Theor Appl Genet (2012) 125:57–70
DOI 10.1007/s00122-012-1816-8

ORIGINAL PAPER

Diversity of North European oat analyzed by SSR, AFLP and DArT markers

Xinyao He · Åsmund Bjørnstad

Received: 20 January 2011 / Accepted: 5 February 2012 / Published online: 18 February 2012

Abstract Oat is an important crop in Nordic countries both for feed and human consumption. Maintaining a high level of genetic diversity is essential for both breeding and agronomy. A panel of 94 oat accessions was used in this study, including 24 museum accessions over 100- to 120-year old and 70 genebank accessions from mainly Nordic countries and Germany, covering different breeding periods. Sixty-one polymorphic SSR, 201 AFLP and 1056 DArT markers were used to evaluate the past and present genetic diversity of the Nordic gene pool. Norwegian accessions showed the highest diversity, followed by Swedish and Finnish, with German accessions the least diverse. In addition, the Nordic accessions appeared to be highly interrelated and distinct from the German, reflecting a frequent germplasm exchange and interbreeding among Nordic countries. A significant loss of diversity happened at the transition from landraces and old cultivars to modern cultivars. Modern oat originated from only a segment of the landraces and left the remainder, especially black oat, unused. However, no significant overall diversity reduction was found during modern breeding periods, although fluctuation of diversity indices was observed. The narrow genetic basis of the modern Nordic gene pool calls for increasing genetic diversity through cultivar introduction and prebreeding based on neglected sources like the Nordic black oat.

This paper is dedicated to Professor emeritus, Dr. Agric. Knut Aastveit, Chair and Professor of Plant Breeding in the Agricultural University of Norway 1968–1988 and a leading force in Nordic plant breeding, on the occasion of his 90th birthday December 12th, 2011.

Communicated by A. Graner.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-012-1816-8) contains supplementary material, which is available to authorized users.

X. He · Å. Bjørnstad (✉)
Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway
e-mail: asmund.bjornstad@umb.no

Introduction

Oat is a minor cereal in global terms, contributing only 0.93% to the total cereal production (FAOSTAT Data 2009). In the cool temperate areas of the northern hemisphere, however, it is a major crop, with Canada, Russia and the Nordic countries as the most important producers. In the latter region it has a history of more than 2,000 years since it became a secondary domesticate in fields of barley and emmer, due to its superior performance in marginal climatic and edaphic conditions. As an excellent horse feed, it was actively promoted by the Roman cavalry in 'Germania' and then 'Britannia', where it became a staple food for Celts (Moore-Colyer 1995).

In the Nordic countries and Northern Europe it became a well-established crop both for food and feed. Mostly, oat was a crop for marginal lands or came last in rotations. Hull colour was a major criterion for variety classification, mainly black, yellow, grey or white. In Sweden, white oat was predominant in the south and west, and black oat was dominant along the east coast. The many black oat varieties had a specific phenology adapted to early summer drought, but there were also landraces suited for intensive management and varieties that matured very early and could be grown in the far north (Mattsson 1997; Olsson 1997).

Around 1900 about 50% of the Swedish cereal crop grown was oat (Jordbruksstatistisk årsbok 2000). Due to the gradual disappearance of horses from agriculture and

transportation services, the importance of oat declined steadily. In 1908 in Germany, oat was next only to rye in importance, but suffered an even stronger decline (Funke 2008). Today, in the broad zone from eastern Norway to Finland, oat is an essential part of the crop rotation with no diseases in common with barley or wheat. In the southernmost areas of Scandinavia, the survival of winter oat would be an agronomic benefit and would make the crop more competitive with winter wheat or rye (Brautigam et al. 2005). In spring oat, the main current challenges in crop improvement are *Fusarium* resistance and quality improvement (e.g. hull percentage and oil content).

Due to its importance, breeding of oat started early in Sweden (Mattsson 1997). In the Swedish Seed Association in Svalöv, starting from 1888 Hjalmar Nilsson did mass and pure line selection in the landraces, but had limited progress (Olsson 1997). Breeding based on introduced varieties was much more successful, especially using the landrace named after the Probsteier estate in Schleswig–Holstein. According to Atterberg (1887a), this was widely distributed in southern and central Sweden and the 'highest yielding of oat forms' due to its large grains with two seeds per spikelet or three on well-fertilised soils. The first release was 'Swedish awnless probsteier-oat' in 1897. Later, pure line selection in the American Probsteier source Milton resulted in the landmark cultivars Gullregn (Gold Rain) in 1903 and Seger (Victory) in 1908. The breeder was H. Nilsson-Ehle, who also initiated recombination breeding through numerous German × Swedish crosses. After this initial burst in breeding and research, the decline in oat acreage led to less intensive breeding, focusing to a large extent on quality and yield. MacKey (1994) showed that yield potential of Swedish oat was virtually unchanged from 1910 to 1960. Since then yield potential increased due to renewed emphasis on harvest index and lodging resistance.

Moreover, in early German breeding Probsteier was an outstanding parental line, but additionally Milton (and its Svalöv derivatives), Ligowo from the Pyrenees and Lochow's Gelbhafer (Yellow Oat) were important in breeding (Funke 2008). The latter became a parent of the first cultivars that were obtained by crossing, notably Flämingsgold, Flämingstreue and a series of later successful cultivars (Bickelmann 1989).

The most extensive diversity study of Nordic oat to date analyzed 64 cultivars and 17 landraces using 4 quantitative traits and 7 SSRs (Nersting et al. 2006). The study found that recent lines were shorter, earlier and had a higher harvest index, but were unchanged in thousand grain weight. The diversity of these traits showed a decline in the pure line era (1898–1920), which was restored through recombination breeding, only to face a similar decline in the past decades. The four polymorphic SSRs used in the study showed a clearly reduced diversity over time. In addition, Achleitner et al. (2008) analyzed AFLP diversity in 114 oat accessions, with 74 originating from Europe (of which 13 from Nordic countries) and 40 from outside Europe, and the results demonstrated a much lower diversity in European accessions than in North and South American accessions. Furthermore, in a recent paper on DArT development in oat (Tinker et al. 2009), the 28 Nordic entries had conspicuously lower diversity than the North American germplasm.

All of these studies indicated a limited diversity in Nordic oat. The competitiveness of Nordic oat in terms of yield, quality and product development depends on continued breeding progress, to which a systematic evaluation of Nordic oat diversity is a prerequisite. However, previous studies were confined either to the limited markers that may not have whole-genome coverage (Nersting et al. 2006) or to the limited number of accessions from Nordic countries (Buerstmayr et al. 2007; Tinker et al. 2009).

The present investigation addresses both historical and regional trends, analyzing a set of 24 museum samples as well as 70 genebank accessions from mainly Sweden, Finland, Norway and Germany spanning more than 120 years, using three different molecular maker techniques.

Materials and methods

Plant materials

A panel of 94 oat accessions was included in this study (Tables 1, 2). The museum materials used to assess the diversity at the onset of the formal breeding era were 24 accessions of a 'collection of typical forms of oats' (*Sammlung typischer Haferformen*) compiled by Dr. Albert Atterberg, head of the Chemical Institute of Kalmar (*Kalmars kemiska anstalt*), in 1891 and supplemented with 4 new in 1908 (Table 1). The samples were in a box with 24 glass containers, each having a label denoting the name and classification identifier. Dr. Atterberg recognised 7 morphological classes in oat based on panicle and seed morphology, seed colour and number of seeds per spikelet. Some documentation is available in publications describing their origins (Atterberg 1887b) and 'an attempt at their classification' (Atterberg 1887a). The papers give a glimpse of the existing landrace diversity and a very active exchange and testing of cultivars from all over northern Europe from France to Russia. Some accessions with the same names are available in the collections of NordGen (Nordic Genetic Resources Centre), such as the plump-seeded 'Potato oat' from the UK. These museum seeds allow a glimpse into the genetic diversity of Nordic oats of more than

Table 1 Museum samples from the Atterberg collection used in this study

Accession name[a]	Country of origin	Age group[b]	Year of release
Kl. 1a. *Gefle svarta Spethafre* (Black sharp oat from Gävle)	Sweden	Museum	Landrace
Kl. 1b. *Grå Spethafre (Skogbohafre) fr. Halland* (Grey sharp oat from Halland (Skogbo))	Sweden	Museum	Landrace
Kl. 1b. *Ölandshafre fr. trädgårdsjord* (Oat from garden soil in Öland)	Sweden	Museum	Landrace
Kl. 2. *Nordisk Hvithafre fr. Mora* (Nordic white oat from Mora)	Sweden	Museum	Landrace
Kl. 2. *Spethafrelik Gråhafre ur Hvithafre fr. Dalarne* (Sharp oat-like grey oat from Dalarna)	Sweden	Museum	Landrace
Kl. 4. *Svart Plymhafre fr. Dalarne* (Black one sided panicle oat from Dalarna)	Sweden	Museum	Landrace
Kl. 4. *Svart Risphafre fr. Östergötland* (Black broad panicle oat from Östergötland)	Sweden	Museum	Landrace
Kl. 7. *Wissefjerda Trindhafre* (Wissefjerda plump oat)	Sweden	Museum	Landrace
Kl. 2c. *Probstejerhafre fr. Stockholm* (Probstejerhafre from Stockholm)	Sweden	Museum	<1892
Kl. 2b. *Championhafre* (Champion oat)	Sweden	Museum	<1894
Kl. 6. *Excelsiorhafre* (Excelsior oat)	Sweden	Museum	<1894
Kl. 2c. *Ligowohafre* (Ligowo oat)	Sweden	Museum	1894
Kl. 2c. *Hvitlinghafre* (Hvitling oat)	Sweden	Museum	1897
Kl. 2b. *Stormogulhafre* (Stormogul oat)	Sweden	Museum	1901
Kl. 2c. *Guldregnhafre* (Goldrain oat)	Sweden	Museum	1903
Kl. 6. *Norsk New-Zeelandshafre fr. Trondhjem* (Norwegian New-Zealand oat from Trondheim)	Norway	Museum	<1894
Kl. 7. *Norsk Potatoëhafre* (Norwegian Potato oat).	Norway	Museum	<1894
Kl. 2. *Nordfinsk Svarthafre fr. Umeå* (North Finnish black oat from Umeå)	Finland	Museum	Landrace
Kl. 5. *Spetskornhafre fr. Wiborg, Finland* (Sharp oat from Wiborg, Finland. (Now Russia))	Finland	Museum	Landrace
Kl. 6. F*insk Kubbhafre* (Finnish plump oat)	Finland	Museum	Landrace
Kl. 3. *Grå engelsk Vinterhafre* (Grey English winter oat)	UK	–	<1894
Kl. 7. *Potatoëhafre fr. England* (Potato oat from England)	UK	–	<1894
Kl. 5. *Österikisk Spetskornhafre, Öppen form* (Austrian sharp oat. Open type).	Austria	–	<1894
Kl. 3. *Rysk Gulhafre fr. Kurland* (Russian yellow oat from Kurland (Latvia))	Latvia	–	<1894

[a] Both the original (in Swedish) and the translated names are presented, and the serial numbers that precede the names are classification identifiers made by Dr. Atterberg (1887a)

[b] Only Nordic accessions were classified

100 years ago (in a few cases like 'Grey English Winter Oat' probably included as a morphological class).

Additionally to the 24 museum accessions, 70 genebank accessions were sourced from NordGen, Graminor (a Norwegian plant breeding company) and Dr. Bernd Rodemann, Julius Kühn-Institut. In the genebank materials, we deliberately included accessions with the same names as those in the museum materials, such as Ligowo and Probsteier, for comparisons. The others were selected to cover major landraces and cultivars released in both different historic periods and different countries (Table 2), with an attempt to make comparable group sizes. A set of more recent German cultivars were included to compare German and Nordic gene pools more than 75 years after the period of active germplasm exchange. In addition, one cultivar from Netherlands once widely grown in Norway and two breeding lines from the US were also included as references from the DArT study (Tinker et al. 2009). The two US accessions, Z595-7 and Z615-4, were half-sib lines with 50% *A. sterilis* parentage (Holland et al. 2000).

Pedigree data of the accessions used in this study are available in a supplementary table (Table S1), which were obtained mostly from the Canadian Pedigrees of Oat Lines (POOL) database (Tinker and Deyl 2005). Other sources used for information on pedigree were breeder's websites (e.g. Graminor), the literature and personal communications.

DNA extraction

Despite their intact appearance, the museum seeds were not viable after 100 years of storage, but the DNA quality was good enough for PCR (Leino et al. 2009). Two morphologically representative seeds of each accession were manually dehulled and ground with mortar and pestle in liquid nitrogen and sand. The powder was then subjected to DNA extraction using E.Z.N.A SP Plant DNA Miniprep Kit following the manufacturer's instructions (Omega Bio-Tek, Norcross, GA). For the genebank accessions, fresh leaf tissues were harvested from 2-week-old single plant progenies and DNA extracted in the same way.

Table 2 List of the 70 genebank accessions used in this study

Accession name	Country of origin[a]	Year of release	Age group[b]	Source
Probsteier	Sweden	<1892	Old	NordGen
Ligowo	Sweden	1894	Old	NordGen
Hvitling	Sweden	1897	Old	NordGen
Stormogul I	Sweden	1901	Old	NordGen
Guldregn I	Sweden	1903	Old	NordGen
Seger I	Sweden	1908	Old	NordGen
Fyris	Sweden	1911	Old	NordGen
Hvit Odal	Sweden	1926	Old	Graminor
Klock Extra	Sweden	1933	Old	NordGen
MK 5-1050 'Sol II'	Sweden	1942	Old	NordGen
Selma	Sweden	1968	Medium	Graminor
Sang	Sweden	1974	Medium	NordGen
Svea	Sweden	1976	Medium	Graminor
Kerstin	Sweden	1988	Medium	NordGen
Freja	Sweden	1991	New	NordGen
Matilda	Sweden	1994	New	NordGen
Belinda	Sweden	1998	New	Graminor
Cilla	Sweden	1998	New	Graminor
SW Betania	Sweden	2004?	New	B. Rodemann
Aveny	Sweden	2008	New	NordGen
Beiar	Norway	Landrace	Old	Graminor
Hird	Norway	<1940	Old	Graminor
Gråkall	Norway	1972	Medium	Graminor
Pol	Norway	1974	Medium	Graminor
A4066	Norway	1980?	Medium	Graminor
A4013	Norway	1980?	Medium	NordGen
Moholt	Norway	1982	Medium	Graminor
Kapp	Norway	1986	Medium	Graminor
Martin	Norway	1988	Medium	Graminor
Grane	Norway	1992	New	Graminor
Bikini	Norway	1997	New	Graminor
Hurdal	Norway	2005	New	Graminor
Nudist	Norway	2007	New	Graminor
Ringsaker	Norway	2008	New	Graminor
Odal NK	Norway	2009	New	Graminor
Ylitornio	Finland	Landrace	Old	NordGen
Rajala	Finland	Landrace	Old	NordGen
N Finnish	Finland	Landrace	Old	NordGen
Puhti	Finland	1978	Medium	NordGen
Veli	Finland	1981	Medium	Graminor
Virma	Finland	1988	Medium	NordGen
Yty	Finland	1989	Medium	NordGen
Sisko	Finland	1993	New	NordGen
Aarre	Finland	1995	New	NordGen
Roope	Finland	1996	New	NordGen
Fiia	Finland	2002	New	Graminor
Steinar	Finland	2009	New	Graminor
Jumbo	Germany	1976	–	B. Rodemann
Alf	Germany	Unknown	–	B. Rodemann
Heinrich	Germany	Unknown	–	B. Rodemann
Flämingsstern	Germany	Unknown	–	B. Rodemann
Neklan	Germany	1997	–	B. Rodemann
Aragon	Germany	Unknown	–	B. Rodemann
Flämingsglanz	Germany	Unknown	–	B. Rodemann
Nelson	Germany	1973?	–	B. Rodemann
Atego	Germany	1996	–	B. Rodemann
Dominik	Germany	Unknown	–	B. Rodemann
Kaplan	Germany	Unknown	–	B. Rodemann
Typhon	Germany	2005	–	B. Rodemann
Flämingsfit	Germany	2005	–	B. Rodemann
Robinson	Germany	Unknown	–	B. Rodemann
Scorpion	Germany	Unknown	–	B. Rodemann
Mozart	Germany	Unknown	–	B. Rodemann
Bessin	Germany	Unknown	–	Graminor
Rasputin	Germany	Unknown	–	B. Rodemann
Eugen	Germany	Unknown	–	B. Rodemann
Pure line potato oat	UK	<1850	–	NordGen
Mustang	Netherlands	1971	–	Graminor
Z595-7	US	1990?	–	Iowa
Z615-4	US	1990?	–	Iowa

[a] Mozart, Neklan and Atego were originated from Czech Republic and Eugen from Austria, but they were all treated as German cultivars in this paper since they were marketed in Germany

[b] Age group: old (cultivars released before 1950 and landraces), medium (cultivars released between 1960 and 1990), new (cultivars released after 1991). Only Nordic accessions were classified into age groups

Molecular marker assay

A set of 216 SSR markers published by Becher (2007) were screened for polymorphism in 24 accessions selected from a wide range of historic periods and countries, and 43 informative SSRs were chosen for genotyping. SSR analysis was performed with fluorescently labelled primers (Schuelke 2000), and the PCR system and the cycling programme followed Becher (2007). All PCRs were performed in an MJ Research Tetrad 2 thermal cycler (MJ Research, Waltham, Mass.). PCR products were subjected to capillary electrophoresis on an ABI 3730 Gene Analyzer and electropherograms were analyzed using GENEMAPPER version 4.0 (Applied Biosystems, Foster City, CA).

For the AFLP assay, the protocol used in Gannibal et al. (2007) was adopted, but instead of *EcoRI*, *Pst*I was used as

digestion enzyme. AFLP products were analyzed in the same way as described for the SSRs. Forty-two *Mse*I and *Pst*I AFLP primer combinations were screened for polymorphism using the 24 accessions and 16 primer pairs were selected for genotyping the entire set of accessions.

DArT markers were genotyped by Diversity Arrays Technology Pty. Ltd., Canberra, Australia (http://www.DiversityArrays.com), using the strategy as described in Tinker et al. (2009). Only the 70 genebank accessions were genotyped, due to DNA degradation of the 24 museum accessions during the non-frozen 7-day shipping to Australia.

Data analyses

The co-dominant SSR data were transformed into dominant data by treating each polymorphic peak as a single locus and coded by 1 (presence) or 0 (absence), and then combined with the AFLP data to create one dataset.

The molecular marker data were analyzed at both population and individual levels. For the former, the plant materials were classified either by country of origin or by year of release (Tables 1, 2). Five country groups were generated, i.e. Norway, Sweden, Finland, Germany and Other (including the accessions from other countries), while four age groups were set up, i.e. Museum (the 24 Nordic accessions from Dr. Atterberg), Old (landraces and cultivars released before 1950), Medium (accessions released between 1961 and 1990) and New (accessions released after 1991). Since this study focused on Nordic materials, only Nordic accessions were included in age groups. However, since 15 accessions in the Museum group were Swedish in origin, the distribution of the countries within each age group was not equal (Tables 1, 2).

The genetic diversity within each group was measured by Shannon's information index (Shannon 1948), the genetic distances among categorical groups were indicated by Nei's distance (Nei 1973), and the variation within and among populations was analyzed by AMOVA (Excoffier et al. 1992). All the indices were calculated using GenAlEx ver. 6.4 (Peakall and Smouse 2006). In addition, based on Nei's genetic distances, principal component analysis (PCA) plots were drawn, using *The Unscrambler* ver. X 10.0.1 (CAMO Software AS, Oslo, Norway), and dendrograms were drawn by UPGMA using DARwin software ver. 5.0.158 (Perrier and Jacquemoud-Collet 2006). The PCA calibration model was validated with a leave-one-sample-out procedure (Geladi and Kowalski 1986), and from the calibration and validation R^2 curve plots with increasing number of principal components (PCs). While the first PC always increased, the latter had a breakpoint, stabilised or even declined, indicating overfitting. This allowed the identification of a subset of markers mainly contributing to the validated PCs, which was based on the 'Correlation loadings' plot, where the PCA loadings were standardised into correlations ranging from $+1$ to -1 (Fig. 1a). An ellipse crossed the PC dimensions at ± 0.7, and for markers outside this, more than 50% of the variation ($0.7^2 \times 100 \approx 50$) was explained by the PCs (Bjornstad et al. 2004). In the plot such markers with high loadings were manually selected for each validated PC and the PCA rerun (Fig. 1a). The optimised plot was compared with the full model and its robustness visualised through stability ('fuzz') plots (Fig. 1b), where less perturbation indicated an improved model.

Results

The 43 SSRs used for genotyping detected 61 polymorphic loci of which 39 were co-dominant and 22 were dominant. Twenty-four of the co-dominant markers appeared biallelic, while the other 15 were multi-allelic, with a highest allele number of six. In 35 instances (found in 23 accessions by 14 SSRs, Table S3) an SSR marker appeared to be heterozygous, with 28 from the genebank and 7 from the museum accessions, accounting for only 1.0 and 0.7% of the co-dominant data points in the two groups, respectively. The 16 primer pairs for AFLP genotyping amplified 201 polymorphic peaks with strong signal, which were then combined with the binarized SSR data to form an SSR_AFLP dataset comprising 333 markers. The binarization and combination treatments did not cause major change in diversity profile (Table S2). As for DArTs, 1,056 loci were found to be polymorphic in the 70 genebank accessions.

For SSR genotyping, 18% of the data points were missing for the museum accessions, probably due to DNA degradation, since only 4% were missing for the genebank accessions. For AFLPs, the problem was less due to the rapid processing from genomic DNA to PCR products, with the proportions 9% in the museum and 3% in the genebank accessions.

Optimization of PCA plots

The validation R^2 curve in all cases indicated that most of the variation was explained by the first two PCs. The correlation loadings plot allowed the markers with the highest loadings (outside the inner ellipse) to be identified (Fig. 1a). Based on this criterion, the model was optimised and became more stable. Taking the PCA using DArTs as an example (Figs. 1, 2), the variations explained by the first two PCs were 13 and 9% before the optimization procedure

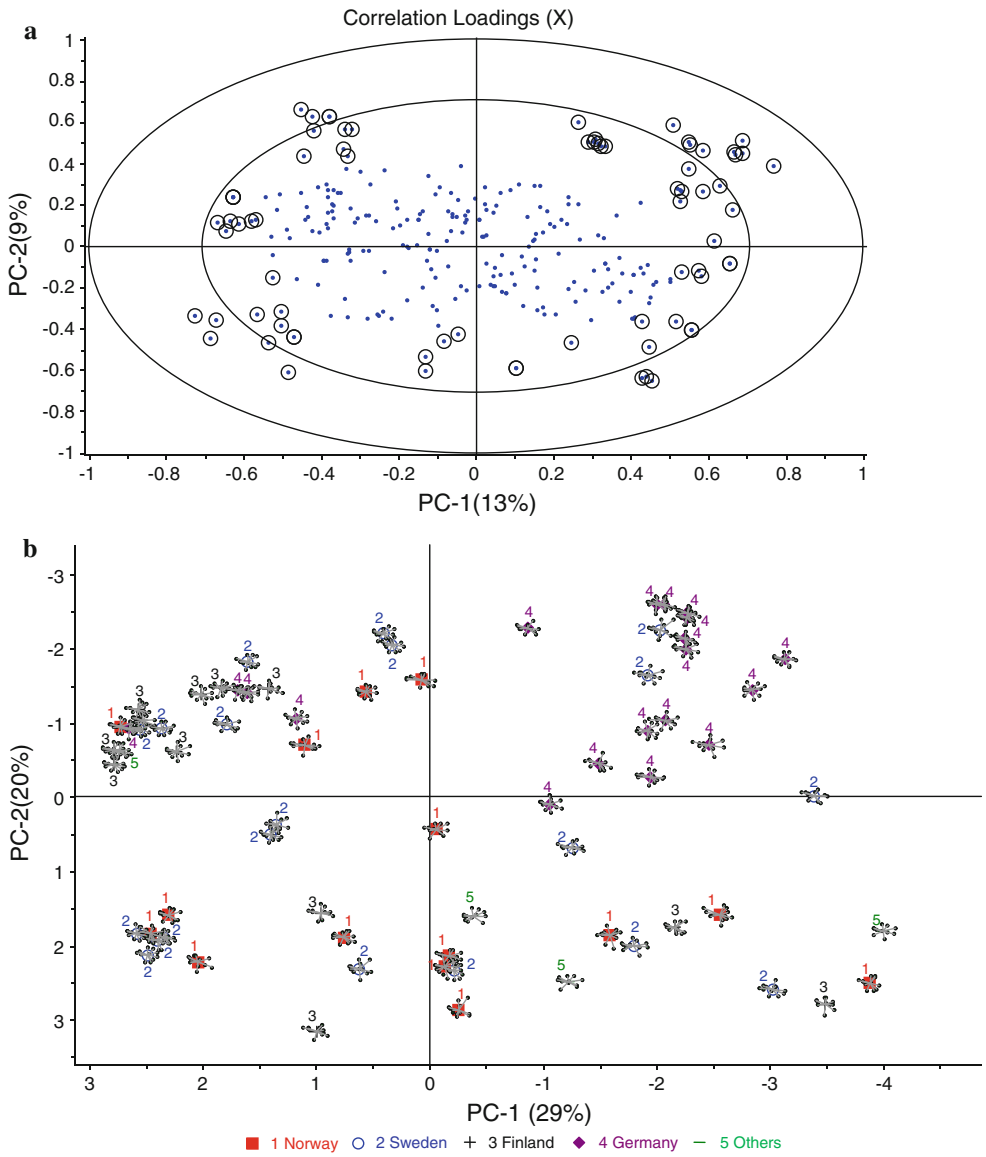

Fig. 1 Correlation loadings plot of the 1,056 DArTs, with the 67 markers with higher loadings selected (**a**), and the stability test for the PCA plots of oat accessions grouped by country of origin using the 67 most informative DArTs (**b**)

and increased to 29 and 20% when only the 67 most informative markers associated with the validated PCs were used. The same pattern remained, except that the sub-clusters became more clear in the optimised model (Fig. 1b) than in the model containing all markers (not shown).

Genetic diversity of oat from different countries

The SSR_AFLP and DArT markers detected similar genetic diversities among and within different country groups. Within groups the Shannon's index detected most SSR_AFLP variation in the other group, with Germany as the least diverse. DArT markers detected most variation in Norway (Table 3). To test whether these differences were caused by the inclusion of the museum accessions in the SSR_AFLP dataset, the indices were re-calculated using only the genebank accessions. The results were then similar to that of the DArT dataset and the ranking order in diversity of the three Nordic groups was identical (data not shown).

For the three Nordic groups, the distribution of age groups within each country group was not even, especially when Museum and Old accessions were included (Tables 1, 2), which may lead to biased results. To address this problem, SSR_AFLP and DArT datasets were merged

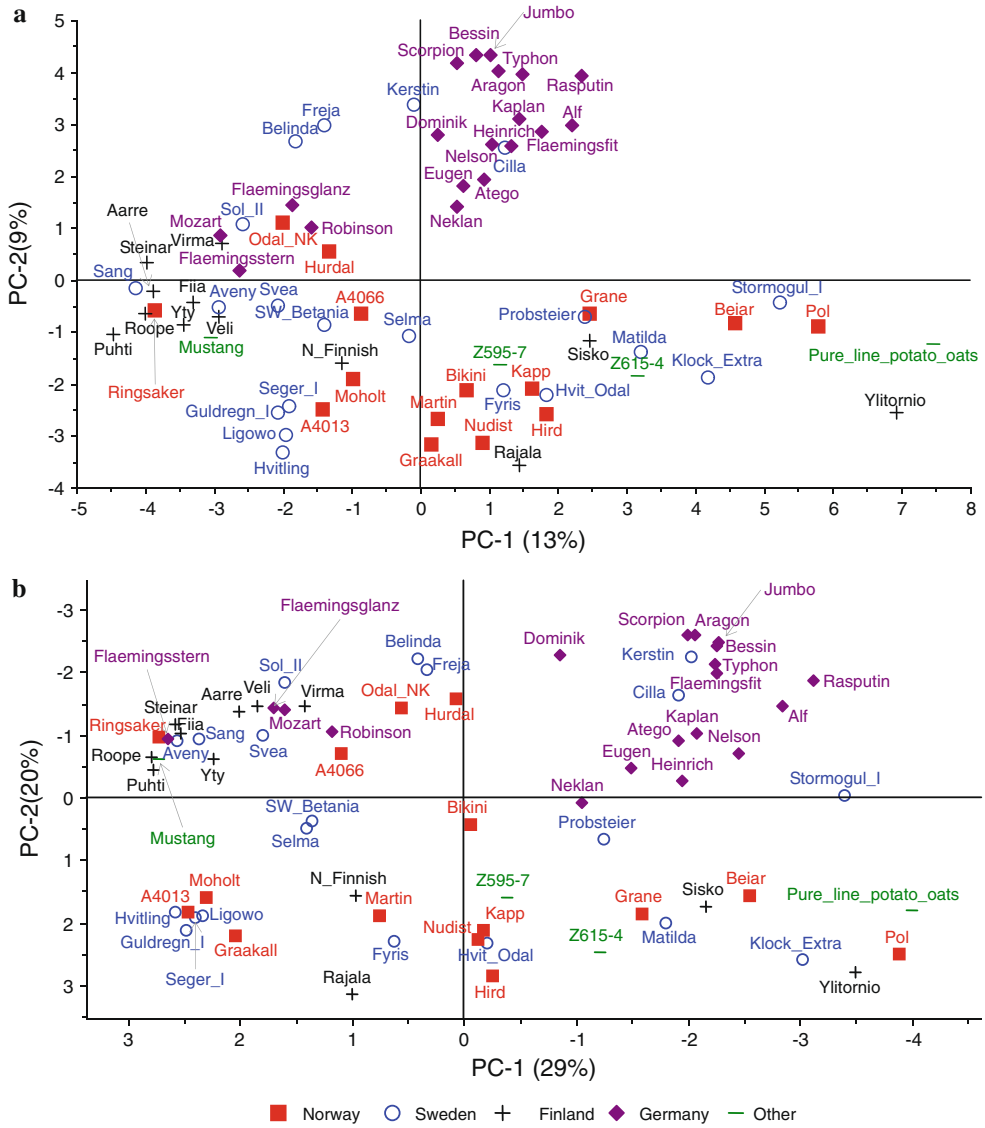

Fig. 2 PCA plots of DArTs for oat accessions grouped by country of origin using all the 1,056 markers (**a**) and the 67 most informative markers (**b**)

together for calculating diversity indices of country groups containing only Medium_New or New accessions. Similar results were obtained (data not shown).

Among country groups the two datasets were also similar, i.e. the three Nordic groups clustered together, keeping clear distances from both Germany and Other groups (Fig. 3a). However, a larger part of variation was found among country groups using DArTs than using SSR_AFLPs (Table 4). Similarly, the pair wise Φ_{pt} comparisons did not reveal significant differences among the three Nordic groups for SSR_AFLPs, but were highly significant for DArTs (Table 3). When only New accessions were compared using the complete dataset, the Nordic groups were not significantly different from each other, making them appear like a single gene pool. This can also be inferred from the pedigree data, which indicated a high frequency of germplasm exchange among Nordic countries (Table S1). The Germany group was always significantly different from Nordic groups with respect to Φ_{pt} (Table 3).

The two PCA plots generated by SSR_AFLPs (not shown) and DArTs (Fig. 2b) showed a similar pattern: most German accessions clustered together, while the Nordic accessions tended to mix with each other. The same trends can also be observed in the dendrogram (Fig. 4). To test whether the low diversity in German accessions was caused by lack of landraces and old cultivars, a comparison was made between German and Nordic accessions from the same period, which indicated a narrow diversity in German

Table 3 Comparisons of genetic diversity indicators of oat originating from different countries

Country	SSR_AFLPs					DArTs				
	No.	Pol. loci[a]	No. uniq.[b]	I[c]	Φ_{pt}^{d}	No.	Pol. loci	No. uniq.	I	Φ_{pt}
Norway	17	279	4	0.39	a	15	889	8	0.43	a
Sweden	35	309	7	0.43	a	20	921	8	0.40	b
Finland	15	251	2	0.35	a	12	766	0	0.35	c
Germany	19	189	3	0.26	b	19	582	8	0.27	d
Other	8	256	2	0.43	–	4	719	5	0.42	–

[a] Polymorphic loci

[b] Number of unique allele, referring to the number of alleles that only found in a specific group

[c] Shannon's information index

[d] Φ_{pt} grouping, different letters indicate highly significant differences between the two groups ($P < 0.01$)

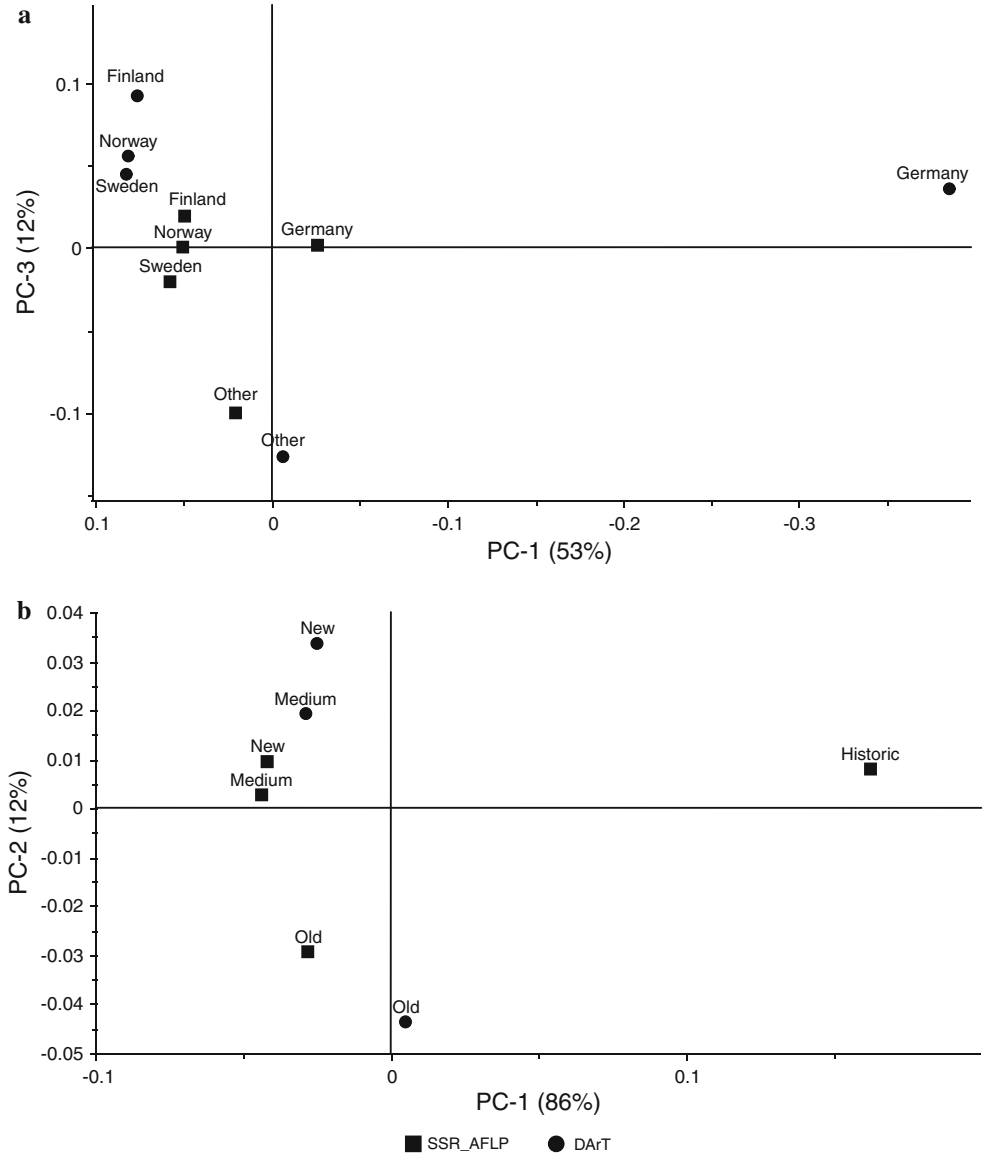

Fig. 3 Principal coordinate analyses for the country groups (**a**) and the age groups (**b**) using SSR_AFLP and DArT markers. In plot **a**, PC-3 was used instead of PC-2, since the latter separates marker groups instead of country groups

Table 4 Analyses of molecular variance (AMOVA) for the distribution of variation among and within categorical groups detected by SSR_AFLPs or DArTs

Marker method	Country groups		Age groups	
	SSR_AFLP	DArT	SSR_AFLP	DArT
Among groups (%)	5.8	31.3	14.8	3.3
Within groups (%)	94.2	68.7	85.2	96.7
P values	<0.01	<0.01	<0.01	<0.01

lines (data not shown), in accordance with the previous results (Table 3; Fig. 2).

Genetic diversity of Nordic oat from different breeding periods

The SSR_AFLP marker set gave evidence of a strong decrease in genetic diversity from Museum to Old and then to Medium periods, with some increase in diversity in the New period. DArTs (which did not include the museum accessions) showed the same trend (Table 5). Apart from the Shannon's index, the 'Number of polymorphic loci' and 'Number of unique alleles' also indicated a clear drop in the Medium group, which had no unique DArT alleles (Table 5). When the two oldest groups were merged, 39 alleles unique to this group were found, which accounted for more than 10% of the total loci, in sharp contrast to the 4 unique alleles in the Medium group and 6 in the New group.

The same picture emerged based on AMOVA with the significantly higher diversity in the Museum group (Table 5).

In the PCA plot (SSR_AFLPs), the first principal component distinguished a big cluster mostly of museum accessions plus one accession from Old (Stormogul I) and one from Medium (Pol). The three museum accessions in the lower-left quadrant clustered as expected with their counterpart genebank accessions (Fig. 5). However, Probsteier from NordGen and the museum Probstejerhafre appeared very different from each other (Fig. 5; Table 6) The position of Pol, a very early North Norwegian cultivar, corresponded to its outlier position in the DArT plot by Tinker et al. (2009) (Fig. 5).

For comparison (results not shown), we regressed the SSR_AFLP dataset on (binarized) age groups using partial least squares (PLS), which allows significant markers to be rigorously identified based on the regression coefficients. In the optimised model the validation and calibration curves *both* explained 25%, showing that PC1 could be fully accounted for by $\sim$10% of the markers in the dataset. Indeed, by selecting manually among these to represent the clusters, as few as a dozen could account for the pattern, with only limited loss in R^2.

Discussion

DNA extracted from museum oat materials

Extracting genomic DNA from ancient materials has been studied extensively. Isolation of PCR-amplifiable DNA from 4,700-year-old maize (Goloubinoff et al. 1993), 3,300-year-old emmer wheat (Allaby et al. 1994) and 2,900-year-old barley (Palmer et al. 2009) has been reported. Compared with these ancient materials, 120-year-old oat seeds are almost 'fresh', and indeed, the AFLP profiles between the museum and the genebank accessions were quite comparable, with no problems in data scoring. As we suspected that DNA samples from the museum accessions might be unstable, samples were divided into small aliquots and kept in the freezer until use to prevent further degradation. The frozen condition could not be maintained during the shipping to Australia for the DArTs analysis and the DNA of these samples degraded. Despite this, the genotyping of the museum accessions was both reliable and reasonable as shown by the clustering or closeness of the accessions with the same names from the museum and the genebank groups (Fig. 5). The genetic differences of the museum Probstejerhafre with its genebank counterpart were probably not caused by DNA degradation but by the diversity present within a landrace. The Atterberg samples were clearly selected 'type specimens' not supposed to represent the diversity in the landrace and the same name was often used for different landraces. Atterberg (1887a, b) described a number of landraces called Probsteier, from which 17 pure line varieties have been selected directly or indirectly (Zade 1918, cited in Funke 2008). The possibility of non-authenticity could not be ruled out (van de Wouw et al. 2011), in which either the museum or genebank Probsteier accession was wrongly labelled.

After this study was completed, the total Atterberg collection of 168 accessions of 'botanical types' was rediscovered in Sweden (Leino 2011), providing a further chance to investigate the oat gene pool of a century ago.

Markers in diversity detection and in PCA optimization

In the current study, SSR_AFLP and DArT datasets generated similar diversity profiles and there was no apparent difference among marker types in discovering genetic variation.

According to Tinker et al. (2009), DArT markers have a wide distribution within the oat genome, although highly clustered, similar to the pattern in wheat reported by Semagn et al. (2006). The very large number of DArTs used in the present study made it reasonable that most chromosomal regions were represented by at least one DArT marker, capturing genetic variation without significant

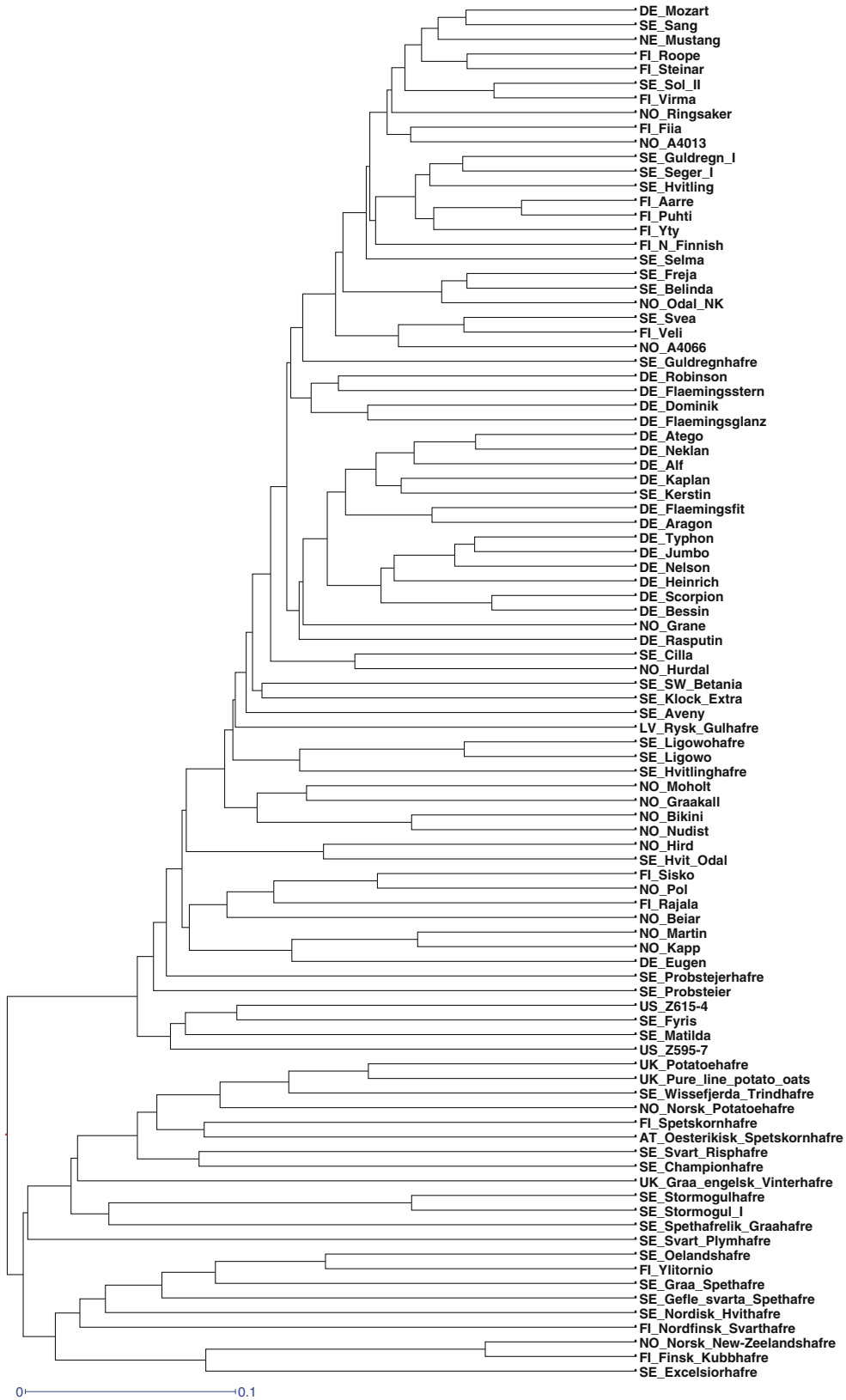

Fig. 4 Dendrogram of the 94 accessions calculated by the unweighted pair group method (UPGMA) using the SSR_AFLP dataset. For each accession, the country of origin was denoted by a prefix, i.e. *AT* stands for Austria, *DE* Germany, *FI* Finland, *LV* Latvia, *NE* The Netherlands, *NO* Norway, *SE* Sweden, *UK* the United Kingdom, *US* the United States

Table 5 Genetic diversity indicators of Nordic oat over different breeding periods

Age group	SSR-AFLPs					DArTs				
	No.	Pol. loci[a]	No. uniq.[b]	I[c]	Φ_{pt}[d]	No.	Pol. loci	No. uniq.	I	Φ_{pt}
Museum	20	283	23	0.45	a	–	–	–	–	–
Old	15	244	2	0.35	b	15	911	25	0.44	a
Medium	15	202	4	0.29	bc	15	784	0	0.37	ab
New	17	230	6	0.30	c	17	910	13	0.40	b

[a] Polymorphic loci

[b] Number of unique allele, referring to the number of alleles that only found in a specific group

[c] Shannon's information index

[d] Φ_{pt} grouping, different letters indicate highly significant differences between the two groups ($P < 0.01$)

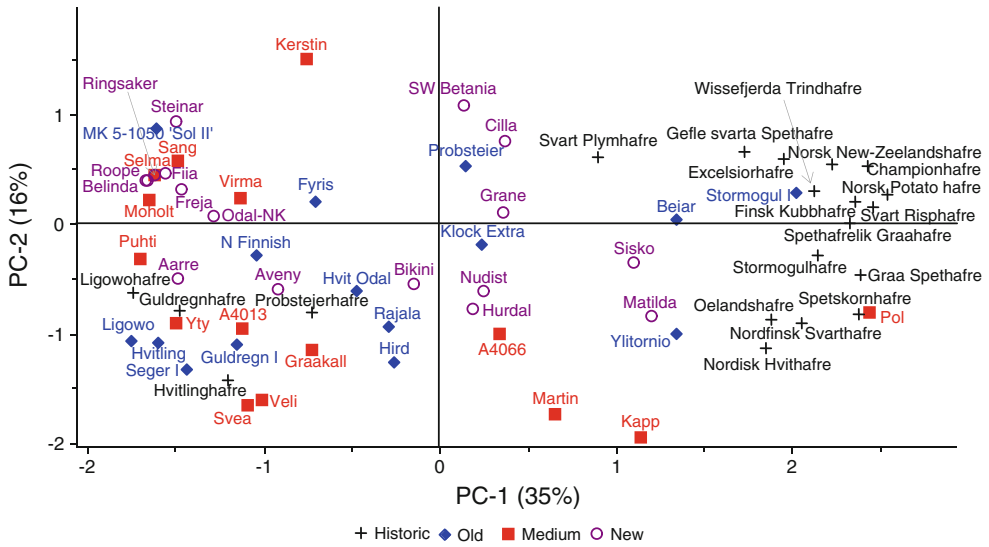

Fig. 5 PCA plot of Nordic oat accessions grouped by breeding periods, using the 28 most informative SSR_AFLP markers

Table 6 Similarities, calculated as the ratio of identical markers of all scored markers of the synonymous museum versus genebank accessions using the complete dataset

	Similarity
Potatohafre vs. Pure line potato oat	0.854
Potatohafre vs. Norsk potatoehafre	0.808
Norsk potatoehafre vs. Pure line potato oat	0.771
Probsteier	0.647
Hvitling	0.881
Ligowo	0.911
Guldregn	0.884
Stormogul	0.880

bias. On the other hand, Groh et al. (2001) have shown that AFLP markers were distributed evenly on their two linkage maps, due to the adoption of a methylation-sensitive enzyme (*Pst*I), which was also used in the present study, implying an even distribution of the AFLPs within the oat genome. As for SSRs, although only 43 polymorphic SSRs were scored, no sign of clustering was observed.

The PC validation procedure and the associated marker optimization showed that a limited number of markers accounted for the PCs. Basically the pattern was one of two validated PCs, the PC1 reflecting age groups, while PC2 mainly distinguished Germany form the Nordic. Inspection of marker clusters showed that the age groups had contrasting SSR alleles, indicating that the selected markers represented alleles unique to or predominant in the age groups. From the existing maps we did not try to identify which markers may be chromosomally linked, but inspecting the plots three-dimensionally indicated multiple clusters contributed to the differences.

Genetic diversity of oat from different countries

According to Tinker et al. (2009), the 28 Nordic accessions in their study were less diverse than the North American

germplasm. This is also reflected in the current study, in which the four genebank accessions from outside Nordic countries and Germany showed a high level of divergence (Table 3). The 10 most important ancestors of modern North American oat cultivars demonstrated a worldwide origin of the modern cultivars (Souza and Sorrells 1989). In addition, a number of crown rust resistance genes and other traits from *A. sterilis* have been introduced into the North American gene pool (Coffman 1977; Fu et al. 2007; Rines et al. 2006). With the exception of Milton, the ancestors of modern Nordic cultivars were mainly from Nordic countries and Northern Germany (Table S1). The Nordic cultivar which differs most from the other cultivars is a high-oil accession (Matilda) based on *Avena sterilis*-derived breeding lines from the US.

Among the three Nordic groups, Sweden showed the largest variation using SSR_AFLP, owing to the contribution of the 15 museum accessions. The low diversity of the Finland group may be due to a closer kinship among accessions, as inferred from pedigree data. On the other hand, the accessions in the Norway and Sweden groups appear less related, although a few frequently used early maturing parental lines may be observed, such as Gråkall and Voll. Seen as a whole, the three Nordic groups are highly related and distinct from Germany and Others (Fig. 3a), confirming the frequent germplasm exchange among the three Nordic countries as is evidenced from known pedigrees. Both marker and pedigree data confirm the limited diversity in Nordic oat observed by Tinker et al. (2009).

German accessions showed consistent lower diversity in this study. According to Bickelmann (1989), most German cultivars can be traced back to two ancestral cultivars, Flämingsgold and Flämingstreue, which are sister lines from a same cross. With the shrinking of oat acreages in the last century, oat breeding in Germany also decreased, leading to a probable loss of genetic diversity (Achleitner et al. 2008; Frese et al. 2009).

German varieties played an important role during the early breeding in Sweden, most notably the variety Probsteier. The later cultivars Hvitling, Seger and Guldregn were selected from the Milton derivative of Probsteier, making it the cornerstone for modern Nordic oat breeding (Åkerman et al. 1938; Coffman 1977; Mattsson 1997). Our marker data confirm this historical picture. German varieties appear less used as parents during the modern breeding period, while some Dutch cultivars have been used (Mattsson 1997). This relative germplasm isolation resulted in the genetic distinctness between the German and Nordic gene pools (Fig. 3a), which, together with the geographical closeness and the climate similarity, makes German varieties a good resource for well-adapted germplasm in Nordic oat breeding.

Genetic diversity of oat over different breeding periods

Genetic erosion due to modern breeding has long been a cause of concern. Studies on temporal trends of genetic diversity show different or even conflicting results (van de Wouw et al. 2010b). Specific to oat, Nersting et al. (2006) reported the reduction in diversity of modern Nordic varieties measured by SSRs and directional changes in agronomic characters. Fu et al. (2003) also found a significant decrease of SSR diversity in Canadian cultivars released after 1970. The same set of 96 Canadian cultivars did not show significant diversity reduction when AFLPs were applied (Fu et al. 2004). These results are not necessarily conflicting for three reasons. First, the numbers of SSRs used in the two former studies were very limited (only 7 and 11 loci, respectively). Second, different populations may exhibit different diversity trends, due to the local breeding strategies and germplasm conservation efforts (van de Wouw et al. 2010b). Third, even unchanged diversity levels may conceal that old alleles are replaced by new ones. In the study of Nersting et al. (2006), it was found that the alleles for tall stature have been effectively removed from modern oat, and according to Frey (1998) modern US cultivars are more drought tolerant than old cultivars.

It is generally accepted that diversity has decreased in modern cultivars compared with landraces (Reif et al. 2005; Tenaillon et al. 2001; van de Wouw et al. 2010a). Moreover in the current study, genetic diversity reduced significantly between the two oldest groups, reflecting the genetic erosion at the switch from landraces or old cultivars to modern cultivars.

Before the modern breeding era, numerous landraces were grown in Nordic countries. The within-landrace variation was high (Olsson 1997). A large number of single plants from three white oat landraces were investigated by Granhall (1938, cited in Olsson 1997). He found that ~20–25% of the lines represented a distinct morphological 'biotype'. Although Dr. Atterberg may have eliminated most of the within-landrace diversity due to his purpose of showing 'typical forms', other landraces studies indicate that sampling single lines from many landraces also captures the within-landrace diversity (Bjornstad and Abay 2010). After the onset of the modern breeding era, the landraces of the black and early types, e.g. potato and club oat lines were not used in later breeding programmes. Indeed, by closer scrutiny of the second and third principal coordinates the Museum group splits into different distinct groups (results not shown). This might be the main reason for the decrease of diversity indices from Museum to Old group.

The fluctuation of diversity in the latter three age groups found in this study was also reported by Nersting et al.

(2006). In their study, Nei's diversity index began to drop from the 1941–1960 period, reached the lowest value during 1961–1980, and increased again from 1990. Recently, van de Wouw et al. (2010a) performed a meta analysis on temporal trends of crop genetic diversity, using data from 44 published papers. Although no overall substantial reduction was observed, genetic diversity decreased significantly in the 1960s, and then recovered gradually and reached a high value in 1990s, regaining the loss from the 1960s. This reflects the increased use of new diversity in breeding efforts. Our results match well with that of the meta analysis. The diversity reduction in the Medium group is probably due to the narrow foundation formed at the early stage of modern breeding, which comprised only a limited number of high performing cultivars and no black oat (Nersting et al. 2006). In the new group, a high number of new alleles were found showing the use of new diversity in breeding.

Implications for Nordic oat breeding

The genetic narrowness of Nordic cultivars inferred from pedigree and marker information in this study supports the results found in previous studies (Achleitner et al. 2008; Tinker et al. 2009), and indicates limited utilisation of non-Nordic germplasm. Thus it is advisable to enrich the Nordic oat gene pool in future breeding work, and there are several ways of reaching this goal.

Only a limited portion of the gene pool of landraces and old cultivars found their way into Nordic oat breeding. Sweden bred black oat until the 1930s, and all three countries bred extremely early northern (mostly white) oat until the 1960s, but as separate breeding programmes (Mattsson 1997). These types are known to possess favourable alleles that have not been utilised, e.g. resistance to frit fly and Mn deficiency in white oat and resistance to early summer drought in black oat (Mattsson 1997). Although quality (hull percentage) was a big hurdle in early breeding, a new look might be beneficial for modern breeding.

Increased introgression from other European countries, especially Germany, would be beneficial. Cultivars from these countries are distinct from the Nordic countries and form a well-adapted source likely to be successful.

Introgression from 'exotic' oat is usually regarded as more long term and risky. Buerstmayr et al. (2007) identified several North American varieties that showed both superior quality traits and good agronomy in Austrian and German environments, and Swedish cultivars like the current Triple Crown are grown in Canada, exhibiting a promising prospect of inter-continent introduction.

During 1990–2000, a population based on intercrossing 5 Nordic, 6 North American and 9 breeding lines from North American × *A. sterilis* backgrounds underwent six cycles of recurrent selection for yield in three environments (Norway, Iowa and Idaho). The results from the three first cycles (Holland et al. 2000, 2002) showed progress in yield, stability and many transgressive segregants in Norwegian trials, showing the potential of base broadening in Nordic oat, exhibiting a feasibility of utilising both North American and *A. sterilis* germplasms in Nordic oat breeding.

Acknowledgments This work was supported by grants from the Norwegian Research Council, Project number 178273, 'Safe grains: Mycotoxin prevention through resistant wheat and oats'. We are very grateful to Prof. H. Martens, Norwegian University of Life Sciences, for his kind help on PCA optimization, and to the anonymous referees for helpful and critical comments.

References

Achleitner A, Tinker NA, Zechner E, Buerstmayr H (2008) Genetic diversity among oat varieties of worldwide origin and associations of AFLP markers with quantitative traits. Theor Appl Genet 117:1041–1053

Åkerman Å, Granhall I, Nilsson-Leissner G, Müntzing A, Tedin O (1938) Swedish contributions to the development of plant breeding. A. Bonniers boktryckeri, Stockholm

Allaby RG, Jones MK, Brown TA (1994) DNA in charred wheat grains from the Iron Age hillfort at Danebury. Engl Antiq 68:126–132

Atterberg A (1887a) Försök till klassifikation af hafrevarieteterna efter deras kornformer. Kongl Lantbruksakademiens handlingar och tidskrift 26:135–160

Atterberg A (1887b) Kort Redogörelse öfver år 1886 insamlade och undersökta hafreprof. Kalmars Kemiska Anstalt, Kalmar

Becher R (2007) EST-derived microsatellites as a rich source of molecular markers for oats. Plant Breed 126:274–278

Bickelmann U (1989) The Pedigree of German oat cultivars (*Avena sativa* L.) and the occurrence of fatuoids. Plant Breed 103:163–170

Bjornstad A, Abay F (2010) Multivariate patterns of diversity in Ethiopian barleys. Crop Sci 50:1579–1586

Bjornstad A, Westad F, Martens H (2004) Analysis of genetic marker-phenotype relationships by jack-knifed partial least squares regression (PLSR). Hereditas 141:149–165

Brautigam M, Lindlof A, Zakhrabekova S, Gharti-Chhetri G, Olsson B, Olsson O (2005) Generation and analysis of 9792 EST sequences from cold acclimated oat, *Avena sativa*. BMC Plant Biol 5

Buerstmayr H, Krenn N, Stephan U, Grausgruber H, Zechner E (2007) Agronomic performance and quality of oat (*Avena sativa* L.) genotypes of worldwide origin produced under Central European growing conditions. Field Crops Res 101:343–351

Coffman FA (1977) Oat history, identification, and classification. Agricultural research service. US Department of Agriculture, Washington

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes—application to human mitochondrial-dna restriction data. Genetics 131:479–491

Frese L, Reinhard U, Bannier H-J, Germeier CU (2009) Landrace inventory in Germany—preparing the national implementation

of the EU Directive 2008/62/EC. In: Veteläinen M, Negri V, Maxted N (eds) European landraces: on-farm conservation, management and use. Bioversity International, Rome, pp 79–96
Frey K (1998) Genetic responses of oat genotypes to environmental factors. Field Crops Res 56:183–185
Fu YB, Peterson GW, Scoles G, Rossnagel B, Schoen DJ, Richards KW (2003) Allelic diversity changes in 96 Canadian oat cultivars released from 1886 to 2001. Crop Sci 43:1989–1995
Fu YB, Kibite S, Richards KW (2004) Amplified fragment length polymorphism analysis of 96 Canadian oat cultivars released between 1886 and 2001. Can J Plant Sci 84:23–30
Fu YB, Chong J, Fetch T, Wang ML (2007) Microsatellite variation in *Avena sterilis* oat germplasm. Theor Appl Genet 114:1029–1038
Funke C (2008) Hafer, *Avena sativa* L. Bemühungen um das ehemals wichtigste Futtergetreide. In: Röbbelen G (ed) Die Entwicklung der Pflanzenzüchtung in Deutschland (1908–2008): 100 Jahre GFP eV—eine Dokumentation. Gesellschaft für Pflanzenzüchtung, Göttingen, pp 312–320
Gannibal PB, Klemsdal SS, Levitin MM (2007) AFLP analysis of Russian Alternaria tenuissima populations from wheat kernels and other hosts. Eur J Plant Pathol 119:175–182
Geladi P, Kowalski BR (1986) Partial least-squares regression—a tutorial. Anal Chim Acta 185:1–17
Goloubinoff P, Paabo S, Wilson AC (1993) Evolution of maize inferred from sequence diversity of an Adh2 gene segment from archaeological specimens. Proc Natl Acad Sci USA 90:1997–2001
Groh S, Zacharias A, Kianian SF, Penner GA, Chong J, Rines HW, Phillips RL (2001) Comparative AFLP mapping in two hexaploid oat populations. Theor Appl Genet 102:876–884
Holland JB, Bjornstad A, Frey KJ, Gullord M, Wesenberg DM, Buraas T (2000) Recurrent selection in oat for adaptation to diverse environments. Euphytica 113:195–205
Holland JB, Bjornstad A, Frey KJ, Gullord M, Wesenberg DM (2002) Recurrent selection for broad adaptation affects stability of oat. Euphytica 126:265–274
Jordbruksstatistisk årsbok (Swedish Agricultural Statistics Yearbook) (2000) Statistiska centralbyrån (SCB), Stockholm
Leino MW (2011) Återupptäckt av en historisk havresamling (Rediscovery of a historical oat seed collection). Sven Bot Tidskr 105(1):1–4
Leino MW, Hagenblad J, Edqvist J, Strese EMK (2009) DNA preservation and utility of a historic seed collection. Seed Sci Res 19:125–135
MacKey J (1994) The history of cereal yield increase. Melhoramento 33:37–53
Mattsson B (1997) Svensk växtförädling av havre (Swedish breeding of oats). In: Olsson G (ed) Den svenska växtförädlingens historia (The history of plant breeding in Sweden). The Swedish Academy of Agricultural and Forest Sciences, Stockholm, pp 205–214
Moore-Colyer RJ (1995) Oats and oat production in history and prehistory. In: Welch RD (ed) The oat crop: production and utilization. Chapman & Hall, Landon, pp 1–34
Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323
Nersting LG, Andersen SB, von Bothmer R, Gullord M, Jorgensen RB (2006) Morphological and molecular diversity of Nordic oat through one hundred years of breeding. Euphytica 150:327–337
Olsson G (1997) Gamla lantsorter—utnyttjande och bevarande (Old landraces—utilization and conservation). In: Olsson G (ed) Den svenska växtförädlingens historia (The History of Pant Breeding in Sweden). The Swedish Academy of Agricultural and Forest Sciences, Stockholm, pp 121–130
Palmer SA, Moore JD, Clapham AJ, Rose P, Allaby RG (2009) Archaeogenetic evidence of ancient nubian barley evolution from six to two-row indicates local adaptation. Plos One 4:e6301
Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295
Perrier X, Jacquemoud-Collet JP (2006) DARwin software. http://darwin.cirad.fr/darwin
Reif JC, Zhang P, Dreisigacker S, Warburton ML, van Ginkel M, Hoisington D, Bohn M, Melchinger AE (2005) Wheat genetic diversity trends during domestication and breeding. Theor Appl Genet 110:859–864
Rines H, Molnar S, Tinker N, Phillips R (2006) Oat. In: Kole C (ed) Genome mapping and molecular breeding in plants, cereals and millets. Springer, Berlin, pp 211–242
Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234
Semagn K, Bjornstad A, Skinnes H, Maroy AG, Tarkegne Y, William M (2006) Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. Genome 49:545–555
Shannon CE (1948) A mathematical theory of communication. At&T Tech J 27:379–423 and 623–656
Souza E, Sorrells ME (1989) Pedigree analysis of North-American oat cultivars released from 1951 to 1985. Crop Sci 29:595–601
Tenaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF, Gaut BS (2001) Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp *mays* L.). Proc Natl Acad Sci USA 98:9161–9166
Tinker NA, Deyl JK (2005) A curated internet database of oat pedigrees. Crop Sci 45:2269–2272
Tinker NA, Kilian A, Wight CP, Heller-Uszynska K, Wenzl P, Rines HW, Bjornstad A, Howarth CJ, Jannink JL, Anderson JM, Rossnagel BG, Stuthman DD, Sorrells ME, Jackson EW, Tuvesson S, Kolb FL, Olsson O, Federizzi LC, Carson ML, Ohm HW, Molnar SJ, Scoles GJ, Eckstein PE, Bonman JM, Ceplitis A, Langdon T (2009) New DArT markers for oat provide enhanced map coverage and global germplasm characterization. BMC Genomics 10:39
van de Wouw M, Kik C, van Hintum T, van Treuren R, Visser B (2010a) Genetic erosion in crops: concept, research results and challenges. Plant Genet Resour-C 8:1–15
van de Wouw M, van Hintum T, Kik C, van Treuren R, Visser B (2010b) Genetic diversity trends in twentieth century crop cultivars: a meta analysis. Theor Appl Genet 120:1241–1252
van de Wouw M, van Treuren R, van Hintum T (2011) Authenticity of old cultivars in genebank collections: a case study on Lettuce. Crop Sci 51:736–746