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Did Modern Plant Breeding Lead to Genetic Erosion in European Winter Wheat Varieties?

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ABSTRACT

The objective of this study was to assess whether modern plant breeding has led to any loss of genetic diversity in modern European winter wheat varieties (*Triticum aestivum* L.). For this purpose, a collection of 511 widely grown winter wheat varieties of Central and Northern Europe was genotyped with 42 microsatellite markers. In the varieties representing the National List of the UK during the 1980s and 1990s the allelic richness and gene diversity were lower than in the varieties of Recommended Lists covering the time period 1945– 2000. However, no apparent quantitative loss of genetic diversity was found by comparing the different decadal groups of varieties present in the Recommended Lists. Analysis of molecular variance (AMOVA) showed that the variance component among varieties within decadal groups accounted for 96.41% of the genetic variation, but among decadal groups only for 3.59%. The *Fst* values increased from the 1950s to the 1990s compared to the 1940s with a slight decrease in the 1970s. These results suggested that modern plant breeding has resulted in changes of alleles present in the germplasm; however, it appears that modern plant breeding has resulted in no apparent loss of allele numbers, or genetic diversity, in the investigated European wheat varieties over time.

Fears have often been expressed that modern intensive plant breeding leads inevitably to genetic erosion (Vellvé, 1993), which if correct, would have serious consequences both for the genetic vulnerability of crops and for their plasticity to respond to changes in the production environment. It is therefore vital for plant breeding programs to maintain sufficient diversity to allow for the production of new varieties able to withstand attack from new races and pathovars of continuously evolving pathogenic microorganisms (Tripp, 1996). The forecast changes in abiotic environmental conditions, including the effects of global warming, altered agricultural practices, and the presence of pollutants in the environment will all play their part in requiring a genetic remodelling of plant varieties.

The risk of erosion implicates the systems that deliver the products of modern plant breeding systems. Within the EU, this includes Plant Breeders' Rights (PBR), National Listing (NL) and selection at regional or national levels in voluntary Recommended List (RL) trials or their equivalent. Before they can be marketed in the member states of the European Union, newly bred varieties of crop plants undergo statutory testing, part of which requires that varieties are distinct (D) uniform (U) and stable (S). This DUS testing also forms the basis for the system of intellectual property protection for plant breeders, known as Plant Breeders' Rights. In many countries there is then a further series of RL trials and tests, either regionally or nationally based, which ensure that farmers have a source of objective advice to direct their choice of variety(ies), in terms of their specific agronomic or commercial attributes. These systems undoubtedly exert a considerable influence on the varieties grown. In the UK, the RL reflects varieties which are considered by the regulatory authorities to have the potential to provide a consistent economic benefit to the whole industry. As a result, the overwhelming bulk of all commercial crops grown in the UK comprise RL varieties, although there is no obligation on producers to restrict varietal choice to these. Crops cannot be commercially traded, however, unless the variety appears on the NL, and to do so they must have been demonstrated as DUS and to be of satisfactory value for cultivation and use. Typically, the RL includes a limited number of cultivars—for example, the 2005/2006 list for UK winter wheat consists of less than 20 entries (www.hgca.com/publications/documents/varieties/ww06rlcand.xls; verified 11 Dec. 2006), while the NL is much wider, as it includes many outclassed but still commercially viable varieties. A comparison of the genetic diversity between RL and NL therefore represents an opportunity to address the issue as to whether the bulk of the UK commercial crop (i.e., the RL) is less or equally diverse as the totality of the crop (i.e., the NL). It has been argued that modern plant breeding and its interaction with the NL and RL systems inevitably cause genetic erosion through convergent breeding, manifested as a temporal decline in crop genetic diversity. However, objective evidence for this view is lacking.

DNA-marker techniques have provided the tools for directly measuring genetic diversity and hence to test for the occurrence of genetic erosion (Almanza-Pinzón et al., 2003). The picture emerging from the analysis of various wheat gene pools is not uniform. Donini et al. (2000) concluded from a set of UK RL winter wheats that changes in the composition and occurrence of alleles rather than the number of alleles characterized the

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Abbreviations: AMOVA, Analysis of molecular variance; DUS, distinct (D) uniform (U) and stable (S); *He*, gene diversity; NA, alleles per locus; NL, National Lists; PBR, Plant Breeders' Rights; PCoA, Principal Coordinate Analysis; PIC, polymorphism information content; RL, Recommended Lists; UPGMA, unweighted pair-group method with arithmetic average.

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flux of genetic diversity over time. While in a study of Nordic spring wheat, Christiansen et al. (2002) concluded that genetic diversity was enhanced by plant breeding in the first quarter of the 20th century, fell during the second quarter, but increased again after 1960. On the other hand, both allelic reduction and genetic shift have been reported for Canadian hard red spring wheat germplasm released from 1845 to 2004 (Fu et al., 2005; 2006). In French bread wheat accessions a 25% decrease in allelic richness was observed by comparing landraces to varieties, that when considering only registered varieties changes in diversity related to temporal trends appeared more qualitative than quantitative (Roussel et al., 2004). Specifically, this means that different sets of alleles appear in genotypes from different eras, but the diversity measured by the total number of alleles and the number of rare alleles did not change. A loss of genetic diversity was also reported for CIMMYT and CIMMYT-related modern wheat cultivars in comparison to *Triticum tauschii* and traditional landrace cultivars (Reif et al., 2005). 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 to 50 yr in four comparable geographical regions in Europe and Asia (Khlestkina et al., 2004). However, one-third of the detected alleles were collection mission-specific.

In the current study we have applied a set of well-characterized genomic microsatellite markers to investigate the genetic diversity of a collection of 511 widely grown Central and Northern European winter wheat varieties grown over the time period 1945–2000. Our first objective was to compare the genetic diversity present in NL versus RL to address the question whether the genetic diversity present in the NL, which include all varieties eligible for growing, is reduced by the implementation of RL which propose only a subset of varieties with the highest agronomic potential and lead to a preferential use of the recommended varieties. The second objective was to investigate whether genetic erosion over time could be detected as a consequence of modern plant breeding.

MATERIALS AND METHODS

Plant Material

The collection of winter wheat varieties was divided into two sets, one the 'Recommended List' reflecting wide usage (at least 5% of the acreage, in at least 2 yr in one of the EU member countries Austria, Belgium Denmark, France, Germany, Great Britain, Netherlands, and Sweden during the period 1945–2000); and the other the 'National List' representing the spectrum of varieties in commerce in the UK in the mid 1990s. The origin and distribution of 282 wheat varieties on the RL from the 1940s to 1990s and 229 wheat varieties on the NL from 1980s to 1990s are listed in Table 1. The variety names are presented in Supplementary Table 1.

DNA Extraction and Microsatellite Analysis

For each line, a bulked sample of about 30 seeds was coarsely ground, and DNA was extracted from the grits using the DNAeasy Qiagen kit (Qiagen, Hilden, Germany). The genotyping set was composed from 42 genomic wheat microsatellites, as described by Röder et al. (1998), and developed by TraitGenetics GmbH, Gatersleben, Germany (Table 2). Each microsatellite maps to a distinct chromosome arm, thereby providing whole genome coverage of the 21 chromosomes of hexaploid wheat. Genotyping was performed with an ABI3100 capillary sequencer system (Applied Biosystems, Foster City, CA) using dye-labeled primers, and following established protocols. Genotyper 3.6 software was used for automated data scoring, followed by a manual check in which all datapoints with ambiguous scoring, all heterogeneities and all null alleles were repeated to confirm authenticity.

Table 1. Origin and distribution of 282 wheat varieties of the Recommended Lists (RL) and 229 wheat varieties of the National Lists (NL) from the 1940s to 1990s.

	RL (282)		NL (229)	
Decade	Origin of varieties	Number of varieties	Origin of varieties	Number of varieties
1940s	A, D, F, GB, NL†	24	–	–
1950s	A, D, F, GB, NL, S	24	–	–
1960s	A, B, D, F, GB, NL	33	–	–
1970s	A, B, D, F, GB, NL, S	54	–	–
1980s	A, B, D, DK, F, GB, NL, S	63	D, F, GB, NL, S	85
1990s	A, D, DK, F, GB	50	D, F, GB, NL,	132
unknown	B, D, S	34	F, GB, NL	12

† Austria (A), Belgium (B), Denmark (DK), France (F), Germany (D), Great Britain (GB), Netherlands (NL), and Sweden (S).

Genetic Analysis

POPGENE version 1.31 (University of Alberta, Edmonton, Alberta, Canada) (Yeh et al., 1997) was used to calculate the number of alleles per locus (NA), the gene diversity (*He*), and unbiased genetic distances (Nei, 1978) for each group. To obtain comparable allele numbers per locus between groups with different sample sizes, we used a resampling method by repeated random sampling of the smaller sample size from the larger groups. The sampling was repeated 1000 times using Matlab program (www.mathworks.com/products/matlab/; verified 12 Dec. 2006) (The MathWorks, Inc., Natick, MA) and the results were averaged. *He* is equivalent to the proportion of heterozygous loci under Hardy-Weinberg expectations (expected heterozygosity) and was calculated by the unbiased method of Nei (1978), which adjusts for sample size. To measure the relative loss of genetic diversity in NL vs. RL, we have defined a parameter ΔHe = 1- (He_{NL}/He_{RL}), where He_{NL} and He_{RL} are, respectively, the genetic diversity in NL and RL. The relative loss of NA is ΔAllele = 1- (NA_{NL}/NA_{RL}), where NA_{NL} and NA_{RL} are the number of alleles in NL and RL, respectively. The paired sample *t* tests were performed using the package SPSS for Windows (SPSS, Chicago, IL). The standard error of a sample with sample size n is the sample's standard deviation divided by $\sqrt{n}$. In this study, n is 42 for 42 microsatellite loci. Since all microsatellite loci represent different chromosome arms linkage disequilibrium among the loci is not expected.

Principal Coordinate Analysis (PCoA) was performed with the NTSYS-pc package (Exeter Software, Setauket, NY) (Rohlf, 1998) based on the Dice similarity index (Dice, 1945). A dendrogram based on unbiased genetic distances (Nei, 1978) was constructed using UPGMA (unweighted pair-group method with arithmetic average). F-statistics include three fixation indices: *Fis*, *Fit*, and *Fst*, where *Fis* measures the de-

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Table 2. Chromosomal location, total number of alleles, number of unique alleles and gene diversity (*He*) for National Lists (NL) and Recommended Lists (RL) based on 42 microsatellite loci.

Genome	Locus	Chr.	Number of alleles				*He*	
			Total	NL-unique	RL-unique	Shared	NL	RL
	Xgwm3094	1AS	17(null)†	1	7	9	0.732	0.763
	Xgwm0357	1AL	5	0	2	3	0.487	0.497
	Xgwm0095	2AS	7	0	0	7	0.592	0.637
	Xgwm0312	2AL	20	3	8	9	0.706	0.790
	Xgwm3092	3AS	15(null)	2	3	10	0.623	0.735
	Xgwm0155	3AL	10	1	3	6	0.599	0.685
A	*Xgwm1531*	4AS	12	1	1	10	0.209	0.583
	Xgwm0610	4AL	7(null)	1	3(null)	3	0.050	0.261
	Xgwm0415	5AS	3	0	1	2	0.449	0.360
	Xgwm0291	5AL	12	1	3	8	0.501	0.699
	Xgwm0334	6AS	9	0	3	6	0.642	0.637
	Xgwm0570	6AL	10	1	3	6	0.642	0.687
	Xgwm0834	7AS	17(null)	0	3(null)	14	0.542	0.770
	Xgwm0631	7AL	8	1	3	4	0.414	0.567
	Total		152	12	43	97	7.188	8.671
	Mean		10.86	0.86	3.07	6.93	0.513	0.619
	Xgwm0018	1BS	11	0	2	9	0.491	0.603
	Xgwm0818	1BL	11	1	3	7	0.597	0.714
	Xgwm1750	2BS	13	1	2	10	0.585	0.667
	Xgwm0619	2BL	12(null)	1	4(null)	7	0.521	0.730
	Xgwm0389	3BS	13(null)	0	2	11(null)	0.682	0.734
	Xgwm3144	3BL	16	1	5	10	0.686	0.693
B	*Xgwm3072*	4BS	9	1	1	7	0.615	0.665
	Xgwm0513	4BL	5	0	1	4	0.285	0.328
	Xgwm0213	5BS	15	1	6	8	0.581	0.719
	Xgwm0408	5BL	12	2	3	7	0.464	0.648
	Xgwm0680	6BS	7	1	3	3	0.464	0.482
	Xgwm0219	6BL	17	1	7	9	0.741	0.783
	Xgwm0046	7BS	13(null)	2	2(null)	9	0.558	0.752
	Xgwm0577	7BL	24(null)	1	6	17(null)	0.759	0.849
	Total		178	13	47	118	8.029	9.367
	Mean		12.71	0.93	3.36	8.43	0.574	0.669
	Xgwm0458	1DS	4	0	0	4	0.630	0.597
	Xgwm0793	1DL	9(null)	1	0	8(null)	0.686	0.818
	Xgwm0261	2DS	7	1	1	5	0.390	0.457
	Xgwm0320	2DL	9(null)	1	2	6(null)	0.656	0.750
	Xgwm0456	3DS	10(null)	0	4(null)	6	0.702	0.676
	Xgwm0003	3DL	5(null)	1(null)	0	4	0.517	0.486
D	*Xgwm0819*	4DS	4	0	1	3	0.442	0.268
	Xgwm1397	4DL	18	0	3	15	0.747	0.862
	Xgwm0190	5DS	8	1	1	6	0.724	0.694
	Xgwm0272	5DL	6	0	1	5	0.393	0.594
	Xgwm0325	6DS	8	0	1	7	0.562	0.612
	Xgwm4787	6DL	6(null)	0	1	5	0.237	0.453
	Xgwm1619	7DS	9(null)	0	3	5	0.409	0.617
	Xgwm0437	7DL	18	2	6	10	0.371	0.566
	Total		121	7	24	89	7.466	8.45
	Mean		8.64	0.50	1.71	6.36	0.533	0.604
Across all genomes	Total		451	32	114	304	22.7	26.5
	Mean		10.74	0.76	2.71	7.24	0.540	0.631

† Null allele was included.

viations from Hardy-Weinberg expectations within populations, *Fit* measures deviations from Hardy-Weinberg expectations within and across populations combined, and *Fst* measures the genetic difference between populations (Weir and Cockerham, 1984). To specify the level of genetic differentiation between decadal groups of RL, group pairwise *Fst* values were calculated based on 1000 permutations using the whole set of microsatellite loci (Slatkin, 1995). To measure goodness of fit for a cluster analysis (dendrogram), the Mantel test (Smouse et al., 1986; NTSYS subroutine MXCOMP) is used to compute and test the linear correlation between Nei's matrix and *Fst* matrix. The Nei's matrix contains estimates of the Nei's genetic distances between the groups obtained using POPGENE version 1.31, whereas the *Fst* matrix

Table 3. Comparison of genetic variation between Recommended Lists (RL) and National Lists (NL) based on 42 microsatellite loci.

	RL	NL	Diversity loss
Sample size	282 (229)†	229	
Total no. of alleles	418 (375)	336	
Average no. of unique alleles	2.71 ± 0.31‡ (2.45 ± 0.26)	0.76 ± 0.11 (0.81 ± 0.18)	
Average no. of alleles per locus (NA)	9.95 ± 0.70 (8.93 ± 0.63)	8.00 ± 0.54	0.20§ ± 0.023 (0.16 ± 0.023)
Gene diversity (*He*)	0.631 ± 0.023	0.540 ± 0.025	0.14¶ ± 0.035

† Results from the resampling method are shown in parenthesis.
‡ Mean ± standard error.
§ The relative loss of alleles ΔAllele = 1- (NA_{NL}/NA_{RL}).
¶ The relative loss of gene diversity ΔHe = 1- (He_{NL}/He_{RL}).

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Table 4. Analysis of molecular variance (AMOVA): National Lists (NL) versus Recommended Lists (RL).

Source of variation	*df*	Variance component	Percentage of variation
Between	1	0.71 **	5.29
Within	509	12.78 ***	94.71
Total	510	13.49	100.00

** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

contains estimates of the *Fst* values between the groups obtained using Arlequin software version 3.01 (Excoffier et al., 2005). Arlequin software version 3.01 was also used to compute the analysis of molecular variance (AMOVA) among and within groups.

RESULTS

Comparison of Genetic Diversity between Varieties in the National Lists and Those in the Recommended Lists

The 511 varieties carried 451 alleles (including null alleles) across the 42 loci (Table 2). Numbers of alleles ranged from 3 for *Xgwm415* to 24 for *Xgwm577,* with a mean of 10.7 (Table 2). Null alleles were found at 14 loci. The highest number of alleles was present in the B genome (12.7), compared to 10.9 and 8.7 in genomes A and D, respectively. This is consistent with the outcome in a different set of genotypes, as reported by Huang et al. (2002). Of the 451 alleles, 304 were shared between entries in the RL and NL (Table 2). Overall, 336 and 375 alleles were detected for the varieties of the NL and RL, respectively (Table 3). The mean number of unique alleles was 0.81 for the NL, but 2.45 for the RL. NL varieties had a lower NA and a lower *He* than the RL varieties. NL varieties showed a loss of diversity as compared with those in the RL. This loss was significant when measured as Δallele (paired samples T-Test, $t = 6.84$, $P < 0.001$) or as Δ*He* ($t = 5.54$, $P < 0.001$). AMOVA indicated that the molecular variance between NL and RL only accounted for 5.3% of the total variation, while 94.7% resided within the sets (Table 4). The genetic distance between NL and RL was 0.087. Overall, these statistics indicate that the RL varieties have more genetic diversity than those in the NL. This can be explained by the fact that the RL set represents several countries and time periods, while the NL set is from 1980s to 1990s and is set in the context of the UK wheat production environment, so that most of the varieties in the NL were bred in the UK (Table 1). The comparison indicates that the use of RL for favorable varieties does not lead to an obvious reduction of genetic diversity present in the widely grown varieties.

Table 5. Comparison of genetic variation within National Lists (NL) based on 42 microsatellite loci.

Decade	Sample no.	Average no. of alleles per locus	Average gene diversity (*He*)
1980s	85	6.55 ± 0.42†	0.57 ± 0.023
1990s	132 (85)‡	5.95 ± 0.46 (5.26 ± 0.34)	0.53 ± 0.028

† Mean ± standard error.
‡ Results from the resampling method are shown in parenthesis.

Table 6. Analysis of molecular variance (AMOVA) in National Lists (NL) and Recommended Lists (RL).

Source of variation	*df*	Variance component	Percentage of variation
NL			
Between decadal groups	2	0.42 **	3.50
Within decadal groups	226	11.51 ***	96.50
Total	228	11.93	100.00
RL			
Between decadal groups	6	0.49 ***	3.59
Within decadal groups	275	13.22 ***	96.41
Total	281	13.71	100.00

** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

Changes in the Genetic Diversity in the National Lists

Two decadal groups, the 1980s and 1990s are present in the NL. The release year of 12 varieties is unknown and these were omitted from the analysis. The 1990s varieties have a lower NA and *He* than those from the 1980s, even though the former are represented by more entries than the latter (Table 5). The genetic distance between the 1980s and 1990s varieties was 0.039. Table 6 clearly shows that the within-decadal group component of the molecular variance dominates overwhelmingly (96.5%). PcoA suggests an overlapping pattern of genetic diversity between the 1980s and 1990s entries, and the first two axes explained 7.9 and 5.7% of the total molecular variance (data not shown).

Temporal Trends in Recommended List Diversity

Six decades from the 1940s to the 1990s are present in the RL. The release year of 34 varieties is unknown and was omitted from the analysis. The mean NA and *He* are listed in Table 7. The lowest *He* is present in the 1940s. From the 1950s to 1990s, gene diversity appeared to be stable, with a slight dip in the 1970s. The genetic distances were calculated for each pair of decadal groups to estimate the extent of their divergence, as summarized in Table 8. The highest genetic distance (0.192) was found between 1940s and 1990s, whereas the lowest (0.029) was observed between the 1970s and 1980s. The dendrogram resulting from the cluster analysis based on Nei's genetic distance is presented in Fig. 1. Varieties in the 1940s clustered separately, whereas the decadal groups from the 1950s to 1990s formed two clusters: the 1950s and 1960s in one cluster, and the 1970s, 1980s, and 1990s in another. This suggests the possible introduction of new alleles after 1980. The Mantel test indicated that the Nei's matrix was very significantly correlated with

Table 7. Comparison of decadal genetic variation within Recommended Lists (RL) based on 42 microsatellite loci.

Decade	Sample no.	Average no. of alleles per locus	Average gene diversity (*He*)
1940s	24	5.26 ± 0.32†	0.56 ± 0.028
1950s	24	6.00 ± 0.36	0.65 ± 0.028
1960s	33 (24)‡	5.83 ± 0.38 (5.25 ± 0.28)	0.63 ± 0.025
1970s	54 (24)	6.71 ± 0.40 (5.56 ± 0.31)	0.63 ± 0.028
1980s	63 (24)	7.31 ± 0.48 (5.47 ± 0.28)	0.64 ± 0.022
1990s	50 (24)	6.55 ± 0.40 (5.53 ± 0.28)	0.65 ± 0.026

† Mean ± standard error.
‡ Results from the resampling method are shown in parenthesis.

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Table 8. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among decadal groups of Recommended Lists (RL).

Decade	1940s	1950s	1960s	1970s	1980s	1990s
1940s	—	0.937	0.896	0.904	0.865	0.825
1950s	0.065	—	0.946	0.944	0.915	0.885
1960s	0.110	0.056	—	0.958	0.943	0.909
1970s	0.101	0.058	0.043	—	0.973	0.945
1980s	0.145	0.088	0.058	0.029	—	0.962
1990s	0.192	0.123	0.095	0.057	0.039	—

the *Fst* matrix ($r = 0.988$, $P < 0.001$), suggesting that the cluster analysis based on Nei's genetic distance was reliable. The PcoA plot resulted in congruent clusters for varieties from the 1940s to 1990s with no obvious separation of decadal groups (data not shown). The proportion of the total variance explained by the first two axes was 7.37 and 4.95%.

The AMOVA showed that the proportion of the variance among varieties within decadal groups accounted for 96.41%, but between decadal groups only for 3.59% of the overall molecular variance (Table 6). *Fst* was calculated from 1950s vs. 1940s to 1990s vs. 1940s. The *Fst* value increased from the 1950s to the 1990s, compared to the 1940s, with a slight decrease in the 1970s. A linear regression ($Y = -0.0098 + 0.0007x$) for the period 1950s to 1990s is depicted in Fig. 2. These results suggest that the genetic diversity in the RL was enhanced by plant breeding from the 1940s to 1990s and do not support the idea that modern plant breeding has led to any reduction in diversity.

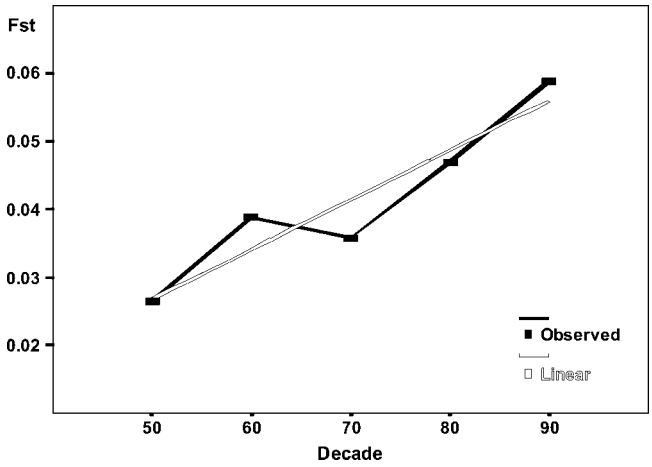

Fig. 2. Changes in the *Fst* from 1950s to 1990s compared to 1940s in the Recommended Lists (RL) (Y = −0.0098 + 0.0007x).

DISCUSSION

The NL collection represented varieties in commerce in the UK in the early 1990s, and its inclusion in the study was to determine whether the restricted 'Recommended List' system, as used in the UK (but replicated over the EU in various forms), led to any narrowing of the diversity present in the crop. At the SSR level, the NL collection was less diverse than the total RL set, even when the differences in sample size of the two groups (282 versus 229 entries) were adjusted by a resampling method. However, the vast majority of the NL entries represented varieties released in the decade up to 1994, while the investigated entries of the RL represented six decades. Only 5.29% of the observed variation was explained by differences between NL and RL (Table 4), indicating that the genetic diversity present in the NL is well represented in the more restricted RL.

Although a slight decrease in allelic richness between the 1980s and the 1990s characterized both the NL (Table 5) and the RL varieties (Table 7), the overall trend was rather an increase in genetic diversity when

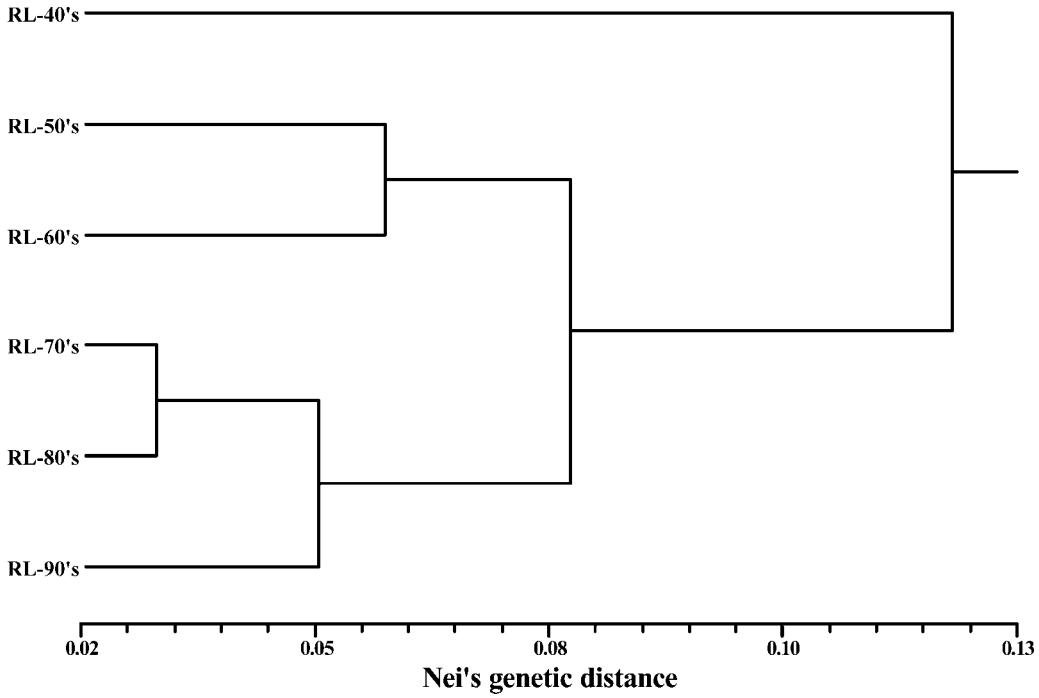

Fig. 1. UPGMA dendrogram of six decadal groups of the Recommended Lists (RL) based on Nei's genetic distance.

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the RL of the 1940s were compared to the later decades (Table 7, Fig. 2). The PCoA clustering failed to identify any significant deviation in the distribution of genetic diversity of the individual decadal groups in either the NL and the RL. The UPGMA dendrogram indicated a close clustering of the varieties from the 1970s to the 1990s while the earlier decadal groups were more distantly related (Table 8, Fig. 1). These observations are supported by the results of AMOVA where only 3.50% of the variation in the NL and 3.59% of the variation in the RL resided between decadal groups (Table 6). Overall, there was no indication of any quantitative genetic erosion in this varietal set, which is highly representative of the actual crop grown over Central and Northern Europe in the period 1950–2000. Rather, these results confirm the notion suggested by Donini et al. (2000) that modern plant breeding has resulted in qualitative rather than quantitative shifts in diversity over time, meaning that changes occurred in the composition and occurrence of alleles rather than in the number of alleles. Because the current study only extended to widely grown varieties, no conclusions can be drawn regarding the genetic diversity residing in landraces versus modern varieties. Roussel et al. (2004) reported a decrease of about 25% in allelic richness between French landraces and varieties, whereas changes in diversity related to temporal trends appeared more qualitative than quantitative, except at the end of the 1960s when a bottleneck might have occurred. In a second study of the same author (Roussel et al., 2005) a clear separation of European wheat varieties before and after 1970 was recorded. Analysis of similarity of the present alleles over time indicated that the more recent the European varieties, the more similar they were to each other. A dip in genetic diversity was observed in the 1960s in this study (Fig. 2) and by Christiansen et al. (2002), however no quantitative loss of genetic diversity was found for the later decades.

The early 1970s featured two seminal events affecting wheat breeding in Europe (and worldwide). One was the dissemination of a wheat-rye translocation (1BL.1RS), which has been associated with improved agronomic performance but reduced bread-making quality (Villareal et al., 1991). The second major breeding event was the switch to semi-dwarfness, which also occurred in this period. This semi-dwarfness was achieved by the introduction of one of the pair of independent major genes *RhtB1b* (old nomenclature: *Rht1*) or *Rht-D1b* (old nomenclature: *Rht2*) (Peng et al., 1999). The gradual increase in diversity in the later period indicates the success of the breeding industry in accessing novel sources of variation, predominantly for disease resistance, but also for yield and quality. Among the known single-resistance genes that have been introduced in this period from wild germplasm are *Lr37* (Bariana and McIntosh, 1993) and *Pch1* (Doussinault et al., 1983; Worland et al., 1988), but much of the improved and sustained resistance of modern varieties is likely to be controlled by as yet unidentified major and minor genes.

Although no indications for genetic erosion have been uncovered in this pool of European wheat varieties, the concepts of using unadapted and wild germplasm for broadening the genetic base of crop plants (Tanksley and McCouch, 1997; Hoisington et al., 1999; Huang et al., 2004) have not lost any of their strategic importance for the maintenance of diversified plant breeding efforts in the future.

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REFERENCES

Almanza-Pinzón, M.I., M. Khairallah, P.N. Fox, and M.L. Warburton. 2003. Comparison of molecular markers and coefficients of parentage for the analysis of genetic diversity among spring bread wheat accessions. Euphytica 130:77–86.

Bariana, H.S., and R.A. McIntosh. 1993. Cytogenetic studies in wheat XV. Location of rust reistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. Genome 36:476–482.

Christiansen, M.J., S.B. Andersen, and R. Ortiz. 2002. Diversity changes in an intensively bred wheat germplasm during the 20th century. Mol. Breed. 9:1–11.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26:297–302.

Donini, P., J.R. Law, R.M.D. Koebner, J.C. Reeves, and R.J. Cooke. 2000. Temporal trends in the diversity of UK wheat. Theor. Appl. Genet. 100:912–917.

Doussinault, G., A. Delibes, R. Sanchez-Monge, and F. Garciaolmedo. 1983. Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. Nature 303:698–700.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Fu, Y.B., G.W. Peterson, K.W. Richards, D. Somers, R.M. DePauw, and J.M. Clarke. 2005. Allelic reduction and genetic shift in the Canadian hard red spring wheat germplasm released from 1845 to 2004. Theor. Appl. Genet. 110:1505–1516.

Fu, Y.-B., G.W. Peterson, J.-K. Yu, L. Gao, J. Jia, and K.W. Richards. 2006. Impact of plant breeding on genetic diversity of the Canadian hard red spring wheat germplasm as revealed by EST-derived SSR markers. Theor. Appl. Genet. 112:1239–1247.

Hoisington, D., M. Khairallah, T. Reeves, J.-M. Ribaut, B. Skovmand, S. Taba, and M. Warburton. 1999. Plant genetic resources: What can they contribute toward increased crop productivity? Proc. Natl. Acad. Sci. USA 96:5937–5943.

Huang, X.Q., A. Börner, M.S. Röder, and M.W. Ganal. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet. 105:699–707.

Huang, X.Q., H. Kempf, M.W. Ganal, and M.S. Röder. 2004. Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109:933–943.

Khlestkina, E.K., X.Q. Huang, F.J.B. Quenum, S. Chebotar, M.S. Röder, and A. Börner. 2004. Genetic diversity in cultivated plants—loss or stability? Theor. Appl. Genet. 108:1466–1472.

Smouse, P.E., J.C. Long, and R.R. Sokal. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. 35:627–632.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Peng, J., D.E. Richards, N.M. Hartley, G.P. Murphy, K.M. Devos, J.E. Flintham, J. Beales, L.J. Fish, A.J. Worland, F. Pelica, D. Sudhakar, P. Christou, J.W. Snape, M.D. Gale, and N.P. Harberd. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261.

Reif, J.C., P. Zhang, S. Dreisigacker, M.L. Warburton, M. van Ginkel,

D. Hoisington, M. Bohn, and A.E. Melchinger. 2005. Wheat genetic diversity trends during domestication and breeding. Theor. Appl. Genet. 110:859–864.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.-H. Tixier, P. Leroy, and M.W. Ganal. 1998. A microsatellite map of wheat. Genetics 149:2007–2023.

Rohlf, F.J. 1998. NTSYS-pc: Numerical taxonomy and multivariante analysis system. Version 2.0. Applied Biostatistics, New York.

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

Roussel, V., L. Leisova, F. Exbrayat, Z. Stehno, and F. Balfourier. 2005. SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. Theor. Appl. Genet. 111:162–170.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462.

Tanksley, S.D., and S.R. McCouch. 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277: 1063–1066.

Tripp, R. 1996. Biodiversity and modern crop varieties: Sharpening the debate. Agric. Hum. Values 13:48–63.

Vellvé, R. 1993. The decline of diversity in European agriculture. Ecologist 23:64–69.

Villareal, R.L., S. Rajaram, A. Mujeebkazi, and E. Deltoro. 1991. The effect of chromosome 1B/1R translocation on the yield potential of certain spring wheats (*Triticum aestivum* L.). Plant Breed. 106:77–81.

Weir, B.S., and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution Int. J. Org. Evolution 38:1358–1370.

Worland, A.J., C.N. Law, T.W. Hollins, R.M.D. Koebner, and A. Guira. 1988. Location of a gene for resistance to eyespot (*Pseudocercosporella herpotrichoides*) on chromosome 7D of bread wheat. Plant Breed. 101:43–51.

Yeh, F.C., R.C. Yang, T. Boyle, Z.H. Ye, and J.X. Mao. 1997. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Edmonton, AB, Canada.

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Supplementary Table 1. List, release decade, origin and distribution of 282 wheat varieties of the Recommended Lists (RL) and 229 wheat varieties of the National Lists (NL).

	Year's	Origin	Variety name	Number
RL (282)	1940's	A, D, F, GB, NL	Alba, Bersee, Criewener 192, Ebersbacher Weiss, Heine 4, Holdfast, Juliana, Kadolzer, Lovink, Mahndorfer Tempo, Mendel, Redman, Rimpaus Bastard 2, Rimpaus Braun, Ritzlhofer, Salzmunder Standard, Schreibers Sturm, Staring, Steadfast, Svalov 0987, Tschermaks Weisser Begrannter Marchfelder, Vague d'épis, Vilmorin 27, Warden	24
	1950's	A, D, F, GB, NL, S	Banco, Cappelle-Desprez, Carstens 5, Carstens 6, Carstens 8, Dr. Lassers Dickkopf, Eros, Etoile de Choisy, Flamingo, Heine 7, Hochland, Leda, Loosdorfer Austro Bankut Grannen, Minister, Muck, Peragis, Pilot (GB), Stamm 101, Strubes Dickkopf 2, Svalov Kronen, Tassilo, Triumph (NL), Vilmorin 53, Werla	24
	1960's	A, B, D, F, GB, NL	Admonter, Apollo (NL), Capitole, Champlein, Cleo, Drauhofener Kolben, Elite Lepeuple, Erla Kolben, Felix, Flevina, Florian, Hubertusweizen, Hybrid 46, Ibis (NL), Joss Cambier, Jubilar, Manella, Maris Widgeon, Moisson, Norda, Orlando, Probstdorfer Stabil, Probus, Professeur Marchal, Rabe, Record, Rémois, Schweigers Taca, Starke, Stella (NL), Tadorna, Thor, Triumph (A)	33
	1970's	A, B, D, F, GB, NL, S	Adam, Alcedo, Almus, Aquila, Armada, Arminda, Atou, Benno, Bouquet, Cama, Caribo, Clément, Courtôt, Cyrano, Danubius, Diplomat, Disponent, Extrem, Fakir, Fanal, Flanders, Flinor, Hardi, Hildur, Hobbit, Holme, Kador, Kawkas, Kinsman, Kormoran, Kranich, Lely, Lutin, Mardler, Maris Freeman, Maris Huntsman, Maris Nimrod, Maris Ranger, Mega, Mironowskaja 808, Mironowskaja Jubilejnaja, Multiweiss, Nautica, Oenus, Okapi, Poros, Solid, Sportsman, Starke II, Talent, Top, Vuka, Walde, Winnetou	54
	1980's	A, B, D, DK, F, GB, NL, S	Agron, Albatross, Anja, Apollo (D), Arkos, Avalon, Beauchamp, Beaver, Bounty, Brigand, Brimstone, Brock, Calif, Camp rémy, Capitaine, Compal, David, Escorial, Fenman, Festival, Fidel, Folke, Galahad, Gamin, Gawain, Granada, Granta, Helge, Hereward, Hornet, Hustler, Iena, Ikarus, Kanzler, Karat, Kosack, Longbow, Martin, Mercia, Miras, Mission, Moulin, Norman, Odeon, Perlo, Pernel, Pontus, Rapier, Récital, Regent, Regina, Rektor, Scipion, Slejpner, Soissons, Sperber, Stetson, Taras, Thésée, Titus, Urban, Virtue, Zemon	63
	1990's	A, D, DK, F, GB	Abbot, Altria, Aztec, Borenos, Brigadier, Buchan, Bussard, Cadenza, Capo, Cézanne, Charger, Claudius, Contra, Equinox, Expert, Faktor, Flair (D), Flame, Florida, Genesis, Georg, Greif, Haven, Hubertus, Hunter, Ibis (D), Isengrain, Kontrast, Lindos, Mikon, Napier, Obelisk, Optimus, Palur, Pastiche, Pegassos, Pepital, Renan, Riband, Ritmo, Savannah, Shamrock, Sidéral, Silvius, Spark, Tambor, Torfrida, Toronto, Trémie, Zentos	50
	unknown	B, D, GB, S	Bledor, Cama (B), Celesta, Chinese Spring, Clovis, Ergo, Eroica, Eroica II, Ertus, Flair (GB), Glicevka, Hesbinion, Jarl, Jason, Marco, Marisa, Meridien, Mina, Mutant Odeon I, Mutant Odeon II, Odin, Orestis, Pony, Prima, Roi Albert, Rufus, Skandia IIIB, Stava, Stella (B), Svale, Terra, Virgo, Virtus, William	34
NL (229)	1980's	D, F, GB, NL, S	Ambassador, Amdon, Angler, Apostle, Avocet, Axial, Banner, Baron, Belplaine, Bert, Boxer, Breval, Captor, Carolus, Challenger, Civic, Club (D), Colombia, Colonel, Conveyor, Corinthian, Coxswain, Crest, Creweau, Custom, Dauntless, Dean, Depot, Druid, Eagle, Emblem, Erland, Feuvert, Flint, Focus, Foreman, Fortress, Fresco, Gambit, Governor, Guardian, Hammer, Hanno, Heinrich, Jeep, Kronjuwel, Legend, Lynx, Magneto, Mandate, Mantle, Meteor, Monitor, Motto, Nougat, Parade, Patience, Peacock, Pennant, Poet, Prospect, Rebel, Renard, Rendezvous, Rifle, Ritz, Rocket, Sabre, Sarsen, Sickle, Sirius, Sniper, Squadron, Stag, Talon, Tandem, Tara, Tasker, Taurus, Token, Trader, Vocal, Voyage, Weaver, Wizard	85
	1990's	D, F, GB, NL	Ability, Access, Acier, Admiral, Adroit, Alert, Andante, Anthem, Aristocrat, Asset, Athlet, Atla, Atoll, Bandit, Beaufort, Bercy, Biscay, Blitz, Bloggo, Brutus, Bryden, Bullet, Buster, Caprimus, Caspian, Catamaran, Caxton, Cheetah, Chianti, Claire, Clove, Club (GB), Coaster, Cobalt, Combat, Commodore, Consort, Contour, Daphne, Deben, Denver, Destroyer, Diablo, Dorby, Drake, Dynamo, Encore, Estica, Fenda, Flash, Fletum, Frista, Fromendor, Futur, Galatea, Galliard, Gondola, Harrier, Hickory, Holster, Hudson, Hussar, Imola, Jaguar, Kontiki, Lancelot, Leo, Lynx, Madrigal, Magellan, Malacca, Mantle, Mars, Massada, Morell, Newhaven, Norseman, Oldier, Optimist, Option, Orqual, Ostara, Piccadilly, Pistol, Profi, Prophet, Puma, Raleigh, Ravel, Reaper, Record, Renown, Rhino, Rialto, Rooster, Rostrum, Russet, Samson, Sarek, Saxon, Semper, Sennet, Shango, Shannon, Sitka, Solstice, Spice, Spitfire, Spry, Stallion, Tanker, Tessa, Texel, Thunder, Tilburi, Tjalk, Tomo, Torch, Tosti, Toucan, Trawler, Trend, Trooper, Turpin, Veritas, Vivant, Warrior, Wasp, Welton, Woodstock, Wykeham, Zodiac	132
	Unknown	F, GB, NL	Bourbon, Capital, Corsaire, Estorial, Jubilatka, Kyalami, Rubens, Soleil, Trafalgar, Victo, Xi 19, Yacht	12