Nineteenth Century Seeds Reveal the Population Genetics of Landrace Barley (*Hordeum vulgare*)

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Abstract

Barley (*Hordeum vulgare*) is a major crop, grown worldwide and in a wide range of climatic conditions. Despite its importance as a crop species, little is known about the population genetics of barley and the effects of bottlenecks, adaptation, and gene flow on genetic diversity within and between landrace populations. In areas with highly developed agriculture, such as Northern Europe, these types of genetic studies are hampered by lack of landraces preserved in situ or ex situ. Here, we report a genetic study of Swedish landrace barley using 113-year-old seed samples. The results demonstrate differing levels of variation with some latitudinal effect. We also detect clear population differentiation and population structure within Sweden into a southern and a northern cluster. These results possibly reflect different introduction routes of barley into Sweden. We thus show that the study of historic material can be an important alternative for regions where no or little extant landrace material is available.

Key words: barley, population structure, population differentiation, diversity, aged DNA, landrace.

Introduction

Barley (*Hordeum vulgare*) is one of the major crop species in the world; it is important as an animal feed crop as well as for human consumption, both directly and through malting. Barley is believed to have been domesticated about 10,000 years ago (Badr et al. 2000; Salamini et al. 2002) possibly multiple times (Morrell and Clegg 2007; Saisho and Purugganan 2007). From the Fertile Crescent, barley subsequently spread across Eurasia, becoming adapted to a wide range of climates ranging from tropical regions to north of the Arctic Circle (Bothmer et al. 2003). At least two separate distribution routes of barley into Europe are well established by archeological evidence, and Jones, Leigh, et al. (2008) suggest that the genotyping of European landraces could further resolve the spread of barley cultivation.

Landrace barley has been described as being locally adapted and more genetically variable than modern cultivars (Fischbeck 2003). Kolodinska Brantestam et al. (2007), comparing barley from different time periods from the Nordic and Baltic countries, found little decrease of genetic diversity over time in the region as a whole. However, when comparing barley from northern and southern regions separately, they could show a decrease in diversity in the southern material during the 20th century. Similar patterns of loss of genetic diversity between samples collected at different times or cultivars of differing ages have been observed elsewhere in the world (Khlestkina et al. 2004; Russell et al. 2004; Malycheva-Otto et al. 2007), whereas other reports suggest little or no loss of genetic diversity (Koebner et al. 2003; Ordon et al. 2005).

Most studies of genetic variation in barley so far have focused on single representatives of sets of cultivars, whereas population-based studies with multiple representatives of the same population (or cultivar) are mostly confined to wild barley (Baek et al. 2003; Hübner et al. 2009). Kolodinska Brantestam et al. (2007) used multiple individuals from modern and landrace cultivars but did not report any intrapopulation analysis. Little is therefore known about within-population diversity in domesticated barley.

Some of the studies of genetic variation in barley indicate clear geographic structure. Malycheva-Otto et al. (2006) could show how modern European cultivars differed genetically from those from the rest of the world. On a finer scale geographic structure has been found within both Himalayan and Spanish landraces (Pandey et al. 2006; Yahiaoui et al. 2007).

For the past 100 years, barley has been subjected to modern plant breeding, leading to the development of genetically uniform elite cultivars with improved yield, quality, and pest resistance traits (Fischbeck 2003). However, breeding has also resulted in the loss of many of the landrace cultivars grown throughout most of human history. In Europe, most landraces were replaced by modern cultivars already in the 1920s (Fischbeck 2003; Jones, Lister, et al. 2008), and the remaining landraces, now preserved in gene bank depositories, provide poor representation of the once cultivated material. Much of the landrace material available in today's gene banks originates from mountainous or otherwise remote areas with no or few cultivars available from main agricultural areas such as northern Europe (Jones,
The integrity of cultivars preserved in gene banks has also been questioned (Börner et al. 2000; Parzies et al. 2000; Chebotar et al. 2002).

A valuable complement to gene bank material is historic specimens conserved at museums or in herbaria. These can be trusted to be bona fide representatives of the species cultivated at the time of collection, and the origin and age of the samples may be well documented, although in other instances information like dating, cultivar name, and growth location is vague or lacking (Jones, Lister, et al. 2008). The major downfall of using herbarium material is, however, the limited amount of material usually available, effectively preventing performing studies on a population level.

The KSLA (Royal Swedish Academy of Agriculture and Forestry) seed collection is an exceptional resource for population genetic studies of agricultural species. Collected in the late 19th and early 20th centuries, before or during early plant improvement, it consists of more than 3,300 seed samples representing 582 agronomically important species (Leino et al. 2009). The seeds have been stored in sealed glass containers, most of them labeled with harvest year, name of cultivar (if applicable), and growth location. Although germinability is lost, DNA can be extracted and analyzed, and the amount of seed of each sample not only means a high likelihood of the sample being representative of the cultivar but also allows for genetic studies on a population scale (Leino et al. 2009). Historic material has been used for population studies in both plants and animals (Flagstad et al. 2003; Cozzolino et al. 2007). However, such studies are hampered by the number of individuals that can be sampled from each population and time point.

In Sweden, barley has been cultivated for at least 5,000 years (Hjelmqvist 1955). Historically, six-row barley has been the most common type, but around the time the KSLA collection was compiled, two-row barley was being introduced in the southern parts of Sweden. Today two-row barley is the most common type except for the northernmost areas where six-row barley is still preferred. We have conducted a population genetic study of 15 populations of Swedish landrace barley cultivated in 1896 and collected “on farm,” with a particular focus on the Norrbotten region in northern Sweden. The northernmost part of the world where barley is being grown and landraces once cultivated here can be expected to have been subjected to strong bottlenecks during their migration north, as well as selection for adaptation to the climate of northern Scandinavia. Although geographic structure has been detected on this scale in barley before (Pandey et al. 2006; Yahiaoui et al. 2007), this has only been done for regions with a good representation of landrace material in gene banks, and there are few, if any, studies of within-population diversity in any barley material. With the combined effects of migration and selection on landrace barley in Sweden and in particular the Norrbotten region, the patterns of genetic diversity should be very different from the patterns in regions closer to the species’ origin or with a climate more similar to that of where barley originated. In this study, we demonstrate the levels of inter and intrapopulation variation in barley and show evidence of geographic structure in Swedish landrace barley.

Materials and Methods

Plant Material

Twelve, or in 2 cases 11, seeds were analyzed from each of 15 populations (samples) of barley, chosen to give a good geographical representation of Sweden (fig. 1, table 1). The populations were chosen to also range from a countrywide geographic scale down to a within-village scale, with a particular focus on the Norrbotten region in northern Sweden (populations Pajala, Kengis, Matarengi, Voullerim, Vuono, Kurrokveik, and Sandön). Seeds from the harvest of 1896 were collected “on farm” and used in germinability assays. The remaining parts of the seed samples were thereafter stored in sealed glass containers (fig. 2) at KSLA until 1963 when they were donated to the Swedish Museum of Cultural History where they have been kept since. Each container holds between 3,000 and 3,300 seeds. Although the original sampling procedure is unknown, we believe that the samples are representative for the populations cultivated in the field and that the effect from sampling is negligible. We also included in the study the modern cultivar ‘Rolfi’ (provided by SW Seed, Svalöv, Sweden) recommended for present-day barley cultivation in northern Sweden.

DNA Analysis

DNA was extracted from single seeds using the FastDNA Spin Kit and the FastPrep Instrument (MP Biomedicals, Solon, OH). With each extraction series, a negative control was performed in parallel, and DNA extraction was carried out in a separate laboratory from that used for subsequent analysis.

Fourteen microsatellite markers (EBmag0705, Bmag0770, Bmag0870, Bmag0135, Bmag0378, Bmag0384, Bmag0749, AF043094A, Bmag0613, Bmag0828, Bmag0387, Bmag0718, Bmag0740, and EBmac0827), two for each chromosome, taken from Varshney et al. (2007) were amplified using polymerase chain reaction (PCR). Forward primers were modified with M13-tails, and PCR amplification was run in two separate rounds, the first with modified forward primers and reverse primers, the second with a fluorescently labeled (FAM or HEX) M13 forward primer and the marker’s reverse primer. PCRs were run in 20 μl reactions containing 0.5 U Taq polymerase (New England Biolabs), 1× New England Biolabs ThermoPol buffer, 0.1 μM each of forward and reverse primers and 0.25 μM of each deoxynucleoside triphosphates (dNTP). One microliter of total DNA template was used for the first round of PCR, and 3 μl of the first PCR reaction was used as template for the second round of PCR.

Overall, poor DNA quality can be a cause of amplification failure, as can the presence of null alleles, where alleles fail to amplify due to mutations in the primer site. To minimize random failure, nonamplifying PCR reactions were repeated with the same annealing temperature and also using a 48°C annealing temperature in the first PCR to accommodate for some mutation in the primer site.
Amplification products were analyzed by capillary gel electrophoresis and confocal laser scanning on a MegaBACE 1000 DNA Analysis System using a 350-bp ROX labeled internal size marker. Sizing of fragments was performed using the software MegaBACE Fragment Profiler 1.2.

Genotyping of Vrs1

The three causative mutations (E152 > F.S., F75 > L, and A40 > F.S) in Vrs1 segregating six-row barley from two-row barley (Komatsuda et al. 2007) were PCR amplified in all populations using the primer pairs Vrs1a1F1 (5’-TGC ACA TCT GCA GTT CCT CT-3’), Vrs1a1R1 (5’-GTG TCC GCC ATC CAC CTT-3’), Vrs1a2a3R1 (5’-GGC GGC CAG ATA CAC CT-3’). PCRs were run in 20 µl reactions containing 1 U DreamTaq polymerase (Fermentas), 1× DreamTaq buffer, 0.1 µM each of forward and reverse primers and 0.2 µM of each dNTP. One to two microliters of total DNA template was used for a first round of PCR, and 2 µl of the first PCR reaction was used as template for a second round of PCR. Amplified products were sequenced (Eurofins MWG Operon, Ebersberg, Germany) using primer Vrs1a1F1 or Vrs1a2a3F1.

Statistical Analysis

Results were analyzed for genetic subdivision by estimating $F_{ST}$ of Wright (1951) using the software Arlequin (ver 3.1) (Excoffier et al. 2005). $F_{ST}$ values were estimated for pairwise populations and also between all Norrbotten populations and populations from the rest of Sweden (excluding Gälltofta). Locus-specific $F_{ST}$ was estimated between ‘Rolfi’ and the Norrbotten populations and ‘Rolfi’ and Gälltofta. A null distribution of $F_{ST}$ values assuming no population subdivision was obtained by coalescent simulation using the software SIMCOAL (version 1.0) (Excoffier 2004) followed by estimation of $F_{ST}$ values in Arlequin. Ten thousand simulations of a single population were run with mutation values ranging from $10^{-3}$ to $10^{-5}$, and population sizes of 10,000 and 1,000 individuals were also simulated. The distribution yielding the highest $F_{ST}$ values, being most conservative for our conclusions (mutation rate $10^{-4}$ and population size 10,000 individuals) was used to obtain significance levels for the $F_{ST}$ values observed in our data set.

Principal component analysis was carried out using the software R (R Development Core Team 2007) using the prcomp command. Allele frequencies for each allele at each locus were calculated for the populations analyzed and treated as independent variables in the analysis.

The software Structure v 2.2 (Pritchard et al. 2000; Falush et al. 2003) was used to analyze the results for geographic clustering. As an inbreeder barley is largely homozygous, and we therefore used a haploid setting. We further used a model with correlated allele frequencies among populations with no admixture. Nonamplifying markers were treated as missing data, but we also repeated the analysis treating nonamplifying markers as a separate “null” allele. The software was run with a burn-in length of 50,000 iterations followed by 20,000 iterations for estimating the parameters. This was repeated 10 times for each $K$ (the number of predetermined clusters) ranging from two to six. The software CLUMPP v1.1 (Jakobsson and Rosenberg 2007) was used to compare the results of individual runs and to calculate similarity coefficients and the average matrix of ancestry. In CLUMPP, the FullSearch algorithm was used for comparing runs with $K = 2$, whereas the Greedy algorithm was used for higher Ks. Graphical representation of the results was obtained using the DISTRUCT v 1.1 software (Rosenberg 2004).

**Fig. 1.** Location of populations included in the study.
Results

We have studied the population genetics of landrace barley using DNA from 19th century seeds. Our study populations included one modern six-row barley cultivar, ‘Rolfi’, 14 six-row barley populations and one two-row barley population, Gälltofta (see further below). Microsatellite markers were successfully amplified for all individuals, and in total, we amplified 14 microsatellite loci in 189 individuals from 16 populations. We have used the resulting data set to evaluate within- and between-population variation in landrace barley and to shed light on barley population structure in 19th century Sweden.

Diversity

All genotyped individuals were homozygous for all loci. The total number of alleles at a locus ranged from 2 to 28. Within a population, the number of alleles ranged from one to eight. For most, but not all loci, ‘Rolfi’ had the lowest number of alleles and was monomorphic (Table 1). The number of pairwise identical individuals was low (two or less) for all populations except ‘Rolfi’ indicating that the studied individuals originate from different ears and can be considered a representative sample of their populations of origin. It further shows that the populations studied consist of a large number of different genotypes. We note, however, that failed amplification could limit our power to detect identical individuals and that the number of identical individuals in our populations may be slightly higher.

Although the number of individuals analyzed from each population was not identical, which makes direct comparisons of allele number difficult, the difference of one individual in sample size should have little effect on the outcome. Diversity across all loci, measured by Nei’s $h$ (Nei 1973), ranged from 0.104 for Gälltofta to 0.368 for Assmundstorp (Table 1). ‘Rolfi’ unexpectedly did not have the lowest diversity (although one of the lowest). This was not due to a single differing individual but to low-frequency alleles present in different individuals at different loci. Populations from the Norrbotten region (Pajala, Kengis, Matarenge, Voullerim, Vuono, Kurrokevik, and Sandön) had on average lower diversity (0.194) than populations from the rest of Sweden (0.240) but not significantly so.

Table 1. Origin and Genetic Diversity of Studied Populations.

<table>
<thead>
<tr>
<th>Label</th>
<th>Modern Name</th>
<th>Cultivar Status</th>
<th>$N$</th>
<th>Latitude</th>
<th>Longitude</th>
<th>County of Origin</th>
<th>Average No Alleles</th>
<th>Nei’s $h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kengis</td>
<td>Modern</td>
<td>11</td>
<td></td>
<td>67°10’60”N</td>
<td>23°30’0”O</td>
<td>Norrbotten</td>
<td>1.429</td>
<td>0.106</td>
</tr>
<tr>
<td>Pajala</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>67°12’45”N</td>
<td>23°22’0”O</td>
<td>Norrbotten</td>
<td>1.500</td>
<td>0.106</td>
</tr>
<tr>
<td>Matarenge</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>66°23’13”N</td>
<td>23°39’15”O</td>
<td>Norrbotten</td>
<td>1.786</td>
<td>0.180</td>
</tr>
<tr>
<td>Wuono</td>
<td>Vuono</td>
<td>12</td>
<td></td>
<td>65°51’10”N</td>
<td>23°9’18”O</td>
<td>Norrbotten</td>
<td>2.429</td>
<td>0.277</td>
</tr>
<tr>
<td>Wuollerim</td>
<td>Vuollerim</td>
<td>12</td>
<td></td>
<td>66°25’44”N</td>
<td>20°37’23”O</td>
<td>Norrbotten</td>
<td>2.429</td>
<td>0.258</td>
</tr>
<tr>
<td>Kuovikoair</td>
<td>Kurrokevik</td>
<td>12</td>
<td></td>
<td>66°3’5”N</td>
<td>17°53’11”O</td>
<td>Norrbotten</td>
<td>2.538</td>
<td>0.252</td>
</tr>
<tr>
<td>Sandön</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>65°32’50”N</td>
<td>22’24’2”O</td>
<td>Norrbotten</td>
<td>2.567</td>
<td>0.146</td>
</tr>
<tr>
<td>Sundby</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>64°17’39”N</td>
<td>21°13’22”O</td>
<td>Västernorrland</td>
<td>1.571</td>
<td>0.143</td>
</tr>
<tr>
<td>Brattbäcken</td>
<td>Landrace</td>
<td>11</td>
<td></td>
<td>64°14’36”N</td>
<td>15°52’0”O</td>
<td>Västernorrland</td>
<td>1.714</td>
<td>0.179</td>
</tr>
<tr>
<td>Ramvik</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>62°49’9”N</td>
<td>17°51’23”O</td>
<td>Västernorrland</td>
<td>2.750</td>
<td>0.333</td>
</tr>
<tr>
<td>Toppmyra</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>59°44’51”N</td>
<td>17°33’11”O</td>
<td>Uppsala</td>
<td>1.714</td>
<td>0.207</td>
</tr>
<tr>
<td>Hylkebo</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>56°35’17”N</td>
<td>15°51’34”O</td>
<td>Kalmar</td>
<td>2.583</td>
<td>0.330</td>
</tr>
<tr>
<td>Nya Skottorp</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>56°27’2”N</td>
<td>13°0’22”O</td>
<td>Halland</td>
<td>2.727</td>
<td>0.355</td>
</tr>
<tr>
<td>Assmundstorp</td>
<td>Landrace</td>
<td>11</td>
<td></td>
<td>57°46’51”N</td>
<td>11°55’51”O</td>
<td>Bohuslän</td>
<td>2.583</td>
<td>0.368</td>
</tr>
<tr>
<td>Gälltofta</td>
<td>Landrace</td>
<td>12</td>
<td></td>
<td>55°58’58”N</td>
<td>14°18’58”O</td>
<td>Skåne</td>
<td>1.625</td>
<td>0.104</td>
</tr>
</tbody>
</table>

Fig. 2. Barley samples from the Norrbotten region stored in their glass containers.
Table 2. \( F_{ST} \) Values Comparing Individual Loci of Modern and Landrace Material.

<table>
<thead>
<tr>
<th>Locus</th>
<th>'Rolfi' versus Norrbotten</th>
<th>'Rolfi' versus Gälltofta</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBmag0705</td>
<td>1.000***</td>
<td>1.000***</td>
</tr>
<tr>
<td>Bmag0770</td>
<td>-0.007</td>
<td>0.905***</td>
</tr>
<tr>
<td>Bmag0870</td>
<td>0.731***</td>
<td>0.033</td>
</tr>
<tr>
<td>Bmag0135</td>
<td>0.008</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bmag0378</td>
<td>n.a.</td>
<td>1***</td>
</tr>
<tr>
<td>Bmag0384</td>
<td>0.006</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bmag0749</td>
<td>0.319***</td>
<td>0.033</td>
</tr>
<tr>
<td>AF043094A</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bmag0613</td>
<td>0.155*</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bmag0828</td>
<td>n.a.</td>
<td>0.901***</td>
</tr>
<tr>
<td>Bmag0387</td>
<td>0.129*</td>
<td>0.455***</td>
</tr>
<tr>
<td>Bmag0718</td>
<td>0.310***</td>
<td>0.615***</td>
</tr>
<tr>
<td>Bmag0740</td>
<td>0.314***</td>
<td>0.388**</td>
</tr>
<tr>
<td>Bmag0827</td>
<td>0.956***</td>
<td>0.917***</td>
</tr>
</tbody>
</table>

*Significant at \( P = 0.05 \), **significant at \( P = 0.01 \), and ***significant at \( P = 0.001 \).

(Population Differentiation)

'Rolfi' was developed using material from Norwegian, Finnish, and Swedish landraces, including the landrace 'Brändö' from Luleå in Norrbotten. To compare 'Rolfi' with barley from this region, we calculated \( F_{ST} \) values between 'Rolfi' and the Norrbotten populations. Most loci had fairly low \( F_{ST} \) values. Two loci, EBmag0705 and EBmac0827, showed very high differentiation, whereas Bmag0870 showed somewhat lower differentiation (table 2). These loci may represent regions where foreign material has been bred into cultivars from northern Sweden. 'Rolfi' also has two-row barley in its pedigree. Comparing 'Rolfi' with the two-row population, Gälltofta showed that the region surrounding the loci Bmag0870 and Bmag0749 in 'Rolfi' might have its origin from two-row barley. These loci showed high \( F_{ST} \) when compared with Norrbotten populations (0.731 and 0.319, respectively) but low when compared with Gälltofta (0.033 for both loci) (table 2).

One of the main aims of this study was to investigate population structure in 19th century landrace barley. We calculated pairwise \( F_{ST} \) values between populations (table 3). Average \( F_{ST} \) across loci ranged from being slightly negative in some comparisons to 0.958 between Gälltofta and Brattbäcken. In general, \( F_{ST} \) values tended to be lower for comparisons between Norrbotten populations (average 0.205) and between populations from outside Norrbotten (average 0.347, excluding Gälltofta) than when pairwise comparing Norrbotten populations with those from the rest of Sweden (average 0.568). All pairwise comparisons across the Norrbotten border were highly significant, whereas those between Norrbotten populations or between populations from outside of Norrbotten often had lower significance values or were not significant. Comparing all Norrbotten populations combined with the combined set of populations from rest of Sweden (excluding Gälltofta) gave a moderately high (0.357) and highly significant value of \( F_{ST} \) (\( P < 0.001 \)). In general, populations located more closely to each other tended to have lower \( F_{ST} \) values than more distantly located populations, consistent with an isolation-by-distance scenario but not in all cases. For example, \( F_{ST} \) is drastically lower (and not significant) between Assmundstorp and Sundby, 870 km apart, than between Sundby and Sandön (highly significant), only 160 km apart, but across the Norrbotten border (table 3).

The morphology of the seeds from Gälltofta suggested that this population was two-row barley in comparison to the other populations that were six-row barleys. To confirm the row phenotype in all populations, we genotyped the three identified causative mutations in the \( Vrs1 \) locus a (Komatsuda et al. 2007). All populations except Gälltofta had a deletion in \( E152 > F.S. \) resulting in a six-row phenotype. In the loci \( F75 > L \) and \( A40 > F.S. \), no differences between populations were found. Gälltofta, being confirmed as a single two-row barley population, was subsequently removed from all further analysis of population structure. We also removed 'Rolfi' from the analysis of population structure as it is not of landrace origin.

Population Structure

To further investigate population structure, principal component analysis was performed for the 14 six-row landrace populations (fig. 3). The first two principal components accounted for 36% of the genetic variance. As suggested by the \( F_{ST} \) values, Toppmyra was found to differ from the other landrace populations. The first two principal components for the rest of the populations showed a high correlation with their geographical origin, though less so for the Norrbotten populations. The Norrbotten populations were clearly separated from populations from the rest of Sweden, but populations from the rest of Sweden were also clearly separated from each other suggesting geographical effects on gene flow.

We also analyzed our data for geographic clustering with the Structure software. When assuming two clusters, we found clear separation of populations from Norrbotten from those of the rest of Sweden (fig. 4). Adding a third cluster did not improve the resolution (supplementary fig. S1, Supplementary Material online), but indicated separate clustering of Toppmyra (supplementary fig. S2, Supplementary Material online). Analyses of subsets of the data were performed to further elucidate any population structure, but we could not find any further structure neither for the Norrbotten populations only nor for the subset of populations from the rest of Sweden.

In conclusion, both principal component and structure analysis supported our \( F_{ST} \) values and showed one clear cluster
for Norrbotten populations and one cluster for the rest of Sweden with some separation of the population Toppmyra.

**Discussion**

Landrace barley is generally described as being very heterogeneous. However, very few studies have been conducted to estimate the within-population diversity by means of molecular markers. Barley from Sardinia has been studied using isozymes and random amplified polymorphic DNA (Papa et al. 1998), and Demissie et al. (1998) used restriction fragment length polymorphism markers to characterize Ethiopian landraces. Both these studies found high levels of within-population diversity in comparison to between-population diversity. The authors of these studies concluded that within these areas a very active seed exchange and mixture must occur. Our results partly contrast this conclusion with fairly modest levels of diversity within populations. For example, the populations from Kengis and Gälltofta had as low diversity as ‘Rolfi’, which contrary to our expectations, was not monomorphic. The low diversity of Kengis is surprising as it has clearly lower diversity than the Pajala population from the same village.

Six-row barley has historically been the dominating type in Sweden with two-row barley being introduced in the southern parts of the country around the time the KSLA collection was compiled. Today six-row barley is only preferred in the northernmost parts of Sweden with two-row barley dominating elsewhere. Clear genetic differentiation between two- and six-row barley has been shown in several studies (e.g., Malysheva-Otto et al. 2006, 2007; Kolodinska Brantestam et al. 2007; Yahiaoui et al. 2007). When selecting our study populations, none of the samples was known to be of the two-row type. However, Gälltofta showed clear differentiation from other populations in terms of $F_{ST}$ values and seed morphology. Genotyping of the Vrs1 locus confirmed the two-row origin of Gälltofta. If Gälltofta is an early developed two-row variety imported to Sweden, that may explain its low diversity.

The Norrbotten populations tended to have on average fewer alleles than more southern populations, though only slightly. Norrbotten populations also had a lower Nei’s $h$ than populations from the rest of Sweden but not significantly so. Although the trend is in agreement with a scenario where more northern populations have been subjected to more severe bottlenecking during their migration north, there is no clearcut geographical trend. We can thus find no severe bottleneck effect of the spread of barley cultivation across Sweden. It is possible that seed trade hasameliorated any bottleneck effect of spread of barley cultivation, but unlike previous studies (Demissie et al. 1998; Papa et al. 1998), we find in many cases, moderate or even high genetic isolation between populations.

We find clear evidence of population structure in our sample. Most evident is the separation of populations from Norrbotten and from those from the rest of Sweden evident in both the structure analysis, principal component analysis, and from $F_{ST}$ values. Historical sources (reviewed
by Lyttkens 1911–1915) mention “stjärnkorn” (star barley) as a separate barley type grown in northernmost Sweden. Stjärnkorn is characterized by uniformly distributed kernels on the spike in comparison to regular six-row barley. We were unable to distinguish between stjärnkorn and normal six-row barley based on morphological seed characters, and as no unthreshed spikes are preserved, we cannot tell if the Norrbotten populations were of the stjärnkorn type. Interestingly, the division of a southern and a northern group is similar to that found in some wild species (see Taberlet et al. 1998 and references therein), where postglacial colonization of Scandinavia has occurred both from the south and the north through Finland. The Torneåalen area (the origin of the populations Pajala, Matarengi, Vuono, and Kengis) is known to have been colonized from Finland and a close relationship exists between domestic animal breeds in northern Fennoscandia (Engelmark R, personal communication). Stjärnkorn is also called “lappkorn” (Lapp­onian barley) or “finnkorn” (Finnish barley) (Lyttkens 1911–1915), which further suggests a Finnish origin.

‘Rolfi’ was developed for cultivation in high-latitude regions and originated in a cross between the cultivars ‘Arra’ and ‘Nord.’ The pedigrees of these cultivars mostly end up in landraces from northern Finland, Sweden, and Norway. However, ‘Nord’ also contains Swedish two-row barley in its pedigree. We could identify possible two-row components in the ‘Rolfi’ genome by comparing the locus specific $F_{ST}$ values between ‘Rolfi’, two-row barley and six-row barley from Norrbotten. Interestingly, ‘Rolfi’ is of the stjärnkorn type, perhaps a heritage from its northern ancestors.

In addition to the major clusters (Norrbotten and the rest of Sweden), our $F_{ST}$ values, principal component
and clustering analysis also suggest that the Toppmyra population differ to some extent from the other populations. Toppmyra is located not far from the Royal Academy of Agriculture in Stockholm and the Agricultural College in Uppsala. Both institutions were active in testing foreign cultivars during the 19th century (Lange 2000). Seed exchange with either of these institutions may have occurred and be responsible for introducing new genetic material. For two of the loci studied, Toppmyra had alleles not present in any other populations, possibly supporting a partly foreign origin.

We successfully amplified markers in all individuals, but many individuals also failed to amplify one or more markers. Although the degradation of DNA with time is well known and also visible in barley from the KSLA collection (Leino et al. 2009), the nonrandom distribution of nonamplifying markers suggests that null alleles has contributed to PCR failure in our sample. The presence of null alleles is a well-known phenomenon among microsatellites (Dakin and Avise 2004). It has been shown that the frequency of null alleles increases as the study population becomes more distantly related to the focal population where the microsatellites were developed (e.g., Li et al. 2003). Kolodinska Brantestam et al. (2007) studying Nordic barley with microsatellite markers found null alleles among cultivars with 4 of the 19 markers used. As we have studied populations ancestral to modern barley where the markers were originally developed, we should expect null alleles to be present in at least some of our loci. If the levels of null alleles are comparable with those found in other studies (Hearnden et al. 2007; Kolodinska Brantestam et al. 2007) two to three markers should exhibit null alleles. As historic material will, by its very nature, often differ from the material where genetic markers originally were developed, the presence of null alleles should be considered in studies such as this. Thus, studies of aged DNA will face the dual challenge of degraded DNA and marker systems developed in modern material, both causing PCR amplification to fail. The exact cause of failure will in many cases be difficult to elucidate.

If nonamplifying samples in our data set are treated as separate alleles (“null alleles”) rather than missing data, additional population structure appear. In the Norrbotten sample, structure analysis suggests a clustering of samples from the Torndalen area (Pajala, Matarengi, Vuono, and part of Kengis populations) (fig. 5a). Similar treatment of the populations outside of Norrbotten indicates a clustering of the more southern populations Assmundtorp, Nya Skottorp, and Hylkebo (fig. 5b). Should the missing data be due to null alleles rather than poor-quality DNA, we can thus identify population structure on an even finer scale.

Conclusions

The geographic structuring of populations detected in this study is on a similar scale as has been detected in other studies of barley landrace material from Spain (Yahiaoui et al. 2007) and the Himalayas (Pandey et al. 2006), both regions where well-documented landrace material is abundant. Lack of gene bank holdings of North European landraces has previously rendered any fine-scale population study in this region impossible, and using modern cultivars only large-scale structure has been shown (Malyshova-Otto et al. 2006). The present study shows how historical material can be used to resolve genetic structuring on a fine geographical scale in regions where landrace material is largely absent. Furthermore, the present study detected geographic structure using far fewer markers than in previous studies. This suggests, together with the structure indicated by analyzing potential null alleles, that it will be possible to detect further population structure with the use of
additional markers and more populations. Further analysis of populations outside of Sweden, in particular from Finland and the continent, is also needed to shed light on the role of different introduction routes of barley into Sweden.

Supplementary Material
Supplementary figures S1 and S2 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

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