Genetic variation in populations of the endemic *Achillea millefolium* ssp. *megacephala* from the Athabasca sand dunes and the widespread ssp. *lanulosa* in western North America

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Abstract: As part of an analysis of genetic diversity in endemic taxa of the Athabasca sand dunes in northern Saskatchewan, Canada, genetic variation was examined by starch gel electrophoresis in six populations of the endemic *Achillea millefolium* ssp. *megacephala*, and 13 populations of the closely related widespread taxon, *A. millefolium* ssp. *lanulosa*. Endemic populations had more alleles per locus, a higher percentage of polymorphic loci, and greater genetic diversity than did populations of the widespread taxon. At polymorphic loci, total gene diversity was comparable in both taxa, although within-population gene diversity was higher in the endemic taxon. Population differentiation (G_{ST}) was considerably lower in ssp. *megacephala* than in ssp. *lanulosa*, although G_{ST} values were reduced when the parameter was calculated separately for geographic subdivisions of the widespread taxon. Our results differ from previous studies in which the endemic is typically depauperate of genetic variation relative to related widespread species. We suggest that obligate sexual reproduction and the absence of long-term asexual reproduction may be one of a number of factors that help populations of ssp. *megacephala* maintain higher levels of genetic variation on the Athabasca sand dunes.

Key words: genetic variation, endemic, rare species, Athabasca sand dunes, *Achillea millefolium*.

Résumé : Dans le cadre d'une analyse de la variation génétique chez les taxons endémiques des dunes de sable de l'Athabasca (au nord de la Saskatchewan, Canada), six populations de l'entité endémique *Achillea millefolium* ssp. *megacephala* et 13 populations du taxon proche à large répartition géographique *A. millefolium* ssp. *lanulosa* ont été étudiées par électrophorèse sur gel d'amidon. Les populations endémiques comportent plus d'allèles par locus, un plus fort pourcentage de loci polymorphes, et une plus grande diversité génétique que ne le font les populations du taxon à large répartition. Aux loci polymorphes, la diversité génétique totale est comparable dans les deux taxons, bien qu'à l'intérieur des populations la diversité génétique est plus importante chez le taxon endémique. La différenciation des populations (G_{ST}) est considérablement plus faible chez la ssp. *lanulosa*, bien que les valeurs G_{ST} soient réduites lorsque le paramètre est calculé séparément pour des subdivisions géographiques du taxon à large répartition. Les résultats des auteurs diffèrent de ceux d'études précédentes dans lesquelles le taxon endémique présente une plus faible diversité génétique comparativement à l'espèce largement répandue. Ils suggèrent que la reproduction sexuelle obligatoire et l'absence d'un processus de reproduction asexuée à long terme pourraient être un des facteurs qui permettent aux populations de la ssp. *megacephala* de maintenir une variation génétique plus importante sur les dunes de sable de l'Athabasca.

Mots clés : variation génétique, endémique, espèce rare, dunes de sable de l'Athabasca, *Achillea millefolium*.
[Traduit par la rédaction]

Introduction

Widespread species of plants must adapt to a broad range of environmental conditions to maintain their large geographic distributions. Consequently, to exist in such an extensive range of habitats, many widespread species have high genetic diversity (Hamrick and Godt 1989) and have evolved into a series of ecological races (Turesson 1922), or exhibit considerable phenotypic plasticity (Schlichting 1986). The experiments of Clausen et al. (1940) were the first to comprehensively demonstrate the importance of both genetic differentiation and phenotypic plasticity for plant species to adapt to and survive in a wide range of environmental conditions. Endemics, which are often restricted to a much narrower range of environmental conditions, may not have the same requirement for considerable genetic diversity, population differentiation, or phenotypic plasticity for survival.

Studies comparing closely related widespread and endemic species showed that endemic species generally have less allozyme diversity (Karron 1987; Loveless and Hamrick 1988; Pleasants and Wendel 1989; Hamrick and Godt 1989; Soltis and Soltis 1991; Purdy 1995). Whether low levels of allozyme variation in endemic species are a cause or a consequence of a restricted geographic distribution is still a subject of debate (Huenneke 1991). While various historical, ecological, and genetic hypotheses have been presented as to the causes of

Received April 3, 1995.

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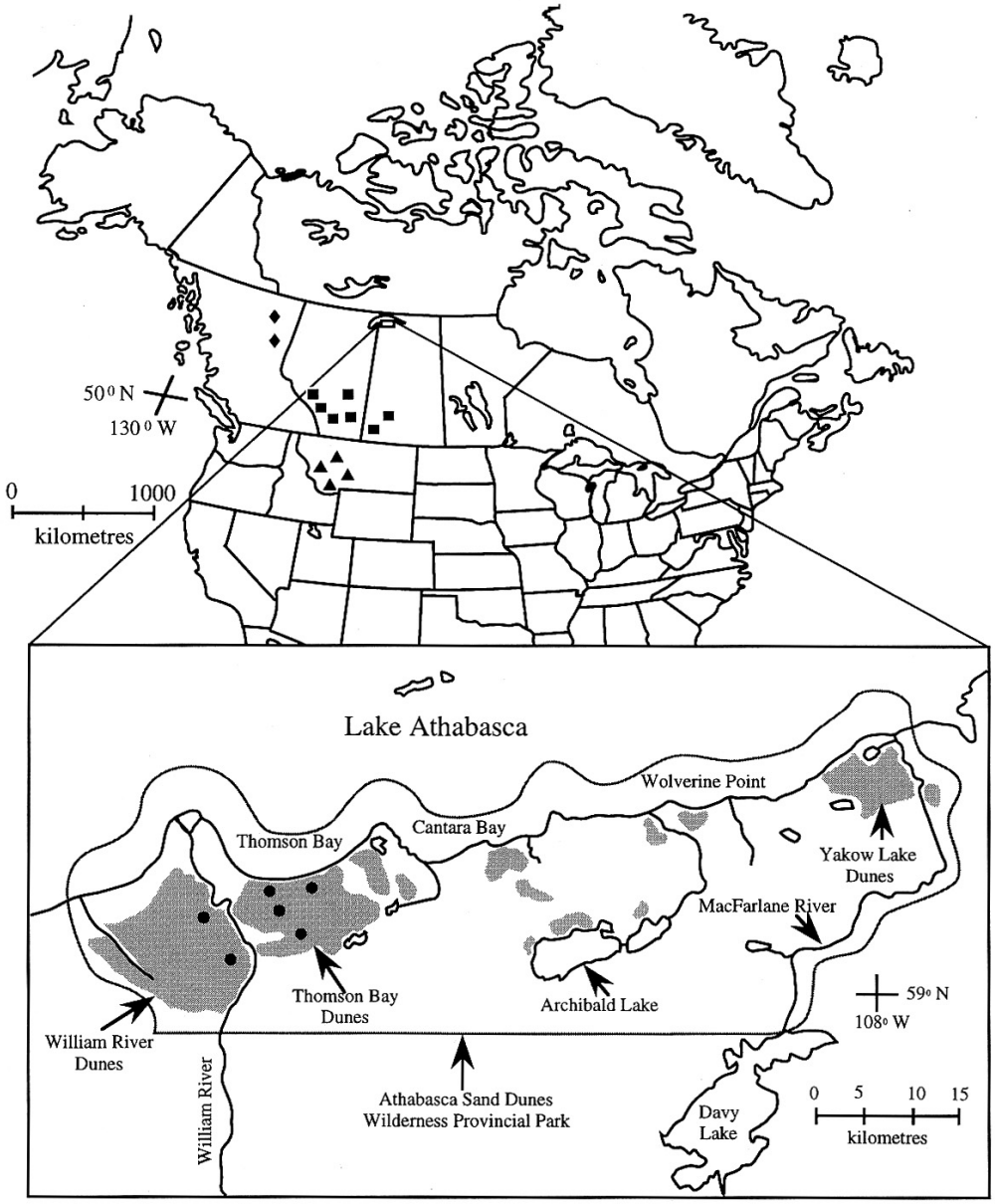
Fig. 1. Location of 13 populations of the widespread *Achillea millefolium* ssp. *lanulosa* from northern (♦), central (■), and southern (▲) regions, and 6 populations of the endemic ssp. *megacephala* (●) sampled in this study. Active sand dunes are shaded on the map.



rarity (Stebbins 1980; Kruckeberg and Rabinowitz 1985), no single explanation can be applied to all species.

The members of the *Achillea millefolium* L. complex occur in many habitats of the temperate and subarctic regions of the northern hemisphere (Clausen et al. 1948). In occupying such a diversity of environments, this complex has evolved a variety of ecological races adapted to specific environmental conditions. Variation in the species is attributable to genetic differentiation, phenotypic plasticity, and polyploidy (Clausen et al. 1940, 1948; Warwick and Briggs 1980; Warwick and Black 1982). Presently, there is no satisfactory taxonomic treatment of the variation within the *A. millefolium* complex in North America (Warwick and Black 1982). Typically, two variants of *A. millefolium* are recognized in the boreal forest of North America, *lanulosa* and *borealis*, which have been variously treated as unique species or as subspecies and varieties of *A. millefolium* (Kartesz and Kartesz 1980).

On the south shore of Lake Athabasca in northwestern Saskatchewan, there is a unique assemblage of sand dune adapted endemic plant taxa (Raup 1936; Raup and Argus 1982), which includes the tetraploid *A. millefolium* ssp.

Table 1. Population designation, collection location, habitat, and sample size for populations of *Achillea millefolium* ssp. *megacephala* (dunes) and ssp. *lanulosa* (north, central, and south).

Population	Location	Habitat	Sample size
Dunes			
ASD1	Central William River dune field; 59°03′N, 109°20′W, elev. 244 m	Active sand dunes	23
ASD2	Southern William River dune field; 58°55′N, 109°12′W, elev. 275 m	Active sand dunes	27
ASD3	Western Thomson Bay dune field; 59°05′N, 109°11′W, elev. 244 m	Active sand dunes	12
ASD4	Eastern Thomson Bay dune field; 59°06′N, 108°59′W, elev. 270 m	Active rolling dunes	24
ASD5	Central Thomson Bay dune field; 59°03′N, 109°05′W, elev. 270 m	Active rolling dunes	22
ASD6	Thomson Bay shoreline; 59°05′N, 109°10′W, elev. 215 m	Disturbed beach ridges	18
North			
BC1	Buckinghorse River, B.C.; 57°25′N, 122°50′W, elev. 1010 m	Gravel and silt floodplain	19
BC2	Toad River, B.C.; 59°10′N, 125°50′W, elev. 855 m	Gravel floodplain	20
Central			
AB1	Gooseberry Lake, Alta.; 52°45′N, 110°50′W, elev. 645 m	Wet parkland meadow	18
AB2	Battle River, Alta.; 52°53′N, 111°02′W, elev. 610 m	Mixed grassland	16
AB3	Ram Mountain, Alta.; 52°20′N, 115°45′W, elev. 1860 m	Open subalpine forest	16
AB4	Red Deer River, Alta.; 50°55′N, 111°53′W, elev. 646 m	Riparian habitat	15
AB5	Waiperous Creek, Alta.; 51°25′N, 115°05′W, elev. 1500 m	Gravel floodplain	14
SK1	Webb, Sask. 50°18′N, 108°13′W, elev. 735 m	Moist prairie	19
SK2	Antelope Lake, Sask.; 50°17′N, 108°24′W, elev. 701 m	Moist prairie	24
South			
MT1	Highwood Mountains, Mont. 47°30′N, 110°32′W, 1372 m	Pine forest	32
MT2	Crazy Mountains, Mont.; 46°18′N, 110°25′W, elev. 1830 m	Pine forest	32
MT3	Branham Lake, Mont.; 45°30′N, 112°00′W, elev. 2680 m	Subalpine meadow	26
MT4	Echo Lake, Mont.; 46°14′N, 113°15′W, elev. 2012 m	Wet willow community	26

megacephala (Raup) Argus. The flora of the Athabasca sand dunes is notable because endemic taxa are rare in boreal and arctic regions (Kruckeberg and Rabinowitz 1985). The endemic ssp. *megacephala* differs from other northern races of *A. millefolium* ssp. *lanulosa* (Nutt.) Piper in having a large involucre and in having considerable pubescence on stems and leaves. Though the taxonomy of the group as a whole is not well understood, ssp. *megacephala* is at least a distinct ecotype and shares characters common to other endemics that are considered adapted to the open sand dune environment of the Athabasca sand dunes, such as increased pubescence (Argus and Steele 1979).

This study was initiated to investigate the allozyme diversity within and among populations of the rare *A. millefolium* ssp. *megacephala*. We were interested in comparing levels of genetic diversity in this endemic with tetraploid populations of *A. millefolium* found in other habitats of western North America. We hypothesized that the amount of genetic diversity within and among populations of *A. millefolium* from the Athabasca sand dunes would not necessarily be different from that found in other races of the species, since *A. millefolium* has demonstrated the capacity to evolve ecological races adapted to a wide variety of habitats in temperate and subarctic regions. Also, since we have begun to collect information on the genetic structure of populations of other Athabasca sand dune endemic plant species, these data provide additional information into the origin of the unique flora of the region.

Materials and methods

Population sampling

The locations of the populations that were sampled for this study are found in Fig. 1 and Table 1. Populations of ssp. *lanulosa* were sampled widely through western North America, whereas populations of ssp. *megacephala* were collected from throughout their range on the Athabasca sand dunes (Fig. 1). The number of populations and individuals examined for each subspecies were as follows: for ssp. *megacephala*, 6 and 126, respectively; for ssp. *lanulosa*, 13 and 277, respectively. The number of populations and individuals collected for ssp. *lanulosa* from different geographic regions were as follows; north, 2 and 39; central, 7 and 122; and south, 4 and 116, respectively.

Live plants, which included leaves and belowground rhizomes, were collected from up to 32 individuals in each population. To ensure the collection of distinct genets, we generally collected individuals that were greater than 3 m apart. The collections were transported, wrapped in live *Sphagnum* and in coolers, to the phytotron at the University of Alberta, where the plants were grown in soil.

Electrophoresis

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

Three buffer systems were used to resolve the isozymes in this study (Purdy et al. 1994) and 15 enzyme systems were resolved. On system I, leucine aminopeptidase (LAP) (EC 3.4.11.1), phosphoglucoisomerase (PGI) (EC 5.3.1.9), aldolase (ALD) (EC 4.1.2.13), aconitase (ACO) (EC 4.2.1.3), and glutamate-oxaloacetic transaminase (GOT) (EC 2.6.1.1) were resolved. On system II, menadione reductase (MNR) (EC 1.6.99.2), glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49), isocitrate dehydrogenase (IDH) (EC 1.1.1.42), shikimate 5-dehydrogenase (SKD) (EC 1.1.1.25), and acid phosphatase (ACP) (EC 3.1.3.2) were resolved. On system III, 6-phosphogluconate dehydrogenase (PGD) (EC 1.1.1.44), NADP malate dehydrogenase (ME) (EC 1.1.1.40), NAD malate dehydrogenase (MDH) (EC 1.1.1.39), phosphoglucomutase (PGM) (EC

Table 2. Allele frequency data for polymorphic loci from populations of *Achillea millefolium* ssp. *megacephala* (dunes) and ssp. *lanulosa* (north, central, and south).

Locus and allele	Dunes						North		Central							South			
	ASD1	ASD2	ASD3	ASD4	ASD5	ASD6	BC1	BC2	AB1	AB2	AB3	AB4	AB5	SK1	SK2	MT1	MT2	MT3	MT4
Got-1																			
a	0.31	0.57	0.23	0.15	0.25	0.28	—	—	—	—	—	—	—	—	—	—	—	—	—
b	—	—	—	—	—	—	0.16	0.07	—	—	—	—	—	—	—	—	—	—	—
c	0.69	0.43	0.77	0.85	0.75	0.72	0.84	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98
d	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.02
Idh-1																			
a	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.23	—
b	—	—	—	—	—	—	0.56	0.50	0.40	0.31	0.47	0.46	0.08	0.36	0.14	0.30	0.39	0.17	0.54
c	1.00	1.00	1.00	1.00	1.00	1.00	0.44	0.50	0.60	0.69	0.53	0.54	0.92	0.64	0.86	0.70	0.61	0.60	0.46
Idh-2																			
a	0.10	0.05	0.05	0.10	0.55	0.15	—	—	—	—	—	—	—	—	—	—	—	—	—
b	0.90	0.95	0.95	0.90	0.43	0.85	0.53	0.57	0.89	0.65	0.85	0.67	0.92	0.89	0.88	0.98	0.86	1.00	0.88
c	—	—	—	—	0.02	—	0.47	0.43	—	—	—	—	—	—	—	—	—	—	—
d	—	—	—	—	—	—	—	—	0.11	0.35	0.15	0.33	0.08	0.11	0.12	0.02	0.14	—	0.12
Lap-2																			
a	—	—	—	—	—	—	—	—	—	—	—	—	0.10	—	—	—	—	—	—
b	0.73	0.62	0.44	0.65	0.40	0.40	0.72	0.75	1.00	1.00	1.00	1.00	0.65	1.00	0.83	0.85	0.88	0.81	0.86
c	0.27	0.38	0.56	0.35	0.60	0.60	0.28	0.25	—	—	—	—	0.25	—	0.17	0.15	0.12	0.19	0.14
Mdh-3																			
a	0.02	—	0.27	0.13	0.25	0.28	—	—	—	—	—	0.37	0.04	—	—	—	—	—	—
b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.09	0.28	—	—
c	0.98	1.00	0.73	0.83	0.75	0.72	1.00	1.00	1.00	1.00	1.00	0.63	0.96	1.00	1.00	0.91	0.72	1.00	1.00
d	—	—	—	0.04	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pgd-1																			
a	0.04	0.04	0.27	0.09	0.25	0.17	0.13	0.11	—	—	—	—	—	—	0.08	—	—	—	—
b	0.96	0.96	0.73	0.91	0.75	0.80	0.87	0.89	1.00	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00	1.00	1.00
c	—	—	—	—	—	0.03	—	—	—	—	—	—	—	—	—	—	—	—	—
Pgi-2																			
a	0.19	0.21	0.16	0.17	0.05	0.32	—	—	—	—	—	—	—	—	—	—	—	—	—
b	—	—	—	—	—	—	0.08	—	—	—	—	—	0.04	—	—	0.03	0.01	0.04	0.02
c	0.02	—	—	—	—	—	—	—	—	—	—	1.00	0.21	—	0.10	0.13	0.05	—	—
d	—	—	0.16	0.04	0.05	0.09	—	—	—	—	—	—	—	—	—	0.84	0.94	0.96	0.96
e	0.79	0.79	0.68	0.79	0.90	0.59	0.92	1.00	1.00	1.00	1.00	—	0.75	1.00	0.90	—	—	—	—
f	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.02
Pgm-2																			
a	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.02
b	0.54	0.62	0.82	0.57	0.74	0.50	0.31	0.04	0.28	0.41	0.41	0.33	0.29	0.41	0.04	0.21	0.37	0.23	0.09
c	0.46	0.38	0.18	0.43	0.19	0.50	0.69	0.96	0.72	0.59	0.50	0.67	0.71	0.56	0.96	0.75	0.63	0.77	0.78
d	—	—	—	—	0.07	—	—	—	—	—	0.06	—	—	0.03	—	0.04	—	—	0.11
e	—	—	—	—	—	—	—	—	—	—	0.03	—	—	—	—	—	—	—	—
Skd-1																			
a	0.96	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	0.94
b	0.04	—	—	—	0.02	—	—	—	—	—	—	—	—	—	—	—	—	—	—
c	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.02	—	—	0.06

5.4.2.2), and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (EC 1.2.1.9) were resolved.

Enzymatic assays followed Soltis et al. (1983), except for MNR (Wendel and Weeden 1989). 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). The isozyme banding patterns conformed to those expected for tetrasomic inheritance. Homozygotes were scored as having four copies of the allele, balanced heterozygotes as having two copies of each allele, unbalanced heterozygotes as having one and three copies of the respective alleles. Banding intensity was used to identify unbalanced heterozygotes. Allele frequencies at each locus were determined for each population.

Genetic analysis

To provide a measure of the level of allozyme variation within populations and subspecies, the following statistics were computed:

Table 3. Summary of allozyme variation for 19 loci within populations of the endemic *Achillea millefolium* ssp. *megacephala* (dunes) and the widespread ssp. *lanulosa* from different geographic regions (north, central, south).

Population	A	A_P	P	H_E	H_O
Dunes					
ASD1	1.47	2.13	26.3	0.123	0.110
ASD2	1.32	2.00	21.1	0.114	0.102
ASD3	1.42	2.14	31.6	0.115	0.139
ASD4	1.47	2.29	36.8	0.112	0.118
ASD5	1.58	2.38	36.8	0.122	0.148
ASD6	1.47	2.29	36.8	0.167	0.157
Mean	1.45	2.21	31.6	0.126	0.129
SE	0.03	0.06	2.7	0.008	0.009
North					
BC1	1.37	2.00	36.8	0.110	0.133
BC2	1.32	2.00	26.3	0.079	0.096
Mean	1.34	2.00	31.6	0.095	0.115
SE	0.08	0.00	5.3	0.016	0.019
Central					
AB1	1.16	2.00	15.8	0.062	0.058
AB2	1.16	2.00	15.8	0.104	0.075
AB3	1.21	2.33	15.8	0.085	0.073
AB4	1.21	2.00	21.1	0.096	0.102
AB5	1.42	2.33	26.3	0.105	0.093
SK1	1.21	2.33	15.8	0.078	0.064
SK2	1.32	2.00	26.3	0.060	0.062
Mean	1.35	2.07	19.6	0.084	0.075
SE	0.05	0.04	1.9	0.007	0.006
South					
MT1	1.47	2.29	26.3	0.090	0.084
MT2	1.37	2.17	31.6	0.083	0.103
MT3	1.26	2.25	15.8	0.062	0.070
MT4	1.53	2.43	26.3	0.088	0.082
Mean	1.41	2.29	25.0	0.081	0.085
SE	0.08	0.11	3.3	0.006	0.007
ssp. *megacephala*					
Population	1.45*	2.21	31.6*	0.126*	0.129*
SE	0.03	0.06	2.7	0.008	0.009
Subspecies	1.74	2.75	42.1	0.134	—
SE	0.05	0.25	—	0.035	—
ssp. *lanulosa*					
Population	1.31*	2.16	23.1*	0.085*	0.084*
SE	0.03	0.05	1.9	0.005	0.006
Subspecies	2.05	3.22	47.4	0.115	—
SE	0.31	0.36	—	0.035	—

Note: Variables are as follows: A, mean number of alleles per locus; A_P, mean number of alleles per polymorphic locus; P, percentage of polymorphic loci (95% criterion); H_E, expected heterozygosity; H_O, observed heterozygosity. *, values differ between taxa for population genetic statistics ($P < 0.05$).

A, the mean number of alleles per locus; A_P, the mean number of alleles per polymorphic locus; P, the proportion of polymorphic loci when the most common allele has a frequency less than or equal to 0.95; and H_E, the expected panmictic heterozygosity. Values for A, A_P, P, and H_E were calculated for each population, which were then averaged for each subspecies or geographic region. Subspecies level statistics were calculated for the four genetic diversity parameters by treating all populations of a taxon as if they were one population and t tests were used to test for significant differences among these parameters. The partitioning of gene diversity within and among populations was analyzed using measures proposed by Nei and Chesser (1983). We also calculated Wright's gene flow estimate (Wright 1951) using G_{ST} instead of F_{ST}, as well as genetic identities unbiased for sample size (Nei 1978).

Population variation statistics and genetic identities were calculated using the BIOSYS program (Swofford and Selander 1989). Gene diversity statistics were calculated using the GENESTAT-PC program (Whitkus 1988). Principal components analysis (PCA) using the SYSTAT program (Wilkinson 1990) helped evaluate the phenetic interpopulational relationships based on allele frequency distributions from polymorphic loci only.

Results

The 15 enzyme systems assayed in this study are coded by 19 putative loci. Some isozymes (ALD, MNR, ME, ACP-2, ACP-3) were excluded from our analysis because they had complex banding patterns, were resolved inconsistently, or overlapped with other loci. Ten of the loci were monomorphic in all populations: *Aco-1*, *Acp-1*, *G3pdh-1*, *G6pdh-1*, *G6pdh-2*, *Lap-1*, *Mdh-1*, *Mdh-2*, *Pgi-1*, and *Pgm-1*. Nine of the loci were polymorphic in at least some populations: *Got-1*, *Idh-1*, *Idh-2*, *Lap-2*, *Mdh-3*, *Pgd-1*, *Pgi-2*, *Pgm-2*, and *Skd-1*. Allele frequencies for polymorphic loci for all populations are presented in Table 2.

There were 35 alleles at the nine polymorphic loci, though only 28 alleles had frequencies greater than 0.05 in any one population (Table 2). Six alleles were restricted to the endemic taxon (*Got-1a*, *Idh-2a*, *Mdh-3d*, *Pgd-1c*, *Pgi-2a*, and *Skd-1b*), though three of these never occurred at frequencies greater than 0.05 (*Mdh-3d*, *Pgd-1c*, and *Skd-1b*). Twelve alleles were restricted to the widespread taxon (*Got-1b*, *Got-1d*, *Idh-1a*, *Idh-1b*, *Idh-2d*, *Lap-2a*, *Mdh-3b*, *Pgi-2b*, *Pgi-2f*, *Pgm-2a*, *Pgm-2e*, and *Skd-1c*), though four of these alleles never occurred at frequencies greater than 0.05 (*Got-1d*, *Pgi-2f*, *Pgm-2a*, and *Pgm-2e*).

At the population level, ssp. *megacephala* had more allozyme variation than ssp. *lanulosa* (Table 3). Mean values for A (1.45 vs. 1.31), P (31.6 vs. 23.1), and H_E (0.126 vs. 0.085) were all significantly higher in ssp. *megacephala* compared with ssp. *lanulosa*. In contrast, at the subspecies level, where all populations of a taxon were considered as if they were one population, ssp. *lanulosa* and ssp. *megacephala* had similar genetic diversity for A, P, and H_E (Table 3).

Total allozyme diversity at polymorphic loci (H_T) in ssp. *megacephala* was 0.296, compared with 0.254 in ssp. *lanulosa* (Table 4). Within-population gene diversity (H_S) was higher in ssp. *megacephala* (0.272) than in ssp. *lanulosa* (0.178). The among-population differentiation (G_{ST}) was lower in the endemic (0.078) than the widespread taxon (0.300) (Table 4). G_{ST} values for geographic subdivisions of ssp. *lanulosa* were lower: north, 0.011; central, 0.222; and south, 0.056. Wright's estimate for gene flow (*Nm*) for ssp. *megacephala* was 2.94 migrants per generation, and for ssp. *lanulosa* it was 0.58 migrants per generation (Table 4). *Nm* values for geographic regions of ssp. *lanulosa* were 23.56 for north, 0.87 for central, and 4.22 for south.

Genetic identity values (I) for all pairs of populations were high. Intra-subspecific I values for populations of ssp. *megacephala* ranged from 0.968 to 0.999, with a mean of 0.988; for ssp. *lanulosa*, I values ranged from 0.919 to 1.000 (Table 5), with a mean of 0.961. The mean inter-subspecific

Table 4. Gene diversity statistics (Nei and Chesser 1983) and estimates of gene flow for populations of the endemic *Achillea millefolium* ssp. *megacephala* and the widespread ssp. *lanulosa* from different geographic regions (north, central, south).

Taxon and location	H_T	H_S	D_{ST}	G_{ST}	*Nm*
ssp. *megacephala*	0.296	0.272	0.023	0.078	2.94
ssp. *lanulosa* (all)	0.254	0.178	0.076	0.300	0.58
North	0.245	0.242	0.003	0.011	23.56
Central	0.204	0.159	0.045	0.222	0.87
South	0.189	0.179	0.011	0.056	4.22

Note: Variables are as follows: H_T, total gene diversity; H_S, gene diversity within populations; D_{ST}, gene diversity among populations; G_{ST}, proportion of gene diversity among populations; *Nm*, number of migrants per generation.

I value between ssp. *lanulosa* and ssp. *megacephala* was 0.943, with values ranging from 0.874 to 0.993. The endemic taxon had higher inter-subspecific *I* values with central and north populations of the widespread taxon.

The PCA is presented with OTUs (populations) plotted onto the derived variables (principal axes) and alleles plotted at the end of vectors of the individual alleles relative to the PCA axes (Fig. 2). The PCA indicates the close association of populations from the sand dunes and their more distant genetic relationship to populations of ssp. *lanulosa* (Fig. 2). Populations from the central and north regions of ssp. *lanulosa* cluster together except for AB4 (Red Deer River, Alberta), which is between the groups formed by the north-central populations and the south populations (Fig. 2). The loci important in differentiating populations of the two taxa are *Lap-2*, *Idh-1*, *Pgm-2*, and *Got-1*. Loci important in differentiating regional groups of ssp. *lanulosa* are *Pgi-2*, *Idh-2*, and *Mdh-3* (Fig. 2).

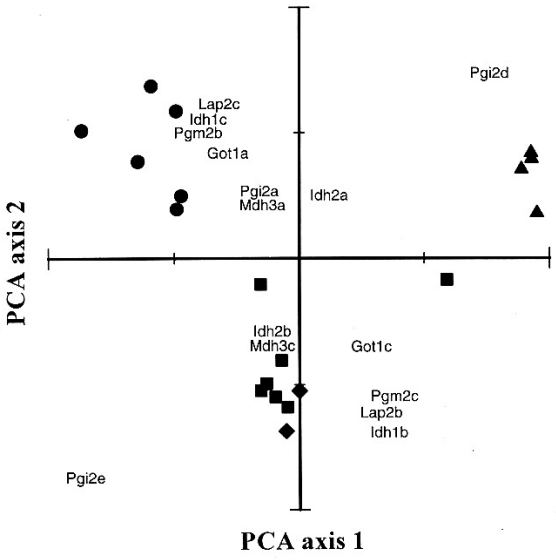
Discussion

Amount and pattern of genetic variation

The endemic *A. millefolium* ssp. *megacephala* has more allozyme variability at the population level than its widespread relative ssp. *lanulosa*. Estimates of the mean number of alleles per locus, percent polymorphic loci, and heterozygosity were all higher in the endemic. Species with restricted geographic distributions typically have fewer polymorphic loci, fewer alleles per locus, and reduced heterozygosity (Gottlieb 1973; Karron 1987; Loveless and Hamrick 1988; Pleasants and Wendel 1989; Baskauf et al. 1994), though there are exceptions (Mashburn et al. 1978; Bayer and Crawford 1986; Karron et al. 1988; Linhart and Premoli 1993). Mean values compiled by Hamrick and Godt (1989) for *A* (1.39), *P* (26.3), and H_E (0.063) in endemic taxa were all lower than the values found in ssp. *megacephala*.

Karron (1987) suggested caution drawing conclusions about associations when comparing population genetic information from unrelated taxa and suggested that closely related widespread congeners be used for comparison with rare species, as they often share ecological and historical traits. We considered the closely related widespread ssp. *lanulosa* as the control to examine aspects of genetic variation for the endemic ssp. *megacephala*, just as the widespread progenitor species served as the control in other studies of genetic diversity in

Fig. 2. Principal components analysis of allele frequencies from populations of the endemic *Achillea millefolium* ssp. *megacephala* (dunes) and the widespread ssp. *lanulosa* (north, central, south). Principal components axes 1 and 2 explain 42.8 and 26.2% of the total variance, respectively. Positions for OTUs (populations) and variables (alleles) are presented. Populations: ●, dunes; ■, central; ▲, south; ♦, north.



Athabasca sand dune endemic species (Purdy et al. 1994; Purdy and Bayer 1995*a*, 1995*b*). Previous studies on the Athabasca sand dune endemics *Stellaria arenicola* (Purdy et al. 1994), *Deschampsia mackenzieana* (Purdy and Bayer 1995*a*), and *Salix silicicola* (Purdy and Bayer 1995*b*) revealed the endemic species to be genetically depauperate relative to closely related widespread taxa. Why then would the relationship between ssp. *megacephala* and its widespread relative be different?

There is considerable evidence that suggests bottlenecks and founder effects play a significant role in governing patterns of genetic variability in colonizing species (see Barrett and Kohn 1991 for review). Theoretical and empirical evidence suggest that genetic bottlenecks have a large effect on allelic diversity and also affect heterozygosity (Wright 1931; Nei et al. 1975; Leberg 1992). Therefore, levels of genetic variation within populations of *A. millefolium* would be dependent on the amount of genetic variation in the founding individuals, the length of time the population remained small, and levels of gene flow.

Once populations are established, individuals of *A. millefolium* can survive and spread by vegetative means as well as by sexually produced seeds (Warwick and Black 1982). Mean values compiled by Hamrick and Godt (1989) for *A* (1.47), *P* (29.4), and H_E (0.103) in taxa with sexual and asexual reproduction were comparable to the values found in ssp. *lanulosa*. On the Athabasca sand dunes, however, the opportunity for continued asexual reproduction in a population is minimal. The nature of the rolling dunes found in the Thomson Bay and William River dune fields, where many of the endemic species are common, ensures that recruitment of

Table 5. Mean genetic identities (Nei 1978) and ranges (in parentheses) for populations of the endemic *Achillea millefolium* ssp. *megacephala* and the widespread ssp. *lanulosa* from different geographic regions (north, central, and south).

Taxon and location	ssp. *megacephala*	North	Central	South
ssp. *megacephala*	0.988 (0.968–0.999)			
North	0.947 (0.926–0.965)	0.999		
Central	0.954 (0.881–0.993)	0.973 (0.919–0.986)	0.977 (0.934–1.000)	
South	0.922 (0.874–0.947)	0.932 (0.926–0.936)	0.943 (0.926–0.961)	0.995 (0.992–0.998)

new individuals occurs by sexual means alone (Hermesh 1972; Raup and Argus 1982). Seedlings are common in dune slacks where the water table is at or near the surface (Raup and Argus 1982). As the rolling dunes shift, the plants established in the dune slacks become buried by sand, from anywhere between 0.5 to 12 m (Hermesh 1972). This burial is one of the most important selection forces determining the persistence of species on the Athabasca sand dunes (Raup and Argus 1982). However, few plants survive the subsequent ablation of sand as the dune moves on (B. Purdy, personal observation). The only possibility for persistence in this habitat is for new individuals to establish from seed in the newly forming dune slacks. Thus, populations of the endemic ssp. *megacephala* may rely more on sexual reproduction than *A. millefolium* populations found in other habitats. This may help explain the greater allozyme variation at the population level found in this endemic.

Subspecies *megacephala* was found to have slightly higher total gene diversity (H_T), and higher within-population gene diversity (H_S), than the widespread ssp. *lanulosa*, different from the pattern found in other studies (Hamrick and Godt 1989). Total and within-population gene diversity in ssp. *lanulosa* was considerably less than that reported for other widespread taxa or for species with sexual and asexual means of reproduction. Some other population genetic studies found the widespread species to be no more genetically diverse than the endemic (Bayer and Crawford 1986; Karron et al. 1988; Linhart and Premoli 1993), though these studies are notable exceptions.

Levels of among-population differentiation (G_{ST}) were considerably lower in ssp. *megacephala* than in ssp. *lanulosa* as one might have expected, since populations of the widespread taxon were collected over a greater geographic area. Geographic subdivisions of ssp. *lanulosa* have lower G_{ST} values, indicating that populations within a region are more similar to one another than populations among regions. Gene flow estimates for the north and south geographic subdivisions of ssp. *lanulosa* and for ssp. *megacephala* were moderate to high for insect-pollinated herbaceous perennials (Hamrick 1987).

Although ssp. *megacephala* has higher population allozyme variation than ssp. *lanulosa*, it has lower total allelic diversity, having 23 alleles at polymorphic loci compared with 30 alleles in the progenitor. However, where polymorphisms do exist at a locus in ssp. *megacephala*, the allele frequencies were more evenly distributed than in ssp. *lanulosa*. Higher levels of sexual reproduction in plant populations on the Athabasca sand dunes may contribute to the broader distribution of allele frequencies found in *A. millefolium* ssp. *megacephala*.

Origin of *Achillea millefolium* ssp. *megacephala*

Recently derived species were predicted to exhibit (*i*) less allozyme variation than the progenitor, (*ii*) a subset of the allelic diversity found in the progenitor with few, if any, unique alleles, and (*iii*) high genetic similarity with the progenitor taxon (Gottlieb 1973; Pleasants and Wendel 1989). These predictions were supported by a number of studies (Gottlieb 1973; Crawford and Smith 1982; Crawford et al. 1985; Gottlieb et al. 1985; Loveless and Hamrick 1988; Pleasants and Wendel 1989). In studies of the genetic relationship between the derivative and progenitor species of other sand dune endemics (Purdy et al. 1994; Purdy and Bayer 1995*a*, 1995*b*), these predictions were supported.

However, in the present study, the relationship between the derivative species and its widespread progenitor is not as clear. Populations of ssp. *megacephala* had higher levels of allozyme variation than populations of ssp. *lanulosa*. In addition, the endemic had six unique alleles, although only three of these had a frequency greater than 0.05 in any one population. The derivative and progenitor taxa did, however, have a high genetic similarity, and ssp. *lanulosa* had higher total allelic diversity than ssp. *megacephala*. Inter-subspecific *I* values between the two taxa were slightly lower than intra-subspecific *I* values found among the geographic subdivisions of ssp. *lanulosa*. Interspecific *I* values between progenitor–derivative species pairs reported for *Stellaria arenicola* (Purdy et al. 1994), *Deschampsia mackenzieana* (Purdy and Bayer 1995*a*) and *Salix silicicola* (Purdy and Bayer 1995*b*) from the Athabasca sand dunes are comparable to the values reported here. The high genetic identities support previous suggestions of a recent Holocene origin for the sand dune endemics (Argus and Steele 1979; Raup and Argus 1982; Macdonald et al. 1987).

Conclusion

Achillea millefolium ssp. *megacephala* differs from other Athabasca sand dune endemics studied previously in that its populations maintain greater allozyme variation than the related widespread taxa. It may be that the reliance on sexual reproduction for persistence on the sand dunes results in the maintenance of higher levels of allozyme diversity in this habitat, although we can only speculate as to the reasons for this pattern. The widespread and ecologically diverse ssp. *lanulosa* had lower levels of allozyme variation than most widespread species, though there are other examples in the literature of this pattern in species with similar geographic distributions and high ecological amplitude, notably species of *Typha* (Mashburn et al. 1978) with no detectable electrophoretic variation.

Populations of ssp. *megacephala* are restricted to the Thomson Bay and William River dune fields on the south shore of Lake Athabasca. The subspecies is listed using nature conservancy rankings as nationally and provincially "extremely rare" (Argus and Pryer 1990) and is a candidate for the endangered species list in Canada, though no status report has been completed to date. The species co-occurs with critical populations of other Athabasca sand dune endemics (B. Purdy, personal observation). Because the entire sand dune region in Saskatchewan has been designated a wilderness provincial park, the most restrictive park status in the province, development pressures are very low. Demographic monitoring of ssp. *megacephala* and other endemics on the large dune fields will be important to ensure the persistence of these species and to increase our understanding of the life history dynamics of endemic species on the Athabasca sand dunes.

Acknowledgements

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 R.J.B. and B.G.P., the Natural Sciences and Engineering Research Council Canada to R.J.B., and a Queen Elizabeth Doctoral Fellowship in Environmental Studies to B.G.P.

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