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# Temporal trends of genetic diversity in European barley cultivars (*Hordeum vulgare* L.)

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**Abstract** The changes of genetic diversity over time were monitored in 504 European barley cultivars released during the 20th century by genotyping with 35 genomic microsatellites. For analysis, the following four temporal groups were distinguished: 1900–1929 (TG1 with 19 cultivars), 1930–1949 (TG2 with 40 cultivars), 1950–1979 (237 cultivars as TG3), and 1980–2000 (TG4 consisting of 208 cultivars). After rarefaction of allelic diversity data to the comparable sample size of 18 varieties, of the 159 alleles found in the first group (TG1) 134 were retained in the last group (TG4) resulting in a loss of only 15.7% of alleles. On the other hand 51 novel alleles were discovered in the group representing the last investigated time period (TG4) in comparison with the TG1. Novel alleles appeared evenly distributed over the genome, almost at all investigated genomic loci, with up to five such novel alleles per locus. Alleles specific for a temporal group were discovered for all investigated time periods, however analysis of molecular variance (AMOVA) did not reveal any significant population structure attributable to temporal decadal grouping. Only 2.77% of the total observed variance was due to differences between the four temporal groups and 1.42% between individual decades of the same temporal group, while 95.81% of the variance was due to variation within temporal groups. The distinction between two-rowed and six-rowe genetic types accounted for 19.5% of the total observed variance by AMOVA, whereas the comparison between 'winter' and 'spring' types accounted for 17% of the total observed variation. The analysis of linkage disequilibrium did not reveal statistically significant differences between the temporal groups. The results indicated that the impact of breeding effort and variety delivery systems did not result in any significant quantitative losses of genetic diversity in the representative set of barley cultivars over the four time periods.

**Keywords** Impact of breeding · Genetic diversity · Genetic erosion · Simple sequence repeats (SSR) · Molecular variance · Linkage disequilibrium · Barley

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## Introduction

It has been suggested that genetic bottlenecks were imposed on crop plants during domestication and through modern plant-breeding practices leading to a

loss of allelic variation (Tanksley and McCouch 1997). Molecular markers provided a new insight to these considerations. For the impact of breeding on genetic diversity a variable picture emerged from studies on several crop species. In 559 French breed wheat accessions, a 25% decrease in allelic richness was observed by comparing landraces to varieties, whereas when considering only registered varieties changes in diversity related to temporal trends appeared more qualitative than quantitative (Roussel et al. 2004). Also for dominant UK winter wheat varieties from the period 1934 to 1994, a qualitative rather than a quantitative shift in the molecular diversity was reported (Donini et al. 2000). No significant differences in both the total number of alleles per locus and in the polymorphism information content (PIC) values were detected for samples of cultivated wheat collected over an interval of 40–50 years in four comparable geographical regions in Europe and Asia (Khlestkina et al. 2004). However, one-third of the detected alleles were collection region or 'mission-specific'. Even an increase in genetic diversity was found for Nordic spring wheat cultivars (Christiansen et al. 2002). No significant reduction in the diversity available for farmers had been observed for maize and peas since the initiation of French Official Catalogue for Plant Varieties and Species (Le Clerc et al. 2006). On the contrary, in Canadian hard red spring wheat germplasm (75 varieties), 19% of the alleles present pre-1910 were undetected in cultivars released after 1990 (Fu et al. 2005). A loss of genetic diversity was also reported for 123 CIMMYT and CIMMYT-related modern wheat cultivars in comparison with traditional landrace genotypes (119 landraces from Mexico and Turkey) (Reif et al. 2005b) and with *Triticum tauschii* (11 accessions from Asia and Near East). In the dent and flint heterotic groups of European maize germplasm, the genetic variation within and among varieties decreased significantly during the past five decades (Reif et al. 2005a). In a study comparing historical US maize inbreds it was concluded that genetic diversity among current inbreds was reduced at the genetic level but not at the population level (Lu and Bernardo 2001).

A comprehensive study of morphological and molecular diversity of UK barley led to the conclusion that systematic plant breeding does not inevitably lead to a reduction in the genetic diversity of agricultural crops (Koebner et al. 2003). For German winter barley cultivars, a slight decrease of genetic diversity over time was found for six-rowed cultivars; whereas in two-rowed cultivars a considerable increase was detected (Ordon et al. 2005). No significant differences for allelic richness and PIC-values were reported for samples of cultivated barley collected in intervals of about 40–50 years in three comparable geographical regions (Khlestkina et al. 2006). On the other hand, a lower level of diversity was reported for modern cultivars in comparison with landrace or 'foundation genotypes' in northern European spring barley germplasm (Russell et al. 2000). In summary, it can be concluded from the cited studies that there is not enough evidence to postulate a negative impact of breeding on crop genetic diversity.

A comprehensive effort to study the impact of modern breeding on the molecular diversity for four important crop species in Europe–barley, wheat, maize, and potato–was undertaken in the international project GEDIFLUX (Reeves et al. 2004). Here, we report in detail the results obtained for widely grown barley cultivars from intensive evaluation trials for statutory purposes 'National Lists' (NL) and the longer and more extensive post-registration trial series of 'Recommended Lists' (RL) of various European countries.

## Materials and Methods

### Plant material and DNA extraction

A set of 504 varieties in total was exclusively prepared for this study and included widely grown barley cultivars from NL of various EU member states to give a balance of individual plant breeders over the period of study, and the commercially successful varieties added to the NIAB RL of the UK. For comparability with other published studies on the observed genetic diversity in barley, as many of these published varieties were included as well. The set collectively represented both the genetic diversity 'available' (NL) and 'exploited' (RL) during the twentieth century in Europe. Most barley cultivars originated from Great Britain, Germany, France, and the Netherlands (Table 1). Approximately, a quarter of the chosen barley cultivars

**Table 1** Overview of barley varieties chosen for the study

| Geographic origin of the investigated varieties | | | | | |
|---|---|---|---|---|---|
| Country of origin | Number of varieties | Country of origin | Number of varieties | | |
| Great Britain | 146 | Spain | 1 | | |
| Germany | 120 | Austria | 9 | | |
| France | 56 | Norway | 6 | | |
| Netherlands | 47 | Czech Republic | 6 | | |
| Sweden | 40 | Belgium | 5 | | |
| Finland | 34 | Hungary | 3 | | |
| Denmark | 24 | Ireland | 3 | | |
| Greek, Italy, Poland, Yugoslavia | | Single varieties | | | |
| Agronomic traits of the investigated varieties | | | | | |
| Period of release | Number of accessions | | | | |
| | Total | Two-rowed | 6-rowed | ''Spring'' | ''Winter'' |
| 1900–1929 (TG1) | 19 | 12 | 6 | 14 | 5 |
| 1930–1949 (TG2) | 40 | 29 | 13 | 30 | 8 |
| 1950–1979 (TG3) | 237 | 169 | 57 | 175 | 54 |
| 1980–2000 (TG4) | 208 | 177 | 27 | 128 | 75 |
| Total | 504 | 387 | 103 | 347 | 142 |

represented the 'winter' type and 103 cultivars were six-rowed (Table 1). The true genetic status of the cultivars in terms of seasonal type, particularly for the older material, is not always clear-cut, however, the best available information on seasonal type was used in this study. Varieties designated as 'winter' were less represented in the beginning of the century. This may be due to the fact that in the early decades of the 20th century 'winter' types were rather adaptable material that could cope with winter sowing, and what material was registered as 'winter' in these decades is likely to be very different from the genuine genetically 'winter' types with requirement for adequate vernalization released in the later decades of the century. For some barley cultivars the information about seasonal type or row type was missing or they were of intermediate type. The seeds were obtained from the Genebank of the John Innes Centre (JIC) in Norwich, UK, from the Genebank of the Leibniz Institute of Plant Genetics (IPK) in Gatersleben, Germany, and from the Nordic Genebank in Alnarp, Sweden.

DNA extraction was performed with a Quiagen kit (Quiagen, Hilden, Germany) from five pooled seedlings for each accession.

## Genotyping of microsatellites

Genomic barley microsatellites were obtained from public sources (Liu et al. 1996; Struss and Plieske, 1998; Ramsay et al. 2000; Li et al. 2003). A total of 35 microsatellites were chosen to evenly cover the seven barley chromosomes and to have good performance in terms of both amplification and detection (Table 2). PCR reactions were performed according to Röder et al. (1998), and microsatellite fragments were detected on an automated laser fluorescence (ALFexpress) sequencer (Amersham Biosciences, UK) and analyzed using the computer program Fragment Analyser v. 1.02 (Amersham Biosciences, UK) by comparison with internal size standards.

Amplification products of different sizes represented different alleles. The allele sizes were transferred into a 1/0 matrix. In case of two or three

**Table 2** Molecular diversity of the SSR loci applied for the construction of the database

| Locus | Chromosome | Allelic richness (AR) | | PIC | Number of unique alleles | | Heterogeneity (%) |
|---|---|---|---|---|---|---|---|
| | | per locus | per chromosome | | per locus | per chromosome | |
| HVM20 | 1H | 6 | 34 | 0.57 | 1 | 5 | 1 |
| Bmag0211 | | 9 | | 0.69 | 2 | | 1 |
| Bmag0382 | | 5 | | 0.47 | 1 | | 2.3 |
| Bmag0718 | | 10 | | 0.52 | 1 | | 2.1 |
| Bmag0579 | | 4 | | 0.56 | | | 2.5 |
| HVM36 | 2H | 9 | 33 | 0.71 | 2 | 6 | 2 |
| GBMS0229 | | 5 | | 0.30 | | | 0.8 |
| GBMS0160 | | 6 | | 0.39 | 1 | | 1.5 |
| HVM54 | | 9 | | 0.72 | 2 | | 2.3 |
| Bmag0749 | | 4 | | 0.65 | 1 | | 3.6 |
| EBmac0705 | 3H | 6 | 49 | 0.30 | 2 | 9 | 1 |
| GBMS0046 | | 10 | | 0.62 | 2 | | 4 |
| Bmag0225 | | 14 | | 0.73 | 3 | | 3.8 |
| GBMS0189 | | 10 | | 0.58 | 2 | | 12.2 |
| Bmag0013 | | 9 | | 0.70 | | | 2.3 |
| HVM40 | 4H | 6 | 37 | 0.58 | 1 | 6 | 4.6 |
| EBmac0906 | | 5 | | 0.73 | | | 3 |
| GBMS0087 | | 8 | | 0.16 | 2 | | 0.4 |
| EBmac0701 | | 12 | | 0.74 | 2 | | 2.3 |
| HVM67 | | 6 | | 0.52 | 1 | | 1.1 |
| GBMS0032 | 5H | 9 | 31 | 0.51 | 2 | 5 | 6.1 |
| EBmac0684 | | 4 | | 0.68 | | | 1.3 |
| HVM30 | | 5 | | 0.50 | 2 | | 0.4 |
| GBMS0119 | | 7 | | 0.50 | 1 | | 1.1 |
| GMS001 | | 6 | | 0.31 | | | 1.5 |
| Bmac0316 | 6H | 12 | 68 | 0.65 | 5 | 12 | 2.1 |
| GBMS0083 | | 6 | | 0.73 | | | 2.9 |
| EBmac0602 | | 9 | | 0.59 | 3 | | 2.8 |
| Bmag0613 | | 18 | | 0.81 | | | 4.8 |
| Bmac0040 | | 23 | | 0.91 | 4 | | 5.4 |
| GBMS0192 | 7H | 6 | 28 | 0.62 | | 4 | 12.2 |
| GBMS0035 | | 5 | | 0.48 | 2 | | 2.1 |
| GBMS0111 | | 3 | | 0.04 | 1 | | 0.4 |
| GBMS0183 | | 5 | | 0.66 | | | 2.5 |
| Bmag0135 | | 9 | | 0.78 | 1 | | 2.9 |
| In total | | 280 | | | 47 | | |
| Average | | 8.0 | | 0.58 | | | |

different alleles at the same locus, the locus was scored as heterogeneous in this accession since DNA was extracted from pooled seedlings. Scoring of multiple peaks followed the quality assurance protocol developed in the framework of the EU project GEDIFLUX. Namely, if the height of the additional peak/peaks was 50% or more of the height of the main peak, it was scored as '1'; if the height of the additional peak/peaks was between 50% and 10% of the height of the main peak, it was scored as '1?';

peaks with a height below 10% of the main peak were score absent '0'. The information concerning the occurrence of additional questionable alleles was included in the database, but was not used for the analysis of genomic diversity. Hence, this analysis gives a cautious or conservative estimate of observed genetic diversity only by analyzing clearly observed alleles as in this regard additional weaker 'peaks' being called as alleles will falsely inflate the computed genetic diversity.

### Data analysis

For analysis, the cultivars were classified into four temporal groups (TGs) according to the year of release: TG1 varieties released during 1900–1929 (19 cultivars); TG2 1930–1949 (40 cultivars); TG3 1950–1979 (237 cultivars); and TG4 1980–2000 (208 cultivars).

Molecular diversity within the whole set of accessions was estimated according to the following parameters: (i) allelic richness as a total number of the detected alleles and a number of alleles per locus; (ii) polymorphism information content (PIC) of all assessed loci computed according to Nei (1973); (iii) occurrence of unique and TG-specific alleles; and (iv) occurrence of heterogeneous loci. Alleles were considered to be unique if they occurred in one accession. TG-specific alleles were defined as alleles exclusively occurring in one of the specified temporal groups. The level of heterogeneity was estimated as percentage of accessions carrying double or triple alleles at the corresponding locus. These statistics and the mean Nei's genetic distance between varieties of the same TG were calculated with the programme GeneFlow V.6 (developed by GENEFLOW Inc., http://www.geneflowinc.com/). For the allelic richness in the TGs, the standard error representing the sample's standard deviation divided by $\sqrt{n}$, and the paired sample *t*-tests were performed using Microsoft Excel with the sample size $n = 35$ for 35 microsatellite loci.

Since for the two groups representing the first half of the twentieth century (TG1 and TG2), less cultivars were available than for the second half of the century (TG3 and TG4), we applied rarefaction method (Petit et al. 1998) to standardize the allelic richness across the TGs. This technique evaluates the expected number of alleles for equalized samples drawn from different TGs. We run the programme given the basic sample size of 18 since the smallest TG1 included 19 varieties. To obtain a corrected number for unique and TG-specific alleles we used the approach described by Roussel et al. (2004). For each TG we calculated the ratio, $R$ which is equal to total allele number after rarefaction divided by total allele number before rarefaction, and then multiplied the detected number of unique and TG-specific alleles by this ratio.

The programmes PopGene (Yeh et al. 1997) and NTSYSpc 2.1 (Rohlf 1998) were applied to cluster the cultivars of temporal groups TG1–TG4 and to perform principal coordinate analysis (PCoA) using a genetic similarity matrix based on genetic similarity according to Nei and Li (1979). To compute AMOVA among and within groups, data were analyzed using Arlequin software (Schneider et al. 2002). Linkage disequilibrium (LD) between pairs of polymorphic loci mapped on the same chromosome as well as on different chromosomes was evaluated using the software package TASSEL developed by the Edward Buckler group (http://www.maizegenetics.net/bioinformatics/). LD was estimated by squared allele-frequency correlations ($r^2$).

## Results

### Changes of molecular diversity over decades during the century

The genotyping of 504 barley cultivars with 35 genomic microsatellite markers resulted in a total of 280 alleles with an average of 8.0 alleles per locus with the highest number of alleles detected for chromosomes 3H and 6H (Table 2). About half of the alleles occurred with a frequency greater than 5%. The average PIC value for all markers was 0.58 ranging from 0.04 (for marker GBMS0111) to 0.91 (for Bmac0040). In total, 41 accessions carried 47 unique alleles, which were detected at 25 marker loci with up to five unique alleles per marker (Bmac0316). All markers displayed some heterogeneous accessions where more than one fragment per locus was observed. The percentage of heterogeneous data points per locus ranged from 0.4% (for markers GBMS0087, HVM30 and GBMS0111) to 12.2% (for markers GBMS0192 and GBMS0189).

**Table 3** Molecular diversity of the investigated varieties related to the period of release

| | Temporal groups (number of varieties) | | | |
|---|---|---|---|---|
| | TG1 (19) | TG2 (40) | TG3 (237) | TG4 (208) |
| Allelic richness (AR) | 161 ± 5.43 (159)[a] | 193 ± 5.44 (171)[a] | 247 ± 5.87 (167)[a] | 214 ± 5.66 (156)[a] |
| Pair-wise *t*-test versus TG1 | | $7.19 \times 10^{-5}$ | $4.25 \times 10^{-7}$ | $3.57 \times 10^{-5}$ |
| TG2 | | | $2.42 \times 10^{-5}$ | 0.0405 |
| TG3 | | | | 0.0013 |
| PIC within the group | 0.59 | 0.59 | 0.56 | 0.55 |
| Number of | | | | |
| unique alleles | 4 (3.9)[a] | 9 (8.0)[a] | 22 (14.7)[a] | 12 (8.6)[a] |
| TG-specific alleles | 4 (3.9)[a] | 9 (8.0)[a] | 29 (21.4)[a] | 18 (13.0)[a] |
| varieties carrying unique alleles | 3 (15.8%) | 8 (19.3%) | 20 (8.4%) | 10 (4.7%) |
| Mean Nei's genetic distance within TG (range of variation) | 0.61 (0.21–0.87) | 0.60 (0–0.87) | 0.56 (0 – 0.94) | 0.55 (0 – 0.94) |

[a] In brackets are given the corrected values after rarefaction to population size of 18 varieties (Petit et al. 1998)

To quantify in detail the qualitative and quantitative changes of allelic diversity over time, the 504 barley cultivars were grouped into four temporal groups (TG1–TG4) according to their year of release (Table 1). The data on allelic flux between temporal groups are summarized in Table 3.

The allelic richness for each temporal group correlated with the number of investigated cultivars ($r = 0.91$). The pair-wise Student's *t*-test showed that differences in the allelic richness were significant for TG1 versus TG2, TG3, and TG4; for TG2 versus TG1, TG3; and for TG3 versus TG1, TG2 ($P < 0.001$ in all cases). Differences in the allelic richness for TG4 versus TG2 and TG3 were insignificant ($P > 0.001$). After equalizing the number of varieties in TGs using Petit's rarefaction method (Petit et al. 1998), the data for the total allelic richness, the number of unique alleles per TG, and TG-specific alleles indicated an increase in allelic diversity from TG1 to TG2 from 159 alleles to 171 alleles, followed by a slight decrease from TG2 to TG4 with 156 alleles. Most unique alleles were observed in the group TG3, however, the highest percentage of cultivars with unique alleles were observed for the first two temporal groups with 15.8% and 19.3%, in TG1 and TG2, respectively (Table 3). TG-specific alleles, in total 60, were either unique, all TG1-TG2-specific alleles and the majority of TG3-TG4-specific alleles, or occurred several times in each of the specified temporal groups, about one third of TG3-TG4-specific alleles. The average PIC value for cultivars of the same temporal group decreased from 0.59 to 0.55 from the first TG1, to the last time period, TG4 (Table 3). This observation may be caused by a higher proportion of related accessions in the later time periods indicated also by the lower mean Nei's genetic distance value between cultivars of the TG4 (0.55 and 0.61 in TG4 and TG1, respectively) (Table 3).

Figure 1 graphically represents the fate of alleles which were present in cultivated varieties in the very beginning of the twentieth century (TG1) and acquisition of novel alleles in the later periods. The presented data were re-calculated using rarefaction

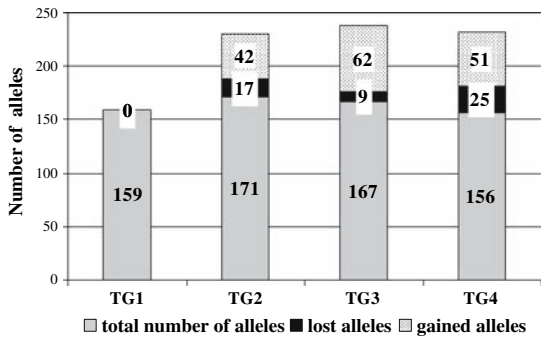



**Fig. 1** Allelic diversity flux over the decades of the 20th century: Changes in the total number of the detected alleles and the number of the gained/lost alleles as compared to the TG1 (varieties released during 1900-1929). The data for the allelic richness in temporal groups was estimated using rarefaction method, and the ratio R was applied to re-calculate the numbers of lost/gained alleles (see Data analysis)

method and the ratio $R$ as described in the data analysis. Most alleles present in the first temporal group TG1 were conserved in the later decades. The last temporal group TG4 still contained 134 (84.5%) of the 159 alleles detected in TG1. The percentages of lost alleles in the three later temporal groups (TG2, TG3, TG4) compared with the first group (TG1) were low with 10.7, 5.7, and 15.70%, respectively. Moreover, 10 alleles that were lost in the TG2 compared with TG1, re-appeared in TG3 or TG4, indicating a dynamic character of the allelic flux. Along with the preservation of the majority of alleles from the TG1 over the century, novel alleles were constantly acquired in the latter decades. Altogether, 128 novel alleles were detected in TG2–TG4 which were absent in TG1. The number of gained alleles was highest in TG3 with 62 novel alleles and declined slightly in the TG4 to 51 alleles. Novel alleles were acquired uniformly over the genome, almost at all investigated genomic loci, with one to five novel alleles per locus (data available on request). An exceptionally high number of gained alleles, in all temporal groups, occurred for locus Bmac0040 on chromosome 6H. The alleles which were lost in sequential decades appear to be well distributed over the genome as well.

## Population structure and molecular variance within the investigated set and temporal groups

The cluster analysis performed with PCoA provided by NTSYS revealed that most of the observed diversity is represented in all specified temporal groups (Fig. 2A). Cultivars belonging to different TGs were scattered over the PCoA axis 1 versus axis 2 space and showed a good overlap between the groups, although in the first two temporal groups (TG1 and TG2) the varieties designated as 'winter' barleys were less represented (Table 1 and Fig. 2A). In the later two TGs, a narrow cluster representing two-rowed spring varieties increased in significance. A good separation between 'spring' versus 'winter' types and two-rowed versus six-rowed barley acces-

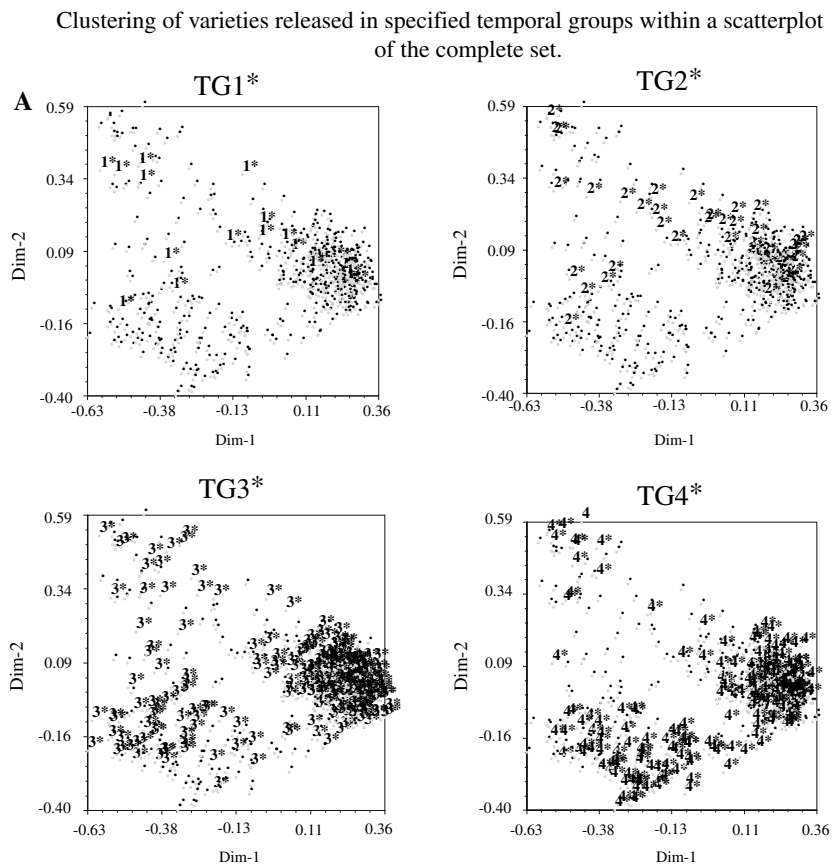
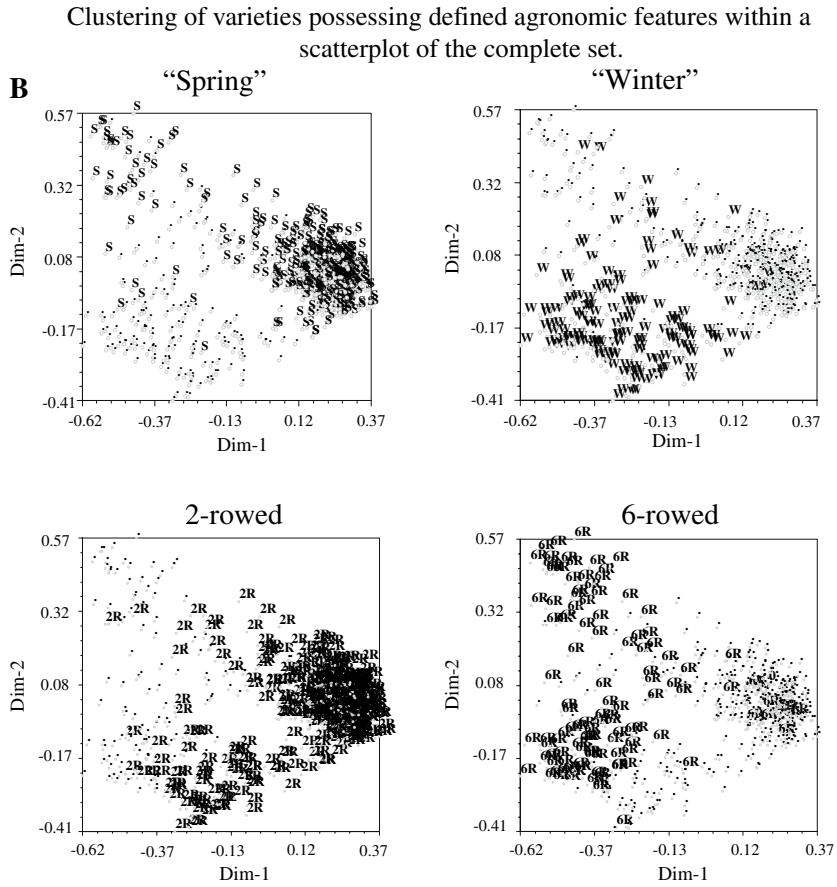



**Fig. 2** Scatter-plots of 504 barley varieties produced by PCoA based on genotyping data at 35 SSR loci with highlights on clustering of varieties released in the specified temporal groups (**A**), and varieties possessing defined agronomic features (**B**). The first two PCoA axes accounted for 13.2% and 5.1% of the total variance

**Fig. 2** continued




*Varieties belonging to the TG1-TG4 were designated on the scatterplots as 1*, 2*, 3* and 4*, respectively.

sions was found in the cluster analysis of all cultivars (Fig. 2B). The first two PCoA axes accounted for 13.2% and 5.1% of the total variance. Within the 504 European barley varieties, no well-defined sub-clusters related to different countries of origin were observed (data not shown), the same was previously reported for the independent set of European barley accessions (Malysheva-Otto et al. 2006).

These results were confirmed by the AMOVA where only variance components of 2.77% and 1.42% were obtained between the temporal groups and between individual decades of the same temporal group, respectively, whereas 95.81% of the variance resided within individual TGs (Table 4). The form of variance partitioning remained the same when only spring barleys were considered (Table 4). However, when the cultivars with different agronomic traits were considered 19.5% of the variance accounted for variation between two-rowed versus six-rowed cultivars, and 17.0% of variance between 'spring' versus 'winter' types (Table 4). The comparison between the individual decades showed that the variance component accounting for inter-decades' variation was significant when comparing each of the decades 1–8 versus decades 7–10, with $F_{st}$ values ranging from 0.0099 to 0.1212 (Table 5). The exceptions were insignificant $F_{st}$ values between populations of varieties released during decades 1 versus 7, 6 versus 7, as well as decade 9 versus 10. The significant $F_{st}$ values increased in dependence of the time interval between the decades compared (Table 5). Variation between the decades of the first half of the century (1–6) was non-significant ($P > 0.05$).

Clustering of four temporal groups in terms of a dendrogram showed that cultivars released in the first half of the twentieth century (TG1 and TG2 totaling 59 varieties) are more closely related than the ones from the second half of the century (TG3 and TG4

**Table 4** Results of AMOVA for the investigated varieties related to the periods of release and to the agronomic features

| Group | Df | Variance component | Variation accounted for (%) |
|---|---|---|---|
| **Breeding period**: | | | |
| Among temporal groups | 3 | 0.281 | 2.77 |
| Among decades within temporal groups | 5 | 0.144 | 1.42 |
| Within decades | 999 | 9.709 | 95.81 |
| **Breeding period (including ''Spring'' varieties only)**: | | | |
| Among temporal groups | 3 | 0.416 (''Spring'') | 4.48 (''Spring'') |
| Within temporal groups | 712 | 8.865 (''Spring'') | 95.52 (''Spring'') |
| **2-rowed versus 6-rowed varieties**: | | | |
| Among populations | 1 | 2.222 | 19.48 |
| Within populations | 998 | 9.242 | 80.62 |
| **''Spring'' versus ''Winter'' varieties**: | | | |
| Among populations | 1 | 1.882 | 16.98 |
| Within populations | 990 | 9.198 | 83.02 |

**Table 5** Matrix of $F_{st}$ (above the diagonal) and $F_{st}$ P-values (below the diagonal) for varieties released in sequential decades during the century

| Decades | 1 | 2–3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | – | – | – | – | – | 0.0576 | 0.0667 | 0.0956 |
| 2–3 | ns | 0 | – | – | – | 0.0573 | 0.0701 | 0.0902 | 0.1212 |
| 4 | ns | ns | 0 | – | – | 0.0414 | 0.0487 | 0.0618 | 0.0827 |
| 5 | ns | ns | ns | 0 | – | 0.0467 | 0.0585 | 0.0726 | 0.0958 |
| 6 | ns | ns | ns | ns | 0 | – | 0.0234 | 0.0397 | 0.0643 |
| 7 | ns | * | * | *** | ns | 0 | 0.0099 | 0.0294 | 0.0471 |
| 8 | * | *** | *** | *** | * | * | 0 | 0.0178 | 0.0307 |
| 9 | ** | ** | *** | *** | *** | *** | *** | 0 | – |
| 10 | *** | *** | *** | *** | *** | *** | *** | ns | 0 |

*$P < 0.05$

**$P < 0.01$

***$P < 0.00001$

totaling 445 varieties). Varieties belonging to TG1–TG2 and to TG3–TG4 clustered more closely together (the dendrogram is available on request). Clustering was based on Nei's genetic distances using UPGMA and 35 SSR loci.

## Analysis of LD among SSR loci

LD evaluated as squared allele-frequency correlations $r^2$ was assessed for 595 combinations of SSR loci for the complete set of varieties and for varieties released in the specified temporal groups TG1–TG4, and for a subpopulation of two-rowed spring varieties to check for the influence of population structure on LD parameters. Since the number of varieties in TG1 and TG2 were low for LD analysis with TASSEL they were combined in one group. As previously suggested (Malysheva-Otto et al. 2006) three criteria were considered: the percentage of loci pairs in significant LD ($P < 0.001$), the value of $r^2$, and the extent of LD on the chromosomes. The results are summarized in Table 6.

For the complete set of investigated varieties, 89% of the assessed loci pairs were in significant LD ($P < 0.001$), the percentage of loci pairs showing elevated levels of $r^2 > 0.05$ equaled 11%, and among

**Table 6** Overview of the genome-wide and intra-chromosomal LD detected for the complete set of investigated varieties, for varieties released in the different time periods (TG1–TG4), and for a subset of two-rowed spring barleys

| Analyzed groups of varieties | Genome-wide LD | | Intra-chromosomal LD | |
|---|---|---|---|---|
| | % of loci pairs in LD[a] with P < 0.001 | % of loci pairs with $r^2 > 0.05$ | % of loci pairs with $r^2 > 0.05$ | Mean $r^2$ (range of variation) |
| TG1–TG4 (504 varieties released in 1900–2000) | 89% | 11% (64 pairs) | 2.0% (12 pairs) | 0.128 (0.054–0.398) |
| TG1 + TG2 (59 varieties released in 1900–1949) | 32% | 24% (140) | 3.1% (20) | 0.148 (0.058–0.305) |
| TG3 (237 varieties released in 1950–1979) | 74.4% | 15.3% (99) | 2.9% (17) | 0.126 (0.055–0.369) |
| TG4 (208 varieties released in 1980–2000) | 59.3% | 14.1% (84) | 2.5% (15) | 0.148 (0.052–0.558) |
| Two-rowed spring sub-population (306 varieties) | 44% | 2.9% (17) | 1.3% (8 pairs) | 0.179 (0.053–0.779) |

[a] In all cases, a total of 595 loci pairs were analyzed

them intra-chromosomal LD accounted for 2% of loci pairs with mean $r^2$ value of 0.128. These LD values were comparable with the previously published data for barley (Kraakman et al. 2004; Malysheva-Otto et al. 2006). The corresponding LD values detected for the groups of varieties released in the specified TGs showed a good correlation with the number of varieties in the respective group with regard to genome-wide LD (Table 6). Intra-chromosomal LD for each considered TG was higher than for the complete set. Ten intra-chromosomal loci pairs in significant LD with $r^2 > 0.05$ were in common across all TGs, and 18 loci pairs revealed LD only in one of the groups. However, the LD parameters for the sub-population of 306 two-rowed spring cultivars did not correlate with the number of cultivars in the set if considered together with the respective values for TGs. This sub-population revealed the highest detected mean value of intra-chromosomal LD in this study with $r^2 = 0.179$, and the lowest number of loci pairs with $r^2 > 0.05$ (17 genome-wide including 8 intra-chromosomal loci pairs) (Table 6).

The plots of intra-chromosomal LD ($r^2$) as a function of genetic distance in centiMorgans (cM) revealed a tendency of LD to decay with the genetic distance (Fig. 3). In different sub-populations, LD extended to distances of up to 50 cM away with $r^2 > 0.2$, indicating that mainly moderately (at 4–9 cM distance) and loosely linked (at 15–50 cM distance) loci revealed LD, although a few unlinked intra-chromosomal loci pairs (at > 50 cM distance) were in LD with $r^2 > 0.15$ (loci linkage classification as in Maccaferri et al. 2005). The most abrupt decay was detected in the sub-population of two-rowed spring cultivars (Fig. 3).

## Discussion

Although much effort has already been expended in quantifying an impact of intensive breeding on the diversity flux of cultivated crops (Donini et al. 2000; Christiansen et al. 2002; Koebner et al. 2003; Roussel et al. 2004, 2005; Fu et al. 2005, 2006; Reif et al.

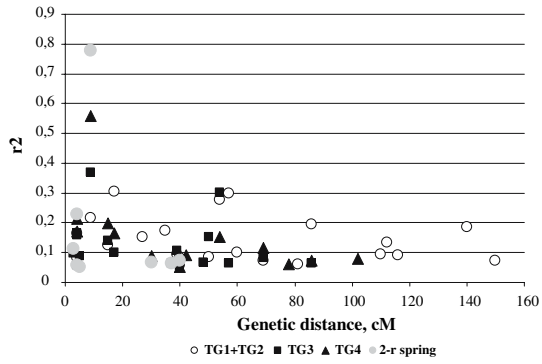



**Fig. 3** The pattern of intra-chromosomal LD detected for varieties released in the different time periods (TG1–TG4), and for a sub-population of two-rowed spring barleys. Plots of LD represented by $r^2$ against genetic distance are shown for varieties released in TG1 and TG2 (○), TG3 (■), TG4 (▲), and for a sub-population of two-rowed spring barleys (●)

2005a,b; Huang et al. 2007), it may be argued that a consensus opinion has not yet been reached. Concerning the importance of preservation of natural diversity and possible genetic erosion as a consequence of intensive breeding, the data obtained in our study were analyzed in relation to two important points. First, if genetic diversity did change over the century and a profound genetic shift occurred, then population stratification imposed by differences between varieties released in various decades should be detected in the investigated set of 504 barley varieties representative of diversity available and exploited in a European agricultural context. Secondly, the tendencies in the changes of allelic diversity, if any, observed in cultivars released in sequential decades during the twentieth century, were investigated.

## Search for evidences indicating population stratification produced by temporal groups

Since breeding may be viewed, in part, as a man-made directional selection, evaluation of population structure can be applied to follow the presence of selection and its degree. Such studies involving computing $F_{st}$ were recently published for humans (Hinds et al. 2005) and *Brassica insularis* (Glemin et al. 2005). To reveal population sub-structures in the complete set of investigated varieties released during the twentieth century, we applied two independent statistical approaches, namely PCoA and AMOVA.

With PCoA, although the portion of the variation accounted for in the first two PcoA axes is modest, the technique is well able to separate 'spring' types from 'winter' types, and to differentiate two-rowed versus six-rowed varieties (Fig. 2B). No such differentiation was evident from the PCoA with respect to cultivars' release periods as the same temporal groups were dispersed over the PCoA diagram and showed complete inter-time group overlap (Fig. 2A). This means PCoA proved the absence of differentiation with regard to the temporal groups. The graphical image was also supported by the calculation of Nei's genetic distance between cultivars of the same temporal group. Individual variety pair-wise genetic distances within every group varied considerably (from 0 to 0.94) with the mean values always higher than 0.55 (Table 3). Comparable values of Nei's genetic distance were reported within temporal groups of wheat accessions, 0.68 and 0.65 for the beginning and the end of the twentieth century, respectively (Roussel et al. 2005).

The results of AMOVA fully supported these conclusions. Most of the diversity resided within decades (95.81%) and not between TGs (2.77%) or between decades within the TGs (1.42%), whereas 17.0% and 19.5% accounted for variation between 'spring' versus 'winter' varieties and two-rowed versus six-rowed varieties, respectively (Table 4). Here again population stratification produced by seasonal growth types rather than temporal grouping was of major impact. Similarly, Roussel et al. (2004, 2005) reported 2.2–3.2% variation accounting for among temporal groups' variances in French bread wheat accessions. Soleimani et al. (2005) reported for barleys grown in the USA and Canada, a 22.6% and 23.2% variance component between two-rowed versus six-rowed and 'spring' versus 'winter' cultivars. Their investigation involved 103 cultivars released between 1956 and 2001. Whereas in our study significant $F_{st}$ values were detected for about half of between-decadal comparisons, the absolute values of $F_{st}$ were always below 0.2 indicating an absence of major population structure on the level of temporal groups (Table 5). In summary, a combination of two statistical approaches commonly applied in population genetics provided convincing evidence for the absence of any significant substructures within the set of analyzed cultivars imposed by temporal groups.

Contrasting results for the diversity changes over the time (years 1845–2004, with eight cultivars released before 1910) were recently published for a set of 75 Canadian hard-red spring wheat cultivars (Fu et al. 2005, 2006). The authors claimed reduction of genetic diversity by the end of the century. They obtained 12.8% variation accounting for among temporal groups variance (AMOVA), and cluster analysis based on group-wise similarities separated accessions belonging to different temporal groups with a threshold genetic similarity of 0.36 and 0.70 for genomic and genic SSRs, respectively (Fu et al. 2005, 2006). However, in their study only Canadian cultivars were assessed and the number of cultivars per breeding period was quite low. Moreover, in the later published review Fu (2006) claimed that genome-wide reduction of genetic diversity appeared to be minor, but allele losses at specific chromosomal

loci could be substantial, because these lost alleles may be associated with undesired traits.

### Temporal flux of allelic diversity

The total number of 280 alleles with an average of 8.0 alleles per locus detected in 504 European varieties was comparable to the reported values for cultivated varieties of barley with 11.3 alleles per locus (Malysheva-Otto et al. 2006), and wheat with 10.5 alleles/marker (Röder et al. 2002). While in a worldwide set of 953 barley accessions, on average 16.7 alleles/marker were found, in the subset of 565 European accessions released over the century 11.3 alleles per locus were reported (Malysheva-Otto et al. 2006).

Allelic richness in the specified temporal groups varied over the century (depicted in Fig. 1 and Table 3). The number of varieties in the first two TGs was low as compared with the TG3 and TG4 (determined by the availability of varieties from the beginning of the twentieth century). However, considering the non-linear increase of the allelic richness in the population with the increase of the population size (described in Malysheva-Otto et al. 2006) and corrected numbers after applying rarefaction method (Petit et al. 1998), we can confirm only slight changes in the allelic richness by the end of the century. Indeed, the majority of the alleles found in TG1 were preserved during the century [134 (84.5%) out of 159 alleles] and were registered in cultivars released after 1980 resulting in a loss of only 15.7% of alleles from TG1. The loss of alleles occurred in every specified temporal group starting from TG2, and no tendency for an expanded on contracted loss of alleles by the end of the century was observed. This may be an indication that loss of alleles detected in our study was a random evolutionary phenomenon rather than a consequence of breeding selection pressure *per se*. Along with the preservation of the majority of alleles from the first temporal group TG1 over the century, novel alleles were constantly acquired in the latter decades, with 24.3, 37.2, and 33.2% of novel alleles in the TG2, TG3, and TG4, respectively. Novel alleles were acquired uniformly over the genome, almost at all investigated genomic loci, with one to five novel alleles per locus. In conclusion, although allelic diversity during the sequential decades of the twentieth century was permanently changing, about 90% of alleles were shared in all temporal groups.

LD which reflects a combination of mutation, selection, and genetic drift can also serve as a parameter for evaluation of the presence of selection, and this was previously demonstrated for wild wheat in the case of natural selection (Li et al. 2000). The assumption made here is that subgroups producing population structure as a result of selection are expected to show a higher degree of LD. In our case the LD detected among assessed genomic loci within the complete set and varieties released during specified temporal groups, supported the conclusion towards absence of the population structures produced by temporal grouping. Hence, in this large set of European barleys which had been deliberately selected to represent both the genetic diversity available in released material as well as the one exploited and utilized in successful commercial varieties, LD in the groups produced by the time period of release was correlated with the number of evaluated varieties and not to temporal grouping (Table 6). However, LD within the group of two-rowed 'spring' barley varieties, which was shown to produce population structure (Fig. 2A), revealed no dependence on the number of involved varieties but elevated value, when compared with other analyzed groups of $r^2$ ($r^2$ = 0.179) (Table 6). An increased value of $r^2$ in the sub-population of two-rowed 'spring' varieties was also reported in a previous study for the independent set of European varieties (Malysheva-Otto et al. 2006).

## Conclusions

In our study we investigated the flux of genetic diversity using two approaches, population structure analysis, and quantification of allelic diversity. In summary, the results indicated that neither the impact of breeding nor the variety evaluation/delivery systems resulted in any apparent quantitative losses of genetic diversity or changes of population structure in the extensive representative set of European barley cultivars over time. No obvious genetic erosion patterns could be detected. AMOVA uncovered strong differentiation among groups of cultivars possessing specific agronomic features but not among temporal groups. Qualitative changes in the allelic diversity were observed only for a small portion of the detected alleles while in quantitative terms there

is no evidence for a contraction or expansion in allelic flux over the time period studied. It should be emphasized, however, that these considerations are only based on data of widely grown cultivars from RL or NL, and the drawn conclusions must not be extended to the relationship of genetic diversity among landraces and released cultivars.

Our results do not imply that genetic diversity of barley cultivars remained fixed over the century, we confirmed both loss and gain of alleles at multiple loci. In general it can be suggested that genetic diversity of barley varieties cultivated in Europe during the last century is a complex dynamic feature which is permanently changing without obvious reduction. The factors affecting diversity could be of economical nature and may be influenced by structural changes in agriculture. New and changing goals for variety development influenced the involvement of accessions with new pedigrees in the breeding process.

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