Variation in populations of the coastal dune building grass Leymus arenarius in Iceland revealed by endospermal prolamins

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Abstract. Variation in the endospermal protein patterns of seeds amongst and between inland and coastal populations of the dune building grass Leymus arenarius was examined in Iceland. Seeds were collected from six coastal populations and five inland populations in Iceland. Endospermal proteins (prolamins) of seeds were extracted with Tris-buffered 2-propanol (50%) and β-mercaptoethanol (0.5% v/v). We used 8% and 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to separate the prolamin protein bands, which were stained with Coomassie Brilliant Blue R. Coastal populations were differentiated from inland populations on the basis of high frequency of high molar weight (HMW) (110 - 150 kg mol^{-1}) prolamins. Coastal populations had significantly higher proportions of their seed with more than two HMW prolamins, than those of inland populations. Subtle differences were found among the HMW prolamins of coastal and inland populations indicating limited intra-population variation. The dynamic environment of sand dunes probably influences the genetic composition of these populations. The results suggest that seeds of the inland and coastal populations should be treated separately in sand dune reclamation and restoration works.

Keywords: Coastal sand dune; Colonization; Ecosystem restoration; Endospermal prolamin; Salt tolerance; SDS-PAGE.

Introduction

The natural distribution of the perennial rhizomatous dune building grass Leymus arenarius (L.) Hochst. (lymegrass) is largely confined to coastal areas of northern Europe (Bond 1952). The distribution of this plant species is, however, not only restricted to coastal areas and inland populations can be found associated with drifting sands in Iceland (Greipsson & Davy 1994a) and along lake-shores in Finland (Suominen 1970). This plant can rapidly colonize newly exposed sandy or gravelly areas (Fridriksson 1987; Ahokas 1992; Greipsson & Davy 1994a). Also, rapid colonization of L. arenarius originating on the beach occurred on gravelly roadsides in Finland (Ahokas 1992; Greipsson et al. 1997). These roadside populations showed high founder effects that was manifested in high (90%) intrapopulation variation and low interpopulation variation in electrophoretic endospermal protein (prolamins) patterns (Ahokas 1992). Furthermore, seed of these roadside populations were found to rapidly loose their salt tolerance at the germinating stage as plant colonization advanced further away from the coast (Greipsson et al. 1997). Seed of coastal populations of L. arenarius showing high salt tolerance was characterized by HMW (110-150 kg mol^{-1}) prolamins (Greipsson et al. 1997). These proteins gave signals of glycosylation and may be linked to salt tolerance (Hurkman et al. 1989; Ahokas & Fredskild 1991; Gao et al. 1995). Variation in seed storage proteins (prolamins) electrophoretic patterns may serve as molecular markers since they are direct products of genetic differentiation (Syros et al. 2003). Such variations have been demonstrated to be useful in population studies (Du Cros et al. 1979; Levy & Feldman 1988).

Interpopulation variation of L. arenarius in Iceland relating to phenology, seed set, seed mass and germination were associated with community succession of the dunes (Greipsson 1991; Greipsson & Davy 1994b, 1995,

1996, 1997). Physiological differences between populations of this species were previously established (Greipsson & Davy 1994b), as for example, seed of coastal populations showed greater germination at high salinity level than those of inland populations. Growth of plants of coastal populations was found to be less affected by moderate and high salinity levels than those of inland populations (Greipsson & Davy 1996).

Dynamic population processes of *L. arenarius* are found on sand dunes where the environment is physically demanding and susceptible to disturbances (Davy & Figueroa 1993; Greipsson 2002). Catastrophic events such as glacier river floods and coastal erosion due to high storm surges and strong wave action of the sea play an important role in the extinction of these populations (Bjornsson 1992; Greipsson & El-Mayas 1996; Gudmundsson et al. 1996). Inland populations are adversely affected by soil erosion due to overgrazing of livestock, adverse climatic conditions and volcanic activities. The extinction of local populations of *L. arenarius* on sand dunes appears to be a stochastic process independent of population size or its genetic composition. Furthermore, community succession of inland and coastal sand dunes can result in decimation of *L. arenarius* populations (Greipsson 1991). Colonization of newly exposed sand plains depends on immigration of seed from nearby populations. Stochastic extinction of local populations and subsequent recolonization of the habitat usually results in a loss of genetic diversity among populations (McCauley 1991).

Leymus arenarius has been used successfully in a large-scale sand reclamation and coastal dune restoration (Greipsson 2002), usually being accomplished by seeding rather than vegetative propagation (Greipsson & Davy 1994b). Maintenance of genetic diversity of the restored population is important in coastal conservation. Seeds are harvested from native populations and information on intra- and interpopulation variation is critical for the restoration work. This study aims at examining variation between and amongst coastal and inland populations of *L. arenarius* in Iceland using electrophoretic endospermal protein patterns.

Methods and Materials

Plant material

Seeds of *Leymus arenarius* were collected from 11 locations around Iceland (63º23' N, 66º32' N and 13º30' W, 24º32' W); six coastal populations and five inland populations (Fig. 1). Low genetic variation at the DNA level was previously detected within populations of *L. arenarius* (Anamthawat-Jonsson et al. 1999). Populations of *L. arenarius* are maintained mainly by rhizomatous clonal growth and recruitment by seedling is rare in established populations. *Leymus arenarius* is considered to be a facultative outbreeder. Therefore, it was decided to sample in the following way to have both selfed and possibly cross-pollinated seeds. Three healthy culms about 100 m away from each other were collected from each population. Culms were air dried and stored in paper bags. Seeds were separated according to their position on the culm (i.e. top, middle or basal). For each population, about 50 seeds were used for endospermal protein analysis. Prior to protein analysis, husks (lemna and palea) were removed from each seed. The seed-endosperm was obtained by removing hairs on the stigmatic end and the embryo was cut off each seed using a scalpel. Each seed was processed individually.

Protein fractionation and analysis

Each seed-endosperm was weighed and placed into an Eppendorf tube (2 ml) with a 3 mm diameter acid-resistant steel ball. Tubes were placed inside a mill (Retsch MM2) and the endosperm was milled for 5 min at speed control 50 (Ahokas & Fredskild 1991).

Proteins were extracted with 450 and 400 μl of Tris-buffered (40 mM, pH 8.0) 2-propanol (50%) and β-mercaptoethanol (0.5% v/v) in the tubes containing the milled endosperms; with incubating for 1 h at 25 °C. Tubes carrying the steel ball were shaken on a test-tube-shaker and centrifuged (5000 rcf) for 10 min. Supernatants (twice ca. 400 ml were precipitated one after the other with 2.5 volumes (1 ml) in a new Eppendorf tube. The methanol-precipitated pellets were dried at room temperature and the residual fractions in a vacuum centrifuge. Sample buffer (Laemmli 1970) without β-mercaptoethanol was added (60 ml for the residual proteins (for endosperms ≤ 12 mg, and for endosperms >12 mg an extra of 3 μl per mg), and 40 μl for the methanol precipitate. Further details can be found in Ahokas (2002).

SDS-PAGE (8% and 12%) was conducted according to Shapiro et al. (1967) and Laemmli (1970). Samples of 40 ml of methanol-precipitated proteins or the residual proteins were run on separate gels, which were stained with Coomassie Brilliant Blue R in acetic 2-propanol. Each seed was fractionated as one lane on the SDS-PAGE gel. The calibration proteins were Combithek (Boehringer Mannheim Biochemica) with marker proteins of 340, 170, 116.4, 85.2, 55.6, 39.2, 26.6, 20.1, 14.3 kg mol^{-1}.

In order to determine significant differences in the number of HMW (in the range of 110 – 150 kg mol^{-1}) prolamins between coastal and inland populations, a χ^2-test was performed as available on the SPSS (vs. 10.1) statistical package.

Results

Variations in the patterns of high molar weight HMW (110-150 kg mol^{-1}) prolamins were found among and between populations (Fig. 2). Frequencies of HMW prolamins were found to differ significantly between coastal and inland populations (Fig. 3). Coastal populations had significantly ($\chi^2 = 128.65$; $P < 0.001$) higher proportions of their seed with more than two HMW prolamins than those of inland populations. In addition, coastal populations had significantly ($\chi^2 = 25.62$, $P < 0.001$) higher proportion of their seed with four and five HMW prolamins than those of inland populations. The HMW prolamins could be separated further apart in the 8% acrylamide than in 12% gels. No significant differences were found in the frequency of HMW prolamins among inland or coastal populations, however, some qualitative exceptions were found. For instance, the coastal population no. 3 (Myrdalssandur) had no prolamins with molar mass about 150 kg mol^{-1} but another coastal population no. 1 (Thorlakshofn) had 100% of seed containing such HMW prolamins. Coastal populations had a significantly ($P < 0.001$) lower numbers (24%) of spikes with monomorphic pattern of prolamins than those of inland populations (44%). Variation in the pattern of HMW prolamins was not significantly related to the position of seed within a culm (i.e. top, middle and base).
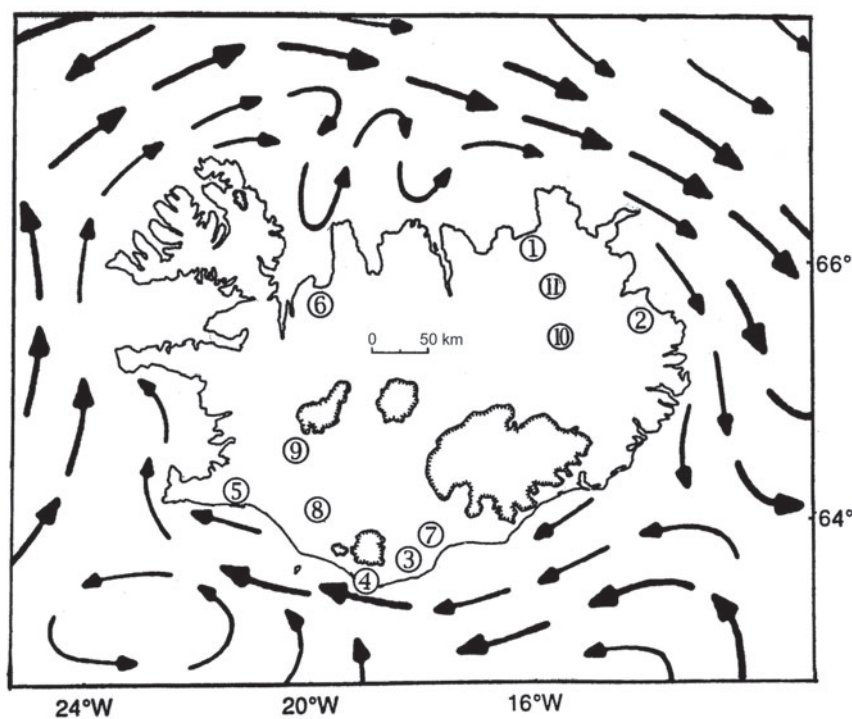
Fig. 1. Map of Iceland showing the location of the 11 populations of *Leymus arenarius* (L.) Hochst. sampled. Hatched areas within the island represent glaciers. Arrows indicate surface sea currents around the coast (Stefansson 1961).

Coastal populations (nos. 1- 6) are:

Vatnsbaeir	(1)
Heradssandur	(2)
Myrdalssandur	(3)
Vik	(4)
Thorlakshofn	(5)
Sigridarstadir	(6)

Inland populations (nos. 7-11) are:

Sudurhagar	(7)
Keldur	(8)
Skjaldbreidur	(9)
Modrudalur	(10)
Holasandur	(11)

Discussion

Intrapopulation variation was established between coastal and inland populations of *Leymus arenarius*. The coastal populations had significantly higher number of HMW prolamins than the inland ones. A significant correlation in populations of *L. arenarius* in Finland was previously found between the number of HMW prolamins in seeds and their ability to germinate at 300 mM NaCl (Greipsson et al. 1997). Therefore, the significantly different distribution of HMW prolamins among the population of *L. arenarius* in Iceland can most likely be explained with the salinity levels of the soils as the principal selective operating agent. Species that are related to *L. arenarius* such as wild barley (*Hordeum vulgare* ssp. *spontaneum*) and wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) were found to have prolamins which were dependent on soil type (Nevo et al. 1983, 1995; Nevo & Payne 1987). Moreover, the DNA diversity of HMW prolamins was shown to be correlated to soil type (Nevo et al. 1995). Studies at the DNA level using specific PCR (polymerase chain reaction) primers have corroborated findings on the genetic diversity of HMW prolamins of natural populations of wild emmer wheat (Pagnotta et al. 1995).

The salinity level of the habitat is probably not the only environmental factor responsible for the population differentiation. Climatic variation is especially pronounced between coastal and inland provenances of Iceland; longer snow cover, shorter growing season but warmer summer days are characteristic of the inland habitats (Einarsson 1991). Furthermore, coastal and inland populations of *L. arenarius* were previously found to vary in their growth responses to sand burial; coastal populations had less numbers of tillers and grew more rapidly upwards through accreting sands compared to those of inland populations (Greipsson 1991). It is likely that this adaptation corresponds to a survival in an environment of rapid sand accretion that can be found on the coast (Greipsson & Davy 1996; Greipsson & El-Mayas 1996).

Low variation in the frequency of HMW prolamins amongst coastal populations confirms previous findings that genetic variation amongst coastal populations of *L. arenarius* is low in Iceland (Anamthawat-Jonsson et al. 1999). The genetic variation among coastal populations was examined using amplified fragment length polymerisms (AFLP) and restriction length polymorphism (RFLP) of the major ribosomal genes (18S-5.8S-26S rDNA) (Anamthawat-Jonsson et al. 1999).
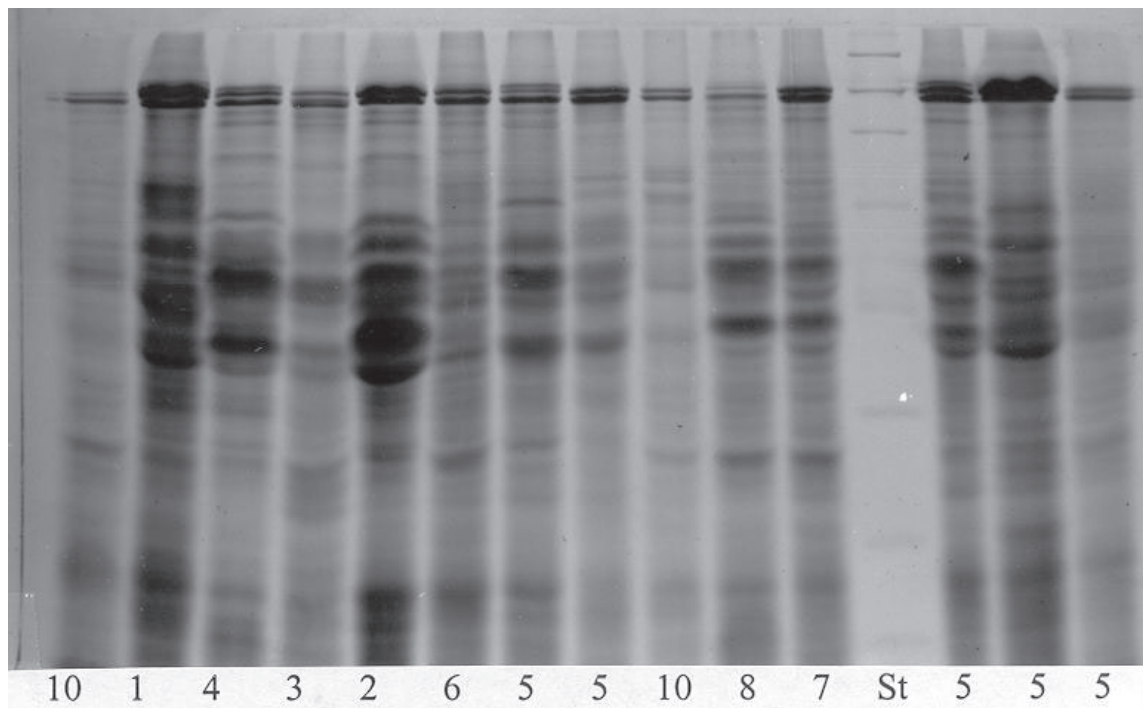
Fig. 2. Variations in the patterns of prolamins found in random samples of *Leymus arenarius* populations. The numbers on the protein tracks refer to the populations listed in Fig. 1. The methanol-precipitated proteins were fractionated on 12% SDS-PAGEs. The track labeled St shows the calibration proteins (Combithek) in the order of 340, 170, 116.4, 85.2, 55.6, 39.2, 26.6, 20.1 kg mol-1 with the highest molar weight at the top of the gel.
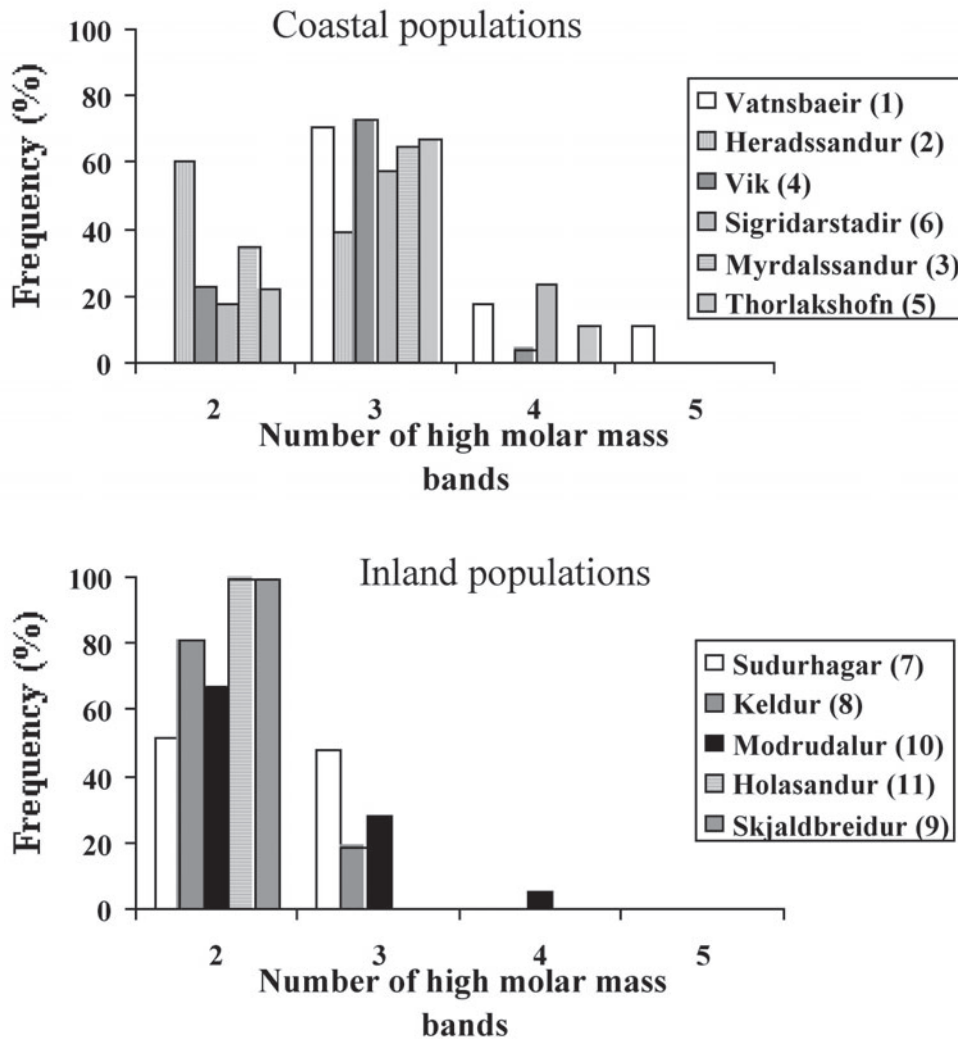
Fig. 3. Frequency of HMW (in the range of 110-150 kg mol-1) prolamins of (a) coastal populations (no. 1-6) and (b) inland populations (no. 7-11).

The limited genetic differentiation observed between coastal populations is possibly the result of a high seed dispersal via surface sea currents that provides constant immigration and consequently gene flow between populations (Fig. 1). Strong surface sea-currents are found around the islands (Fig. 1). The seed of *L. arenarius* is buoyant in water and seed can consequently be dispersed for long distances in the sea via surface currents. Effective seed dispersal via sea was demonstrated when *L. arenarius* was found to be the first vascular plant to colonize the newly created volcanic island of Surtsey, Iceland (Fridriksson 1987). Furthermore, seed of *L. arenarius* that was immersed in sea-water for three months before they were allowed to germinate showed rapid and high final germination percentages (Clarke 1964). A colonizing population of *L. arenarius* may be influenced by the founder effect and hence by genetic drift. However, constant introduction of new seed from nearby constellation populations reduces the chance of a genetic drift. It is unlikely that genetic differentiation of populations occurs when the immigration rate is high. Phenotypic plasticity is another key factor that could limit genetic differentiation between populations. Populations of *L. arenarius* were found to respond to different local environmental conditions by phenotypic plasticity (Greipsson 1991; Greipsson & Davy 1995). In fact, plants showing high phenotypic plasticity are more likely to survive in an unstable environment such as sand dunes (Jefferies 1984).

Community succession is generally found to play an important role in maintaining genetic diversity within and between populations (Scheiner & Teeri 1986, 1987; Gray 1987; Jerling 1988). Interpopulation variation of *L. arenarius* in seed mass and germination behaviour was related to the successional status of populations (Greipsson & Davy 1995). Succession can also play a role in extinction of local plant populations (Giles & Goudet 1997). Successional adaptation is especially important on sand dunes; grasses thrive on the dunes when sand is drifting but as soon as the sand stabilizes other species colonize the dunes and may eventually replace the dune building grasses (Greipsson 2002). A population's persistence time is therefore influenced by the succession rate of an ecosystem. In our study, little variation was found in HMW (about 150 kg mol^{-1}) prolamins within coastal population no. 3 (Myrdalssandur). This particular population grows on the large sand plain Myrdalssandur and it stretches from the shoreline to about 10 km inland. Various stages of succession within this large population could influence its genetic composition. We collected seed about 5 km away from the coast where plants are not exposed directly to seawater. It was previously established that a direct exposure to seawater is needed for a population to maintain its salt tolerance (Greipsson et al. 1997) indicating that salt tolerance is actually a genetic load in non-saline environments.

The results of this work could have practical implications in restoration of coastal and inland sand dunes. Local populations of plants are assumed to be better adapted to their environment than exotic populations (Gray 2002). Furthermore, the current paradigm in restoration ecology centres on the use of locally adapted genotypes in restoration practices (Gray 2002; Greipsson 2002). The work reported here demonstrates that inland and coastal populations differed strikingly on the basis of HMW prolamins. These findings are supported by studies on physiological differences between inland and coastal populations (Greipsson & Davy 1994b, 1996). Furthermore, a local inland population of *L. arenarius* was more persistent in producing seed in a stringent climate compared to those of an exotic population (Greipsson 1991). Therefore, seed of inland and coastal populations should be treated separately in the restoration work. However, it is evident that the genetic differences between coastal populations are subtle and coastal populations could possibly be considered as a metapopulation (*sensu* Freckleton & Watkinson 2002). However, using seed of a local population in the restoration work might be a 'fine adaptive tuning' and thus avoid introduction of completely inappropriate genotypes.

Conclusion

Intra- and interpopulation variations of the dune building grass *Leymus arenarius* were deciphered using pattern of HMW prolamins. Compared with other molecular techniques available the SDS-PAGE is rapid and inexpensive. Coastal populations of *L. arenarius* were characterized by a distinct pattern of HMW prolamins compared to inland ones. Low variation was found in HMW prolamins among coastal populations. We suggest that surface sea currents efficiently disperse seed along the coast and therefore maintain gene flow between those coastal populations. Stochastic population turnover due to natural catastrophes probably reduce differentiation between populations. It is possible that coastal populations could be considered as a metapopulation. However, more work is required to confirm this.

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