Theor Appl Genet (2005) 111: 162–170
DOI 10.1007/s00122-005-2014-8

ORIGINAL PAPER

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# SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000

Received: 23 September 2004 / Accepted: 21 March 2005 / Published online: 11 May 2005


**Abstract** A sample of 480 bread wheat varieties originating from 15 European geographical areas and released from 1840 to 2000 were analysed with a set of 39 microsatellite markers. The total number of alleles ranged from 4 to 40, with an average of 16.4 alleles per locus. When seven successive periods of release were considered, the total number of alleles was quite stable until the 1960s, from which time it regularly decreased. Clustering analysis on Nei's distance matrix between these seven temporal groups showed a clear separation between groups of varieties registered before and after 1970. Analysis of qualitative variation over time in allelic composition of the accessions indicated that, on average, the more recent the European varieties, the more similar they were to each other. However, European accessions appear to be more differentiated as a function of their geographical origin than of their registration period. On average, western European countries (France, The Netherlands, Great Britain, Belgium) displayed a lower number of alleles than southeastern European countries (former Yugoslavia, Greece, Bulgaria, Romania, Hungary) and than the Mediterranean area (Italy, Spain and Portugal), which had a higher number. A hierarchical tree on Nei's distance matrix between the 15 geographical groups of accessions exhibited clear opposition between the geographical areas north and south of the arc formed by the Alps and the Carpathian mountains. These results suggest that diversity in European wheat accessions is not randomly distributed but can be explained both by temporal and geographical variation trends linked to breeding practices and agriculture policies in different countries.

**Electronic Supplementary Material** Supplementary material is available for this article at http://dx.doi/10.1007/s00122-005-2014-8

Communicated by A. Charcosset

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## Introduction

There have been many reports recently on the impact of plant breeding on crop genetic diversity as determined by means of molecular markers. Some of the investigators have suggested that the reduction in genetic diversity accompanying plant improvement has been very limited. For example, with respect to cereal crops, Donini et al. (2000) analysed 55 UK wheat accessions released from 1934 to 1995 with six amplified fragment length polymorphism (AFLP) and 14 simple sequence repeat (SSR) loci, while Koebner et al. (2003) studied 134 UK barley varieties registered over the period 1925–1995. Both authors reported that in the United Kingdom, plant breeding has resulted in a qualitative rather than a quantitative shift in the diversity of these crops over time.

In contrast, Fu et al. (2003) analysed 96 Canadian oat cultivars released from 1886 to 2001 with 30 SSRs and detected a significant decrease in allele diversity at specific loci after the 1970s. They linked these changes to breeding practices. More recently, Roussel et al. (2004) analysed 559 French bread wheat accessions (landraces and cultivars from 1800 to 2000) with a set of 42 microsatellite markers and clearly demonstrated a significant decrease in allelic diversity at the end of the 1960s. In fact, as suggested by Fu et al. (2003), these differences gauging the effects of plant breeding could be explained both by the sample size of the accessions examined and the use of different molecular markers. As already demonstrated in oats, the evaluation of allelic diversity requires effective molecular tools such as SSR markers

in sufficient numbers rather than AFLPs with limited polymorphism per locus. In the paper by Roussel et al. (2004), the results were obtained with quite a large number of markers on a large set of accessions, but all of the accessions originated from the same geographical area (France). This naturally lead to the following question of whether the decrease in diversity was the same on a wider geographical scale.

We report here the results of a genetic study of 480 European bread wheat accessions released from 1840 to 2000 using 39 microsatellite loci. The objectives of this study were: (1) to characterise the allelic diversity of the whole set of accessions; (2) to assess changes in allelic diversity in European bread wheat cultivars over time; (3) to compare the level and the distribution of genetic diversity in 480 European wheat varieties as a function of their geographical origin.

## Materials and methods

### Plant material

A set of 480 bread wheat accessions were chosen from fifteen different European countries or groups of geographical areas and seven different periods of registration or temporal groups (Table 1). In order to facilitate pair-wise comparison, the accessions were selected to obtain a similar number of accessions (66–72) for each temporal group and exactly the same number of accessions (32) for each geographical origin. A list of the names of the accessions and their respective breeder(s) (when known) is provided as electronic supplementary material (see ESM). Seeds were obtained both from the Centre of Biological Resources on Cereal Crops (INRA-Clermont-Ferrand) and the Gene bank of the Research Institute for Crop Production (RICP-Prague). The accessions were selected from the available seed samples of these two gene banks on the basis of meeting, as much as possible, a combination of the following three criteria: (1) equal sample size for each geographical origin; (2) equal sample size for each period of registration; (3) good sample and repartition between breeders' origin within a same geographical origin, to avoid a sampling of accessions with pedigrees that were too similar. As shown in Table 1, the two first objectives were almost perfectly met, except for the sample size of last registration period (1990–1999) where four regions are not represented. Unfortunately, because both the specific organisation of the companies involved in breeding tasks in some countries was limited (sometimes only one public institute per country) and the fact that a lot of passport data were unknown (breeders' origin, pedigrees), it was not always possible to fully satisfy the third criterium. However, the fact that some countries are only represented by a few breeders is an historical reality and cannot be considered to be a factor that carries a risk of biased sampling.

### DNA isolation and fragment analysis

All of the seeds used for DNA extraction were obtained from self-pollinated ears. Five to six plants per accession were pooled, and genomic DNA was isolated using the Genelute Plant Genomic DNA kit (G2N-350; Sigma, St. Louis, Mo.). As fragment analysis was performed in two independent laboratories (RICP and INRA) using different sequencers but similar technologies (ABI PRISM 310 by RICP and ABI PRISM 3100 by INRA; both capillary methodologies produced by Applied Biosystems, Foster City, Calif.), 42 wheat SSR markers (those

**Table 1** Distribution of the 480 accessions as a function of their geographical origin and their registration period

| Origin[a] | Registration period | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | 1840–1929 | 1930–1949 | 1950–1959 | 1960–1969 | 1970–1979 | 1980–1989 | 1990–1999 | |
| AUT/CHE | 7 | 4 | 4 | 6 | 4 | 4 | 3 | 32 |
| BEL | 2 | 5 | 10 | 7 | 4 | 4 | | 32 |
| BGR/ROM | | | 3 | 4 | 11 | 12 | 2 | 32 |
| CSK | 3 | 8 | 2 | 2 | 3 | 3 | 11 | 32 |
| DEU | 4 | 4 | 3 | 5 | 4 | 5 | 7 | 32 |
| ESP/PRT | 11 | | 3 | 9 | 2 | 4 | 3 | 32 |
| FIN/DNK | 2 | 9 | 4 | 4 | 8 | 5 | | 32 |
| FRA | 6 | 6 | 6 | 2 | 2 | 2 | 8 | 32 |
| GBR | 8 | 4 | 1 | 2 | 4 | 4 | 9 | 32 |
| HUN | 2 | 3 | 7 | 3 | 4 | 4 | 9 | 32 |
| ITA | 6 | 7 | 7 | 4 | 3 | 3 | 2 | 32 |
| NLD | 4 | 3 | 2 | 6 | 4 | 4 | 9 | 32 |
| POL | 3 | 5 | 10 | 6 | 4 | 4 | | 32 |
| SWE | 7 | 8 | 3 | 5 | 3 | 3 | 3 | 32 |
| YUG/GRC | 3 | 2 | 1 | 4 | 11 | 11 | | 32 |
| Total | 68 | 68 | 66 | 69 | 71 | 72 | 66 | 480 |

[a]AUT/CHE, Austria/Switzerland; BEL, Belgium; BGR/ROM, Bulgaria/Romania; CSK, Czech and Slovak Republics; DEU, Germany; ESP/PRT, Spain/Portuga; FIN/DNK, Finland/Denmark; FRA, France; GBR, Great Britain; HUN, Hungary; ITA, Italy; NLD, The Netherlands; POL, Poland; SWE, Sweden; YUG/GRC, former Yugoslavia/Greece

used in Roussel et al. 2004) were first tested in both laboratories on a common set of about 50 varieties as controls. Subsequently, only primers that amplified with readable products and for which the two laboratories agreed on the allelic composition were selected. Table 2 gives the final list of the 39 polymorphic loci used in the present analysis and their location on the chromosome map. Each chromosome is represented by at least one marker. The whole set of accessions was shared between both laboratories where PCR analyses were performed according to Röder et al. (1998), also using the 50 first tested cultivars as controls. Finally, fragment analysis was carried out in each laboratory, and fragment sizes were calculated using the same GENESCAN and GENOTYPER software, where different alleles were represented by different amplification sizes for tandem repeats. After allele sizes had been checked on the controls, the two datasets were merged into one.

**Table 2** Chromosome location, total number of alleles, number of rare alleles and Nei's diversity index (*H*) for 39 microsatellite loci

| Locus | Location | Number of alleles | Number of rare alleles | *H* |
|---|---|---|---|---|
| Xgwm99 | 1A | 13 | 9 | 0.575 |
| Xgwm135 | 1A | 26 | 21 | 0.726 |
| Xgwm11 | 1B | 14 | 9 | 0.796 |
| Xgwm413 | 1B | 18 | 14 | 0.786 |
| Xgwm642 | 1D | 11 | 8 | 0.612 |
| Xgwm337 | 1D | 18 | 13 | 0.807 |
| Xgwm312 | 2A | 28 | 22 | 0.871 |
| Xgwm372 | 2A | 24 | 17 | 0.899 |
| Xgwm257 | 2B | 7 | 5 | 0.567 |
| Xgwm120 | 2B | 19 | 12 | 0.841 |
| Xgwm539 | 2D | 30 | 25 | 0.876 |
| Xgwm261 | 2D | 19 | 15 | 0.695 |
| Xgwm2 | 3A | 7 | 4 | 0.483 |
| Xgwm480 | 3A | 10 | 9 | 0.212 |
| Xgwm285 | 3B | 40 | 36 | 0.750 |
| Xgwm566 | 3B | 8 | 4 | 0.750 |
| Xgwm664 | 3D | 4 | 1 | 0.211 |
| Xgwm341 | 3D | 19 | 13 | 0.854 |
| Xgwm610 | 4A | 15 | 12 | 0.578 |
| Cfd71A | 4A | 9 | 8 | 0.284 |
| Xgwm251 | 4B | 19 | 14 | 0.841 |
| Xgwm149 | 4B | 9 | 6 | 0.507 |
| Cfd71D | 4D | 17 | 11 | 0.859 |
| Xgwm415 | 5A | 6 | 3 | 0.626 |
| Xgwm186 | 5A | 17 | 11 | 0.838 |
| Xgwm408 | 5B | 19 | 15 | 0.785 |
| Xgwm234 | 5B | 12 | 4 | 0.843 |
| Xgwm272 | 5D | 9 | 5 | 0.634 |
| Xgwm190 | 5D | 13 | 8 | 0.677 |
| Xgwm427 | 6A | 20 | 14 | 0.853 |
| Xgwm219 | 6B | 23 | 18 | 0.860 |
| Xgwm626 | 6B | 11 | 9 | 0.536 |
| Xgwm469 | 6D | 14 | 9 | 0.838 |
| Xgwm325 | 6D | 12 | 7 | 0.742 |
| Xgwm260 | 7A | 22 | 18 | 0.765 |
| Xgwm400 | 7B | 17 | 13 | 0.782 |
| Xgwm46 | 7B | 19 | 14 | 0.849 |
| Xgwm44 | 7D | 15 | 9 | 0.851 |
| Xgwm437 | 7D | 22 | 16 | 0.808 |
| Total | | 635 | 461 | |

### Data analysis

Standard statistics for characterising genetic variability were first computed for each locus and for the whole set of 480 accessions: the total number of alleles, then the number of rare alleles ($P < 0.05$) and the genetic diversity index (*H*) of Nei (1973) were calculated. This index, which is equivalent to the Polymorphic Information Content (PIC) index, was calculated for each locus, according to the formula:

$$\mathrm{H} = 1 - \sum pi^2$$

where $p_\mathrm{I}$ is the frequency of the *i*th allele.

Accessions were then grouped according to either their period of registration or their geographical origin in order to calculate the exact number of alleles and Nei's average gene diversity across loci for each group. The dataset was analysed using ARLEQUIN software (Schneider et al. 1996) to compute molecular variance (AMOVA) between and within groups. Finally, in order to examine the population structure, Nei's genetic distance (Nei 1973) was calculated between each temporal and geographical group of accessions using GENETIX software, and the robustness of the genetic distance between each group was tested by a permutation test. A hierarchical tree was performed on each matrix of distance using the 'S' programming environment (Becker et al. 1988).

## Results

### Global diversity

Five to six seeds from self-pollinated plants were used for DNA extraction, and most of the amplification products revealed only one allele per locus. When two different alleles were present at a single locus, data were considered to be missing, since it was not possible to distinguish between a DNA mixture of heterogeneous varieties or heterozygosity. Nevertheless, even in these conditions, the global percentage of missing data was lower than 5% and related with neither the geographical origin nor the date of release of the accessions.

Standard statistics are summarised in Table 2: a total of 635 alleles were detected from the 39 amplified loci. Primer pair gwm285 detected 40 alleles (the largest number), and gwm664 only four alleles, while the average number of alleles detected per locus was 16.4. The number of rare alleles also varied considerably, ranging from one for gwm664 to 36 for gwm285. The total number of rare alleles (461) represented about 73% of the total number of alleles. The microsatellite markers used showed different levels of gene diversity: the genetic diversity index of Nei (*H*) ranged from 0.211 to 0.899, with an average of 0.650 for all markers. As this index is equivalent to the PIC index, these results also provide an estimate of the discriminatory power of each microsatellite locus.

## Temporal variations

Table 3 gives the results of AMOVA with respect to the effect of the temporal group. Although highly significant ($P < 0.001$), the between-variance component represents only 2.23% of the total variation. Consequently, only a small proportion of the overall variance appears to be the result of any temporal drift between groups.

As a means to characterise the genetic diversity of the seven different temporal groups and to identify the evolution of diversity over time, Fig. 1 displays the total number of alleles per group and Fig. 2 shows the evolution of Nei's average gene diversity. As the number of accessions was high and quite similar per group, it was not necessary to use Petit's rarefaction allele method (Petit 1998), and so the number of alleles per group could be compared directly (Fig. 1). It can be seen that the number of alleles was quite stable for the first three periods (about 380/group), then decreased regularly after the 1960s to reach 313 in the last decade of the twentieth century, i.e. a decrease of about 18% in allelic richness between these periods. Figure 2 shows a much more erratic evolution of the gene diversity index over time: there was a low average gene diversity from 1840 to 1929, then a strong increase in this parameter from 1930 to 1949 and finally a tendency for average gene diversity to decrease from 1950 to 2000, with a slight increase between 1970 and 1989.

In order to identify qualitative variations in allelic diversity over time, the number of alleles was also analysed in each temporal group. On the basis of both the total number of alleles present in the whole set of 480 accessions and two successive temporal groups, I and j, we calculated the four values, namely *a*, *b*, *c* and *d*, where *a* is the percentage of alleles present in both groups I and j, *b* is the percentage of alleles present in I and absent in j, *c* is the percentage of alleles present in j and absent in I and, finally, *d* is the percentage of alleles absent both in i and j. Figure 3 shows the evolution over time of *a*, *b*, *c* and *d* in successive temporal groups pairs. The *a* values are quite constant over time, except perhaps in the last period (1980–1990/1990–2000). In contrast, the negative slope of the *b* and *c* curves and, consequently, the opposing increase of *d* values over time show that global differentiation between successive temporal groups has been slower in more recent times relative to the earliest periods. In other words, the more recent the European varieties, the more they resemble each other in their global allelic composition. Furthermore, the dramatic increase in the *d* values (percentage of absent alleles) at the end of the twentieth century confirms the results observed on the decrease in average gene diversity for the same period (Fig. 2). The hierarchical tree resulting from cluster analysis on the Nei's distance matrix between temporal groups is shown in Fig. 4: temporal groups are clearly and significantly separated into two clusters: before and after 1970.

**Table 3** Analysis of molecular variance (AMOVA): effect of temporal group

| Source | *df* | Variance component | Variation accounted for (%) |
|---|---|---|---|
| Between temporal groups | 6 | 0.23*** | 2.23 |
| Within temporal groups | 473 | 10.23*** | 97.77 |
| Total | 479 | 10.46 | 100.00 |

***Significant at $P < 0.001$

## Geographical variations

The results of AMOVA of the geographical group effect are shown in Table 4. The two sources of variation are highly significant, but the within-group component is still dominant. However, in our present study, the between-group component accounted for 7.75% of the total variation, indicating that the whole set of 480 European accessions appears to be more differentiated as a function of their geographical origin than of their registration period.

Figure 5, which shows the exact number of alleles per geographical area, highlights the significant variation between geographical origins. As the number of accessions and loci used in the present study are the same for each geographical group, the total number of alleles can be compared directly. Based on a total of 321 alleles, the accessions from Spain and Portugal appear to have the highest level of allelic richness, while French accessions have a lower level (227 alleles). When we consider the

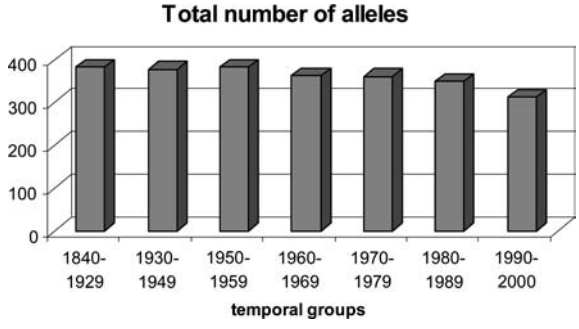


**Fig. 1** Total number of alleles per registration period

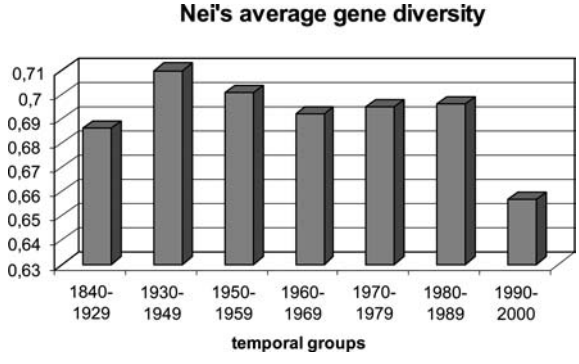


**Fig. 2** Average gene diversity per registration period

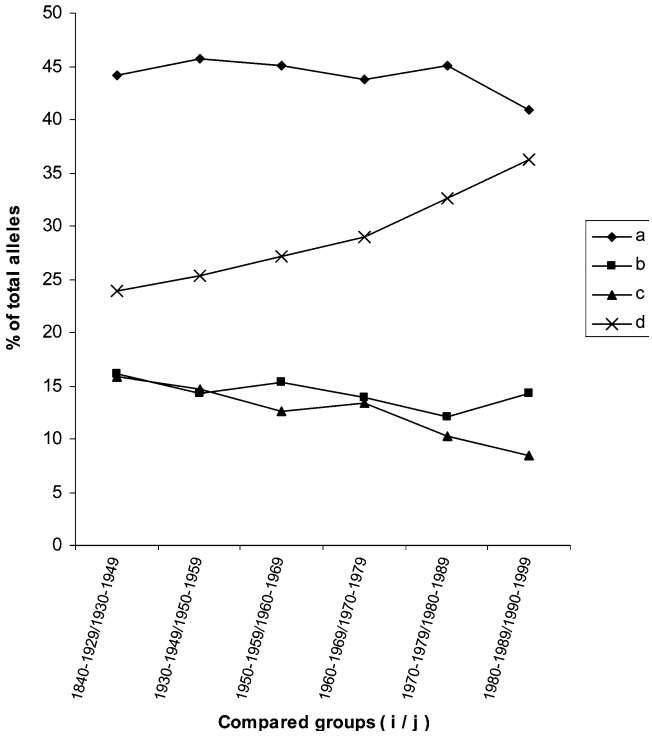


**Fig. 3** Evolution of percentage of total alleles in two successive temporal groups, I and j. *a* percentage of alleles present in both groups I and j, *b* percentage of alleles present in I and absent in j, *c* percentage of alleles absent in I and present in j, *d* percentage of alleles absent in both groups I and j

**Table 4** Analysis of molecular variance (AMOVA): effect of geographical group

| Source | *df* | Variance component | Variation accounted for (%) |
|---|---|---|---|
| Between geographical groups | 14 | 0.81*** | 7.75 |
| Within geographical group | 465 | 9.67*** | 92.25 |
| Total | 479 | 10.48 | 100.00 |

***Significant at $P < 0.001$

overall mean of total alleles per geographical area (about 262), it can be seen that, on average, western European countries (France, The Netherlands, Great Britain and Belgium) have fewer alleles than the south-eastern European countries (former Yugoslavia, Greece, Bulgaria, Romania and Hungary) and the Mediterranean region (Italy, Spain and Portugal), with the latter region having the highest number.

Figure 6, which shows the hierarchical tree resulting from cluster analysis on the Nei's distance matrix between geographical groups, supports our earlier results: the 15 geographical areas are clearly separated into two main clusters. The first cluster comprises geographical areas located south of the arc formed by the Alps and the Carpathian mountains (Italy, former Yugoslavia/Greece, Bulgaria/Romania and Hungary); the second cluster comprises all of the other geographical areas. At a lower level of clustering, western European countries (France, The Netherlands, Great Britain, Belgium,

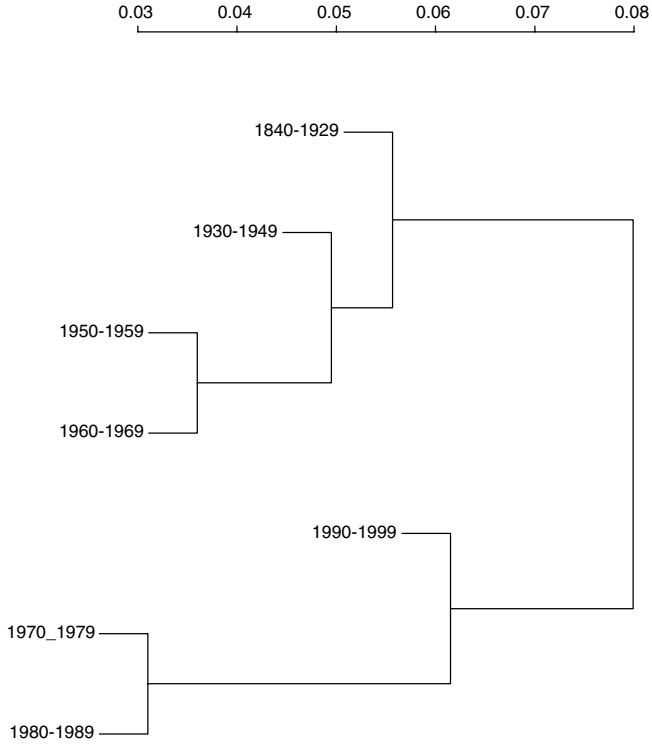


**Fig. 4** Cluster analysis of Nei's matrix distance between seven temporal groups of accessions

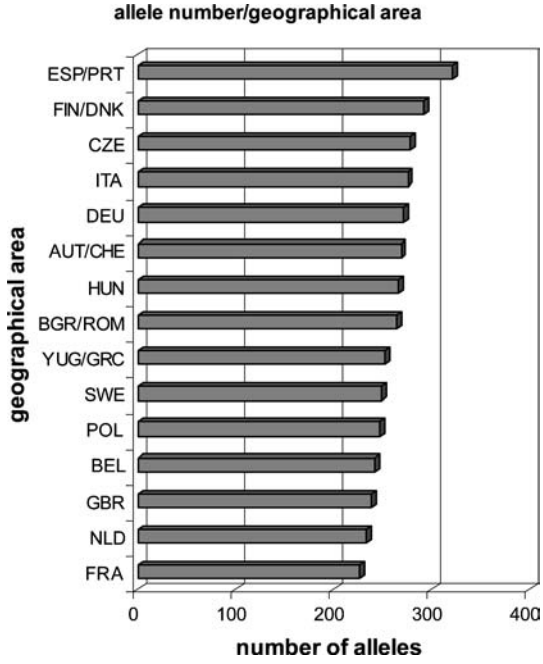


**Fig. 5** Number of alleles per geographical areas. *AUT/CHE* Austria/Switzerland, *BEL* Belgium, *BGR/ROM* Bulgaria/Romania, *CSK* Czech and Slovak Republics, *DEU* Germany, *ESP/PRT* Spain/Portugal, *FIN/DNK* Finland/Denmark, *FRA* France, *GBR* Great Britain, *HUN* Hungary, *ITA* Italy, *NLD* The Netherlands, *POL* Poland, *SWE* Sweden, *YUG/GRC* former Yugoslavia/Greece

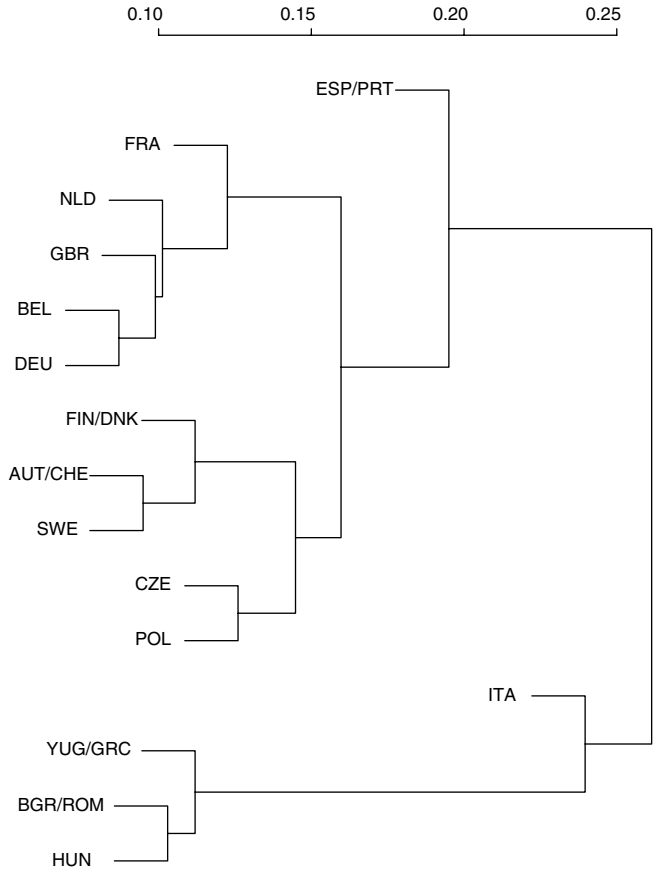


**Fig. 6** Cluster analysis of Nei matrix distance between 15 geographical groups of accessions

Germany) are separated from northern and central European countries (Finland/Denmark, Sweden, Austria/Switzerland, Poland, the Czech and Slovak Republics), while Spain/Portugal is apart.

## Discussion

One main goal of this research was to characterise global genetic diversity in a whole set of European accessions using microsatellite markers. A great deal of effort has already been put into characterising genetic diversity in wheat, and at the present time, the usefulness of using SSRs to detect DNA polymorphism in this species is universally recognised (Plaschke et al.1995; Prasad et al. 2000; Manifesto et al. 2001; Ben Amer et al. 2001; Leisova and Ovesna 2001; Zhang et al. 2002). Röder et al. (2002), analysed 502 recent European wheat varieties using 19 SSRs and detected a total of 199 alleles. In the present study, 635 alleles were investigated with twice the number of microsatellite markers. The apparent lower diversity in Röder's study can probably be explained by the fact that the accessions used were more recent and that the number used for each country was not the same. Compared with the results of Roussel et al. (2004), which were obtained on 559 French accessions released during the same period of time and analysed with a similar number of loci as we have used in the present investigation, we observed, on average, a higher diversity in our European varieties: the mean number of alleles was 16.4, against 14.5 for the French accessions. Despite the fact that a greater number of landraces were sampled in Roussel's study and that the percentage of rare alleles was equivalent in both studies (73%), we observed an increase in the number of common alleles in European accessions, which explains their higher diversity.

Our results on the effect of the release period on diversity and, consequently, on the impact of breeding programmes on European bread wheat diversity are in agreement with those observed for the French sample analysed by Roussel et al. (2004). (1) The between-group component of AMOVA (Table 3) is significant, but the major part of the diversity is due to the genetic variation between accessions within temporal groups. (2) The decrease in the total number of alleles in the European varieties (Fig. 1) from 1840 until 2000 appears to be of the same magnitude as for French varieties during the same period. As suggested by Christiansen et al. (2002), the erratic evolution of average gene diversity (Fig. 2), which was also observed by these authors in Nordic Spring wheat during the twentieth century, may be probably explained by the history of breeding in Europe during the same period of time. We can consider the following hypotheses. (1) The strong increase in average gene diversity observed from 1930 to 1949 in European varieties could be due to the development of new and important breeding programmes by an increasing number of different companies, but also by the introduction and use of new germplasm issued from hybrids created in the previous period. (2) The evolution (decreasing diversity) observed during the 1950s and the 1960s might be explained by an intensive use of a small number of identical varieties as genitors in the different European breeding programmes (bottleneck effect). (3) The slight increase in diversity during the1970s and the 1980s might be related to the introduction of original germplasm (exotic germplasm and interspecific crosses), including some specific genes (i.e. semi-dwarf genes) and interesting new introgressions (i.e. the *Pch1* gene) or translocations (i.e. 1B/1R). (4) The drastic reduction in average gene diversity observed during the last decade can be a consequence of both a reduction in the number of breeding companies at the European level and, simultaneously, the development of integrated breeding programmes on a European scale. These hypotheses are reinforced by the recent results by Fu et al. (2003), who also identified that the different patterns of allelic changes in Canadian oat germplasm could be linked with the impact of breeding. It is very likely that selection pressure has not been identical in the different Europeans countries over the period of time under study. Unfortunately, as we do not have a complete list of the breeders involved for each variety in each country, it was difficult to compare breeding programmes within

any one country. Figure 2 shows a drastic decrease in average gene diversity over the last decade, likely reflecting the impact of recent reorganisations, as previously suggested. Although four different regions (BEL, FIN/DNK, POL, YUG/GRC) were not represented in the present study during the period of 1990–1999, this sampling effect would most likely not have a strong influence on gene diversity decrease, since, as shown in Fig. 5, three of these regions (BEL, POL, YUG/GRC) already show a lower number of alleles (243, 247 and 252, respectively) than the overall mean (262). Figure 3 shows that, on average, new European varieties appear to be increasingly similar to each other with respect to the global composition of their alleles. The hierarchical tree clearly separates the European varieties into two groups (Fig. 4): one cluster that includes accessions registered before 1970 and a second cluster that includes accessions registered during the last three decades of the twentieth century. This last result is exactly the same as that observed by Roussel et al. (2004) on French cultivars released during the same period. Their explanation of the results is based on a temporal drift by a bottleneck effect linked to the 'green revolution' period that occurred following the Second World War. The fact that the same result was also observed on a large sample of European accessions originating from 15 different geographical areas supports this hypothesis: (1) after the Second World War, European countries needed to increase food production; (2) breeding programmes created new and much more productive varieties which were probably bred during the 1960s using a reduced number of genitors chosen among their previously registered varieties; (3) this produced a bottleneck effect. The combination of this bottleneck effect and the introduction of completely new germplasm in some European countries during the 1970s and 1980s (i.e. the *Rht* genes, genitors introgressed from *Aegilops* sp., etc.) is likely responsible for the clustering of accessions in the two main groups. The observation that the 1990–1999 varieties are separated from the 1970–1980 varieties may indicate a recent change in breeding goals. As indicated by NIRS analyses (Roussel et al., unpublished data), the increasing use of some glutenin alleles as quality markers in wheat breeding after 1985 certifies that bread making quality is an increasingly important goal for breeders. This fact may be responsible for such specific clustering.

These varying results on temporal variations led us to conclude that, at the European level, changes in genetic diversity of bread wheat varieties related to breeding practices are firstly quantitative and secondly qualitative. This conclusion is slightly different from those of Donini et al. (2000) and Koebner et al. (2003) on UK wheat and barley, respectively: both authors concluded that plant breeding has resulted, over time, in a qualitative, rather than a quantitative, shift in the diversity of the respective species studied. These investigators probably arrived at this conclusion because their respective studies involved fewer polymorphic markers and only a small number of accessions originating from the same homogenous geographical area and, consequently, from related breeding programmes.

Only two previous studies have assessed diversity in a large number of wheat accessions originating from a wide range of geographical origins: those of Röder et al. (2002) and Huang et al. (2002). In both investigations, the authors analysed and compared genetic diversity indices (number of alleles and average gene diversity) for geographical groups of accessions without taking into account the considerable difference in the number of accessions per group. However, as indicated by Petit et al. (1998), variations in the number of accessions result in a bias in population statistics, and if this number is not correctly estimated using the allele rarefaction method, it is not really possible to compare such indices between themselves. The allele rarefaction method was used by Roussel et al. (2004) to compare groups of different sizes and to support their results. In the present study, accessions were sampled specifically in order to avoid this problem: as the number of accessions was exactly the same for the 15 geographical groups, we were able to compare gene diversity indices without bias. Some of the SSRs used in the different studies, such as the gwm261 marker, cannot really be considered to be neutral (Korzun et al. 1998). In the present study however, the larger number of markers used (39) allowed more dependable calculation and comparison of mean indices per locus.

AMOVA (Table 4) indicated that a significant proportion of total diversity can be explained by differences in the geographical origin of the accessions. When the data in Table 4 is compared with those in Table 3, the geographical factor appears to be more important than the temporal factor in explaining genetic structure; the organisation and practices of breeders over time in each particular country may be a factor to explain limited exchanges between areas. Another complementary reason could be the relatively narrow adaptation of wheat varieties to regional agro-ecological conditions.

The significant differences in the total number of alleles per geographical areas shown in Fig. 5 are in agreement with the results from the studies of Röder et al. (2002) and Huang et al. (2002): in particular, greater allelic richness is to be found in south-eastern Europe than in northern and western Europe. This difference in allelic richness may be a reflection of wheat breeding practices: intensive selection pressure in wheat breeding began earlier in northern and western Europe than in south-eastern Europe, which had other targets. When we consider the high level of correlation between the number of alleles per geographical area and corresponding average gene diversity ($r = 0.90$, $P < 0.01$), our results (not shown here) on average gene diversity reinforce the contrasts between different groups of countries in Europe. The dendrogram (Fig. 6) summarises our previous observations on the geographical structure of diversity in European wheat varieties The contrast between areas north and south of the arc formed by the Alps and the Carpathian mountains is obvious and can

be explained by the adaptation of initial wheat germplasm to different climatic and environmental conditions: north-western countries have large agro-ecological areas that are relatively homogenous with respect to soil and climatic characteristics, whereas countries located in the Mediterranean regions possess relatively limited agro-ecological areas and these are subject to highly diversified climate conditions (temperature, rainfall, etc.) and soil characteristics. In addition, the genetic characteristics of the initial wheat germplasm, which was intensively used by European breeders for two centuries to create their own varieties, are still present in the adapted germplasm of the former group. However, the differences between European countries could also reflect the oldest migration pathways of the initial wheat germplasm brought by the first farmers from the Middle East to western Europe, which would have occurred during the Neolithic period. In this hypothesis, the Alps and the Carpathians would have acted as a natural barrier to human and gene flow. As for the relative position of accessions from Spain and Portugal with respect to the other Western European countries, it is possible that the Mediterranean geographical position of these countries, (isolation), added to the climatic conditions (drought, etc.), also played a major role in the differentiation of initial wheat germplasm. Finally, the structure and the organisation of the companies involved in the breeding tasks are likely responsible for a part of the genetic structure observed in the present studies. For example, during the twentieth century, it is more than likely that Yugoslavia, Bulgaria, Romania and Hungary, which are former communist countries with only government-run breeding institutions at the time, preferably exchanged germplasm between themselves (this will explain their closeness in the hierarchical tree). In contrast, during this same time period, breeding programmes in western European countries were organised as collaborations between government-run institutions and various private companies of medium size. However, a deeper analysis on the breeder origins of a larger number of accessions would be necessary to draw any conclusion on this point. Unfortunately, our sample size and the missing data on the origin of some varieties do not allow such analysis at the present time.

In conclusion, we have shown that diversity in wheat European accessions is not randomly distributed but can be explained both by temporal and geographical variations. At the European level, the effects of breeding practices and agriculture policies in the different countries led to a slight but significant evolution in wheat diversity, which is not only qualitative but also quantitative. The distinct contrast in diversity observed between North–West and South–East European accessions might be the result of the combined effects of both adaptation by the initial germplasm to different environmental conditions and specific breeding practices. The main consequence of these results concerns present-day European wheat breeder, who should increase their exchange of genetic resources in order to expand genetic material and improve new cultivars. Otherwise, the present evolution could be prejudicial to the long-term maintenance of wheat genetic diversity in Europe.

**Acknowledgements** The authors would like to thank the two anonymous reviewers for their very constructive comments to improve this paper. They also thank M. Ambrose (UK), A. de Keyser (Belgium), I. Faberova (Czech Republic), D. Fossati (Switzerland), G. Ittu (Romania), W. Kainz (Austria), J. Koenig (France), J.A. Oliveira (Spain) and L. Van Soest (Netherlands) for their help in recovering passport data. This research was realised as part of 'Barrande' project between the Czech Republic and France.

## References

Becker RA, Chambers JM, Wilks AR (1988) The new S language. A programming environment for data analysis and graphics. Wadsworth and Brooks/Cole Advanced Books and Software, Pacific Grove, Calif.

Ben Amer IM, Borner A, Röder MS (2001) Detection of genetic diversity in Lybian wheat genotypes using wheat microsatellite markers. Genet Res Crop Evol 48:579–585

Christiansen MJ, Andersen SB, Ortiz R (2002) Diversity changes in an intensively bread wheat germplasm during the 20th century. Mol Breed 9:1–11

Donini P, Law JR, Koebner RM, Reeves JC, Cooke RJ (2000) Temporal trends in the diversity of UK wheat. Theor Appl Genet 100:912–917

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

Huang XQ, Borner A, Roder MS, Ganal MW (2002) Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor Appl Genet 105:699–707

Koebner RM, Donini P, Reeves JC, Cooke RJ, Law JR (2003) Temporal flux in the morphological and molecular diversity of UK barley. Theor Appl Genet 106:550–558

Korzun V, Röder MS, Ganal MW, Worland AJ, Law CN (1998) Genetic analysis of the dwarfing gene Rht8 in wheat Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum*). Theor Appl Genet 96:1104–1109

Leisova L, Ovesna J (2001) The use of microsatellite analysis for the identification of wheat varieties. Czech J Genet Plant Breed 37:29–33

Manifesto MM, Schlatter AR, Hopp HE, Suarez HE, Dubcovsky J (2001) Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci 41:682–690

Nei M (1973) Analysis of gene diversity in subdivised populations. Proc Natl Acad Sci USA 70:3321–3323

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. Cons Biol 12:844–855

Plaschke J, Ganal MW, Roder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK (2000) The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet 100:584–592

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Röder MS, Wendehake K, Korzun V, Bredemeijer G, Laborie D, Bertrand L, Isaac P, Rendell S, Jackson J, Cooke RJ, Vosman B, Ganal MW (2002) Construction and analysis of a microsatellite-based database of European wheat varieties. Theor Appl Genet 106:67–73

Roussel V, Koenig J, Beckert M, Balfourier F (2004) Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes. Theor Appl Genet 108:920–930

Schneider S, Kneffer JM, Roessle D, Excoffier L (1996) ARLEQUIN. Genetics and biometry laboratory, Department of Anthropology, University of Geneva, Switzerland

Zhang XY, Li CW, Wang LF, Wang HM, You GX, Dong YS (2002) An estimation of the minimum number of SSR alleles needed to reveal genetic relationships in wheat varieties I. Information from large-scale planted varieties and cornerstone breeding parents in Chinese wheat improvement and production. Theor Appl Genet 106:112–117