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ORIGINAL PAPER

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Molecular diversity of local Norwegian meadow fescue (*Festuca pratensis* Huds.) populations and Nordic cultivars—consequences for management and utilisation

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Abstract Genetic diversity and relatedness were studied in 30 Norwegian local populations of meadow fescue (*Festuca pratensis* Huds.) using amplified fragment length polymorphism (AFLP) markers. The populations were also compared with 13 Nordic meadow fescue cultivars in order to analyse the distribution of variation in local populations and cultivars and to elucidate relationships between local populations and cultivars. Analysis of molecular variance (AMOVA) analysis showed that most of the variation was present within populations and that little variation was found between local populations and cultivars. Separate AMOVA analyses of local populations and cultivars revealed a higher level of variation within registered cultivars than within local populations. A cluster analysis based on corrected average pairwise differences between populations showed that the populations could be divided into three clusters, of which one also contained the cultivars. These results were supported by principal coordinates analysis. The results indicate that the Norwegian meadow fescue has a narrow genetic basis and that the local populations in Norway can be divided into three groups following the most probable routes of introduction of the species into Norway. The inland populations are closely related to the cultivars and have most probably been established as a result of migration from sown meadows. The western and southern populations probably originate from human activity—for example, trade—to the coastal western and northern parts of the country and to the central parts of southern Norway.

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Introduction

Since Vavilov (1940) first focused on the prospects of using wild relatives of crops as sources of genes for crop improvement, a great deal of effort has been expended in establishing germplasm collections of plant species. The conservation of genetic diversity of domesticated and wild crops is especially important in cases where the original gene pool of domesticated crops has been genetically eroded during years of selection and cultivation. Extensive and well-characterised collections of important species can thus provide unique raw materials for the production of new cultivars or act as sources for new traits that can be introduced into already existing breeding materials. The genetic potential of a major portion of the germplasm held in many gene banks is, however, largely unknown because it is poorly characterised. As a step towards the proper utilisation and management of gene banks, a good genetic characterisation of accessions is needed.

Meadow fescue (*Festuca pratensis* Huds.) is one of the most widely used forage grass species in the Nordic region due to its superior combination of quality and winter-hardiness. It is distributed both in both older and newer meadows and as feral populations in all parts of the Nordic countries, however it occurs less frequently in the northern parts. Although widespread in Norway, meadow fescue is not considered indigenous. Elven (1994) proposed that it was introduced as a forage grass in sown meadows. Although systematic use of meadow fescue in lays and pasture in Norway started at the end of the 19th century, breeding not beginning until the 1920s. However, meadow fescue was widespread in large parts of Norway in 1861 (Blytt 1861). Thus, it is not certain when and how meadow fescue was introduced into Norway.

In Europe, the abundance of meadow fescue in grasslands has decreased over the years, and today it is only rarely present in intensively managed grasslands although it is commonly found in species-rich permanent pastures and hay fields in alpine regions and in eastern Europe. Although not widely used as a forage grass species, there has been a growing interest in meadow fescue during the last decade in Europe, North America and Japan. Important factors contributing to this increased interest is its generally high level of stress tolerance and its ability to hybridise with perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (*L. multiflorum* Lam.). Breeding programmes for *Festulolliums* are currently being run in both Europe (Humphreys et al. 2004) and Japan (Yamada and Momotaz 2004). With the growing interest in the use of meadow fescue, detailed genetical and morphological characterisation of potentially useful germplasm is essential. The investigation reported here is part of an effort to completely characterise all meadow fescue accessions (approximately 170) in the Nordic Gene Bank (NGB).

Meadow fescue is a diploid ($2n$, $2x = 14$) outbreeder with a strong gametophytic self-incompatibility system (Lundqvist 1962). As a consequence of this, large genetic heterogeneity is expected to persist within populations. We chose to use amplified fragment length polymorphism (AFLP) markers for the analysis of genetic diversity in meadow fescue (Vos et al. 1995) as this method generates a large number of markers that are reliable and reproducible and which require only nanograms of DNA (Karp et al. 1996). Several studies have shown that AFLP technology is very well suited to characterise and quantify genetic diversity (Cresswell et al. 2001; Guthridge et al. 2001; Nybom 2004). A major drawback is the dominant nature of AFLPs that makes it difficult to estimate proper population genetic parameters since heterozygotes cannot be distinguished from homozygotes. However, several methods have been proposed to circumvent this problem (Excoffier et al. 1992; Lynch and Milligan 1994; Stewart and Excoffier 1996; Zhivotovsky 1999).

We report a genetic characterisation of 30 Norwegian local populations of meadow fescue based on AFLP markers and compare these results with the results of a previous study of 13 Nordic meadow fescue cultivars (Fjellheim and Rognli 2005). The aims of the investigation were to evaluate genetic diversity in the meadow fescue accessions of local populations relative to that of cultivars and to estimate genetic relationships between the local populations and the Nordic cultivars.

Materials and methods

Plant materials

Thirty local populations of meadow fescue (*Festuca pratensis* Huds.) covering a wide range of geographical variation—in latitude, longitude and altitude—were chosen for the investigation (Fig. 1). Seed samples of these populations were obtained from the NGB, Alnarp, Sweden (http://www.ngb.se). Using these accessions, we produced three datasets: the first comprises 15 populations from which we extracted DNA from the seeds; the second comprises 15 populations from which DNA was extracted from the leaves (subsequently referred to as dataset "seed" and "leaf", respectively); a third dataset, denoted "seed cultivars", was constructed in which the dataset "seed" was compared to an AFLP-marker dataset of Nordic meadow fescue cultivars (Fjellheim and Rognli 2005). Twenty individuals were initially included from each population. However, some of the individuals were subsequently excluded from the analysis due to a lack of amplification. The total number of individuals analysed was thus 825. Details on the accessions analysed are presented in Table 1.

DNA extraction

DNA extraction followed the protocol of Sharp et al. (1988). From the populations in dataset "seed" and "seed cultivars", DNA was extracted from whole seeds; from the dataset "leaf", DNA was extracted from 70 mg to 90 mg of fresh leaves. Both seeds and leaves were ground in 1.5-ml Eppendorf tubes containing liquid nitrogen and extra pure sea sand. The use of seeds for DNA extraction eliminates the establishment of large numbers of plants in the greenhouse, but at the same time it excludes further analysis of specific genotypes.

AFLP analysis

AFLP analysis was performed as described by Fjellheim and Rognli (2005). For selective amplification, primer combinations were chosen based on a combined restriction fragment length polymorphism (RFLP) and AFLP linkage map of meadow fescue developed by Alm et al. (2003). Three primer combinations (P77M72, P77M66 and P64M17), which produce markers distributed throughout the linkage groups of meadow fescue, were chosen. Primer sequences were as listed in Alm et al. (2003).

Data analysis

All analyses were done in three parallel experiments based on the three AFLP marker datasets produced (dataset "seed", "leaf" and "seed cultivars"). Dataset "leaf" was separated from the two others because AFLP patterns obtained from the DNA extracted from different organs can be different (Donini et al. 1997) and are thus not directly comparable.

Genetic diversity indices and frequency of polymorphic loci were calculated using the programme

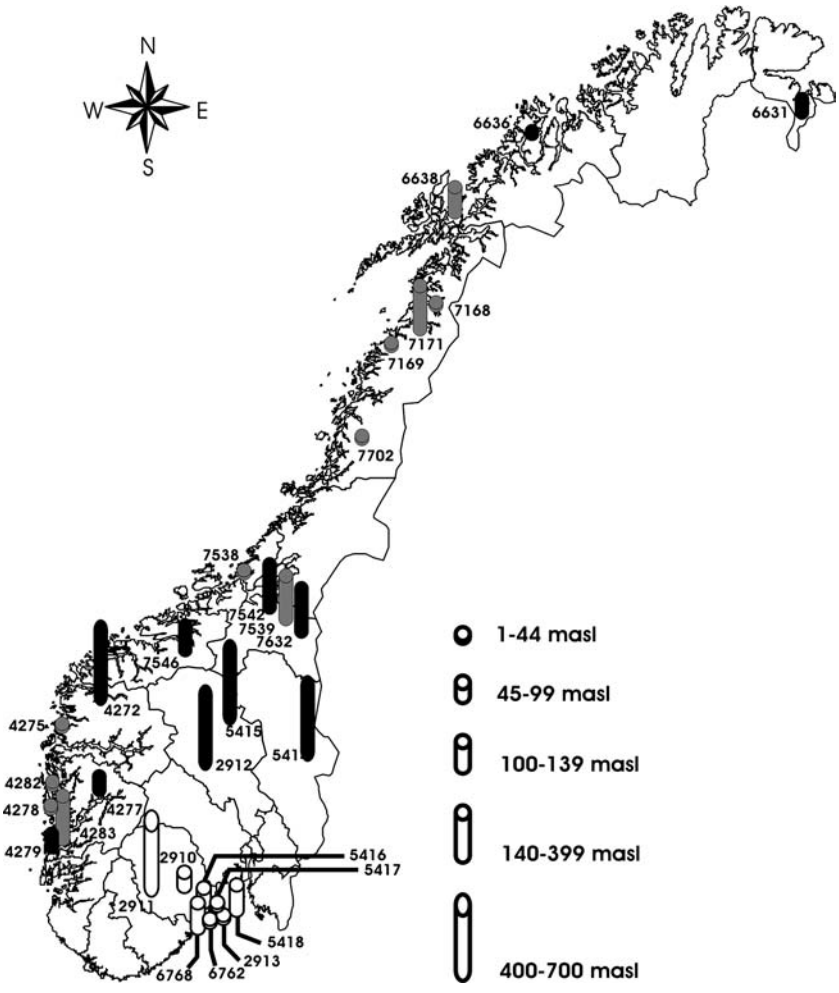

Fig. 1 Collection sites with elevation (masl; meters above sea level) for 30 populations of meadow fescue (*Festuca pratensis* Huds.) in Norway. For a detailed description of the accession indicated by the *numbers* refer to Table 1. *Black labels* Inland populations, *grey labels* western populations, *white labels* southern populations

ARLEQUIN 2.0 (Schneider et al. 2000; http://anthro.unige.ch/arlequin). Two diversity indices were calculated: (1) average difference between all genotypes in the population (Tajima 1983, 1993); (2) average gene diversity over loci (Tajima 1983; Nei 1987).

Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was carried out using the ARLEQUIN 2.0 programme. Separate analyses were conducted for dataset "leaf", "seed", and the cultivars. An ARLEQUIN 2.0 analysis of dataset "seed cultivars" was performed in which local populations and cultivars were treated as separate groups.

A matrix of corrected average pair-wise differences between all populations in each dataset (output from ARLEQUIN 2.0) was used for unweighted pair group method with arithmetic mean (UPGMA) clustering and principal coordinates (PCO) analysis. A minimum spanning tree (MST) was calculated. PCO analyses based on individual plants were also performed. The latter PCO analyses were performed using four different similarity or distance coefficients (Dice, Simple matching, Jaccard and Euclidean distance). As the coefficients provided similar results, we only present the analyses based on Jaccard similarity, given by $a/(a+b+c)$, where a is the number of shared bands and b and c are the number of bands present in one sample but absent in the other sample.

All UPGMA clustering and PCO analyses were performed using NTSYS-PC ver. 2.1 (Rohlf 2000).

Results

The three AFLP primer combinations produced between 80 and 103 markers with less than 5% missing data that could be unambiguously scored. In dataset "seed", a total of 103 scorable markers were produced, of which seven were monomorphic in all populations and thus excluded from the analysis, leaving 96 polymorphic markers for analysis. AFLP analysis of dataset "leaf" produced 80 scorable markers, six of which were monomorphic across populations, leaving 74 polymorphic markers. A matrix was constructed containing common markers from dataset "seed", and the markers were used in the analysis of Nordic meadow fescue cultivars (Fjellheim and Rognli 2005). This set contained 95 polymorphic markers (dataset "cultivars seed"). No genotypes were identical in any of the datasets.

Table 1 Geographical origin, number of individuals analysed and year of collection of 30 local populations of meadow fescue (*Festuca pratensis* Huds.). The accession number is from the Nordic gene bank (NGB)

Dataset	Accession no.	Location	Latitude	Longitude	Altitude (m)	Number of plants	Year of collection
Seed	NGB 2912	Aurdal	60°57′N	9°20′E	420	20	1980
	NGB 4283	Etne	59°45′N	5°45′E	250	20	1980
	NGB 5415	Sel	61°50′N	9°35′E	300	19	1981
	NGB 5418	Nevlunghavn	58°59′N	9°52′E	1	20	1981
	NGB 6631	Svanvik	69°38′N	30°03′E	45	20	1973
	NGB 6636	Tromsø	69°40′N	18°56′E	10	20	1973
	NGB 6638	Harstad	68°48′N	16°30′E	–[a]	20	1973
	NGB 6762	Habbestad	58°47′N	9°55′E	100	20	1981
	NGB 6768	Risør	58°47′N	9°39′E	20	20	1981
	NGB 7538	Bjugn	63°45′N	9°45′E	20	20	1981
	NGB 7539	Selbu	63°13′N	11°03′E	300	18	1981
	NGB 7542	Trondheim	63°22′N	10°48′E	150	20	1981
	NGB 7546	Sunndal	62°40′N	8°36′E	100	16	1981
	NGB 7632	Tydal	63°04′N	11°34′E	550	17	1980
	NGB 7702	Mosjøen	65°47′N	13°18′E	30	19	1976
Leaf	NGB 2910	Bø	59°25′N	9°02′E	80	20	1980
	NGB 2911	Seljord	59°30′N	8°36′E	430	20	1980
	NGB 2913	Skien	59°13′N	9°40′E	120	14	1980
	NGB 4272	Stryn	61°50′N	6°30′E	470	20	1980
	NGB 4275	Askvoll	61°20′N	5°15′E	5	19	–[a]
	NGB 4277	Voss	60°40′N	6°30′E	70	16	1980
	NGB 4278	Bergen	60°20′N	5°15′E	5	19	1981
	NGB 4279	Litlabø	59°45′N	5°30′E	50	20	1981
	NGB 4282	Austvoll	60°15′N	5°15′E	20	19	1981
	NGB 5414	Nybergsund	61°16′N	12°29′E	550	20	1981
	NGB 5416	Gjerpen	59°14′N	9°32′E	60	19	1981
	NGB 5417	Bamble	59°01′N	9°40′E	5	19	1982
	NGB 7168	Straumen	65°09′N	15°33′E	10	20	1978
	NGB 7169	Inndyr	67°04′N	14°03′E	15	19	1976
	NGB 7171	Finneid	67°12′N	15°27′E	150	20	1976
		Cultivar	Country of origin				Year of release
Cultivars	NGB14160	Norild	Norway	Norwegian Crop Research Institute		20	2001
	NGB 2185	Løken	Norway	Norwegian Crop Research Institute		20	1927[b]
	NGB 4269	Fure	Norway	Norwegian Crop Research Institute		20	1989
	NGB 4506	Petursey	Iceland	Locally propagated		20	
	NGB 1681	Leto Dæhnfeldt III	Denmark	L. Dæhnfeldt A/S, Marslev		19	1961
	NGB 8379	Pajbjerg	Denmark	Pajbjergfonden, Odder		19	1961
	NGB 4097	Balder	Denmark	L. Dæhnfeldt A/S, Marslev		20	1982
	NGB 2604	Tammisto	Finland	Hankkija Plant Breeding Station, Hyrylä		20	1928
	NGB 4075	Paavo	Finland	Agricultural Research Centre, Jokioinen		19	1948
	NGB 4074	Kalevi	Finland	Agricultural Research Centre, Jokioinen		19	1979
	NGB 2764	Svalöfs Sena	Sweden	Svalöf AB, Svalöv		20	1917
	NGB 2765	Bottnia II	Sweden	Svalöf AB, Svalöv		20	1956
	NGB 2766	Boris	Sweden	Svalöf AB, Svalöv		17	1971

[a]Not known
[b]This is the first registered reference to Løken

Genetic diversity indices

Genetic diversity indices based on molecular markers are given in Table 2 together with frequency of polymorphic loci. Populations NGB5415, NGB5414 and cv. Bottnia II had the highest frequencies of polymorphic loci in datasets ''seed'', ''leaf'' and cultivars (0.66, 0.71 and 0.62, respectively), whereas populations NGB6762, NGB4278 and cv. Norild had the lowest frequencies (0.34, 0.26, and 0.35, respectively). Mean values for frequencies of polymorphic loci were computed for the two datasets and for the cultivars. For dataset ''seed'', it was 0.46 ± 0.04; for dataset ''leaf'', it was 0.39 ± 0.07 and for the cultivars, it was 0.51 ± 0.04. In dataset ''seed'' and ''leaf'', populations NGB6762 and NGB4278 had the lowest average difference between all pairs of genotypes in the population (10.56 and 5.95, respectively), whereas populations NGB5415 and NGB4277 had the

Table 2 Genetic diversity indices in 30 Norwegian local populations and 13 Nordic cultivars of meadow fescue. The indices calculated are frequency of polymorphic loci, mean number of pairwise differences between individuals in populations and average gene diversity over loci

Dataset	Population (NGB no.)	Frequency of polymorphic loci	Average difference	Average gene diversity
Seed	7546	0.47	14.07	0.1513
	7542	0.52	14.73	0.1583
	7539	0.49	14.60	0.1641
	7538	0.38	11.28	0.1213
	6768	0.36	10.59	0.1127
	6762	0.34	10.56	0.1123
	6638	0.46	13.15	0.1429
	6636	0.49	12.57	0.1412
	6631	0.46	12.80	0.1376
	5418	0.40	10.86	0.1143
	5415	0.66	18.09	0.1988
	4283	0.44	13.24	0.1393
	2912	0.53	13.84	0.1457
	7632	0.45	11.29	0.1228
	7702	0.40	12.42	0.1411
	Average	0.46 (±0.04)[a]	12.94 (±1.11)	0.1402 (±0.013)
Leaf	4282	0.36	8.19	0.1187
	4275	0.35	7.02	0.0567
	4278	0.26	5.95	0.0863
	5417	0.38	7.75	0.1231
	7169	0.32	8.02	0.1163
	7168	0.30	7.91	0.1084
	5416	0.48	10.74	0.1512
	2910	0.32	7.72	0.1043
	4279	0.41	9.33	0.1278
	4277	0.56	16.92	0.2418
	7171	0.29	7.82	0.1204
	2913	0.34	9.34	0.1262
	2911	0.30	6.88	0.0930
	5414	0.71	14.83	0.2149
	4272	0.46	8.16	0.1127
	Average	0.39 (±0.07)	9.11 (±1.66)	0.1268 (±0.026)
Cultivars	Norild	0.35	10.31	0.1066
	Løken	0.50	15.91	0.1692
	Fure	0.53	13.84	0.1488
	Petursey	0.50	13.30	0.1447
	Leto Dæhnfeld IIII	0.49	12.70	0.1477
	Pajberg	0.44	13.17	0.1447
	Balder	0.56	14.66	0.1629
	Tammisto	0.53	14.57	0.1567
	Paavo	0.46	14.19	0.1576
	Kalevi	0.58	16.65	0.1851
	Svalöfs Sena	0.51	15.31	0.1664
	Bottnia II	0.62	17.50	0.1923
	Boris	0.51	16.09	0.1828
	Average	0.51 (±0.04)	14.48 (±1.15)	0.1589 (±0.013)

[a]95% confidence interval

Table 3 Analyses of molecular variance (AMOVA) in 30 Norwegian local populations and 13 Nordic cultivars of meadow fescue

Dataset	Source of variation	*df*	Variance components	Percentage of variation
Seed	Among populations	14	2.96	30.8
	Within populations	274	6.65	69.2
Leaf	Among populations	14	2.15	31.0
	Within populations	268	6.79	69.0
Cultivars	Among populations	12	1.94	20.4
	Within populations	240	7.54	79.6
	Among groups[a]	1	0.35	3.6
Cultivars seed	Among populations	26	2.49	25.1
	Within populations	514	7.07	71.3

[a]The two groups are cultivars and local populations

highest (18.09 and 16.92, respectively). Among the cultivars, the lowest and highest mean differences between all pairs of genotypes were found in Norild (10.31) and Bottnia II (17.50), respectively. Mean values were computed for the two datasets and for the cultivars. For dataset "seed", it was 12.94 ± 1.11; for dataset "leaf", it was 9.11 ± 1.66; for the cultivars, it was 14.48 ± 1.15. Populations NGB6762 and NGB4275 had the lowest average gene diversity over loci in dataset "seed" and "leaf" (0.112 and 0.057, respectively), whereas NGB5415 and NGB4277 had the highest (0.199 and 0.242, respectively). Among the cultivars, Norild had the lowest average gene diversity (0.107), whereas Bottnia II had the highest (0.192). When the means of average gene diversity between the two datasets and the cultivars were compared, dataset "seed" and "leaf" had mean values of 0.140 ± 0.013 and 0.127 ± 0.026, respectively, and the cultivars had an average of 0.159 ± 0.013.

Distribution of genetic diversity

AMOVA analysis revealed the same level of intrapopulation variation in both dataset "seed" and "leaf" (69.2% and 69.0%, respectively; Table 3). A higher intrapopulation variation was found within the cultivars (79.6%) than within the local populations (69.2%). A combined AMOVA was carried out on the dataset "cultivars seed" to check for variation among groups (cultivars and local populations), which was 3.6%.

UPGMA analyses

The clustering of the populations in both the UPGMA and PCO analyses suggested the separation of the populations into three groups according to geographic origin—inland, western and southern. These groups will be used in subsequent discussions of the UPGMA and PCO results. In order to aid visual interpretation, each group were given characteristic labels: inland populations have black labels, western populations have grey labels, southern populations have black, open labels and the cultivars are labelled with small, black dots. The different labels correspond in the datasets and in the PCO and UPGMA analyses.

In both dataset "seed" and "leaf", the UPGMA analyses identified three clusters (inland, western and southern populations), with the western and southern populations clustering together (Fig. 2a, b). There were two exceptions in dataset "leaf"; the southern population NGB5417 clustered with the inland populations, and the western population NGB7168 clustered with the southern populations.

An UPGMA analysis performed on dataset "cultivars seed" confirmed the grouping of the local populations established in the separate UPGMA analysis of dataset "seed" (Fig. 2c). The cultivar Norild was completely distinct from all other local populations and cultivars, while all of the other cultivars clustered together with the inland populations.

PCO analyses

PCO analyses of both dataset "seed" and dataset "leaf" divided the populations into two groups with minor overlaps along the first axis (10.1% and 9.3%, respectively, Fig. 3a, b). One cluster contained inland populations, whereas the other contained the western and southern populations. The first cluster was also retained along the second axis (5.9% and 6.3%, respectively), whereas the second cluster separated into two slightly overlapping groups: one containing the western populations and the other containing the southern ones. The populations NGB4283 and NGB7702 (dataset "seed") were overlapping with both western and southern populations, but based on results from the UPGMA analysis (Fig. 2), they were classified as western populations.

In a PCO analysis of dataset "cultivars seed" (Fig. 3c), the local populations showed the same grouping as in the separate analysis of the "seed" dataset (Fig. 3a). The majority of the cultivars grouped most closely with the inland populations.

PCO analyses were also performed using corrected average pair-wise differences between populations as distance measures. Only the PCO analysis of dataset "leaf" is presented, as it was the only analysis that added information to the PCO analyses of the individuals and the UPGMA analyses. The MST of the populations in dataset "leaf" shows that population NGB7168 had its closest connection to the western populations. Furthermore, the populations NGB4279 and NGB5417 form the connection between the inland populations and southern populations (Fig. 4).

Discussion

Genetic diversity

Most of the diversity was found within the populations, which was to be expected in an outcrossing species. Similar estimates were obtained by Balfourier et al. (1998) in *Lolium perenne* L., a species that can be compared with meadow fescue as it has a similar breeding system.

A significantly higher level of genetic diversity was found in dataset "seed" than in the dataset "leaf". Donini et al. (1997) investigated AFLP patterns in template DNA extracted from different plant organs and concluded that the observed dissimilarities most likely arise as a result of DNA methylation differences between organs and that DNA from mature seeds in wheat is less methylated than DNA from young leaves. This explanation is consistent with results obtained from other studies (Theiss and Follmann 1980; Hepburn et al. 1987) and can probably

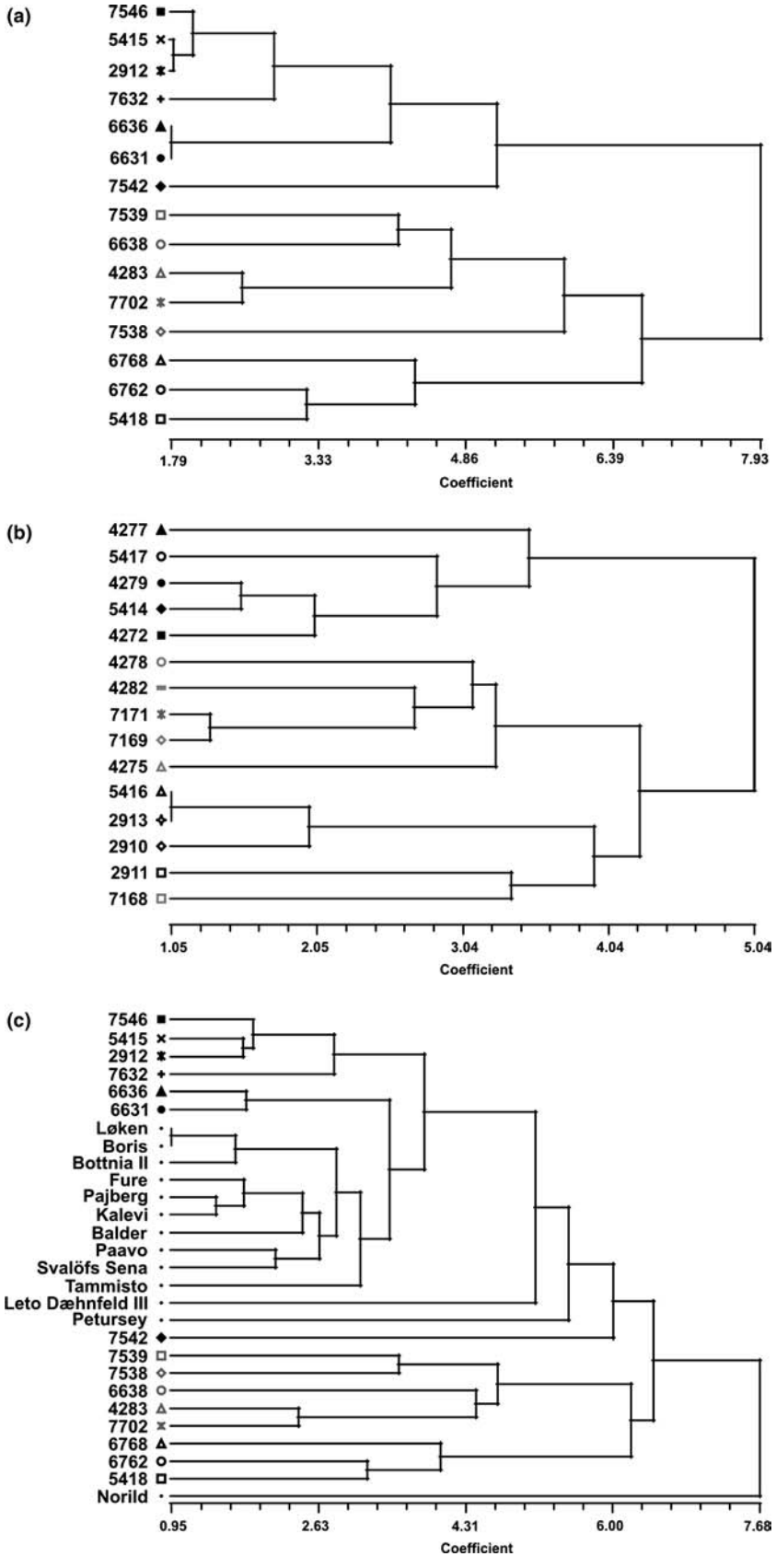

Fig. 2 Unweighted pair group method with arithmetic mean (UPGMA) clustering of 30 Norwegian natural populations and 13 Nordic cultivars of meadow fescue. **a** Dataset "seed" based on 96 polymorphic AFLP markers, **b** dataset "leaf" based on 74 polymorphic AFLP markers, **c** dataset "cultivars seed" based on 95 polymorphic AFLP markers. All analyses are based corrected average pairwise differences. *Black labels* Inland populations, *grey labels* western populations, *black open labels* southern populations. *Small dots* represent Nordic cultivars

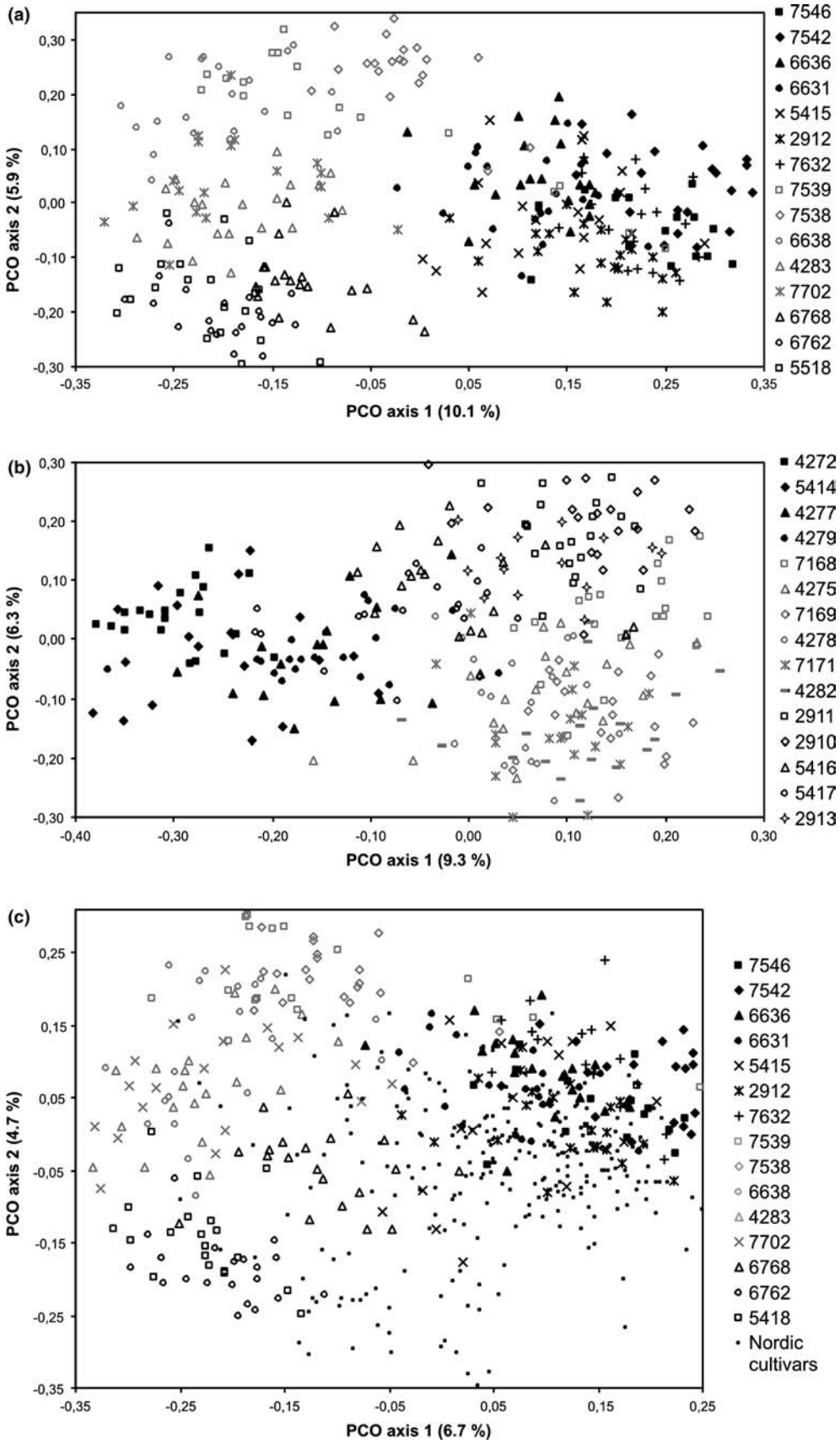

Fig. 3 Principal coordinates analysis (PCO) of 825 individual genotypes from 30 Norwegian natural populations and 13 Nordic cultivars of meadow fescue. **a** Dataset "seed" based on 96 polymorphic AFLP markers, **b** dataset "leaf" based on 74 polymorphic AFLP markers, **c** dataset "cultivars seed" based on 95 polymorphic AFLP markers. All analyses are based on the Jaccard similarity index. *Black labels* Inland populations, *grey labels* western populations, *black open labels* southern populations. *Small dots* represent Nordic cultivars

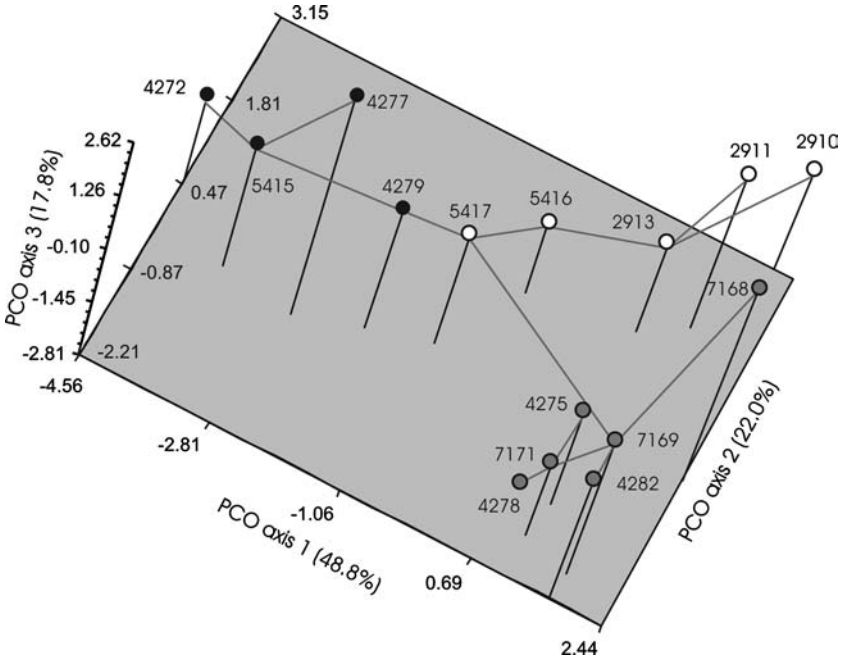

Fig. 4 Principal coordinates (PCO) analysis of 15 local Norwegian populations of meadow fescue from dataset "leaf". The analysis is based on 74 polymorphic AFLP markers as well as on corrected average pairwise distances. A MST is superimposed on the plots. *Black labels* Inland populations, *grey labels* western populations, *black open labels* southern populations

also clarify the differences in level of variation between dataset "seed" and "leaf" in this study, as AFLP profiles were generated using the methylation sensitive restriction enzyme *Pst*I. In addition, the fact that the major part of the seeds is triploid and leaves are diploid might have contributed to the differences, especially since meadow fescue is an obligate outbreeder. Although the level of variation detected in the populations is different in the two datasets, the partitioning of variation within each dataset is similar. In dataset "seed" and "leaf", 69.2% and 69.0%, respectively, of the variation was found within the populations.

We found a lower level of genetic diversity in the local Norwegian populations than in the Nordic cultivars, which is in contrast to the results reported by Kölliker et al. (1998) based on their study of meadow fescue from Switzerland. This may indicate that the cultivars have a wide genetic base due to the use of introduced material for breeding (see Fjellheim and Rognli 2005). Alternatively, it could indicate that the Norwegian meadow fescue populations have a narrow genetic base due to recent introduction of meadow fescue to this area and possible associated bottlenecks. Several studies have described a decreasing level of genetic diversity with increasing geographic distance to centres of diversity of the species (Chowdhury and Slinkard 2000; Grivet and Petit 2003). It has been shown that *Lolium perenne* populations from Scandinavia show reduced levels of diversity compared to populations originating closer to the putative centre of diversity of this species (Balfourier et al. 1998). No centres of diversity have been postulated for meadow fescue, but Norway is at the outskirts of the geographic distribution area of meadow fescue, so the possibility of a bottleneck is highly likely. However, this needs further clarification by diversity analysis of populations from the entire distribution area of meadow fescue.

In addition to a lower level of genetic diversity, the local populations exhibit larger differentiation than the cultivars, as shown by the higher level of among-population variation. This may be a result of more gene flow between cultivars than between local populations, which could have counteracted genetic differentiation between countries. Much of the meadow fescue breeding in the Nordic countries has been based on the exchange of breeding material and seed between countries (see Fjellheim and Rognli 2005), whereas gene flow, at least through pollen, has been shown to be rather restricted between natural populations of meadow fescue (Rognli et al. 2000). This means that pollination between populations that are so geographically dispersed as the present ones must be very restricted.

Genetic relationships between local populations and cultivars

Although dataset "seed" and "leaf" cannot be considered together, comparable results were found in the PCO and UPGMA analyses. Based on these analyses, the populations were divided into three groups: southern populations (black, open labels), western populations (grey labels) and inland populations (black labels, Fig. 1). Southern populations have a clear geographic connection to the central parts of southern Norway, whereas the western populations are distributed along the western coast of Norway, and the inland populations have a broad distribution, with the majority of the populations in inland regions. In the PCO and UPGMA analyses that also included the Nordic cultivars, inland populations clustered together with the cultivars (small, black dots), and these inland populations are clearly more related to the cultivars than to the western and southern popula-

tions. In ''Flora for Norway'' by Blytt (1861), meadow fescue is described as being common in all parts of southern Norway up to the pine line, and in northern parts of Norway up to Bodø (67°N)—but only in the coastal areas. The inland populations correspond geographically to the areas in Norway where Blytt did not find meadow fescue—i.e., areas at higher latitudes and altitudes. In these areas, natural populations of meadow fescue are not stable over time. However, breeding for adaptation to the climate of continental, high-altitude regions started in the beginning of the 20th century and resulted in a systematic use of meadow fescue for forage production in these areas as well. Migration from sown meadows can most probably explain the presence of local populations here and the close genetic relationship between the inland populations and the cultivars observed in this study. However, the inland populations do not overlap completely with the cultivars in the PCO and UPGMA analyses, which may be due to selection and drift in the local populations. These populations will exhibit a different selection pressure than the cultivars, which is kept genetically stable over time due to a requirement for varietal stability.

The western and southern populations are located in areas where Blytt did find meadow fescue in 1861. At this time, meadow fescue was widely distributed in Norway, and it can be assumed that it was introduced to Norway well ahead of mid19th century. The two groups of populations have separate geographic patterns; western populations are distributed along the western coast of Norway up to the Arctic Circle, and southern populations are found in the central parts of southern Norway. The two groups correspond geographically to two areas of Norway that throughout history have experienced a large amount of human activity, mostly related to shipping and trading. The western coast of Norway has been an area of extensive sailing and shipping for more than 1,000 years. When the distribution pattern of the coastal populations is examined, it can be seen that it fits well with the pattern of trading in the Norwegian Saga era. People were travelling between western Norway and Scotland, the Faroe Island and the Shetland Islands. Among the things they brought with them were cattle. As some fodder for the cattle had to be brought as well, it is reasonable to believe that several plant species were introduced unintentionally this way. Several other plants—for example, *Bromus benekenii* Lange, *Scilla verna* Huds., *Leontodon hispidus* L., *Hypochoeris radicata* L., *Centaurea nigra* L.—have a similar distribution pattern, which is also assumed to be related to connections between Norway and Great Britain during Viking times (Reidar Elven, personal communication; Fægri 1960).

The central part of southern Norway, in the areas around the Oslo fjord, was also a main site for both Viking activity and Hanseatic trading, mainly with connections to Denmark and Germany. The southern populations could have been introduced as a result of this activity. Alternatively, it could be related to the large amount of seed import that occurred in this area relative to the western coast. From about the beginning of the 18th century onwards, a great effort was made to improve the quality of meadows, and large amounts of seeds of foreign origin were imported. Due to climate and topography, sown meadows were not widely used in western parts of Norway before 1900 (Hasund 1925), thus the need for seed for meadows was not great in that area. Although meadow fescue specifically was not used until the late 19th century, it is reasonable to believe that it could have been introduced with imported seed, as these often was not very pure (Glærum 1914).

As an alternative, the clusters of western and southern populations can be a result of ecophysiological differentiation. The areas covered by the two groups of populations belong to different climatic zones; western parts of Norway have an oceanic climate, and central parts of southern Norway have a more continental climate.

Concluding remarks

In this study, we provide a genetic characterisation of 30 accessions of Norwegian local populations of meadow fescue from the NGB. A knowledge of the genetic diversity and relatedness between the accessions can be used for a more efficient management of the accessions and will assist breeders who want to use gene bank accessions as starting materials for breeding. The chances of finding new, unique and potentially useful alleles for agronomic traits will be greatest if populations that are genetically distinct from already existing cultivars are being mined. In the case of present-day meadow fescue, this means that emphasis should be put on the conservation, description and utilisation of the western and southern populations. Inland populations will be less interesting for breeding purposes, as they are most closely related to existing cultivars. For even more powerful analyses, molecular variation can be combined with phenotypic data on morphological and agronomic characters to screen populations for markers linked to traits of interest. This would assist breeders in selecting markers that can be mapped and used for marker-assisted selection, and to select populations that can form basis for a breeding programme.

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References

Alm V, Fang C, Busso CS, Devos KM, Vollan K, Grieg Z, Rognli OA (2003) A linkage map of meadow fescue (*Festuca pratensis* Huds.) and comparative mapping with other Poaceae species. Theor Appl Genet 108:25–40

Balfourier F, Charmet G, Ravel C (1998) Genetic differentiation within and between natural populations of perennial and annual ryegrass (*Lolium perenne* and *L. rigidum*). Heredity 81:100–110

Blytt MN (1861) Norges flora, eller beskrivelser over de I Norge vildtvoxende karplanter: tilligemed angivelser af de geographiske forholde, under hvilke de forekomme: 1ste deel. Kongelige Norske Videnskabers Selskab, Christiania

Chowdhury MA, Slinkard AE (2000) Genetic diversity in grasspea (*Lathyrus sativus* L.). Genet Resour Crop Evol 47:163–169

Cresswell A, Sackville Hamilton NR, Roy AK, Viegas MF (2001) Use of amplified length polymorphism markers to assess genetic diversity of *Lolium* species from Portugal. Mol Ecol 10:229–241

Donini P, Elias ML, Bougourd SM, Koebner RMD (1997) AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. Genome 40:521–526

Elven R (1994) Lid & Lids Norsk flora, 6th edn. Det Norske Samlaget, Oslo, Norway

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

Fægri K (1960) Maps of distribution of Norwegian plants 1. The coast plants. Skrifter Universitetet I Bergen 26, Oslo University Press, Oslo

Fjellheim S, Rognli OA (2005) Genetic diversity within and among Nordic meadow fescue (*Festuca pratensis* Huds.) cultivars based on AFLP markers. Crop Sci (in press)

Glærum O (1914) Engdyrkning og enkulturforsøk. In: Ødegaard N, Vik K, Klokk O (eds) Norsk Forsøksarbeid I Jordbruket. Grøndahl & Søns Forlag, Kristiania

Grivet D, Petit RJ (2003) Chloroplast DNA phylogeography of the hornbeam in Europe: evidence for a bottleneck at the outset of postglacial colonization. Conserv Genet 4:47–56

Guthridge KM, Dupal MP, Kölliker R, Jones ES, Smith KF, Forster JW (2001) AFLP analysis of genetic diversity within and between populations of perennial ryegrass (*Lolium perenne* L.). Euphytica 122:191–201

Hasund S (1925) Norges Landbruk 1875–1925. In: Lantbruket I Norden 1875–1925. Göteborgs Litografiska Aktiebolag, Göteborg, Sverige, pp 329–449

Hepburn AG, Belanger FC, Mattheis JR (1987) DNA methylation in plants. Dev Genet 8:475–493

Humphreys M, Humphreys J, Donnison I, King I, Thomas HM, Ghesquiere M, Durand JL, Rognli OA, Zwierzykowski Z, Rapacz M (2004) Molecular breeding and functional genomics for tolerance to abiotic stress. In: Hopkins A, Wang ZY, Mian R, Sledge M, Barker RE (eds) Molecular breeding of forage and turf. Dev Plant Breed 11:61–80

Karp A, Seberg O, Buiatti M (1996) Molecular techniques in the assessment of botanical diversity. Ann Bot 78:143–149

Kölliker R, Stadelmann FJ, Reidy B, Nosberger J (1998) Fertilization and defoliation frequency affect genetic diversity of *Festuca pratensis* Huds. in permanent grasslands. Mol Ecol 7:1557–1567

Lundqvist A (1962) The nature of the two-loci incompatibility system in grasses. II. Number of alleles at the incompatibility loci in *Festuca pratensis* Huds. Hereditas 48:169–181

Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. Mol Ecol 3:91–99

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York, NY, USA

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143–1155

Rognli OA, Nilsson NO, Nurminiemi M (2000) Effects of distance and pollen competition on gene flow in the wind-pollinated grass *Festuca pratensis* Huds. Heredity 85:550–560

Rohlf FJ (2000) NTSYS-PC. Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, New York, USA

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN ver. 2.0: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of beta amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290

Stewart CN, Excoffier L (1996) Assessing population genetic structure and variability with RAPD data: Application to *Vaccinium macrocarpon* (American Cranberry). J Evol Biol 9:153–171

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437–460

Tajima F (1993) Measurement of DNA polymorphism. In: Takahata N, Clark AG (eds) Mechanisms of molecular evolution. Introduction to molecular paleopopulation biology. Sinauer Associates, Sunderland, pp 37–59

Theiss G, Follmann H (1980) 5-Methylcytosine formation in wheat embryo DNA. Biochem Biophys Res Commun 94:291–297

Vavilov NI (1940) The new systematics of cultivated plants. In: Huxley J (ed) The new systematics. Clarendon press, Oxford, pp 549–566

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP—a new technique for DNA-fingerprinting. Nucleic Acids Res 23:4407–4414

Yamada T, Momotaz A (2004) Usefulness of simple sequence repeat (SSR) polymorphism for the breeding programs in *Festulolium*. In: Yamada T, Takamizo T (eds) Development of a novel grass with environmental stress tolerance and high forage quality through intergeneric hybridization between *Lolium* and *Festuca*. National Agriculture and Bio-oriented Research Organization, pp 43–48

Zhivotovsky LA (1999) Estimating population structure in diploids with multilocus dominant DNA markers. Mol Ecol 8:907–913