. 108° 10′ W, elev. 220 m. Active sand dunes.
YLD2	Central Yakow dune field. Lat. 59° 10′ N, Long. 108° 00′ W, elev. 240 m. Active sand dunes.
	S. alaxensis
NBC1	Summit Lake, B.C. Lat. 58° 38′ N, Long. 124° 41′ W, elev. 1,450 m. Rocky shoreline of lake.
NBC2	Mt. St. Paul, B.C. Lat 58° 40′ N, Long. 124° 46′ W, elev. 1,540 m. Gravel alluvium of unnamed creek.
NBC3	Mile One Thirteen Creek, B.C. Lat. 58° 42′ N, Long. 124° 50′ W, elev. 1,300 m. Disturbed creek bed.
NBC4	Trout River, B.C. Lat. 59° 14′ N, Long. 125° 50′ W, elev. 1,200 m. Disturbed creek bed.
NBC5	Toad River, B.C. Lat. 58° 47′ N, Long. 125° 34′ W, elev. 1,290 m. Gravel alluvium.

For enzymes with more than one putative locus, the staining zones were numbered sequentially, with the most anodally migrating isozyme designated as 1, the next 2, and so on. Similarly, the most anodal allozyme of a gene was labeled A, etc. Bands were categorized as isozymes and allozymes by observing segregation of bands among individuals in the populations sampled in light of the typical subunit structure and subcellular compartmentalization (Gottlieb 1981; Weeden and Wendel 1989). Genotype frequencies at each locus were determined for each population.

Data analysis. Levels of allozyme variation were estimated at the species level and for individual populations. Genetic diversity was measured by four parameters: percent polymorphic loci (P), average number of alleles per locus (A), average number of alleles per polymorphic locus (A_P), and expected heterozygosity (H_E). The four diversity parameters were calculated for each population and were averaged across all populations for each species. T-tests were performed to test for significant differences among these population parameters. Species-level statistics were calculated for the four genetic diversity parameters by treating all populations of a species as if they were one population. A locus was considered polymorphic if more than one allele was detected. Fixation indices (F), which reflect deviations of genotypic frequencies from Hardy-Weinberg expectations, were measured as well (Wright 1922).

The partitioning of genetic diversity within and among populations was analyzed with measures proposed by Nei (1973). The parameter H_T is the total allelic diversity at each polymorphic locus, H_S is the allelic diversity within populations, and D_{ST} is the allelic diversity among populations. The coefficient of gene differentiation (G_{ST}), is the ratio of among population genetic diversity relative to the total genetic diversity. F-statistics (Wright 1969) elucidated how the detected heterozygosity was structured. The F-statistics include F_{IS}, an index of inbreeding, F_{ST}, an index of reduced heterozygosity due to population subdivision, and F_{IT}, the overall inbreeding coefficient (Wright 1965). We also calculated Wright's gene flow estimate [Nm_W, where $Nm_W = (1\text{-}G_{ST})/4G_{ST}$]. Genetic identities, I, (Nei 1972) were calculated as well. Population variation statistics (A, A_P, P, and H_E), fixation indices, F-statistics, and standard genetic identities were calculated with the BIOSYS program (Swofford and Selander 1981). Genetic diversity statistics (H_T, H_S, D_{ST}, and G_{ST}) were calculated with the GENESTAT-PC program (Whitkus 1988). A UPGMA phenogram derived from the matrix of the genetic identities was produced using SYSTAT (Wilkinson 1990).

TABLE 2. Allele frequency data for polymorphic loci from eight populations of *Salix silicicola* and five populations of *S. alaxensis*. * indicates alleles unique to *S. alaxensis*, ** indicates the allele unique to *S. silicicola*.

Locus/ allele	*S. silicicola*								*S. alaxensis*				
	WRD1	WRD2	TBD1	TBD2	CAD1	CAD2	YLD1	YLD2	NBC1	NBC2	NBC3	NBC4	NBC5
G3pdh-2													
*a**	—	—	—	—	—	—	—	—	0.018	—	—	—	—
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.982	1.000	1.000	1.000	1.000
G6pdh-2													
a	0.438	0.288	0.615	0.313	0.476	0.750	0.444	0.719	0.250	0.167	0.333	0.200	0.217
*b**	—	—	—	—	—	—	—	—	0.214	0.222	0.333	0.600	0.333
c	0.562	0.712	0.385	0.687	0.524	0.250	0.556	0.281	0.464	0.555	0.334	0.200	0.283
*d**	—	—	—	—	—	—	—	—	—	0.056	—	—	0.167
*e**	—	—	—	—	—	—	—	—	0.072	—	—	—	—
Gdh-1													
a	0.854	0.769	0.962	0.958	1.000	0.979	0.667	1.000	0.893	0.944	1.000	0.700	0.583
*b**	—	—	—	—	—	—	—	—	—	—	—	0.200	0.333
c	—	—	—	0.042	—	—	—	—	0.107	0.056	—	0.100	0.084
*d***	0.146	0.231	0.038	—	—	0.021	0.333	—	—	—	—	—	—
Got-2													
a	0.104	0.288	0.135	0.250	0.119	0.125	0.796	0.688	0.375	0.574	0.500	0.200	0.450
b	0.896	0.712	0.885	0.750	0.881	0.875	0.204	0.312	0.625	0.426	0.500	0.800	0.550
Idh-2													
*a**	—	—	—	—	—	—	—	—	—	—	0.100	0.300	0.150
b	0.021	—	0.154	—	—	0.063	—	—	0.286	0.259	—	0.300	0.450
*c**	—	—	—	—	—	—	—	—	0.286	0.315	0.400	0.100	0.050
d	—	0.212	—	—	0.143	0.145	—	0.188	—	0.185	0.400	0.100	0.050
e	0.979	0.788	0.846	1.000	0.857	0.792	1.000	0.812	0.375	0.185	0.100	0.100	0.100
*f**	—	—	—	—	—	—	—	—	0.053	0.056	—	0.100	0.200
Lap-1													
*a**	—	—	—	—	—	—	—	—	0.393	0.555	0.600	0.300	0.333
b	—	0.058	0.269	—	—	—	—	0.188	0.107	—	—	—	—
c	1.000	0.942	0.731	1.000	1.000	0.979	1.000	0.812	0.393	0.056	0.400	0.700	0.667
d	—	—	—	—	—	0.021	—	—	—	0.389	—	—	—
*e**	—	—	—	—	—	—	—	—	0.107	—	—	—	—
Pgd-3													
*a**	—	—	—	—	—	—	—	—	0.214	0.333	0.200	0.233	0.217
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.786	0.667	0.800	0.767	0.783
Pgi-1													
*a**	—	—	—	—	—	—	—	—	0.036	—	—	—	—
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	1.000	1.000	1.000	1.000
*c**	—	—	—	—	—	—	—	—	0.035	—	—	—	—
Pgi-2													
*a**	—	—	—	—	—	—	—	—	—	0.019	—	—	—
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	0.981	1.000	0.900	1.000
*c**	—	—	—	—	—	—	—	—	0.071	—	—	0.100	—
Skd-1													
*a**	—	—	—	—	—	—	—	—	0.072	—	0.100	0.200	—
b	—	—	0.037	—	—	—	—	—	—	—	—	0.100	0.050
c	0.250	0.192	0.423	0.271	—	0.146	0.167	0.281	0.071	0.111	—	—	—
d	0.750	0.808	0.538	0.729	1.000	0.854	0.833	0.719	0.857	0.778	0.900	0.700	0.650
*e**	—	—	—	—	—	—	—	—	—	0.111	—	—	0.300

TABLE 3. Summary of allozyme variation for 17 putative loci within eight populations of *Salix silicicola* and five populations of *S. alaxensis*. N = sample size, A = mean number of alleles per locus, A_P = mean number of alleles per polymorphic locus, P = % polymorphic loci, and H_E = mean expected heterozygosity. * and ** indicate that values differ between species at $P < 0.05$ and $P < 0.01$, respectively.

Population	N	A	A_P	P	H_E
		S. silicicola			
WRD1	24	1.3	2.0	29.4	0.081
WRD2	26	1.4	2.0	35.3	0.116
TBD1	26	1.4	2.2	35.3	0.116
TBD2	24	1.2	2.0	23.5	0.077
CAD1	21	1.2	2.0	17.6	0.057
CAD2	24	1.4	2.3	35.3	0.076
YLD1	27	1.2	2.0	23.5	0.092
YLD2	32	1.3	2.0	29.4	0.110
Mean	25.5	**1.30	**2.05	**28.7	**0.073
(SE)	1.1	0.04	0.05	2.3	0.022
Species level	204	*1.59	*2.67	35.3	0.108
(SE)	—	0.21	0.21	—	0.041
		S. alaxensis			
NBC1	28	2.1	3.0	58.8	0.215
NBC2	27	1.9	3.2	47.1	0.201
NBC3	30	1.5	2.6	35.3	0.168
NBC4	30	1.9	3.0	47.1	0.212
NBC5	30	1.9	3.3	41.2	0.225
Mean	29.0	**1.85	**2.85	**45.9	**0.195
(SE)	0.7	0.13	0.18	3.9	0.027
Species level	145	*2.53	*3.60	58.9	0.220
(SE)	—	0.42	0.48	—	0.069

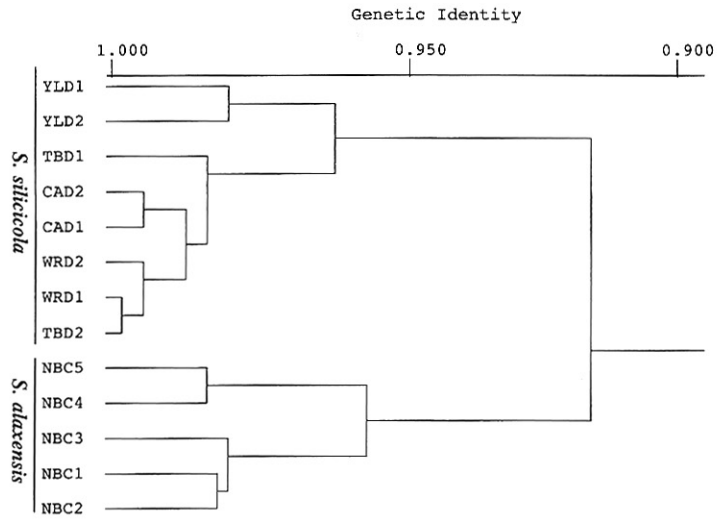

FIG. 3. UPGMA cluster analysis of eight populations of *Salix silicicola* and five populations of *S. alaxensis* based on Nei's (1972) measure of genetic identity (I). The cophenetic correlation coefficient was 0.892.

Results

The 11 enzyme systems assayed in this study are governed by 17 putative loci. Some isozymes were excluded from genetic analysis because they could not be resolved consistently, or had uninterpretable banding patterns (ACP and MNR). The putative loci *G3pdh-1, G6pdh-1, Got-1, Idh-1, Lap-2, Pgd-1,* and *Pgd-2* were monomorphic in all populations. Ten loci (*G3pdh-2, G6pdh-2, Gdh-1, Got-2, Idh-2, Lap-1, Pgd-3, Pgi-1, Pgi-2,* and *Skd-1*) were polymorphic in at least some populations (Table 2). There were 37 alleles at the 10 putative polymorphic loci (Table 2). Alleles unique to a species occurred at frequencies less than 0.50 (Table 2). *Gdh-1d* was the only allele restricted to populations of *Salix silicicola*, whereas 17 alleles (*G3pdh-2a, G6pdh-2b, G6pdh-2d, G6pdh-2e, Gdh-1b, Idh-2a, Idh-2c, Idh-2f, Lap-1a, Lap-1e, Pgd-3a, Pgi-1a, Pgi-1c, Pgi-2a, Pgi-2c, Skd-1a,* and *Skd1-e*) were restricted to populations of *S. alaxensis*. Overall, *Salix silicicola* differed from *S. alaxensis* by the most common allele at a locus at only two loci (*G6pdh-2* and *Idh-2*; Table 2).

At the species level *Salix silicicola* had considerably less genetic variation than did *S. alaxensis*. Only six loci were polymorphic in *S. silicicola*, whereas in *S. alaxensis*, 10 loci were polymorphic. The mean number of alleles per locus, A, (1.59 vs. 2.53), mean number of alleles per polymorphic locus, A_P, (2.67 vs. 3.60), percent polymorphic loci, P, (35.3 vs. 58.9) and average heterozygosity, H_E, (0.108 vs. 0.220) were lower in *S. silicicola* than in *S. alaxensis* (Table 3). On the average, population level genetic variation was also much lower in *Salix silicicola* than in *S. alaxensis*. Populations of the endemic species had lower mean A (1.30 vs. 1.85), A_P (2.05 vs. 2.85), P (28.7 vs. 45.9) and H_E (0.073 vs. 0.195) than did populations of the presumed progenitor species (Table 3, all $P < 0.01$).

Fixation indices suggest contrasting levels of inbreeding in the two species. In *Salix silicicola*, seven of 39 (18%) of the fixation indices are significant and positive, suggesting a deficiency of heterozygotes (Table 4). In *S. alaxensis*, 14 of 38 (37%) of the F values are significant, three positive and 11 negative, suggesting an excess of heterozygotes (Table 4). Values obtained for F_{IS} and F_{IT} indicate that, at five of the six polymorphic loci in *S. silicicola*, there is a deficiency of heterozygotes, whereas values obtained for

TABLE 4. Fixation indices (F) for *Salix silicicola* and *S. alaxensis*. The asterisk indicates that fixation indices differ significantly from Hardy-Weinberg expectations ($P \leq 0.05$), a dash indicates that loci were monomorphic in the population.

	S. silicicola							*S. alaxensis*				
Locus	WRD1	WRD2	TBD1	TBD2	CAD1	CAD2	YLD1	YLD2	NBC1	NBC2	NBC3	NBC4	NBC5
G3pdh-2	—	—	—	—	—	—	—	—	−0.017	—	—	—	—
G6pdh-2	0.407*	0.224	0.216	0.162	0.236	0.180	−0.169	−0.123	−0.384*	−0.273	−0.500	−0.429*	−0.210*
Gdh-1	0.033	−0.300	−0.067	−0.091	—	−0.032	−0.380	—	−0.120	−0.059	—	−0.304*	−0.538*
Got-2	0.233	−0.031	−0.123	−0.011	−0.143	0.429	−0.125	0.446*	0.067	0.100	0.067	−0.250	0.100
Idh-2	0.395	−0.031	−0.185	—	−0.171	0.317	—	0.590*	−0.112*	−0.433	−0.515*	−0.250*	−0.351*
Lap-1	—	−0.061	0.439*	—	—	−0.015	—	0.590*	0.566*	0.099	0.000	0.167	0.000
Pgd-3	—	—	—	—	—	—	—	—	0.409	0.357	0.608*	0.418*	—
Pgi-1	—	—	—	—	—	—	—	—	−0.057	—	—	—	—
Pgi-2	—	—	—	—	—	—	—	—	−0.037	−0.059	—	0.111	—
Skd-1	0.556*	0.500*	−0.414	0.051	—	−0.132	0.357	−0.205	−0.057	−0.091	−0.111	−0.304*	−0.443*

F_{IS} and F_{IT} in *S. alaxensis* indicate that, at seven of the 10 polymorphic loci, there is an excess of heterozygotes (Table 5).

Total genetic diversity at polymorphic loci (H_T) in *Salix silicicola* was 0.305, compared to 0.384 in *S. alaxensis* (Table 6). The among population differentiation (G_{ST}) was higher in the endemic (0.159) than it was in the presumed progenitor (0.097). Estimates of gene flow were moderate in both species. Wright's estimate (Nm_W) for *Salix silicicola* was 1.32 migrants per generation and for *S. alaxensis* it was 2.33 migrants per generation (Table 6).

Genetic identity values (I) for all pairs of populations were high. Intraspecific I values for populations of *Salix silicicola* ranged from 0.954 to 0.998 with a mean of 0.981; for *S. alaxensis*, I values ranged from 0.947 to 0.987 with a mean of 0.973. The mean of genetic identity values between *S. silicicola* and *S. alaxensis* was 0.932, and ranged from 0.902 to 0.963. A UPGMA cluster analysis based on genetic identity values depicts the close genetic relationship among populations of each species and a more distant genetic relationship among populations of the two species (Fig. 3).

Discussion

Genetic Diversity. The plant allozyme literature indicates that the values of several population genetic parameters are correlated with narrow endemism (Karron 1987; Hamrick and Godt 1989; Hamrick et al. 1991). Species with restricted geographic distributions generally have fewer polymorphic loci, fewer alleles per locus, and reduced heterozygosity (Gottlieb 1973; Karron et al. 1988; Loveless and Hamrick 1988; Pleasants and Wendel 1989; Bayer 1992; Sherman-Broyles et al. 1992; Purdy et al. 1994) when compared to widespread congeners.

The results of this study are consistent with previous investigations on genetic diversity in endemic taxa. *Salix silicicola* has considerably less species-level and population-level genetic variation than does its widespread presumed progenitor, *S. alaxensis*. The mean number of alleles per locus, mean number of alleles per polymorphic locus, percent polymorphic loci, and heterozygosity were all lower in the endemic taxon. Allozyme studies on other Athabasca sand dune endemics (Purdy et al. 1994; Purdy and Bayer 1995) have found endemic taxa to have less genetic variation than do their related widespread progenitor taxa, though the relationship between the progenitor and derivative species differ from the present study. *Deschampsia mackenzieana* is a reproductively isolated, tetraploid endemic that occurs in sympatry with its diploid progenitor, *D. cespitosa*. A genetic bottleneck at the time of origin during polyploidization was thought to be the factor responsible for the significantly lower genetic diversity in *D. mackenzieana* (Purdy and Bayer 1995). Although *Stellaria arenicola* had less genetic diversity than *S. longipes*, sympatric populations of the progenitor were thought to provide additional genetic variation through interspecific gene flow because the two taxa are sympatric and hybridize in a number of locations on the Athabasca sand dunes (Purdy et al. 1994).

Mean values compiled from Hamrick and Godt (1989) for A (1.39), P (26.3), and H_E (0.063) in endemic taxa resembled those found in *Salix silicicola* (A = 1.30; P = 28.7; and H_E = 0.073). Values for the same parameters in widespread plant taxa (A = 1.72; P = 43.0; and H_E = 0.159), taxa with animal-outcrossed breeding system (A = 1.54; P = 35.9; and H_E = 0.124), or wind-dispersed seeds (A = 1.70; P = 42.9; and H_E = 0.123), resembled those for *S. alaxensis* (A = 1.85; P = 45.9; and H_E = 0.195).

In the only other study of allozyme variation in the genus, Brunsfeld et al. (1991) generally found that widespread *Salix* species of the sect. *Longifoliae* had the highest mean values for A, P, and H_E. Although levels of population genetic variation in *S. exigua* Nutt. and *S. interior* Rowlee (Brunsfeld et al. 1991), two geographically widespread species, were high and resembled the values obtained for *S. alaxensis* in this study, *S. silicicola*, had less population genetic variation than did *S. fluviatilis* Nutt. and *S. sessifolia* Nutt., two species with narrow geographic ranges. Other factors, such as the evolutionary nature of populations at the margin of their range or species with small population sizes but extensive distributions (e.g., *S. taxifolia* H.B.K.) affected levels of genetic variation (Brunsfeld et al. 1991). Factors that promote high levels of genetic diversity within populations in *Salix* species include dioecism, high fecundity, wind-dispersed seeds and long-lived clonal growth (Hamrick and Godt 1989).

Although *Salix silicicola* lacks much of the ge-

TABLE 5. Summary of *F*-statistics for *Salix silicicola* and *S. alaxensis*.

Locus	*S. silicicola*			*S. alaxensis*		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
G3pdh-2	—	—	—	−0.017	−0.003	0.013
G6pdh-2	0.144	0.326	0.213	−0.355	−0.269	0.064
Gdh-1	−0.201	−0.029	0.144	−0.355	−0.155	0.148
Got-2	0.112	0.271	0.179	0.036	0.098	0.064
Idh-2	0.175	0.270	0.114	−0.281	−0.138	0.112
Lap-1	0.413	0.503	0.154	0.192	0.304	0.138
Pgd-3	—	—	—	0.437	0.480	0.075
Pgi-1	—	—	—	−0.057	−0.011	0.043
Pgi-2	—	—	—	−0.081	−0.031	0.046
Skd-1	0.048	0.134	0.091	−0.274	−0.139	0.106
Mean	0.101	0.241	0.156	−0.116	−0.007	0.098

netic variation found in *S. alaxensis*, its genetic diversity was high at polymorphic loci (H_T = 0.305). The G_{ST} values indicate that most of the genetic diversity occurs within populations for both species (Table 6), although G_{ST} values were higher in *S. silicicola*. Gene flow estimates based on G_{ST} values were moderate for both species, which is consistent with the outcrossed dioecious breeding system and wind-dispersed seeds characteristic of *Salix* species. The low amount of among population differentiation is reflected in the high intraspecific genetic identity values obtained.

At individual loci, there was an excess of heterozygotes over that expected under random mating in *Salix alaxensis*, but a deficiency of heterozygotes in *S. silicicola* (Table 5). The value of F_{IS} was −0.116 in *S. alaxensis*, indicating an 11.6% excess of heterozygotes, relative to Hardy-Weinberg expectations. Heterozygosity may confer a fitness advantage, or provide physiological plasticity in fluctuating environments (Huenneke 1991). Levels of heterozygosity are positively correlated with radial growth in *Populus tremuloides* Michx. (Mitton and Grant 1980), a member of the Salicaceae.

Although the levels of heterozygosity in *Salix alaxensis* were higher than expected, departures from Hardy-Weinberg equilibrium values on a locus by locus basis were not consistent. An absence of any strong singular pattern in *F*-statistics for *S. alaxensis* suggests several forces are probably acting, which differentially affect loci in the populations. There is a consistent deficiency of heterozygotes in *S. silicicola* at five of the six polymorphic loci. The F_{IS} values indicate that there was a 10.1% deficiency of heterozygotes relative to Hardy-Weinberg expectations. The difference in F_{IS} values between *S. alaxensis* and *S. silicicola* may suggest heterosis is not a force acting upon the endemic *S. silicicola* populations, or that directional selection is still occurring at other loci linked with the isozyme loci investigated here. Alternatively, the observed deficit of heterozygotes in *S. silicicola* may be caused by the Wahlund effect, or by biparental inbreeding.

***Origin of* Salix silicicola.** Several predictions have been made concerning the relationship between progenitor and derivative taxa (Gottlieb 1973; Pleasants and Wendel 1989); all of them were met in this study. First, the allelic diversity of *Salix silicicola* was a subset of the gene pool of *S. alaxensis*, and it included only one unique allele. Second, there was less genetic variation in *S. silicicola* than was found in *S. alaxensis*. Third, the intraspecific genetic iden-

TABLE 6. Genetic diversity statistics (Nei 1973), and estimates of gene flow for *Salix silicicola* and *S. alaxensis*. Genetic diversity parameters were calculated for polymorphic loci only. H_T = total gene diversity, H_S = gene diversity within populations, D_{ST} = gene diversity among populations, G_{ST} = the proportion of gene diversity apportioned among populations, and Nm_W = Wright's gene flow estimate.

Species	Number of loci	H_T	H_S	D_{ST}	G_{ST}	Nm_W
S. silicicola	6	0.305	0.256	0.048	0.159	1.32
S. alaxensis	10	0.384	0.347	0.037	0.097	2.33

tities among populations of *S. silicicola* resembled the interspecific genetic identities with populations of *S. alaxensis*. These three expectations concerning the genetic relationship between progenitor-derivative species pairs were met in other studies of endemics from the Athabasca sand dunes (Purdy et al. 1994; Purdy and Bayer 1995).

Mean intraspecific genetic identities for *Salix* species in the sect. *Longifoliae* ranged from 0.987 for *S. fluviatilis* to 0.875 for *S. melanopsis* Nutt. (Brunsfeld et al. 1991). Mean intraspecific *I* values for *S. silicicola* and *S. alaxensis* were 0.981 and 0.973 respectively, values that fall within the range of values reported for other *Salix* species. Mean interspecific *I* values for *Salix* species in the sect. *Longifoliae* ranged from 0.580 to 0.961 (Brunsfeld et al. 1991), although most values were less than 0.900. Interspecific genetic identities between populations of *S. silicicola* and *S. alaxensis* were high compared to those values (mean = 0.932; range = 0.902 to 0.963). The high genetic identities suggest a recent origin for the endemic *S. silicicola*. Other recent progenitor-derivative species pairs exhibit similar values (Gottlieb 1973; Crawford and Smith 1982a, 1982b; Crawford et al. 1985; Gottlieb et al. 1985; Loveless and Hamrick 1988; Purdy et al. 1994; Purdy and Bayer 1995).

The Athabasca sand dune endemic taxa are believed to have evolved after the sand dunes were formed following the Pleistocene, 8,000 years ago (Raup and Argus 1982). The ecology, population genetics, and systematics of Athabasca sand dune endemics that are sympatric with their progenitor species have been the subject of previous investigations (Argus and Steele 1979; Macdonald et al. 1987; Purdy et al. 1994; Purdy and Bayer 1995). However, populations of *Salix alaxensis*, the presumed progenitor taxon of *S. silicicola*, occur near to and above treeline in the Northwest Territories, 300 km north of the dunes (Porsild and Cody 1980). Putative allopatric progenitor populations of two other Athabasca sand dune endemic taxa also derived from arctic ancestors, *Armeria maritima* (Miller) Willd. ssp. *interior* (Raup) Porsild and *Silene acaulis* L. f. *athabascensis* Argus, are found in the same region (Argus and Steele 1979).

Salix silicicola differs from *S. alaxensis* by its very dense and persistent vestiture that covers the leaves and branches (Raup 1959). Tomentose plants of *S. alaxensis* have been collected only from Pelly Lake, in the district of Keewatin, N.W.T. (Argus and Steele 1979). This is the same location where populations of *S. planifolia* Pursh ssp. *planifolia*, the progenitor of another sand dune endemic willow, *S. planifolia* ssp. *tyrrellii* (Raup) Argus, occur. Argus and Steele (1979) speculated that relics of these arctic gene pools became isolated in the active sand dunes during the thermal maximum about 5,000 years B.P.

Because isozyme data from populations of *Salix alaxensis* from the Keewatin are not available, we do not know how much genetic variation occurs in the populations thought to have been the progenitor gene pool for *S. silicicola*. Populations of *S. alaxensis* from northern British Columbia examined in this study maintain much higher amounts of genetic variation than do *S. silicicola*. Theoretical and empirical evidence suggests that genetic bottlenecks have a large effect on allelic diversity and also affect heterozygosity (Wright 1931; Nei et al. 1975; Leberg 1992). Populations of *S. silicicola* from different dune fields are genetically similar in terms of their genetic identities, which suggests either that significant genetic drift has not occurred since the endemic became abundant or that gene flow is occurring among populations. We hypothesize that founding populations of the endemic were small, and that ensuing genetic bottlenecks and strong directional selection for sand dune-adapted traits caused the observed reduction of genetic diversity in the endemic. It would be of interest to investigate the population genetics of *S. alaxensis* and other progenitor taxa from the Keewatin district of the Northwest Territories to test whether this region is, in fact, the relictual progenitor gene pool for several endemic taxa from the Athabasca sand dunes.

ACKNOWLEDGMENTS. This paper presents part of a Ph.D. dissertation to be submitted by BGP to the Department of Biological Sciences at the University of Alberta. The authors thank Shawn Francis for assistance in the field and Ellen Macdonald and two anonymous reviewers for comments on an earlier version of the manuscript. This research was supported by funding from the Endangered Species Recovery Fund (World Wildlife Fund Canada), the Department of Indian and Northern Development (Northern Scientific Training Program), and the Canadian Circumpolar Institute (University of Alberta) to RJB and BGP, the Natural Sciences and Engineering Research Council (Canada) to RJB, and a Queen Elizabeth Doctoral Fellowship in Environmental Studies to BGP.

Literature Cited

Argus, G. W. 1973. *The genus Salix in the Alaska and Yukon.* Ottawa, Ontario: National Museum of Natural Sciences Publications in Botany no. 2.

——— and J. W. Steele. 1979. A reevaluation of the taxonomy of *Salix tyrrellii*, a sand dune endemic. Systematic Botany 4: 163–177.

Bayer, R. J. 1992. Allozyme variation, genecology, and phytogeography of *Antennaria arcuata* (Asteraceae), a rare species from the Great Basin and Red Desert with small disjunct populations. American Journal of Botany 79: 872–881.

Brunsfeld, S. J., D. E. Soltis, and P. S. Soltis. 1991. Patterns of genetic variation in *Salix* section *Longifoliae* (Salicaceae). American Journal of Botany 78: 855–869.

Cardy, B. J., C. W. Stuber, and M. M. Goodman. 1983. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Institute of Statistics Mimeograph series No. 1317. North Carolina State University, Raleigh, North Carolina.

Crawford, D. J. and E. B. Smith. 1982a. Allozyme divergence between *Coreopsis basalis* and *C. wrightii* (Compositae). Systematic Botany 7: 359–364.

——— and ———. 1982b. Allozyme variation in *Coreopsis nuecensoides* and *C. nuecensis* (Compositae), a progenitor-derivative species pair. Evolution 36: 379–386.

———, R. Ornduff, and M. C. Vasey. 1985. Allozyme variation within and between *Lasthenia minor* and its derivative species *L. maritima* (Asteraceae). American Journal of Botany 72: 1177–1184.

Edwards, A. L. and R. Wyatt. 1994. Population genetics of the rare *Asclepias texana* and its widespread sister species, *A. perennis*. Systematic Botany 19: 291–307.

Gottlieb, L. D. 1973. Genetic differentiation, sympatric speciation, and the origin of a diploid species of *Stephanomeria*. American Journal of Botany 60: 545–553.

———. 1974. Genetic confirmation of the origin of *Clarkia lingulata*. Evolution 28: 244–250.

———. 1981. Electrophoretic evidence and plant populations. Progress in Phytochemistry 7: 1–46.

———, S. I. Warwick, and V. S. Ford. 1985. Morphological and electrophoretic divergence between *Layia discoidea* and *L. glandulosa*. Systematic Botany 10: 484–495.

Hamrick, J. L. and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pp. 43–63 in *Plant population genetics, breeding and genetic resources*, eds. A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir. Sunderland, Massachusetts: Sinauer Associates, Inc.

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

Huenneke, L. F. 1991. Ecological implications of variation in plant populations. Pp. 31–44 in *Genetics and conservation of rare plants*, eds. D. A. Falk and K. E. Holsinger. New York: Oxford Univ. Press.

Karron, J. D. 1987. A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. Evolutionary Ecology 1: 47–58.

———, Y. B. Linhart, C. A. Chaulk, and C. A. Robertson. 1988. Genetic structure of populations of geographically restricted and widespread species of *Astragalus*. American Journal of Botany 75: 1114–1119.

Kruckeberg, A. R. and D. Rabinowitz. 1985. Biological aspects of endemism in higher plants. Annual Review of Ecology and Systematics 16: 447–479.

Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution 46: 477–494.

Linhart, Y. B. and A. C. Premoli. 1993. Genetic variation in *Aletes acaulis* and its relative, the narrow endemic *A. humilis* (Apiaceae). American Journal of Botany 80: 598–605.

Loveless, M. D. and J. L. Hamrick. 1988. Genetic organization and evolutionary history in two North American species of *Cirsium*. Evolution 42: 254–265.

Macdonald, S. E., C. C. Chinnappa, D. M. Reid, and B. G. Purdy. 1987. Population differentiation of the *Stellaria longipes* complex within Saskatchewan's Athabasca sand dunes. Canadian Journal of Botany 65: 1726–1732.

Mitton J. B., and M. C. Grant. 1980. Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range. American Journal of Botany 67: 202–209.

Nei, M. 1972. Genetic distance between populations. American Naturalist 106: 283–292.

———. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences U.S.A. 70: 3321–3323.

———, T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29: 1–10.

Pleasants, J. M. and J. F. Wendel. 1989. Genetic diversity in a clonal narrow endemic, *Erythronium propullens*, and its progenitor, *Erythronium albidum*. American Journal of Botany 76: 1136–1151.

Porsild, A. E. and W. J. Cody. 1980. *Vascular plants of continental Northwest Territories, Canada.* Ottawa, Ontario: National Museums of Canada.

Purdy, B. G., R. J. Bayer, and S. E. Macdonald. 1994. Genetic variation, breeding system evolution, and

conservation of the narrow sand dune endemic *Stellaria arenicola* and the widespread *S. longipes* (Caryophyllaceae). American Journal of Botany 81: 904–911.

——— and R. J. BAYER. 1995. Genetic diversity in the tetraploid sand dune endemic *Deschampsia mackenzieana* and its widespread diploid progenitor *D. cespitosa* (Poaceae). American Journal of Botany 82: 121–130.

RAUP, H. M. 1936. Phytogeographic studies in the Athabaska—Great Slave Lake region. I. Catalogue of the vascular plants. Journal of the Arnold Arboretum 17: 180–315.

———. 1959. The willows of boreal western America. Contributions from the Gray Herbarium of Harvard University 185: 3–95.

——— and G. W. ARGUS. 1982. *The Lake Athabasca sand dunes of northern Saskatchewan and Alberta, Canada. I. Land and vegetation.* Ottawa, Ontario: National Museum of Natural Sciences Publication no. 12.

SHERMAN-BROYLES, S. L., J. P. GIBSON, J. L. HAMRICK, M. A. BUCHER, and M. J. GIBSON. 1992. Comparisons of allozyme diversity among rare and widespread *Rhus* species. Systematic Botany 17: 551–559.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, and G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal 73: 9–27.

SOULÉ, M. E. and K. A. KOHM. 1989. *Research priorities for conservation biology.* Washington, D.C.: Island Press.

SWOFFORD, D. L. and R. B. SELANDER. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72: 281–283.

WEEDEN, N. F. and J. F. WENDEL. 1989. Genetics of plant isozymes. Pp. 46–72 in *Isozymes in plant biology*, eds. D. E. Soltis and P. S. Soltis. Portland, Oregon: Dioscorides Press.

WENDEL, J. F. and N. F. WEEDEN. 1989. Visualization and interpretation of plant isozymes. Pp. 5–45 in *Isozymes in plant biology*, eds. D. E. Soltis and P. S. Soltis. Portland, Oregon: Dioscorides Press.

WHITKUS, R. 1988. Modified version of GENESTAT: a program for computing genetic statistics from allelic frequency data. Plant Genetics Newsletter 4: 10.

WILKINSON, L. 1990. *SYSTAT: the system for statisics.* Evanston, Illinois: Systat, Inc.

WRIGHT, S. 1922. Coefficients of inbreeding and relationship. American Naturalist 56: 330–338.

———. 1931. Evolution in Mendelian populations. Genetics 28: 114–138.

———. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19: 395–420.

———. 1969. *Evolution and genetics of populations, vol. 2. The theory of gene frequencies.* Chicago, Illinois: Univ. of Chicago Press.