

Genetic and morphologic variation in *Phyllodoce empetriformis* and *Phyllodoce glanduliflora* (Ericaceae) in Mount Rainier National Park, Washington

Regina M. Rochefort and David L. Peterson

Abstract: Genetic and morphological diversity of *Phyllodoce empetriformis* (Sw.) D. Don and *Phyllodoce glanduliflora* (hook.) Cov. were surveyed in Mount Rainier National Park in the Cascade Mountains of Washington State. Paired populations at high and low elevations were sampled at three study areas between 1720- and 2451-m elevation. Allozyme analysis of four polymorphic loci indicates high levels of genetic diversity within populations (*P. empetriformis* = 94.2% and *P. glanduliflora* = 93.4% of total diversity) and significant differences in allele frequencies among populations and study areas. Individual populations are composed of multiple clones with high ratios of local to widespread genotypes. The proportion of distinguishable clones ranges from 32 to 83% within individual populations. Within individual populations, 18–67% of genotypes were restricted to one population. Patterns of morphologic variation, estimated through measurements of leaf width, leaf length, stem extension, and plant height paralleled those displayed by allozyme analysis. Significant differences were found in leaf width and stem length for *P. empetriformis* and among greenhouse populations for leaf width (*P. empetriformis*) and leaf length (*P. glanduliflora*). Species conservation strategies for *Phyllodoce* should concentrate on the maintenance of within-population levels of diversity, protection of adjacent populations, and protection of safe sites for recruitment of new populations.

Key words: conservation, Ericaceae, genetic diversity, morphologic variation, *Phyllodoce*.

Résumé : Les auteurs ont observé la diversité morphologique et génétique chez le *Phyllodoce empetriformis* (Sw.) D. Don et le *P. glanduliflora* (hook.) Cov., au parc national de Mount Rainier dans les Montagnes Cascades, de l'état de Washington. Ils ont prélevé des échantillons parallèles à haute et basse altitudes dans trois stations d'études situées entre 1720 et 2451 m d'altitude. L'analyse allozymique de quatre loci polymorphiques indique de hauts degrés de diversité génétique à l'intérieur des populations (*P. empetriformis* = 94,2 % et *P. glanduliflora* = 93,4 %), ainsi que des différences significatives dans les fréquences des allèles, selon les populations et les stations d'étude. Les populations individuelles sont composées de multiples clones avec de forts rapports entre génotypes locaux et étendus. La proportion de clones discernables va de 32 à 83 %, pour une population individuelle. De 18 à 67 % des génotypes dans les populations individuelles sont restreints à une population. Les patrons de variation morphologique, évalués à partir de la largeur des feuilles, de la longueur des feuilles, de la longueur des tiges et de la hauteur des plantes sont parallèles à ceux obtenus par analyse allozymique. On observe des différences significatives dans la largeur des feuilles et la longueur des tiges chez le *P. empetriformis* et parmi des populations venant en serre quant à largeur des feuilles (*P. empetriformis*) et la longueur des feuilles (*P. glanduliflora*). Les stratégies de conservation de l'espèce pour le *Phyllodoce* devraient se concentrer sur le maintien de la diversité au niveau de la population, de la protection des populations adjacentes, et de la protection de sites sûrs pour le recrutement de nouvelles populations.

Mots clés : conservation, Ericaceae, diversité génétique, variation morphologique, *Phyllodoce*.

[Traduit par la Rédaction]

Introduction

Heathers are common shrubs in high-elevation plant communities of the Pacific Northwest. *Phyllodoce empetriformis* (Sw.) D. Don and *Phyllodoce glanduliflora* (hook.) Cov. are members of the eight-species genus *Phyllodoce* within the

Ericaceae. All members of this genus are categorized as subshrubs and are distributed in arctic–alpine habitats (Good 1926). *Phyllodoce empetriformis* and *P. glanduliflora* are endemic to western North America (Fig. 1). Studies of these species have focused on plant community description, habitat distribution (Kuramoto and Bliss 1970; Douglas 1972;

Received May 18, 2000. Published on the NRC Research Press website on February 12, 2001.

R.M. Rochefort.^{1,2} Mount Rainier National Park, Tahoma Woods, Star Route, Ashford, WA 98304, U.S.A.

D.L. Peterson. Field Station for Protected Area Research, University of Washington, Box 352100, Seattle, WA 98195, U.S.A.

¹Corresponding author (e-mail: regina_rochefort@nps.gov).

²Present address: North Cascades National Park Service Complex, 2105 State Route 20, Sedro-Woolley, WA 98284, U.S.A.

Henderson 1974; Edwards 1980; Franklin and Dyrness 1987; Ingersoll 1991; Ingersoll and Wilson 1993), and environmental influences on reproduction and physiology (Olmsted 1975; Edwards 1980; Ingersoll 1991). *Phyllodoce empetriformis* is distributed from approximately 1500- to 2000-m elevation, while *P. glanduliflora* is commonly found above 1950 m (Watson 1977). Little information is available on the population dynamics or reproductive biology of the species. However, as recreational demands on natural areas escalate, the potential for damage to these populations also increases. Development of protection guidelines may be necessary for the long-term survival of these species and effective guidelines require a better understanding of the population dynamics.

Heather populations establish by seed on moist, well-drained sites. Viable seed production appears to be high, but seedling recruitment is infrequent even under favorable environmental conditions (Edwards 1980; Ingersoll 1991). Floral morphology, pollination surveys (Macior 1994), and the occurrence of a hybrid, *Phyllodoce intermedia*, (Watson 1977) indicate that the species are outcrossing and bee-pollinated, but no studies have documented whether they are self-compatible. Ring counts of annual growth have documented the life-span of individual stems to be up to 70 years (Olmsted 1975; Edwards 1980), but no data on annual rates of recruitment or mortality are available. Additionally, there are no published studies on genetic diversity of these species and few on species within the Ericaceae (Floyd 1972; Vander Kloet 1976; Bruederle et al. 1991; Strand and Wyatt 1991; Denton 1997; Lim 1999).

Over the past century, recreational use of alpine and sub-alpine areas in North America has grown, becoming an increasingly destructive influence on heather populations (Cole 1996). Many high-elevation populations are located in wilderness areas where there are few maintained trails and campsites. Off-trail use of these areas often results in a proliferation of informal campsites and social trails in vegetated areas. The prostrate, woody stems of heather plants are easily crushed or broken by trampling. Even if the stems are not immediately killed, crushed leaves have depressed photosynthetic activity, and plants may die later in the growing season or after several seasons of repeated trampling (Hylgaard and Liddle 1981; Cole 1993). Human use may cause fragmentation within populations through mortality of individuals or among heather populations through extinction of entire populations. Fragmentation can alter demographic processes and genetic diversity of plant populations and subsequently influence their long-term survival (Wilcox and Murphy 1985; Derda and Wyatt 1990; Foré et al. 1992; Ledig 1992).

Long-term survival of heather metapopulations involves a complex balance of persistence of some populations, periodic establishment of new populations, and extinction of others through succession, displacement by trees, or catastrophic events such as landslides, avalanches, or volcanic eruptions (Rochefort 1995). Although short-term population viability may be primarily influenced by demographic processes, genetic variability may be the critical factor in determining long-term population or species survival (Menges 1990). Studies in a wide range of plant taxa have demonstrated significant variation in genetic, morphologic, and

physiologic characteristics over very short distances in response to environmental differences (e.g., Antonovics 1968; McGraw and Antonovics 1983; Linhart and Grant 1996). Genetically based adaptive variation provides species with the resources to persist through changing environmental conditions (Frankel 1983; Namkoong 1983; Schonewald-Cox 1983; McGraw 1995). Long-term species survival may rely on both among- and within-population diversity. Diversity among populations, such as ecotypes in different habitats, enables species to respond to changing environmental conditions through time (Mooney and Billings 1961; Callaghan 1974; McGraw 1995; Menges 1990). Diversity within populations enables specific populations to persist based on adaptive differences among individuals (McGraw 1995).

As recreational use of natural areas increases, the need for scientifically based resource protection guidelines becomes increasingly important. In this study, we quantified genetic and morphological diversity of *P. empetriformis* and *P. glanduliflora* to better understand their sensitivity to environmental and human induced change. Genetic diversity was examined because it is important in long-term survival and because both species have long-lived individuals with infrequent reproduction, which makes demographic study difficult. This information can then be used to infer reproductive and demographic patterns, develop strategies to protect undamaged populations, and to restore impacted populations. Specific goals were to (i) quantify the range of diversity in natural populations, (ii) determine which environmental factors are associated with patterns of diversity, and (iii) interpret these findings for National Park Service resource managers for use in species and habitat protection guidelines.

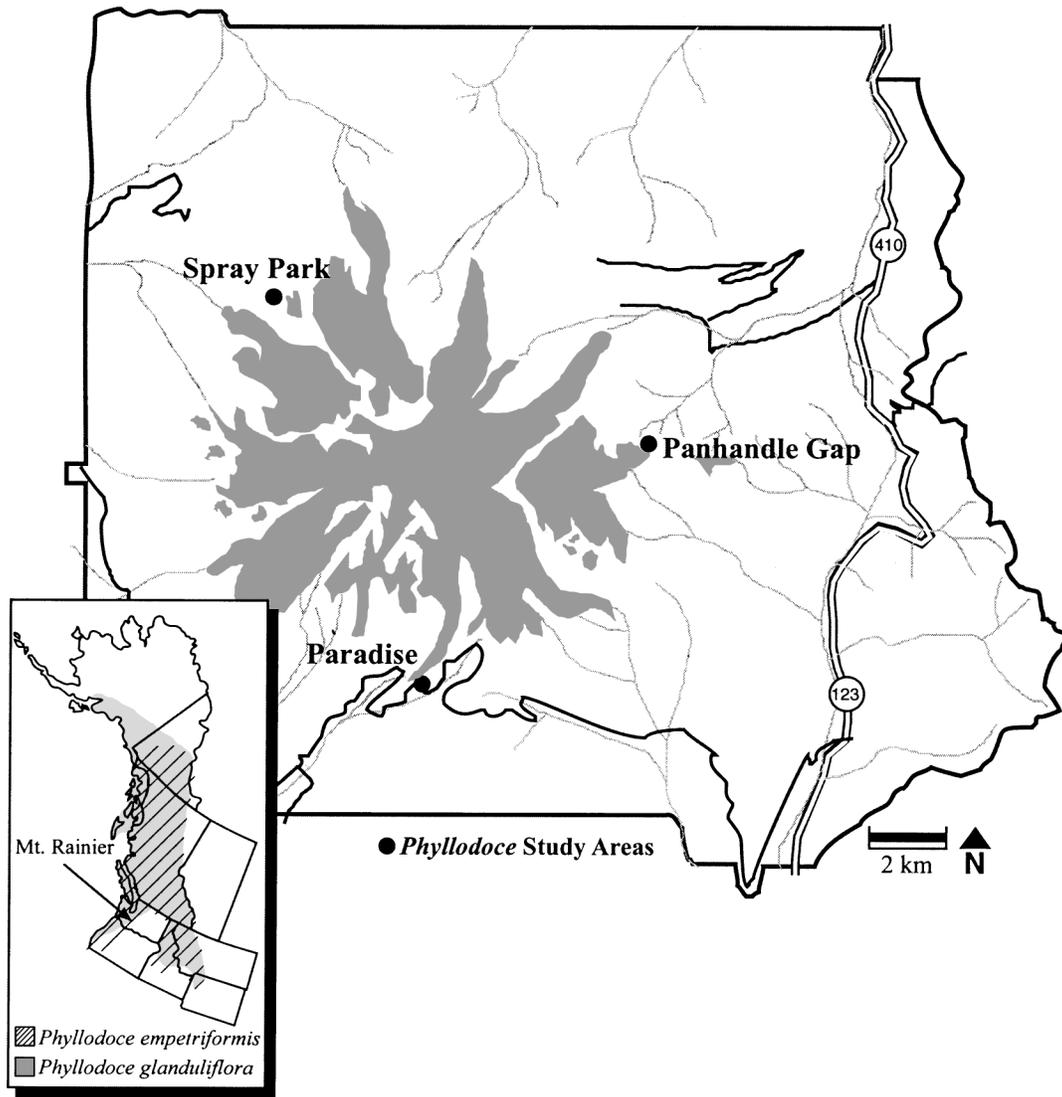
Materials and methods

Study areas and plant collection

Mount Rainier National Park is located on the western slope of the Cascade Range, 100 km southeast of the Seattle-Tacoma metropolitan area (Washington state, U.S.A.). It encompasses 95 389 ha and extends from low-elevation, late-successional coniferous forest (530-m elevation) through subalpine and alpine communities to the summit of Mount Rainier (4400 m). Climate is temperate maritime with cool, wet winters (-2.9 to 3.4°C) and mild, dry summers (7.9 to 16°C). Most of the annual precipitation falls as snow between October and May. Precipitation is generally higher on the west side of the park and increases with elevation up to about 3000 m. Average annual precipitation ranges from 205 cm at 842-m elevation to 269 cm at 1676-m elevation.

Study areas were established in three locations within Mount Rainier National Park: Paradise, Spray Park, and Panhandle Gap (Fig. 1, Table 1) in the summers of 1992 and 1993. The sampling scheme was designed to cover the range of temperature and precipitation gradients within the park because environmental heterogeneity often influences genetic patterns (Linhart and Grant 1996). Study areas were established on west (wet) and east (dry) sides of the park and sample populations selected across a range of elevations. Two to four populations were sampled within each area. Paired populations at high and low elevations were subjectively identified and located within each study area to sample the range of genetic diversity. Each study area included populations in both subalpine (forest-meadow mosaic) and alpine (treeless) areas. Sample populations were located at the highest and lowest elevational extent of *P. empetriformis* and *P. glanduliflora*. At Spray Park and

Fig. 1. Locations of study areas in Mount Rainier National Park (shaded areas indicate glaciers on Mount Rainier), with range of *Phyllodoce* species (as in Good 1926) indicated in the inset.



Paradise, four populations of each species were sampled, two high-elevation and two low-elevation populations. At Panhandle Gap, four populations of *P. empetriformis* (two high-elevation and two low-elevation) and two populations of *P. glanduliflora* (two high-elevation populations, no low-elevation populations) were sampled. Sampled populations were separated by more than 100 m. One study plot was established within each population; each plot was 400 m² (20 × 20 m) and subdivided into quarters. Fifty plants (12–13 per quarter) were randomly selected within the plot for genetic analysis. Stems with 1- and 2-year-old leaves were collected for electrophoretic analysis.

Electrophoresis

Phyllodoce leaves and stems were placed in plastic bags and kept moist and cold in a refrigerator (4°C) until they were prepared for electrophoresis. Leaf tissues were ground with a mortar and pestle under liquid nitrogen (Mittton et al. 1979), and the frozen leaf powder was mixed with a Tris-HCl grinding buffer PVP solution (Soltis et al. 1983). The homogenate was immediately transferred into microtiter trays and stored at -70°C).

Homogenates were thawed, applied to paper wicks, and inserted into 12.5% starch gels. Plant material was initially tested for activity

on 26 enzyme systems (Rocheftort 1995). Only five loci for each species exhibited consistent, scorable resolution. Five putative loci were resolved on three systems for *P. glanduliflora*. Phosphoglucose isomerase (PGI) (two loci) and uridine diphosphoglucose pyrophosphorylase (UGP) were resolved on a continuous morpholine citrate system, pH 8.1 (system E in Conkle et al. 1982). Shikimate dehydrogenase (SKD) was run on morpholine citrate, pH 6.1 (system D in Conkle et al. 1982). Phosphoglucomutase (PGM) was resolved on a discontinuous histidine citrate system, pH 7.0 (Werth 1985). Five putative loci were resolved on two systems for *P. empetriformis*. Phosphoglucose isomerase (PGI) (two loci) was resolved on a continuous morpholine citrate system, pH 8.1 (system E in Conkle et al. 1982). Uridine diphosphoglucose pyrophosphorylase (UGP-1, UGP-2) and phosphoglucomutase (PGM) were scored on a discontinuous histidine-HCl, pH 6.5 (system 11 in Soltis et al. 1983). Only loci polymorphic at the 95% level were utilized for genetic analysis; PGI-1 was monomorphic for both species and therefore was not analyzed.

Morphological variation

Morphological variation was surveyed on field populations and greenhouse plants grown from cuttings of field plants. Four

Table 1. Site designations, descriptions, and locations for 10 populations of *Phyllodoce glanduliflora* (PG) and 12 populations of *Phyllodoce empetriformis* (PE) in Mount Rainier National Park.

Population number	Location		Elevation (m)	Aspect	UTM co-ordinates ^a	
	Site	Elevation			North	East
<i>Phyllodoce empetriformis</i>						
PE1	Paradise	Low	1743	SW	5182.6	596.4
PE2	Paradise	Low	1720	SW	5182.6	596.9
PE3	Paradise	High	2036	SW	5184.4	596.8
PE4	Paradise	High	1950	S	5183.4	597.6
PE5	Panhandle Gap	High	2018	S	5189.7	603.8
PE6	Panhandle Gap	High	2006	S	5189.6	603.4
PE7	Panhandle Gap	Low	1573	N	5186.5	603.8
PE8	Panhandle Gap	Low	1560	N	5186.6	603.8
PE9	Spray Park	Low	1731	S	5196.8	588.6
PE10	Spray Park	Low	1786	SW	5197.2	588.8
PE11	Spray Park	High	2054	NW	5196.4	590.6
PE12	Spray Park	High	2115	NW	5195.8	590.8
<i>Phyllodoce glanduliflora</i>						
PG1	Paradise	Low	2036	S	5184.0	596.8
PG2	Paradise	Low	1950	S	5183.4	597.6
PG3	Paradise	High	2427	S	5185.4	597.6
PG4	Paradise	High	2451	S	5185.5	597.5
PG5	Panhandle Gap	High	1970	NW	5190.3	603.4
PG6	Panhandle Gap	High	2073	NW	5189.8	603.8
PG7	Spray Park	Low	1920	N	5196.9	589.9
PG8	Spray Park	Low	1970	N	5196.8	590.1
PG9	Spray Park	High	2116	NW	5197.8	590.8
PG10	Spray Park	High	2158	N	5195.6	590.8

^aUTM, universal transverse mercator; all sites are in zone 10.

morphologic characters were measured on 25 plants in each of the field populations: leaf width, leaf length, plant height, and mean annual stem growth (i.e., growth as measured between bud scars). Plant height was measured at the tallest portion of the plant. Annual stem growth, leaf width, and leaf length were measured with a micrometer (vernier caliper); values for each character are the average of three measurements for each plant. Leaf width was measured at the widest portion of each leaf. Greenhouse plants were propagated only from cuttings of plants in the Paradise study populations. Cuttings were collected in the autumn of 1992; rooted cuttings were transplanted into 10-cm diameter pots in the spring of 1993 and arranged in a completely randomized design. Plants were moved from the greenhouse to a shadehouse in June 1994, prior to bud break. Leaf width and length were measured in 1995 on leaves formed in 1994.

Data analysis

Genetic data were analyzed using Biosys-1, version 1.7 (Swofford and Selander 1989), and Genestat (Lewis and Whitkus 1989). Genetic variability was characterized by calculating allele frequencies, observed heterozygosity (H_o , direct count), expected heterozygosity (H_e , Nei's 1978 unbiased estimate), and Hamrick and Godt's (1990) H_T (Nei and Chesser 1983). Calculating Wright's (1943, 1951) F -statistics, Nei's genetic identity (1978), and Nei's genetic distance (1978) examined diversity within and among populations. Hierarchical F statistics were used to estimate the contribution of the elevation class (F_{ET}), study area (F_{AR}), populations within study areas (F_{PA}), and populations within elevation class (F_{PE}) to species diversity within Mount Rainier National Park. Phenograms were produced to visualize similarities between populations by conducting a cluster analysis using UPGMA (unweighted

pair-group method, arithmetic average) and Nei's genetic identity. The relationship between genetic distance and geographic distance between populations was examined using the Mantel test (Manly 1991). Chi-square contingency analysis was used to test for significant differences in allele frequencies among populations and study areas (Snedecor and Irwin 1933; Workman and Niswander 1970).

In addition, because *P. glanduliflora* and *P. empetriformis* propagate vegetatively, genetic diversity was examined by calculating the number of genotypes, proportion of clones distinguishable, and the number of local and widespread genotypes per population (Ellstrand and Roose 1987). The number of genotypes was calculated by using only those individuals for which all four loci had been scored. The proportion distinguishable was calculated by dividing the number of genotypes by the sample size. Local and widespread genotypes were calculated within study areas and across all populations. Local genotypes were defined as types found in only one population within the study area. Widespread genotypes were defined as types found in 75% of the populations within the study area or when looking at all populations, 75% of sampled populations.

Morphologic characters of greenhouse plants (leaf width and length) were analyzed with a one-way analysis of variance ($p = 0.05$). If heteroscedasticity was encountered, Kruskal-Wallis procedures were followed. When the null hypothesis was rejected, the Tukey HSD test was applied ($p = 0.05$). Plant height, leaf width, annual stem growth (length), and leaf length of field plants were examined using a two-way analysis of variance. A completely randomized design with three geographic areas as random blocks, two elevation classes as fixed factors, and two populations as replicates within each block and elevation class was used. Differences between blocks and factors were analyzed at the $p = 0.05$ significance level. Correlation analysis ($p = 0.05$) was used to examine the rela-

tionship of morphologic characteristics with elevation and aspect. Relative percentages of among- and within-population variances were calculated for each character (Sokal and Rohlf 1981).

Results

Loci and alleles scored

Resolution of loci for both species was difficult. Although four grinding buffers were tested, many loci expressed activity inconsistently and others displayed a double-banded phenotype. Four loci were utilized to analyze genetic structure in *P. glanduliflora*: PGI-2, PGM, SKD, and UGP. Four alleles were observed at PGI-2 and two at the remaining three loci: SKD, UGP, and PGM. Both PGM and SKD exhibited a double-banded phenotype as homozygotes and were 4-banded as heterozygotes. Four loci were also used to describe the genetic structure of *P. empetriformis*: PGI-2, UGP-1, UGP-2, and PGM. PGM, UGP-1, and UGP-2 exhibited double-banded phenotypes. Three alleles were observed at the UGP-1 and UGP-2 loci and four at PGI-2 and PGM.

Large-scale genetic variability

Total genetic diversity estimates (H_T) are high for both species: 0.434, SE = 0.093, for *P. empetriformis* and 0.4009, SE = 0.0752, in *P. glanduliflora*. In both species, most alleles are shared by all populations (Appendices A and B). In *P. empetriformis*, exceptions are the occurrence of allele 3 of UGP-1 in only populations PE-1 and PE-8, allele 3 of UGP-2 in only PE-1 and PE-8, and allele 4 of PGM in population PE-12. However, in *P. glanduliflora*, allele 4 at the PGI-2 locus is unique to populations from Spray Park (PG7, PG8, PG9, and PG10). In addition, population PG6 (Panhandle Gap) is fixed for allele 1 at the UGP locus. There were no trends in allele frequencies that could be correlated with high or low elevations in either species (see Appendices A and B).

Observed heterozygosity ranges from 0.139 to 0.599 in *P. empetriformis* and is less than the expected heterozygosity in three of the four loci surveyed. Observed heterozygosity ranges from 0.195 to 0.333 in *P. glanduliflora* and is less than the expected heterozygosity at all four loci (Table 2). Analyses of individual populations of each species also indicate a deficiency of heterozygotes. This pattern could indicate inbreeding or may be an underestimate of heterozygosity owing to the Wahlund effect from repeated sampling of individuals that have propagated vegetatively (Weber and Stettler 1981).

Genetic variation among population

Mean F_{ST} values indicate that genetic subdivision is present in both species at relatively low levels. Variation among populations is 6.6% for *P. glanduliflora* and 5.8% for *P. empetriformis*. Partitioning of variation by elevation class, study area, populations within elevation class, or populations within study areas reveals that most of the variation was among populations rather than among study areas or elevation classes. In *P. glanduliflora*, higher F values were calculated among populations within areas ($F_{PA} = 0.035$) and elevation classes ($F_{PE} = 0.033$) than among elevation classes ($F_{ET} = 0.024$) or among study areas ($F_{AT} = 0.027$). The contrasts were greater for *P. empetriformis*: variation among populations within areas ($F_{PA} = 0.047$) and elevation classes

($F_{PE} = 0.040$) was much greater than among elevation classes ($F_{ET} = 0.007$) or among study areas ($F_{AT} = 0.001$). Although most of the genetic diversity within these species is within populations (93.4 and 94.2%, respectively), allele frequencies among populations of each species were significantly different at all loci (Table 2).

Genetic identity values for *P. empetriformis* range from 0.871 to 1.00 and values for *P. glanduliflora* range from 0.900 to 1.00. Genetic distance was positively correlated with geographic distance for both, but there was considerable scatter in the points and the relationship between genetic and geographic distance was especially weak for *P. empetriformis* (*P. glanduliflora*, $r = 0.583$, $p < 0.001$; *P. empetriformis*, $r = 0.262$, $p = 0.023$). Phenograms produced from UPGMA cluster analysis provide a graphic representation of patterns among populations (Figs. 2 and 3). Two broad groups of populations are displayed for *P. glanduliflora*: Paradise – Panhandle Gap and Spray Park. No clear patterns are evident in the phenogram for *P. empetriformis*.

Variation within sites

Allele frequencies of *P. empetriformis* populations differ significantly at three loci in the Paradise area, all loci in Spray Park and at two loci in the Panhandle Gap study area. Chi-square contingency analyses of heterogeneity indicate that allele frequencies are significantly different among populations of *P. glanduliflora* at three of four loci in the Paradise study area, three loci in the Spray Park study area, and one locus in the Panhandle Gap study area (Table 3).

Clonal diversity

Analysis of clonal diversity reveals that, in both species, all sampled populations are composed of multiple clones (Table 4). The proportion of distinguishable clones ranges from 0.32 to 0.83 in *P. empetriformis* and from 0.32 to 0.73 in *P. glanduliflora*. In both species, local genotypes are an important component of population diversity. Analysis of *P. empetriformis* documented 116 genotypes among a total of 419 individuals; 52 (45%) of these are local genotypes (i.e., occurring in one population) and 4 (3%) are widespread. Within study populations, local genotypes range from 39 to 67%, and widespread genotypes range from 6 to 32% of all genotypes present. A total of 460 individuals of *P. glanduliflora* were scored for all four loci within the three study areas combined. Of the 98 genotypes observed, 47 (48%) are local and only 4 (4%) are widespread types. Local genotypes ranged from 11 to 67% within populations and widespread ranged from 28 to 44%.

Morphological variation

Comparison of morphologic characteristics among field populations reveals significant variation in stem length between elevation classes ($F = 26.688$, $df = 1$, $p = 0.035$) and between study areas for leaf width ($F = 26.247$, $df = 2$, $p = 0.037$) for *P. empetriformis*, but no significant differences were found for *P. glanduliflora* (Figs. 4 and 5). However, leaf width of *P. glanduliflora* was positively correlated with elevation ($p = 0.019$, $r = 0.720$). Plant height ($p < 0.001$, $r = -0.848$) and stem length ($p = 0.009$, $r = -0.713$) were negatively correlated with elevation for *P. empetriformis*. Addi-

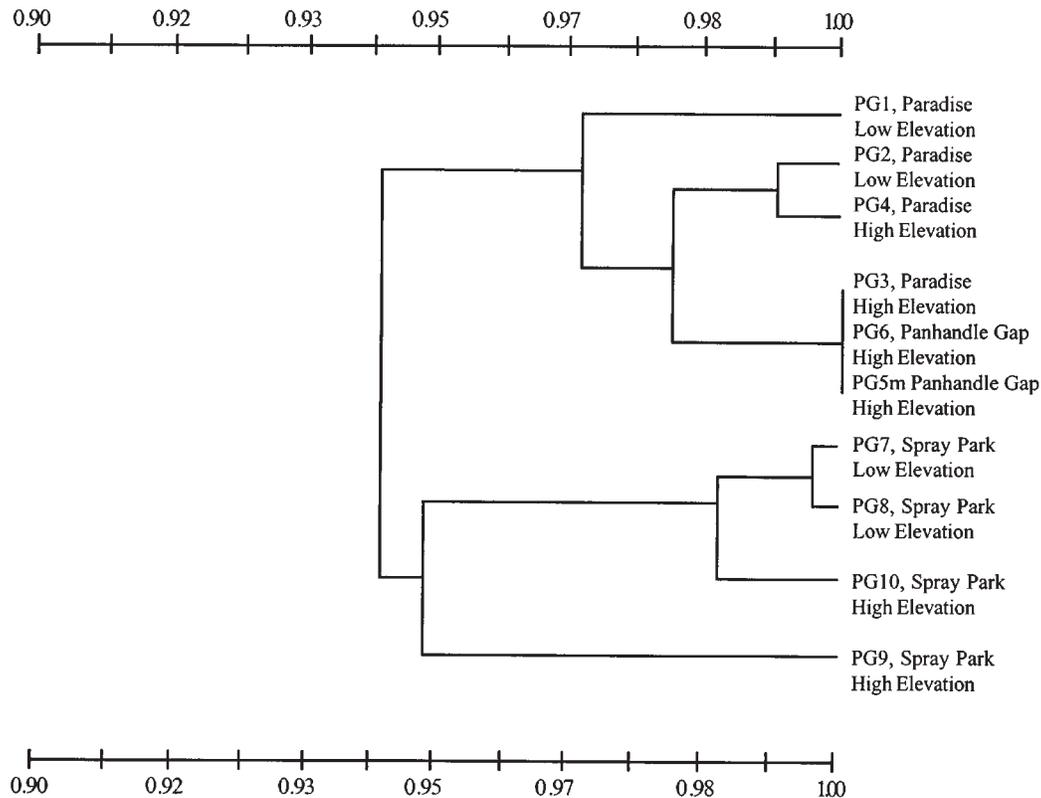
Table 2. Summary of genetic diversity and fixation indices at four polymorphic loci in 12 populations of *Phyllodoce empetriformis* and 10 populations of *Phyllodoce glanduliflora* in Mount Rainier National Park.

Locus	Chi-square ^a (df)	H _o	H _e	F _{IS}	F _{IT}	F _{ST} ^b
<i>Phyllodoce empetriformis</i>						
PGI-2	101.611* (33)	0.599	0.649	0.033	0.074	0.043*
UGP-1	106.996* (22)	0.139	0.228	0.358	0.400	0.065*
UGP-2	75.817* (22)	0.347	0.343	-0.050	-0.001	0.048*
PGM	122.689* (33)	0.305	0.517	0.325	0.380	0.082*
Mean		0.347	0.434	0.144	0.194	0.058
<i>Phyllodoce glanduliflora</i>						
PGI-2	127.720* (27)	0.412	0.501	0.108	0.177	0.077*
UGP	72.717* (9)	0.138	0.179	0.171	0.234	0.076*
PGM	51.968* (9)	0.191	0.427	0.524	0.549	0.053*
SKD	59.749* (9)	0.357	0.497	0.231	0.280	0.064*
Mean		0.274	0.401	0.266	0.314	0.066

^aAn asterisk (*) indicates significant differences in allele frequencies between all populations at the $p < 0.0001$ level.

^bAn asterisk (*) indicates that F_{ST} values are significantly different from zero at the $p < 0.0001$ level.

Fig. 2. Cluster analysis of 10 populations of *Phyllodoce glanduliflora* in Mount Rainier National Park. Analysis and phenogram are based on Nei's (1978) genetic identity.



tionally, plant height ($p = 0.039$, $r = -0.6$), stem length ($p = 0.031$, $r = -0.622$), and leaf width ($p = 0.018$, $r = -0.666$) were negatively correlated with aspect. Aspect was analyzed using a 1 to 8 numerical scale ranging from north = 1 clockwise to northwest = 8. As with genetic variation, most among-population variation is substantially less than within-population variation. Estimates of among-population variation for *P. empetriformis* are annual stem growth, 24.7%; leaf width, 17.9%, and leaf length, 40.3%. However, among population variation was greater than within population variation for plant height (62.8%). Estimates of among popula-

tion variation for *P. glanduliflora* are annual stem growth, 25.5%; plant height, 9.4%; leaf width, 15%, and leaf length, 7.3%. Plant height and leaf lengths are more variable in *P. empetriformis* than *P. glanduliflora*, but variation in annual stem growth and leaf width are similar.

Patterns of variation for greenhouse plants are different for each species. *Phyllodoce empetriformis* plants differ significantly among populations for leaf width ($p = 0.019$) but not for leaf length ($p = 0.29$) (Fig. 6). Relative amounts of among-population variation are 10.8% for leaf width and 1.3% for leaf length. *Phyllodoce glanduliflora* populations

Fig. 3. Cluster analysis of 12 populations of *Phyllodoce empetriformis* in Mount Rainier National Park. Analysis and phenogram are based on Nei's (1978) genetic identity.

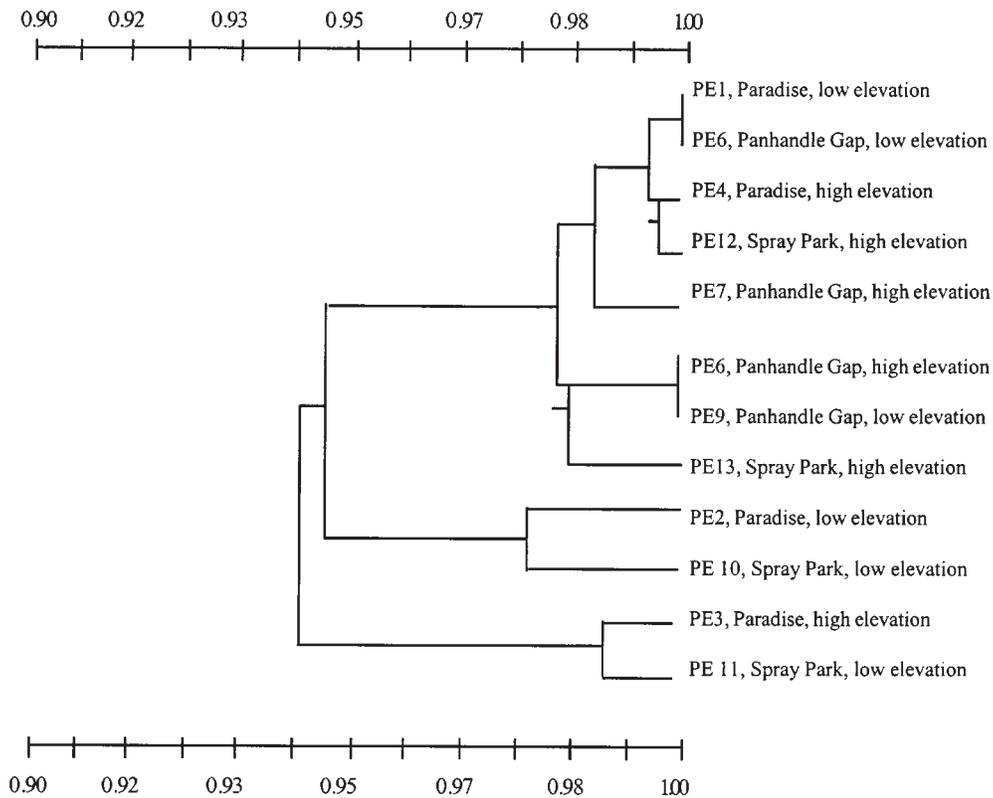


Table 3. Chi-square contingency analysis of heterogeneity among populations of *Phyllodoce empetriformis* and *Phyllodoce glanduliflora* within each study area.

Locus	Paradise			Panhandle Gap			Spray Park		
	Chi-square	df	P	Chi-square	df	P	Chi-square	df	P
<i>Phyllodoce empetriformis</i>									
PGI-2	38.80	9	<0.001	12.41	9	0.191	37.64	9	<0.001
UPG-1	38.45	6	<0.001	19.17	6	0.004	26.81	3	<0.001
UPG-2	12.50	6	0.052	22.60	6	0.001	23.95	3	<0.001
PGM	19.79	6	0.003	9.18	6	0.164	27.99	9	0.001
Total	109.54	27	<0.001	63.36	27	<0.001	116.39	24	<0.001
<i>Phyllodoce glanduliflora</i>									
PGI-2	18.8	6	0.004	0.14	2	0.30	22.05	9	0.009
UPG	27.98	3	<0.001	10.54	1	0.001	21.61	3	<0.001
PGM	4.90	3	0.179	0.03	1	0.865	7.65	3	0.057
SKD	10.14	3	0.017	0.03	1	0.562	29.16	3	<0.001
Total	64.82	15	<0.001	11.05	5	0.050	80.47	18	<0.001

have significantly different leaf lengths ($p < 0.001$) but no significant differences in leaf width ($p = 0.56$). Among-population variation is 22.0% for leaf length and 4.8% for leaf width.

Discussion

Conservation of genetic diversity in native plant communities is a critical mandate for the management of parks and protected areas. This is particularly true for subalpine and alpine plant species that are sensitive to impacts from human

activities and potential climatic change. Knowledge of patterns of genetic variation within and among populations may be useful in understanding present-day ecology of the species, past patterns of population development (Loveless and Hamrick 1988), and in developing both protection and restoration strategies for heather populations. Protection of biodiversity at all levels—landscape, species, and genetic diversity—requires knowledge of “natural” levels of diversity and the forces that influence these levels.

The results of this study indicate that *P. empetriformis* and *P. glanduliflora* have high levels of total genetic diversity as

Table 4. Clonal diversity within populations and study areas for *Phyllodoce empetriformis* and *Phyllodoce glanduliflora*.

Population		Number of		No. of local	No. of widespread	Proportion
Site	Number	genotypes	Sample size	genotypes (%)	genotypes (%)	distinguishable
<i>P. empetriformis</i>						
Paradise	PE1	28	42	16 (57)	6 (21)	0.67
	PE2	32	43	18 (56)	7 (22)	0.74
	PE3	24	32	12 (50)	7 (32)	0.75
	PE4	27	51	11 (41)	7 (26)	0.53
Panhandle Gap	PE5	18	29	11 (61)	4 (22)	0.64
	PE6	25	34	11 (44)	6 (24)	0.74
	PE7	23	43	9 (39)	5 (22)	0.53
	PE8	24	43	10 (42)	4 (16)	0.56
Spray Park	PE9	11	28	6 (54)	3 (27)	0.39
	PE10	15	18	10 (67)	1 (6)	0.83
	PE11	15	25	6 (40)	3 (20)	0.60
	PE12	16	31	9 (56)	4 (25)	0.52
All populations		116	419	52 (45)	4 (3)	0.32
<i>P. glanduliflora</i>						
Paradise	PG1	21	35	3 (14)	10 (48)	0.60
	PG2	28	50	6 (21)	11 (39)	0.56
	PG3	22	45	7 (32)	9 (41)	0.49
	PG4	32	50	6 (18)	11 (34)	0.64
Panhandle Gap	PG5	18	49	12 (67)	6 (33)	0.37
	PG6	16	50	10 (62)	6 (38)	0.32
	PG7	28	48	11 (39)	10 (36)	0.58
	PG8	18	46	2 (11)	8 (44)	0.39
	PG9	36	49	16 (44)	10 (28)	0.73
	PG10	21	38	8 (38)	6 (28)	0.55
All populations		98	460	47 (48)	4 (4)	0.34

compared with other woody perennials (Hamrick and Godt 1990). Hamrick and Godt (1990) reported mean H_T values of 0.298 (SE = 0.012) for long-lived woody perennials and $H_T = 0.272$ (SE = 0.014) for plants with boreal–temperate distributions. We found total genetic diversity estimates of $H_T = 0.430$ (SE = 0.0938) for *P. empetriformis* and $H_T = 0.4009$ (SE = 0.0752) for *P. glanduliflora*. Although the values we calculated might be influenced by small sample size (i.e., 4 loci vs. 17 uses by Hamrick and Godt (1990)), they are similar to those found in other long-lived species in the Ericaceae (Ng and Corlett 2000a, 2000b).

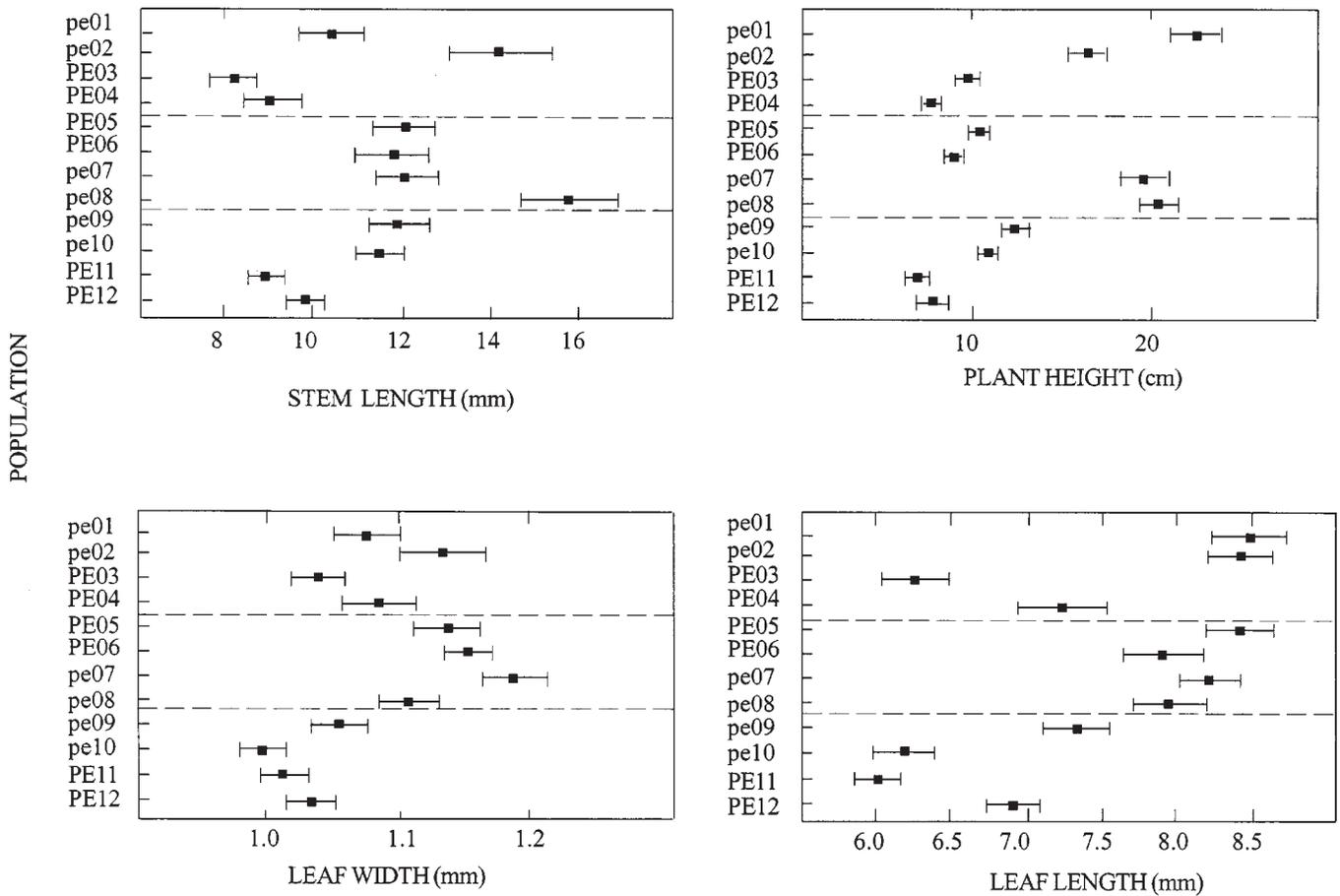
Analysis of genetic structure demonstrates that 93.4% of *P. glanduliflora* and 94.2% of *P. empetriformis* total variation is found within populations. Most populations of each species share common alleles, but allele frequencies among populations and study areas are significantly different. High within population diversity is characteristic of out-crossing species (heather species are bee-pollinated) and may also be the result of recruitment over long time periods and diverse climatic conditions (Mulcahy 1975; Brubaker 1986). Further partitioning of variation reinforced that variation among populations (i.e., among all populations (5.8%), within elevation classes (4%), or within study areas (4.7%)) contributed more to total variation than did elevation classes (0.7%) or study areas (0.1%) for *P. empetriformis*. However, elevation (2.4%) and study area (2.7%) did explain more of the variation in *P. glanduliflora* but still to a lesser degree than among population variation (6.6%; among populations within

elevation classes (3.3%), among population within study areas (3.5%)). Although these results suggest that among population differences may be the result of isolation by distance or adaptive selection (in particular for *P. glanduliflora*), we cannot identify the primary influences without further studies, such as reciprocal transplants.

Clonal and genotype population structure provides a more detailed understanding of within-population diversity. This analysis shows that individual populations are multiclonal, and a large proportion of the individuals have local genotypes. The proportion of distinguishable genotypes (genotypes divided by sample size) ranges from 0.32 to 0.75 for *P. empetriformis* and 0.32 to 0.73 for *P. glanduliflora*. Local genotypes were a significant component of each species composition (average values were 48% for *P. glanduliflora* and 45% for *P. empetriformis*). Our calculations may underestimate these proportions because we only used four loci. We would expect the number of genotypes and perhaps the number of local genotypes to increase as the number of loci surveyed increased. For example, using just two loci of each species, the percent local genotypes calculated for *P. glanduliflora* would be 33 versus 48% with four loci and 9 versus 45% for *P. empetriformis*. These values are comparable to those for populations founded by sexual propagules, but for which additional periods of sexual recruitment into the established populations were rare (Ellstrand and Roose 1987).

Patterns of allele frequencies and the sharing of uncommon alleles (e.g., PGI-2, allele 4) among populations of

Fig. 4. Means and standard errors for four morphologic characters for 12 field populations of *Phyllodoce empetriformis*. Labels for low elevation populations are in lowercase letters and dotted lines separate study areas.



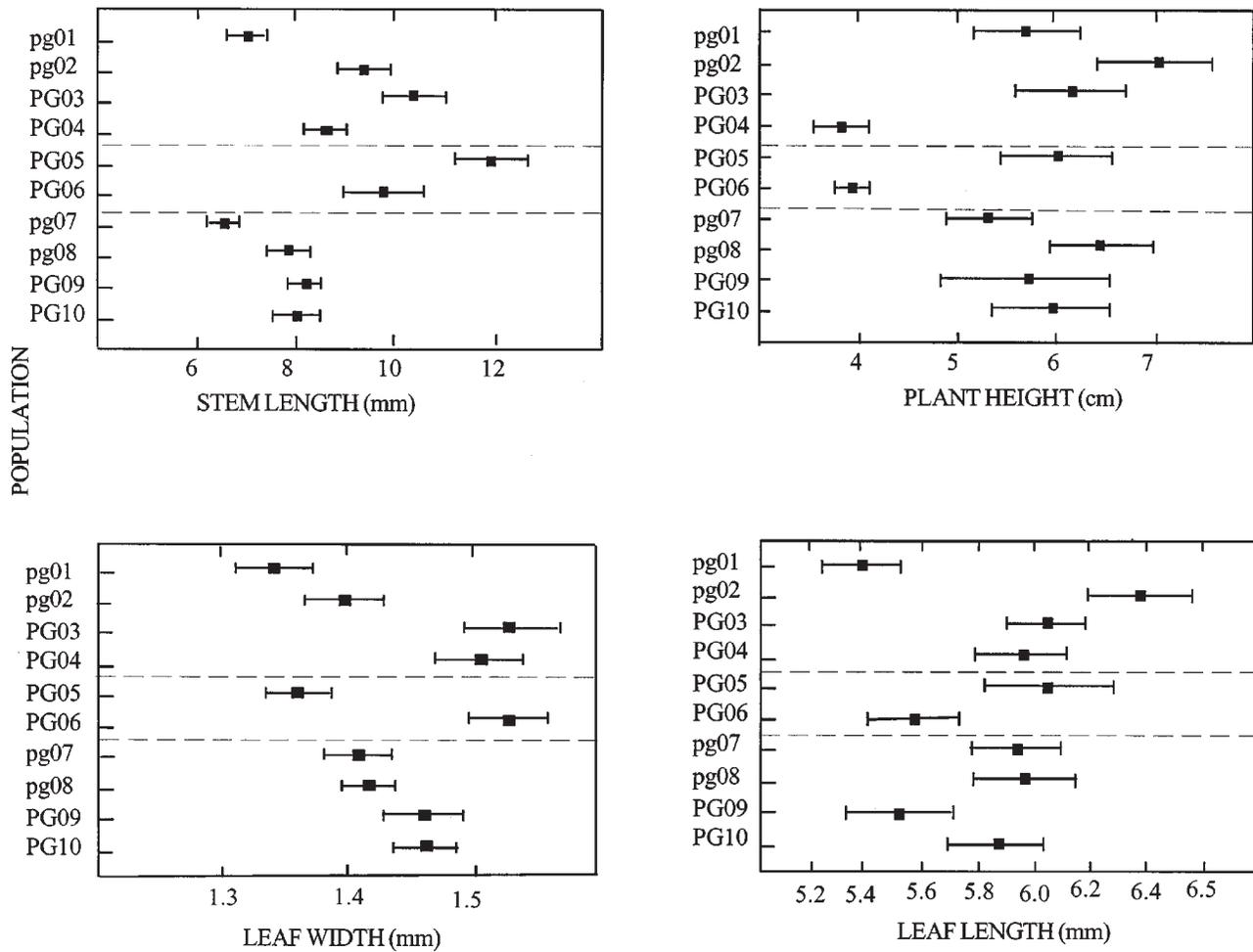
P. glanduliflora suggest that gene flow occurs among populations within the study areas. The positive correlation of genetic distance with geometric distance may be a result of reduced gene flow with distance. In addition, cluster analysis (UPGMA) indicates that populations within study areas are more similar to one another than to populations in other study areas. This result is reasonable because each of the study areas is essentially a ridge surrounded by glaciers on three sides and forests below (at lower elevation sites). These barriers limit long-distance dispersal of pollen by bees (Macior 1994).

Patterns of morphologic variation parallel those displayed by genetic analysis. For most characters observed, variation within populations was greater than among populations. One exception to this is plant height of *P. empetriformis*, which displayed greater among than within population variation and significant negative correlation with elevation. This result may reflect a phenotypic response to habitat rather than reflecting gene flow or selection patterns. The height of heather plants appears to vary with their position on the landscape—taller plants are found in protected sites such as next to rocks or trees or in small depressions where they are sheltered from the wind. Shorter plants were found in exposed areas or higher elevations (often ridges) where wind often blows away snow cover. The alpine habitats of *P. glanduliflora* have few trees and the populations we sam-

pled were often found in relatively even terrain or on ridges that are often snow free in the winter. The results of greenhouse plant measurements indicate that observed variation in leaf length (*P. glanduliflora*) and leaf width (*P. empetriformis*) may include a genetic component, although a larger sample size and testing of additional environmental conditions would be necessary to confidently ascertain this fact.

Data on the genetic diversity of *Phyllodoce* species can be used to develop both conservation strategies to enhance survival of pristine populations and restoration strategies to repair degraded populations. Protection of natural evolutionary processes and minimization of human interference with evolving genetic diversity are mandates of the National Park Service and a necessary component of a realistic protection plan for these species (National Park Service 1988). For *P. glanduliflora* and *P. empetriformis* specifically, protection must focus on population processes and habitat conditions that have resulted in extant patterns such as high within-population levels of diversity and high proportions of local genotypes with populations. This means preservation of individuals within populations, protection of adjacent populations, and preservation of safe sites for establishment of new populations. Protection of individuals within populations and adjacent populations will facilitate outcrossing and continued development of high levels of diversity within new populations. Loss of individual plants in heather populations,

Fig. 5. Means and standard errors for four morphologic characters for 10 field populations of *Phyllodoce glanduliflora*. Labels for low elevation populations are in lowercase letters and dotted lines separate study areas.



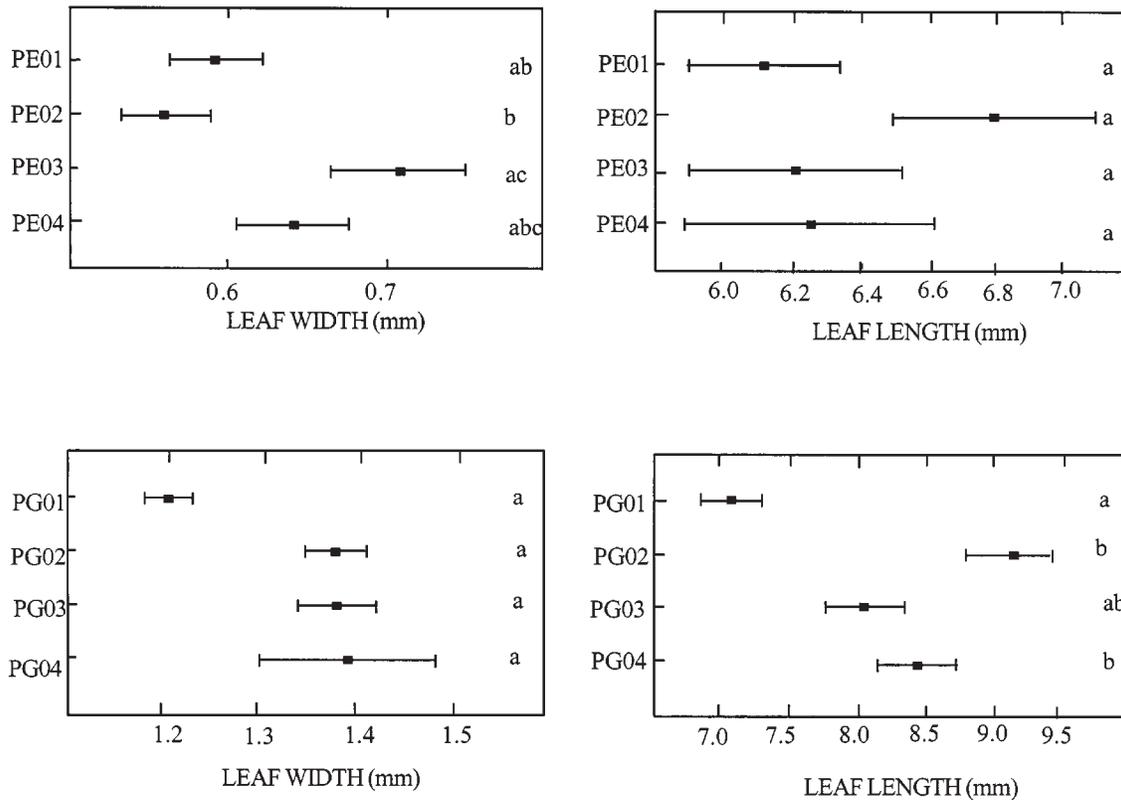
without establishment of new seedlings, may significantly alter within-population diversity. If trampling and mortality of individuals are minimized, within-population levels of diversity can be protected. Protection of entire plant populations will conserve genetic diversity within the populations and maintain within-population demographic processes. Although both species have high within population diversity levels, protection of adjacent populations is important not just for demographic processes but because of the high levels of local genotypes. Diversity across the landscape (or within a study area, management zone, or ecosystem) is influenced by unique and local genotypes. However, the range of local genotypes per population was quite wide: 39–61% in *P. empetriformis* and 11–67% in *P. glanduliflora*. Protection of one or two populations might conserve common alleles but would not assure protection of local genotypes.

Protection of safe sites across the landscape is necessary because new populations generally establish on sites away from mature populations (Edwards 1980). Long-term protection of landscapes and habitats is recommended because new populations appear to establish infrequently, as is evidenced by high diversity levels and field observations of limited numbers of young plants. Protection by National Park managers must be based on time frames of decades rather than

annual budget cycles. Each of our study areas was essentially an ecosystem composed of an alpine ridge subtended by a subalpine meadow. Gene flow appears to occur throughout this ecosystem and less frequently between adjacent ecosystems or ridges that are separated by glaciers and forests. Efforts should be made to protect habitat conditions both within ecosystems and to protect adjacent ecosystems. In the case of *P. glanduliflora*, loss of one ecosystem or ridge (the Spray park) would have resulted in the loss of a unique allele and lowered the general populations' genetic diversity.

Knowledge of genetic diversity can also aid in restoring degraded heather populations. Restoration of severely damaged areas can require the establishment of entire heather populations. Extant populations in this study contain 11–36 genotypes, depending on their size. Restoration efforts should consider planting populations with offspring propagated from many individuals or genotypes. This is extremely important because sexual recruitment into mature heather populations is infrequent owing to the difference in habitats optimal for seedling recruitment and established populations (Edwards 1980). Successfully restored populations (and genetic diversity) are likely to persist for long time periods and will provide the genetic legacy for future generations of heather.

Fig. 6. Means and standard errors for two morphologic characters for greenhouse populations of *Phyllodoce empetriformis* (PE) and *Phyllodoce glanduliflora* (PG). Populations with the same letter do not differ significantly when compared using Tukey's HSD test ($p < 0.05$).



Acknowledgements

The authors thank Y. Linhart, R. Stettler, B. Freet, K. Jope, A.J. Shaw, and two anonymous reviewers for constructive criticism of the manuscript and L. Brubaker, C. Halpern, and P. Kareiva for reviewing an earlier version of the work. We thank D. Delaney for instruction on isozyme analysis and G. Ettl for assistance on interpreting and screening isozyme results. D. Billheimer, D. McKenzie, and K. Jenkins assisted with the statistical analysis of the data. We thank B. Rocheftort for developing the graphics in this article. The National Park Service, the Mazamas, and the School of Forest Resources, University of Washington, provided funding for this research.

References

Antonovics, J. 1968. Evolution in closely adjacent plant populations. V. Evolution of self-fertility. *Heredity*, **23**: 219–238.
 Brubaker, L.B. 1986. Responses of tree populations to climate change. *Vegetatio*, **67**: 119–130.
 Bruederle, L.P., Vorsa, N., and Ballington, J.R. 1991. Population genetic structure in diploid blueberry *Vaccinium* section *Cyanococcus* (Ericaceae). *Am. J. Bot.* **78**: 230–237.
 Callaghan, T.V. 1974. Intraspecific variation in *Phleum alpinum* L. with specific reference to polar populations. *Arct. Alp. Res.* **13**: 83–94.
 Cole, D.N. 1993. Trampling effects on mountain vegetation in Washington, Colorado, New Hampshire, and North Carolina.

Ogden, Utah. USDA Forest Service Intermountain Research Station. Research paper No. INT-64.
 Cole, D.N. 1996. Wilderness recreation use trends 1965 through 1994. Ogden, Utah. USDA Forest Service Intermountain Research Station. Research paper No. INT-RP-488.
 Conkle, M.T., Hodgskiss, P.D., Nunnally, L.B., and Hunter, S.C. 1982. Starch gel electrophoresis of conifers: laboratory manual. Pacific Southwest Forest and Range Experiment Station, Forest Service, US Department of Agriculture, Berkeley, Calif. General technical report No. PSW-64.
 Denton, A.L. 1997. The evolution of RNA polymerase II introns: ancient polymorphism and paralogy in the genus *Rhododendron* (Ericaceae). Ph.D. dissertation, Department of Botany, University of Washington, Seattle, Wash.
 Derda, G.S., and Wyatt, R. 1990. Genetic variation in the common hair-cap moss, *Polytrichum commune*. *Syst. Bot.* **15**: 592–605.
 Douglas, G.W. 1972. Subalpine plant communities of the Western North Cascades, Washington. *Arc. Alp. Res.* **4**: 147–166.
 Edwards, O.M. 1980. The alpine vegetation of Mount Rainier National Park: structure, development, and constraints. Ph.D. dissertation, College of Forest Resources, University of Washington, Seattle, Wash.
 Ellstrand, N.C., and Roose, M.L. 1987. Patterns of genotypic diversity in clonal plant species. *Am. J. Bot.* **74**: 123–131.
 Floyd, J.W. 2000. Phylogenetic and biogeographic patterns in *Gaylussacia* (Ericaceae) based on morphological, nuclear DNA, and chloroplast DNA variation. Ph.D. dissertation, Department of Botany, North Carolina State University, Raleigh, N.C.
 Foré, S.A., Hickey, R.J., Vankat, J.L., Guttman, S.I., and Schaffer, R.L. 1992. Genetic structure after fragmentation: a landscape ecology perspective on *Acer saccharum*. *Can. J. Bot.* **70**: 1659–1668.

- Frankel, O.H. 1983. The place of management in conservation. *In* Genetics and conservation: a reference for managing wild animal and plant populations. Edited by C.M. Schonewald-Cox, S.M. Chambers, B. MacBryde, and W.L. Thomas. The Benjamin/Cummings Publishing Co, Menlo Park, Calif. pp. 1–14.
- Franklin, J.F., and Dyrness, C.T. 1987. Natural vegetation of Oregon and Washington. Oregon State University Press, Corvallis, Ore.
- Good, R.D.O. 1926. The genera *Phyllodoce* and *Cassiope*. *J. Bot.* **64**: 1–10.
- Hamrick, J.L., and M.J. W. Godt. 1990. Allozyme diversity in plant species. *In* A plant population genetics, breeding, and genetic resources. Edited by H.D. Brown, M.T. Clegg, A.L. Kahler, and B.S. Weir. Sinauer Associates, Inc., Sunderland, Mass. pp. 43–63.
- Henderson, J.A. 1974. Composition, distribution and succession of subalpine meadows in Mount Rainier National Park. Ph.D. dissertation, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Ore.
- Hylgaard, T., and Liddle, M.J. 1981. The effect of human trampling on a sand dune ecosystem dominated by *Empetrum nigrum*. *J. Appl. Ecol.* **18**: 559–569.
- Ingersoll, C.A. 1991. Plant reproductive ecology and community structure along a subalpine snowmelt gradient. Ph.D. dissertation, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Ore.
- Ingersoll, C.A., and Wilson, M.V. 1993. Buried propagule bank of a high subalpine site: microsite variation and comparisons with aboveground vegetation. *Can. J. Bot.* **71**: 712–717.
- Kuramoto, R.T., and Bliss, L.C. 1970. Ecology of subalpine meadows in the Olympic Mountains, Washington. *Ecol. Monogr.* **40**: 317–345.
- Ledig, F.T. 1992. Human impacts on genetic diversity in forest ecosystems. *Oikos*, **63**: 87–108.
- Lewis, P., and Whitkus, R. 1989. Genestat for microcomputers. ASPT Newsletter, **2**: 15–16.
- Lim, C.C. 1999. Physiological and genetic mechanism(s) of cold acclimation in *Rhododendron*. Ph.D. dissertation, Department of Plant and Soil Sciences, West Virginia University, Morgantown University, Morgantown, W.Va.
- Linhart, Y.B., and Grant, M.C. 1996. Evolutionary significance of local genetic differentiation in plants. *Ann. Rev. Ecol. Syst.* **27**: 237–277.
- Macior, L.W. 1994. Pollen-foraging dynamics of subalpine bumblebees (*Bombus* Latr.). *Plant Species Biol.* **9**: 99–106.
- Manly, B.F. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, London.
- McGraw, J.B. 1995. Patterns and causes of genetic diversity in arctic plants. *In* Arctic and alpine biodiversity: patterns, causes, and ecosystem consequences. Edited by F.S. Chapin, III, and C. Korner. Springer-Verlag, New York. pp. 33–43.
- McGraw, J.B., and Antonovics, J. 1983. Experimental ecology of *Dryas octopetala* ecotypes I. Ecotypic differentiation and life-cycle stages of selection. *J. Ecol.* **71**: 879–897.
- Menges, E.S. 1990. Population viability analysis for an endangered plant. *Conserv. Biol.* **4**: 52–62.
- Mitton, J.B., Linhart, Y.B., Sturgeon, Y.B., and Hamrick, J.L. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *J. Hered.* **70**: 86–89.
- Mooney, H.A., and Billings, W.D. 1961. Comparative physiological ecology of arctic and alpine populations of *Oxyria digyna*. *Ecol. Monogr.* **31**: 1–29.
- Mulcahy, D.L. 1975. Differential mortality among cohorts in a population of *Acer saccharum* (Aceraceae) seedlings. *Am. J. Bot.* **62**: 422–426.
- Namkoong, G. 1983. Preserving natural diversity. *In* Genetics and conservation: a reference for managing wild animal and plant populations. Edited by C.M. Schonewald-Cox, S.M. Chambers, B. MacBryde, and W.L. Thomas. The Benjamin/Cummings Publishing Co, Menlo Park, Calif. pp. 317–334.
- National Park Service. 1988. Management policies. US Department of the Interior, Washington, D.C.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583–590.
- Nei, M., and Chesser, R.K. 1983. Estimation of fixation indices. *Evol.* **40**: 643–645.
- Olmsted, I.C. 1975. Environmental factors influencing the distribution of *Phyllodoce* in the mountains of western North America. Ph.D. dissertation, School of Forestry and Environmental Studies, Duke University, Durham, N.C.
- Ng, S.C., and Corlett, R.T. 2000a. Genetic variation and structure in six *Rhododendron* species (Ericaceae) with contrasting local distribution patterns in Hong Kong, China. *Mol. Ecol.* **9**: 959–969.
- Ng, S.C., and Corlett, R.T. 2000b. Comparative reproductive biology of the six species of *Rhododendron* (Ericaceae) in Hong Kong, South China. *Can. J. Bot.* **78**: 221–229.
- Rocheftort, R.M. 1995. Ecology and conservation of heather in Mount Rainier National Park. Ph.D. dissertation, College of Forest Resources, University of Washington, Seattle, Wash.
- Schonewald-Cox, C.M. 1983. Conclusions: guidelines to management: a beginning to attempt. *In* Genetics and conservation: a reference for managing wild animal and plant populations. Edited by C.M. Schonewald-Cox, S.M. Chambers, B. MacBryde, and W.L. Thomas. The Benjamin/Cummings Publishing Co, Menlo Park, Calif. pp. 414–445.
- Snedecor, G., and Irwin, M.R. 1933. On the χ^2 test for homogeneity. *Iowa State Coll. J. Sci.* **8**: 75–81.
- Sokal, R.F., and Rohlf, F.J. 1981. Biometry, the principles and practice of statistics in biological research. W. H. Freeman and Company, New York.
- Soltis, D.E., Haufler, C.H., Darrow, D.H., and Gastony, G.J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.* **72**: 9–27.
- Strand, A.E., and Wyatt, R. 1991. Geographical variation and biosystematics of sand myrtle, *Leiophyllum buxifolium* (Ericaceae). *Syst. Bot.* **16**: 529–545.
- Swofford, D.L., and Selander, R.B. 1989. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72**: 281–283.
- Vander Kloet, S.P. 1976. A novel approach to sampling *Vaccinium* populations. *Can. J. Bot.* **54**: 669–671.
- Watson, T.J.J. 1977. An analysis of populations formed by hybridization between *Phyllodoce empetrififormis* and *P. glanduliflora* (Ericaceae). *Rhodora*, **79**: 1–16.
- Weber, J.C., and Stettler, R.F. 1981. Isozyme variation among ten populations of *Populus trichocarpa* Torr. et Gray in the Pacific Northwest. *Silv. Genet.* **30**: 32–37.
- Werth, C.R. 1985. Implementing an isozyme laboratory at a field station. *Va. J. Sci.* **36**: 53–76.
- Wilcox, B.A., and Murphy, D.D. 1985. Conservation strategy: the effects of fragmentation on extinction. *Am. Nat.* **125**: 879–887.
- Workman, P.L., and J.D. Niswander. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *J. Hum. Genet.* **22**: 24–49.
- Wright, S. 1943. Isolation by distance. *Genetics*, **28**: 114–138.
- Wright, S. 1951. The genetical structure of populations. *Annals of Eugenics*, **15**: 323–354.

Appendix A. Isozyme allele frequencies in 12 populations of *Phyllodoce empetriformis*.

Isozyme	Allele	Population											
		PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	PE9	PE10	PE11	PE12
PGI-2	<i>n</i> =	56	47	41	52	37	45	50	48	37	35	42	49
	1	0.143	0.117	0.390	0.269	0.135	0.144	0.140	0.219	0.081	0.414	0.250	0.153
	2	0.536	0.596	0.280	0.471	0.622	0.600	0.570	0.521	0.662	0.314	0.393	0.612
	3	0.170	0.181	0.280	0.183	0.203	0.200	0.140	0.156	0.135	0.157	0.190	0.153
	4	0.152	0.106	0.049	0.077	0.041	0.056	0.150	0.104	0.122	0.114	0.167	0.082
UGP-1	<i>n</i> =	55	47	49	52	32	42	47	47	31	48	42	48
	1	0.064	0.319	0.102	0.077	0.172	0.214	0.064	0.138	0.065	0.031	0.048	0.229
	2	0.927	0.681	0.898	0.923	0.828	0.786	0.936	0.819	0.935	0.969	0.952	0.771
	3	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000
UGP-2	<i>n</i> =	54	46	48	51	37	43	50	47	31	49	42	50
	1	0.750	0.848	0.719	0.873	0.662	0.884	0.770	0.723	0.952	0.827	0.798	0.640
	2	0.241	0.152	0.281	0.118	0.338	0.116	0.230	0.234	0.048	0.173	0.202	0.360
	3	0.009	0.000	0.000	0.010	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000
PGM	<i>n</i> =	48	44	52	52	36	43	50	48	34	35	25	32
	1	0.552	0.364	0.413	0.615	0.722	0.581	0.620	0.698	0.265	0.271	0.480	0.500
	2	0.417	0.614	0.538	0.385	0.264	0.419	0.380	0.302	0.735	0.700	0.440	0.484
	3	0.031	0.023	0.048	0.000	0.014	0.000	0.000	0.000	0.000	0.029	0.080	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016

Appendix B. Isozyme allele frequencies in 10 populations of *Phyllodoce glanduliflora*.

Isozyme	Allele	Population									
		PG1 (45) ^a	PG2 (51)	PG3 (51)	PG4 (51)	PG5 (49)	PG6 (49)	PG7 (50) ^b	PG8 (49)	PG9 (50)	PG10 (50) ^c
PGI-2	1	0.856	0.716	0.765	0.618	0.765	0.786	0.410	0.510	0.470	0.610
	2	0.056	0.098	0.078	0.069	0.051	0.051	0.080	0.031	0.060	0.120
	3	0.089	0.186	0.157	0.314	0.184	0.163	0.500	0.429	0.420	0.260
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.031	0.050	0.010
PGM	1	0.422	0.471	0.333	0.461	0.224	0.235	0.327	0.214	0.163	0.230
	2	0.578	0.529	0.667	0.539	0.776	0.765	0.673	0.786	0.837	0.770
SKD	1	0.514	0.735	0.574	0.588	0.561	0.602	0.367	0.388	0.680	0.342
	2	0.486	0.265	0.426	0.412	0.439	0.398	0.633	0.612	0.320	0.658
UGP	1	0.711	0.863	0.960	0.912	0.898	1.000	0.900	0.980	0.810	0.960
	2	0.289	0.137	0.040	0.088	0.102	0.000	0.100	0.020	0.190	0.040

^aSample size for SKD = 35.

^bSample size for PGM and SKD = 49.

^cSample size for SKD = 25.