Theor Appl Genet (1999) 99: 210–217

O. Díaz · B. Salomon · R. von Bothmer

Genetic variation and differentiation in Nordic populations of *Elymus alaskanus* (Scrib. ex Merr.) Löve (Poaceae)

Received: 23 October 1998 / Accepted: 19 December 1998

Abstract To gain information on the extent and nature of genetic variation in *Elymus alaskanus*, levels and distribution of genetic variation were assessed within and among 13 populations originating from Iceland, Norway, Sweden and Russia using allozymes. The results showed that four (30.7%) of the 13 loci were polymorphic within the species, while the mean percentage of polymorphic loci within the populations was 1.9%. The mean number of alleles per locus for the species was 1.8 and 1.02 across the populations. Genetic diversity at the species level was low ($H_{es} = 0.135$), and mean population diversity was notably lower ($H_{ep} = 0.005$). A high degree of genetic differentiation was observed among populations. The salient points emerging from this study are: (1) statistically significant differences were found in allele frequencies among populations for every polymorphic locus ($P < 0.001$), (2) the high mean coefficient of gene differentiation (G_{ST}) showed that 95% of the total allozyme variation was attributable to differences among populations, and (3) relatively high genetic distances between the populations were obtained (mean D = 0.16). The Norwegian populations had the highest genetic diversity as compared with the other populations. Geographical comparisons revealed three different groups of populations clearly differentiated, i.e. Scandinavia (Norway and Sweden), Iceland and Russia. Cluster and principal coordinates analyses revealed the same genetic patterns of relationships among populations. Generally, this study indicates that *E. alaskanus* contains low allozymic variation in its populations. The implications of these results for the conservation of the species are discussed.

Key words Triticeae · *Elymus alaskanus* · Allozymes · Genetic differentiation · Diversity

Communicated by P. M. A. Tigerstedt

O. Díaz (✉) · B. Salomon · R. von Bothmer
Department of Plant Breeding Research, The Swedish University of Agricultural Sciences, S-268 31, Svalöv, Sweden
Fax: +46 418 667081
E-mail: oscar.diaz@vf.slu.se

Introduction

Elymus L. is the largest genus in the tribe Triticeae, with some 150 species distributed in most temperate regions of the world (Dewey 1984). At present, except for a few species, very little is known about the extent and nature of genetic diversity within and among populations in this genus, or the genetic structure in different geographic areas, (Díaz et al. submitted; Sun et al. 1998, 1999 a, b). The *Elymus* species are related to wheat, barley, rye, and several important forage grasses. Thus, they may serve as a source of desirable traits for the improvement of these crops. For most *Elymus* species, however, their potential use in plant breeding is still unknown and needs to be investigated.

Elymus alaskanus (Scribn. & Merr.) Löve, as delineated by us, is an arctic-alpine species complex with a northern circumpolar distribution, which includes the Nordic region, northern Russia, Siberia, Alaska, northern Canada, and Greenland. The current taxonomic classification is still under discussion and the complex has previously been treated as several different taxa (see Barkworth 1997). *E. alaskanus* is a perennial, self-fertilising, and allotetraploid species which grows on limestone outcrops, screes, moraines, dry meadows and similar low-competition habitats.

In the Nordic region, *E. alaskanus* is found in northern Iceland, the Scandinavian mountains and northern Finland. Usually, it has a patchy distribution pattern with distinct populations of varying size. Within this area, *E. alaskanus* is morphologically relatively variable in spike characters (e.g. glume and lemma size, shape,

and vestiture). Based on these differences it has been suggested that the Nordic populations of *E. alaskanus* be divided into three subspecies, namely, subsp. *islandicus* (Meld.) Löve & Löve in Iceland and subsp. *subalpinus* (Neuman) Löve & Löve and subsp. *scandicus* (Nevski) Meld. in Scandinavia (Melderis 1978; Löve 1984). Whereas *E. alaskanus* subsp. *islandicus* is rather distinct, the independent status of the latter two subspecies is quite dubious and subsp. *scandicus* was included in subsp. *subalpinus* by Tzvelev (1973). All Scandinavian populations used in the present study belong to subsp. *subalpinus* in the narrow sense.

To effectively conserve and utilise the genetic resources of *E. alaskanus*, information about its genetic population structure, which is to-date unknown, is required. One way to obtain this information is by using allozyme markers, which have proved to be useful tools for assessing levels of genetic variation as well as degrees of differentiation in plant populations (Brown 1978; Brown and Weir 1983). Therefore, the objectives of the present study were: (1) to quantify the amount and distribution of genetic variability in 13 populations of *E. alaskanus* using allozyme analysis, and (2) to utilise this information to develop appropriate conservation strategies for this species in the Nordic region.

Materials and methods

Thirteen populations of *E. alaskanus* were sampled from different locations in Iceland, Norway, Sweden and Russia (Table 1). The sampling procedure followed that described by Díaz et al. submitted. The allozyme analysis was carried out using horizontal starch-gel electrophoresis. The enzyme systems assayed were: aconitase (ACO, EC 4.2.1.3), diaphorase (DIA, EC 1.8.1.4), glucose phosphate isomerase (GPI, EC 5.3.1.9), malate dehydrogenase (MDH, EC 1.1.1.37), phosphoglucomutase (PGM, EC 5.4.2.2) and shikimate dehydrogenase (SKD, EC 1.1.1.25). Methods of enzyme extraction, horizontal starch-gel electrophoresis, and enzyme staining were all according to Díaz et al. (1998). The genetic interpretation of the zymograms was inferred as in Díaz et al. submitted. Each locus was numbered sequentially, beginning with the most anodal migrating locus designated as 1, the next 2, and so on (e.g. *Aco-1*, *Aco-2*, etc.). The electrophoretic variants referred to as alleles were labelled alphabetically, starting with the letter "a" for the most anodal allele. Only loci that exhibited consistent activity and clear bands were considered in the analyses.

The amount of genetic variation was measured both at the species and population levels using three parameters; the mean number of alleles per locus (A), the proportion of polymorphic loci (P) and the gene diversity index (H_e). Parameters subscripted with the letter "s" represent within-species values and those subscripted with the letter "p" are mean within-population values. The mean number of alleles per locus was determined by summing all the alleles observed at all loci analysed within the species or population and dividing this sum by the total number of loci analysed. A locus was considered polymorphic if the frequency of its most common allele does not exceed 0.95. The proportion of polymorphic loci was calculated by dividing the number of loci polymorphic within the species or within a population by the total number of loci considered. The gene diversity index (H_e) is equivalent to the expected heterozygosity and was calculated by the unbiased method of Nei (1978), which adjusts for sample sizes. H_e was calculated for each locus including monomorphic and polymorphic loci. Mean genetic diversity at the species or population level was obtained by averaging H_e over all loci. Mean within-population parameters were obtained by averaging over individual populations for P, A and H_e. The computations were carried out with the computer program BIOSYS-1 (Swofford and Selander 1989).

To study the distribution of genetic variation in the populations, Nei's (1973) gene-diversity statistics was employed. At each polymorphic locus, the total gene diversity for the species is presented by H_T, which is partitioned into the mean gene diversity within populations (H_S) and the gene diversity among populations (D_{ST}). These quantities are related by the expression $H_T = H_S + D_{ST}$. The proportion of total genetic variation residing among populations was estimated using the coefficient of gene differentiation (G_{ST}), which is calculated as the ratio D_{ST}/H_T. The G_{ST} value can vary between 0 and 1, where a value of $G_{ST} = 1$ indicates that the populations are fixed for different alleles. An overall population differentiation was estimated from the mean G_{ST} value. Gene flow among populations was estimated by the indirect method of Slatkin and Barton (1989),

Table 1 List of the *E. alaskanus* populations included in this investigation with their population designations, sample size and geographical origin (the numbers in brackets refer to the accession code of our own collections)

Population designation	Sample size	Geographical origin	Latitude[a]	Longitude[a]	Altitude (m)
SWE-1	138	Sweden, Norrbotten province (H 10356)	68 °14′N	18 °50′E	540
SWE-2	10	Sweden, Västerbotten province (H 10372)	64 °32′N	16 °57′E	550
NOR-1	15	Norway, Troms province (H 10368)	69 °15′N	19 °54′E	5
NOR-2	50	Norway, Troms province (H 10369)	69 °14′N	19 °47′E	80
NOR-3	50	Norway, Oppdal province (H 10574)	62 °14′N	9 °44′E	–[b]
NOR-4	36	Norway, Oppdal province (H 10576)	62 °3′N	9 °29′E	–[b]
NOR-5	56	Norway, Oppdal province (H 10577)	61 °51′N	9 °18′E	–[b]
ICE-1	50	Iceland, Eyjafjardarsysla (H 10357)	65 °19′N	17 °57′W	20
ICE-2	13	Iceland, Eyjafjardarsysla (H 10358)	65 °12′N	18 °5′W	30
ICE-3	50	Iceland, Skagafjardarsysla (H 10365)	65 °23′N	19 °10′W	140
ICE-4	49	Iceland, Skagafjardarsysla (H 10366)	65 °7′N	19 °9′W	100
ICE-5	10	Iceland, Hunavatnssysla (H 10367)	65 °36′N	19 °30′W	30
RUS-1	22	Russia, Far East, Kamchatka (H 10458)	53 °36′N	157 °43′E	700

[a] Approximation
[b] No information available

which is based on the average number of migrants (Nm) between any pair of populations. Since G_{ST} is equivalent to Wright's between-population differentiation coefficient F_{ST} (Nei 1973), Nm was calculated as follows: $Nm = (1-G_{ST})/4G_{ST}$, where N is the effective population size and m the rate of migration.

Population differentiation was also estimated by using Nei's unbiased genetic distance (Nei 1978). To graphically display the genetic relationship of the populations, a dendrogram was generated from a pairwise distance matrix using the unweighed pair group method with arithmetic average (UPGMA) (Sneath and Sokal 1973). A principal coordinates analysis (PCoA) (Gower 1966) was also calculated from the distance matrix using the double-center and EIGEN procedures in the NTSYS-pc computer program (Rohlf 1993). This multivariate analysis was chosen to provide a complementary picture of the genetic relationships among populations given by UPGMA clustering, because UPGMA has a higher resolution for the analysis of closely related populations, while PCoA is more informative regarding distances among major groups (Hauser and Crovello 1982). A two-dimensional projection of the populations on the two PCo axes was done to illustrate the relationships among the populations.

Results

Patterns and levels of genetic variation

The six enzyme systems assayed in this study were found to be encoded by 14 loci. The *Gpi-2* locus showed a fixed heterozygous phenotype with the same alleles in all individuals analysed. Since no variation was found, this locus was not considered in the calculations to avoid an over-estimation of the genetic variation. Of the remaining 13 loci, nine were monomorphic for the same allele (*Aco-1*, *Aco-4*, *Dia-1*, *Gpi-1*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Pgm-1* and *Pgm-2*) and four were polymorphic (*Aco-2*, *Aco-3*, *Skd-1* and *Skd-2*). All polymorphic loci were scored as diallelic, except for *Aco-2* which displayed three alleles. At each polymorphic locus, all individuals were homozygous for one of the alleles, and no heterozygotes were observed. The allele frequencies at polymorphic loci in the populations show almost exclusive fixation for different alleles (Table 2).

At the population level (Table 3), the mean number of alleles per locus (A_p) was 1.02, ranging from 1.0 to 1.2. The percent of polymorphic loci (P_p) ranged from 0% to 15.4%, with a mean of 1.9%. The mean population gene diversity (H_{ep}) was 0.005, ranging from 0 to 0.049. The highest A_p, P_p and H_{ep} values were always observed in the populations NOR-5 and NOR-3. The remaining populations showed apparently no diversity at all. At the species level (i.e. treating the populations as one unit, Table 3), the mean number of alleles per locus (A_s) was 1.8, the percent of polymorphic loci (P_s) was 30.7% and the gene diversity index (H_{es}) was 0.135.

Distribution of genetic variation and differentiation among populations

Results of chi-square tests for heterogeneity of allele frequencies and hierarchical partitioning of the gene diversity in the species are presented in Table 4. Statistically significant differences ($P < 0.001$) in allele frequencies among populations existed for all polymorphic loci. The average total genetic diversity (H_T) was 0.44, ranging from 0.29 to 0.49. The mean within-population component of diversity (H_S) was 0.02, ranging from 0.0 to 0.03. The mean gene diversity among populations (D_{ST}) was 0.42, ranging from 0.26 to 0.49. The proportion of total genetic variation among populations (G_{ST}) was, on average, 0.95. This reveals that the within-population component accounted for 5% of the total gene diversity at polymorphic loci while 95% of the total was due to the interpopulational component. This also indicates a high degree of differentiation among the populations. The number of migrants per generation among populations (Nm), based on the mean G_{ST} for all polymorphic loci, was 0.01.

Genetic distances were calculated for each pair of populations to estimate the extent of their divergence. The mean genetic distance (D) among populations was 0.16. The lowest genetic distance (D = 0.00) was found

Table 2 Allele frequencies at four polymorphic loci analysed in 13 populations of *E. alaskanus*

Locus	Allele	Population[a]												
		SWE-1	SWE-2	NOR-1	NOR-2	NOR-3	NOR-4	NOR-5	ICE-1	ICE-2	ICE-3	ICE-4	ICE-5	RUS-1
Aco-2	a	1.00	1.00	–	–	0.90	–	0.89	1.00	1.00	1.00	1.00	1.00	–
	b	–	–	1.00	–	0.10	–	0.11	–	–	–	–	–	1.00
	c	–	–	–	1.00	–	1.00	–	–	–	–	–	–	–
Aco-3	a	1.00	1.00	1.00	1.00	–	1.00	0.67	1.00	1.00	1.00	1.00	1.00	–
	b	–	–	–	–	1.00	–	0.33	–	–	–	–	–	1.00
Skd-1	a	–	–	–	–	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–	–	–
Skd-2	a	–	–	–	–	–	–	–	1.00	1.00	1.00	1.00	1.00	1.00
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	–	–	–

[a] Population designations refer to Table 1

Table 3 Levels of allozyme variation detected in 13 populations of *E. alaskanus*. A = mean number of alleles per locus, P = percentage of polymorphic loci and H_e = gene diversity (equivalent to the expected heterozygosity under panmixia, Nei 1978)

Population	A	P	H_e
SWE-1	1.0	0.0	0.000
SWE-2	1.0	0.0	0.000
NOR-1	1.0	0.0	0.000
NOR-2	1.0	0.0	0.000
NOR-3	1.1	7.7	0.014
NOR-4	1.0	0.0	0.000
NOR-5	1.2	15.4	0.049
ICE-1	1.0	0.0	0.000
ICE-2	1.0	0.0	0.000
ICE-3	1.0	0.0	0.000
ICE-4	1.0	0.0	0.000
ICE-5	1.0	0.0	0.000
RUS-1	1.0	0.0	0.000
Mean	1.02	1.9	0.005
Species[a]	1.8	30.7	0.135

[a] Analysis was performed by treating all populations as one unit

Table 4 Number of alleles at each polymorphic locus, heterogeneity chi-square values (with degrees of freedom) and gene diversity analyses (Nei 1973) of 13 *E. alaskanus* populations[a]

Loci	Allele	χ^2 (*df*) [S][b]	H_T	H_S	D_{ST}	G_{ST}
Aco-2	2	1850.764 (24)*	0.49	0.03	0.46	0.94
Aco-3	3	902.375 (12)*	0.29	0.03	0.26	0.89
Skd-1	2	1078.000 (12)*	0.49	0.00	0.49	1.00
Skd-2	2	1078.000 (12)*	0.49	0.00	0.49	1.00
Mean	2.25		0.44	0.02	0.42	0.95

[a] H_T = total gene diversity; H_S = gene diversity within populations; D_{ST} = gene diversity among populations and G_{ST} = coefficient of gene differntiation
[b] [S] Significance: $*P < 0.001$

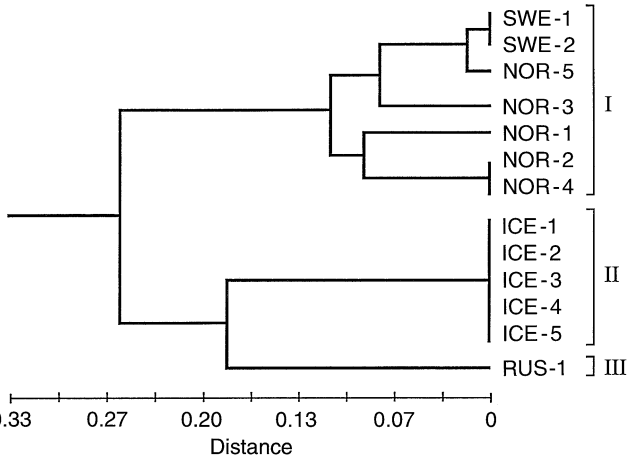

Fig. 1 UPGMA dendrogram of 13 *E. alaskanus* populations using Nei's (1978) genetic distances. Population designations refer to Table 1. The groups are shown in Roman numbers. Co-phenetic correlation coefficient 0.90

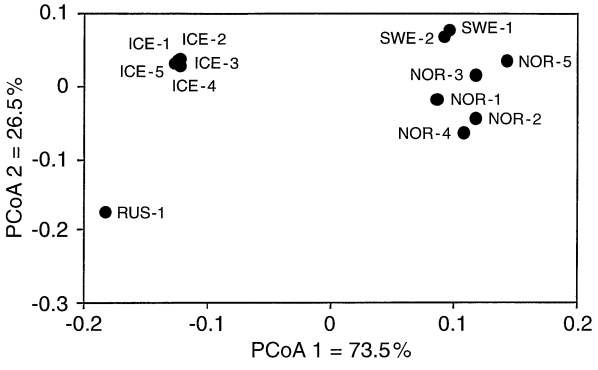

Fig. 2 Patterns of relationships of 13 *E. alaskanus* populations revealed by the principal coordinates analysis. Proportion of the total variance explained by the first two axes is 100%. Population designations refer to Table 1

between populations in Sweden (SWE-1 and SWE-2) and between populations in Iceland (ICE-1–ICE-5). The highest genetic distance (D = 0.241) was found between the Icelandic and Russian populations and the rest. The UPGMA dendrogram revealed a clear geographical pattern in the relationships of populations (Fig. 1). Three major groups were observed at the 50% level. The first included the populations from Sweden and Norway, while the second comprises all the Icelandic populations. The last group was formed by the single Russian population. The co-phenetic correlation coefficient for this dendrogram was 0.90, indicating that the structure of the dendrogram is a very good representation of the genetic relationships among populations (Rohlf 1993).

The principal coordinates analysis (PCoA) showed the same pattern of interpopulational relationship obtained by cluster analysis (see Fig. 2), but with a clearer differentiation of the Scandinavian populations according to their geographical origin. The Russian and Icelandic populations were separated from the Scandinavian populations as two distinct groups. Among the Scandinavian populations, the Swedish ones appeared to be slightly separated from the Norwegian. The first two axes explained 100% of the total allozyme variation, with PCo1 accounting for 73.5% and PCo2 for 26.5%.

Discussion

Genetic variation at species and population levels

Plant isozyme variation has usually been estimated at the population level. However, for species with considerable population differentiation, as is expected for self-pollinating taxa, population means may not accurately reflect total genetic diversity (see Hamrick et al. 1991 for an explanation). Therefore, assessing

levels of genetic diversity at both the population and the species levels has been suggested (Hamrick and Godt 1989, 1997).

The levels of allozyme variation maintained at the species level by *E. alaskanus* are very close to the values obtained for other self-fertilising species (Hamrick and Godt 1989). For instance, the average number of alleles per locus ($A_s = 1.8$) and the mean gene diversity ($H_{es} = 0.135$) were slightly higher in *E. alaskanus* than for the other self-fertilising species ($A_s = 1.69$ and $H_{es} = 0.12$). The proportion of polymorphic loci ($P_s = 30.7\%$) was, on the other hand, slightly lower than the average value for the other selfers ($P_s = 41.8\%$). When comparing these results with those obtained for *Elymus fibrosus* ($A_s = 1.5$, $H_{es} = 0.025$, $P_s = 50\%$, in Díaz et al. submitted), *E. alaskanus* has higher values for A_s and H_{es}, but lower for P_s.

At the population level, considerably lower genetic variation was detected in *E. alaskanus* ($A_p = 1.02$, $P_p = 1.9\%$ and $H_{ep} = 0.005$, Table 3), as compared with the means reported for other self-pollinating taxa ($A_p = 1.31$, $P_p = 20\%$, and $H_{ep} = 0.07$, in Hamrick and Godt 1989). Similar results were obtained for 63 populations of *Elymus canadensis* L. ($A_p = 1.09$, $P_p = 9.2\%$ and $H_{ep} = 0.026$) by Sanders et al. (1979), and for 14 populations of *E. fibrosus* ($A_p = 1.05$, $P_p = 4.8\%$ and $H_{ep} = 0.007$) by Díaz et al. submitted. Furthermore, low levels of polymorphism have been reported for accessions of *Elymus trachycaulus* (Link) Gould ex Shinners and *Elymus transbaicalensis* (Nevski) Tzvel. (Díaz et al. 1998). The levels of allozyme variation in *E. alaskanus* are still lower than those detected for these two *Elymus* species.

Distribution of genetic variation and differentiation among populations

Genetic variation is non-randomly distributed among populations, species and higher taxa (Hamrick et al. 1979; Nevo 1998). This distribution of alleles and genotypes in space or in time is often referred to as the genetic structure of a population (Loveless and Hamrick 1984). In the present study, the results indicate that *E. alaskanus* maintains (at polymorphic loci) a high allozyme variation and 95% of this variation is found among populations ($H_T = 0.44$, $H_S = 0.02$, $D_{ST} = 0.42$ and $G_{ST} = 0.95$). This pattern corresponds to what was previously reported for other *Elymus* species in particular (Sanders et al. 1979; Díaz et al. submitted) and selfing species in general (Hamrick and Godt 1989, 1997).

Population genetic structure can be either continuous or fragmented and a species may be genetically homogeneous or differentiated into genetically distinct populations, with all combinations and grades between the extremes. Species that occur as small isolated patches scattered over large geographic areas, such as *E. alaskanus*, are expected to show genetic drift and to exhibit high levels of population divergence (Godt and Hamrick 1993). In this respect, three main features of genetic differentiation among *E. alaskanus* populations are notable. First, statistically significant differences were found in allele frequencies among populations for every polymorphic locus ($P < 0.001$). Second, the high G_{ST} value shows that 95% of the total allozyme variation was due to differences among populations. This level of divergence is almost twice the mean reported for selfing species (Hamrick and Godt 1989) and is the highest reported for *Elymus* species (Hamrick et al. 1979; Hamrick 1983; Díaz et al. submitted). Third, relatively high genetic distance values were obtained between populations (mean D = 0.16), which correspond well to the degree of population divergence observed in this study. Obviously, the drastic differences found were due to the exceptionally high degree of fixation found at different loci.

The migration of genes among populations has an important effect on the population differentiation (Wright 1978; Hamrick 1987; Hamrick and Godt 1989). Species with a limited potential for gene flow show more differentiation among populations than do species with high levels of gene flow (Govindajaru 1989; Hamrick et al. 1991). Direct estimation of gene flow in natural populations is generally difficult and, therefore, indirect methods have usually been employed (Hamrick 1987). We estimated gene flow among *E. alaskanus* populations by the indirect measure of Nm (Slatkin and Barton 1989). In *E. alaskanus*, the Nm value (0.01) suggests that the gene flow between populations is too low to affect population differentiation. Gene flow can occur most likely only between adjacent populations like NOR-3 and NOR-5 which also have similar allele frequencies at the *Aco-2* locus. Apparently, this level of gene flow is not sufficient to counterbalance the effects of genetic drift and/or bottleneck and founder effects. Genetic drift is considered to be the main factor responsible for population differentiation when $Nm < 1$ (Slatkin 1987). The low allelic variation within populations and the very high G_{ST} estimates at every polymorphic locus indicate that some kind of stochastic forces, such as genetic drift, may have been a major factor in the population differentiation in *E. alaskanus*.

Natural selection, on the other hand, may be another important factor influencing the genetic structure and differentiation of the *E. alaskanus* populations. The fact that all Icelandic populations were "a-a-a-a" (their genotype at the four polymorphic loci), all the Swedish ones were "a-a-b-b", two Norwegian populations were "c-a-b-b" and the others were either "b-a-b-b", "a/b-b-b-b" or "a/b-a/b-b-b", while the Russian population was "b-b-a-a", shows that different alleles were mostly fixed in geographically (and probably environmentally) different regions. This observation may indicate selection pressures on multiloci. Natural selection can operate on multilocus allelic combinations as a unit

(i.e. co-adapted gene complexes) and leads to stable superior multilocus genotypes adapted to specific habitats (see Allard et al. 1972; Pérez de la Vega et al. 1994; Allard 1997; Nevo 1998). Whether these allele combinations are actually selected by specific environments is not known. There is not enough information to conclude one way or another. Further studies are needed to demonstrate the possible effect of selection pressures on the isozymes analysed in the present study.

An association between geographic origin and genetic relationships was revealed by both the cluster and principal coordinates analyses (Figs. 1 and 2). Three different groups of populations were clearly identified, i.e. Russia, Iceland and Scandinavia (= Sweden and Norway). Within each group, no correlation between geographic distance and genetic divergence was found. The Russian group (with only one population, RUS-1) showed the largest genetic distance when compared to the Scandinavian group, and showed the highest isozyme similarity with the Icelandic populations. Within the Scandinavian group, two Norwegian populations (NOR-3 and -5) exhibited the highest levels of genetic variation (A_p, P_p and H_{ep}). The divergence of the populations NOR-3 and -5 was principally due to the presence of the *Aco-3(b)* allele. In addition, the *Aco-2(c)* allele appears to be specific to the Norwegian populations NOR-2 and NOR-4.

Based on morphological characteristics, the Islandic populations of *E. alaskanus* have been designated as *E. alaskanus* subsp. *islandicus* whereas the Scandinavian populations used in this study belong to *E. alaskanus* subsp. *subalpinus*. A corresponding genetic divergence was clearly observed when comparing the Islandic populations with those from Scandinavia, especially at the *Skd-1* and *Skd-2* loci. Hence, the allozyme differentiation supports the morphological differentiation.

All the Icelandic populations were genetically identical, with no intrapopulational variation. This suggests that these populations originated from a (few) individual(s) of an ancestral population (founder effect) and thus genetic drift may have been an important factor regulating the gene content and causing low allozymic variability.

Low levels of genetic variation within populations

The cause of the low allozyme variability observed within the *E. alaskanus* populations (A_p, P_p and H_{ep}) could mainly be explained by a predominantly self-pollinated breeding system. A relationship between autogamy and low levels of allozyme variation has been demonstrated in various plant species (Brown 1979; Brown and Moran 1981; Hamrick 1983). Although no analysis of the breeding system has been performed in *E. alaskanus*, the floral structures (e.g. small anthers and comparatively closed flowers) indicate that this species is highly self-pollinated. In selfing species, high levels of inbreeding result in homozygosity and decreased genetic variation within groups and increased variance between groups. Populations can also be monomorphic at almost all loci over their geographic range (Selander 1983; Crawford 1990; Tayyar and Waines 1996). A restricted intrapopulational variation and high levels of divergence ($G_{ST} = 0.95$) were marked features of the *E. alaskanus* populations analyzed in this study. Furthermore, most of the loci were monomorphic and no heterozygous individuals were observed at polymorphic loci. If we compare the level of gene flow with estimates averaged over a number of species (Sanders et al. 1979; Govindajaru 1989; Díaz et al. submitted), we again find that *E. alaskanus* with $Nm = 0.01$ fits well within the category of self-pollinated species.

Implications of genetic structure for conservation

The present survey, although based on only a few polymorphic loci, demonstrates that *E. alaskanus* has a population genetic structure of a typically highly self-pollinated species. Relatively high total genetic variation (H_T) was observed at polymorphic loci in *E. alaskanus*, with high levels of genetic differentiation and low levels of gene flow among populations. From a conservation perspective, the results suggest that as many populations as possible, but with only small sample sizes (e.g. about five individuals), would be enough to preserve most of the genetic variability in the Nordic region. This suggestion is relevant for Scandinavia, where the genetic variation and differentiation were higher as compared to Iceland, where the populations were identical. On Iceland, one or two selected populations with small sample sizes should be enough to capture the genetic diversity of *E. alaskanus* as revealed by isozymes.

The preservation of these populations should be achieved by both in situ and ex situ methods. Noteworthy, the two methods are complementary rather than antagonistic (Falk 1987; Given 1987; Nevo 1998). Since a high degree of differentiation was found among populations in the Scandinavian region, further collecting in Norway, Sweden and Finland is needed for ex situ conservation. Especially, populations of typical subsp. *scandicus* should be collected to assess genetic differences with the other two subspecies. In situ conservation should also be emphasised for the Norwegian populations that displayed relatively higher levels of variation and unique genetic structures. A sweeping recommendation can not be made on the basis of isozymes alone. Although isozyme diversity has provided a valuable insight into the genetic structure of this species in the Nordic region, it would be necessary to use DNA markers, phenology and morphology to obtain a broader picture of genetic diversity, and such

combined data would possibly allow the formulation of a sound sampling strategy.

Acknowledgements This study was supported by World Wildlife Foundation (WWF), The Swedish Council for Forestry and Agricultural Research, The Nordic Gene Bank (NGB) and Nilsson-Ehle Foundation. We are grateful to Ann-Sofie Fält for her excellent laboratory assistance.

References

Allard RW (1997) Genetic basis of the evolution of adaptedness in plants. In: Tigerstedt PMA (ed) Adaptation in plant breeding. Kluver Academic Publishers, The Netherlands, pp 1–11

Allard RW, Babbel GR, Clegg MT, Kahler AL (1972) Evidence for coadaptation in *Avena barbata*. Proc Natl Acad Sci USA 69 : 3043–3048

Barkworth M (1997) *Elymus alaskanus*. Nomenclatural set. Internet address: http://www.biology.usu.edu/herbarium/virtualherbarium/elymalnm.html

Brown AHD (1978) Isozymes, plant population genetic structure, and genetic conservation. Theor Appl Genet 52 : 145–157

Brown AHD (1979) Enzyme polymorphism in plant populations. Theor Appl Genet 15 : 1–42

Brown AHD, Moran GT (1981) Isozyme and the genetic resources of forest trees. In: Conkle MT (ed) Proc Symp Isozymes of North American Forest trees and Forest Insects. Gen Tech Rep PSW-48. USDA Forest Service, PSW Forest and Range Experiment Station, Berkeley, California, pp 1–10

Brown AHD, Weir BS (1983) Measuring genetic variability in plant populations. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding, part A. Elsevier, Amsterdam, pp 219–239

Crawford DJ (1990) Plant molecular systematics: macromolecular approaches. Wiley Interscience, New York

Dewey DR (1984) The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: Gustafson JP (ed) Gene manipulation in plant improvement. Plenum Publishing Corporation, New York, pp 209–280

Díaz O, Salomon B, Bothmer R von (1998 a) Description of isozyme polymorphisms in *Elymus* species using starch gel electrophoresis. In: Jaradat AA (ed) Triticeae III. Science Publishers Inc, Enfield, New Hampshire, USA. pp. 199–207

Falk DA (1987) Integrated conservation strategies for endangered plants. Natl Areas J 7 : 118–123

Given DR (1987) What the conservationist requires of ex situ collections. In: Branwell D, Hamann O, Heywood, Synge H (eds) Botanical gardens and the world conservation strategy. Academic Press, London, pp 103–116

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53 : 325–338

Godt MJW, Hamrick JL (1993) Genetic diversity and population structure in *Tradescantia hirsuticaulis* (Commelinaceae). Am J Bot 80 : 959–966

Govindajaru DR (1989) Variation in gene flow levels among predominantly self-pollinated plants. J Evol Biol 2 : 173–181

Hamrick JL (1983) The distribution of genetic variation within and among natural populations. In: Schowald-Cox CM, Chambers SM, MacBryde B, Thomas L (eds) Genetics and conservation. Benjamin-Cummings, Menlo Park, California, pp 335–348

Hamrick JL (1987) Gene flow and distribution of genetic variation in plant populations. In: Urbanska KM (ed) Differentiation patterns in higher plants. Academic Press, Orlando, Flaorida, pp 53–68

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding and genetic resources. Sinauer Associates Inc, Sunderland, Massachusetts, USA, pp 43–63

Hamrick JL, Godt MJW (1997) Allozyme diversity in cultivated crops. Crop Sci 37 : 26–39

Hamrick JL, Linhart YB, Mitton JB (1979) Relationship between life history characteristics and electrophoretically detectable genetic variation in plants. A Rev Ecol Syst 10 : 173–200

Hamrick JL, Godt MJW, Murawski DA, Loveless MD (1991) Correlations between species traits and allozyme diversity: implications for conservation biology. In: Falk DA, Holsinger KE (eds) Genetics and conservation of rare plants, Oxford University Press, New York, pp 75–86

Hauser LA, Crovello TJ (1982) Numerical analysis of generic relationships in *Thelypodieae* (*Brassicaceae*). Systematic Bot 7 : 249–268

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. Annu Rev Ecol Syst 15 : 65–96

Löve A (1984) Conspectus of the Triticeae. Feddes Rep 95 : 425–521

Melderis A (1978) Taxonomic notes on the tribe Triticeae (Gramineae), with special reference to the genera *Elymus* L. *sensu lato*, and *Agropyron* Gaertner *sensu lato*. Bot J Linn Soc 76 : 368–384

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70 : 3321–3323

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89 : 583–590

Nevo E (1998) Genetic diversity in wild cereals: regional and local studies and their bearing on conservation ex situ and in situ. Genet Res Crop Evol 45 : 355–370

Nordström H (1990) Gräs. Natur och Kultur (in Swedish). Helsinborg, Sweden

Perez de la Vega M, Saenz-de-Miera LE, Allard RW (1994) Ecogeographical distribution and differential adaptedness of multilocus allelic associations in Spanish *Avena sativa* L. Theor Appl Genet 88 : 56–64

Rohlf FJ (1993) Numerical taxonomy and multivariate analysis system. Version 1.80. Exeter Software, Setauket, New York

Sanders TB, Hamrick JL, Holden LR (1979) Allozyme variation in *Elymus canadensis* from the tallgrass prairie region: geographic variation. Am Midl Nat 101 : 1–12

Selander RK (1983) Evolutionary consequences of inbreeding. In: Schonewald-Cox CM, Chambers SM, MacBryde B, Thomas L (eds) Genetics and conservation. Bejamin/Cummings, Menlo Park, California, pp 201–215

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236 : 778–792

Slatkin M, Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43 : 1349–1368

Sneath PH, Sokal RM (1973) Numerical taxonomy. The principles and practice of numerical classification. Freeman and Co, San Francisco

Sun GL, Díaz O, Salomon B, Bothmer R von (1999 a) Genetic diversity in *Elymus caninus* as revealed by isozyme, RAPD and microsatellite markers. Genome (in press)

Sun GL, Salomon B, Bothmer R von (1998) Characterization of microsatellite loci from *Elymus alaskanus* and length polymorphism in several *Elymus* species (Triticeae: Poaceae). Genome 41 : 455–463

Sun GL, Díaz O, Salomon B, Bothmer R von (1999 b) Microsatellite variation and its comparison with isozyme and RAPD variation in *Elymus fibrosus* (Poaceae). Hereditas (in press)

Swofford DL, Selander RB (1989) BIOSYS-1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1–7 (DL Swofford publisher) Illinois Nat Survey, Champaign, Illinois

Tayyar RI, Waines JG (1996) Genetic relationships among annual species of *Cicer* (Fabaceae) using isozyme variation. Theor Appl Genet 92 : 245–254

Tzvelev NN (1973) Conspectus specierum tribus Triticeae Dum. familie Poaceae in flora URSS. Nov Syst Pl Vasc 10 : 19–59

Wright S (1978) Evolution and the genetics of populations, vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago