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P. Donini · J.R. Law · R.M.D. Koebner · J.C. Reeves
R.J. Cooke

Temporal trends in the diversity of UK wheat

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Abstract The common assertion that scientific plant breeding leads to a narrowing in crop diversity has been examined. We have characterised the dominant UK winter wheat varieties from the period 1934–1994 using two types of PCR-based DNA profiling (AFLPs, amplified fragment length polymorphisms, and SSRs, simple-sequence repeats, microsatellites), seed storage protein analysis and morphological descriptors. The varieties were grouped into a series of decadal groups on the basis of their first appearance on the 'Recommended List', and by analysis of molecular variance it was shown that an overwhelming proportion of the overall observed variance occurred within, rather than between, decades. A further range of statistical indices provided little evidence for any significant narrowing of overall diversity over the time studied. Principal co-ordinate analysis showed that the diversity in the time periods overlapped and that the most modern group of varieties encompassed the majority of the diversity found in earlier decades. The consistent indication is that plant breeding has resulted, over time, in a qualitative, rather than a quantitative, shift in the diversity of winter wheat grown in the UK.

Key words *Triticum aestivum* · Morphological characters · Molecular markers · Genetic diversity · Plant breeding

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P. Donini · R.M.D. Koebner
John Innes Centre, Norwich NR4 7UH, UK

J.R. Law · J.C. Reeves · R.J. Cooke (✉)
National Institute of Agricultural Botany,
Cambridge CB3 0LE, UK
e-mail: robert.cooke@niab.com

Present address: P. Donini
National Institute of Agricultural Botany,
Cambridge CB3 0LE, U.K.

Introduction

Public concern is often expressed that the practice of modern intensive plant breeding leads inevitably to a reduction in the genetic diversity of crops (Vellvé 1993; Clunies-Ross 1995). Such an erosion could have serious consequences, both on the genetic vulnerability of crops to changes in the spectrum of pests and diseases and on their plasticity to respond to future changes in climate or in agricultural practices (Clunies-Ross 1995; Tripp 1996; Smale 1996; FAO 1998). It is clearly necessary, therefore, to attempt to quantify the genetic diversity of major agricultural crops on both scientific and socio-economic grounds (Tripp 1996; Smale 1996; Duvick 1984; Swanson 1996). To date, objective assessments of diversity have been hampered by confusions in terminology and by a lack of suitable data (Tripp 1996; Smale 1996). However, polymerase chain reaction (PCR)-based molecular marker technologies, currently dominated by AFLP (amplified fragment length polymorphisms, Vos et al. 1995) and SSR (simple-sequence repeats, microsatellites, Morgante and Olivieri 1993), largely overcome these limitations (Karp et al. 1997), even in species such as bread wheat (*Triticum aestivum* L. emend. Fiori et Paol.*)* which show relatively poor levels of molecular variation (Gale et al. 1990).

Assessments of genetic diversity in wheat have been reported previously using restriction fragment length polymorphisms (RFLPs, Kim and Ward 1997), random amplified polymorphic DNAs (RAPDs, Joshi and Nguyen 1993) and SSRs (Plaschke et al. 1995). The success of these approaches has been limited by a lack of polymorphism (RFLP, RAPD) or by the relatively low number of loci assayed (SSR). Both these limitations are removed with the use of AFLPs, with its high effective multiplex ratio (Milbourne et al. 1997) and its flexibility in increasing the numbers of polymorphisms by generating additional profiles via amplification with further primer pairs (Law et al. 1998). Thus, Barrett and Kidwell (1998) were able to survey 229 polymorphic AFLP products and used these to infer relationships between 54 USA

wheat varieties. Since 45 of these varieties were released post-1975, however, they were not able to address the issue of possible temporal erosion of diversity.

We report here the use of AFLPs and SSRs, in addition to seed storage proteins and morphological descriptors, to assess the diversity of the UK winter wheat crop during the period 1934–1994. During this time, varietal choice in the UK has been largely guided by the NIAB 'Recommended Lists', which highlight the most widely grown varieties at any given period. Hence, by selecting fully Recommended varieties from different times, we were able to represent the bulk of the UK crop. We have used a range of statistical tools to explore any temporal trends in diversity and to test for evidence of erosion of diversity. To our knowledge this represents the first report of the use of these methods for this purpose and the first attempt to examine any changes in diversity over time in an objective manner.

Materials and methods

Plant material

Seed of winter wheat was obtained from the reference collections of NIAB and/or the BBSRC Cereals Collection held at the John Innes Centre. The varieties (55 in total) were chosen from the NIAB 'Recommended Lists' for 1934 onwards, taken every 10 years (1934, 1944, etc.), to represent the major part of the UK acreage over each decade. The varieties (Table 1) were only included in the decade of their first listing and not in subsequent periods. Thus, any changes in diversity due to the release of new varieties could be readily identified.

Table 1 Wheat varieties used for analysis of diversity

Decade	Variety	Decade	Variety
1930 s	Iron III	1980 s	Armada
	Little Joss		Avalon
	Standard Red		Aquila
	(Squareheads Master)		Brigand
	Wilhelmina (Victor)		Fenman
	Yeoman (Yeoman II)		Galahad
1940 s	Bersee (Jubilege)		Hustler
	Holdfast		Longbow
	Juliana		Mission
	Redman		Norman
	Steadfast		Rapier
	Vilmorin 27		Stetson
	Warden		Virtue
1950 s	Cappelle-Desprez	1990 s	Admiral
	Hybrid 46		Apollo
	Minister		Beaver
	Pilot		Brigadier
	Staring		Cadenza
1960 s	Champlein		Flame
	Elite Lepeuple		Genesis
	Flamingo		Haven
	Maris Widgeon		Hereward
	Professeur Marchal		Hunter
	Thor		Mercia
1970 s	Bouquet		Riband
	Maris Huntsman		Spark
	Maris Nimrod		Torfrida
	Maris Ranger		
	West Desprez		

Generation of diversity data

Genomic DNA was extracted from the flour of 2 g of seeds, as described in Donini et al. (1997). AFLP and SSR profiles were generated as described elsewhere (Donini et al. 1997,1998; Law et al. 1998). AFLP profiles were manually scored and analysed as in Law et al. (1997, 1998), whilst for SSRs, the allele constitution at each microsatellite locus in all varieties was scored.

Seed storage proteins (gliadins and high-molecular-weight glutenins) were extracted and analysed using the procedures described in Cooke and Law (1998). The allelic constitution at the *Gli –1, Gli-2 and Glu-1* loci was determined as in Cooke and Law (1998).

The morphological data were collated from existing historical records held at NIAB and from National List trials (and hence are not publicly available currently). A total of 14 characters (plant growth habit, plant height, straw wall thickness, ear colour, ear scurs, ear shape, ear length, ear density, beak length, spikelet frequency, shoulder width, internal hairs, internal imprint and grain colour) was used for which data were available for all varieties. A numerical score of 1–9 (UPOV Notes, as defined in the UPOV Guidelines 1994) was assigned for each character.

Statistical analysis

The polymorphic AFLP bands were transformed into Jaccard distances using the RAPDIST software package (Armstrong et al. 1993). Microsatellite and seed storage protein alleles were analysed using the City Block approach. Morphological data were treated as Euclidean for analysis purposes. Two-dimensional data matrices were constructed, comprising the bands/alleles/characters present in each variety.

The datasets were subjected to a number of statistical treatments, as described previously (Law et al. 1997; Cooke and Law 1998). Analysis of molecular variance (AMOVA, Excoffier et al. 1992), Theta statistics (Tajima 1989a,b) and diversity per character scores (Theta Pi/number of characters) were obtained by submitting the raw data to the software package ARLEQUIN (Schneider et al. 1996). The AMOVA was carried out without multiple data points for each variety by treating varieties as sub-units of the decadal groups and then amalgamating these into broader classes (Law et al. 1997).

Principal Co-ordinate (PCO) analysis (Gower 1966) was carried out using the inter-variety distances as data units. Pair-wise similarities (1-distance) were calculated from the raw data matrices using the Jaccard band matching coefficient (Sneath and Sokal 1973) and were used as input into the Statistica (1996) statistical package to conduct the PCO analysis and generate the graphical output. The resulting ordering was such that the centroid of the PCO plot is at the origin, and points were arranged relative to the principal axes, so that the first dimension of the fitted solution gave the best one-dimensional fit and the first two dimensions gave the best two-dimensional fit.

Multidimensional scaling (MDS, Kruskal 1964) was also used to reveal patterns of relatedness within the similarity matrices by assigning co-ordinates to the variance represented within each decade. The co-ordinates were used to create scatterplots and calculate pair-wise distance estimates between decades.

Results

The six AFLP primer combinations employed generated 84 non-redundant polymorphic bands from the set of 55 varieties (Law et al. 1998). SSR analysis provided information with respect to 14 loci, with numbers of al-

Table 2 Summary of AMOVA for different types of data from 55 wheat varieties from decadal groups

	Nested AMOVA variance components:					
	Between decadal groups[a]	Between varieties within decadal groups	Within decadal groups			
	V(a)%	V(b)%	V(c)%	F(st)	F(sc)	F(ct)
AFLP	6.30	0.93	92.77	0.072	0.010	0.063
SSR	10.08	11.38	78.54	0.215	0.127	0.101
Storage proteins	4.12	13.29	82.59	0.174	0.139	0.041
SSR + storage proteins	6.38	1.40	92.22	0.078	0.015	0.064
AFLP + SSR + storage proteins	4.63	1.14	94.24	0.058	0.012	0.046
Morphology (i)[b]	9.98	2.61	87.41	0.126	0.029	0.100
Morphology (ii)	7.43	2.59	89.99	0.100	0.028	0.074
Morphology (iii)	31.66	2.77	65.57	0.344	0.041	0.317
AFLP + SSR + storage proteins + morphology	6.41	1.63	91.96	0.080	0.017	0.064

[a] Decades grouped into 1930 s + 1940 s + 1950 s (‘old’ – 17 varieties), 1960 s + 1970 s (‘intermediate’ – 11 varieties) and 1980 s + 1990 s (‘new’ – 27 varieties)
[b] (i), All 14 characters; (ii), plant height excluded; (iii), plant height alone

leles varying from 2 to 9 (Donini et al. 1998) and a total of 53 alleles, whilst seed storage protein profiles allowed the identification of 37 alleles at 9 loci (with from 2 to 8 alleles per locus) across all varieties (Cooke and Law 1998). The raw data are available from the authors on request.

Analysis of diversity trends

AMOVA allows an analysis of variance-like approach to the study of patterns of genetic variation within and between groups. We have previously shown that in AMOVA, the influence of individual entries in decades with relatively poor representation can be minimised by grouping decades together (Law et al. 1997). Appropriate groupings were established by choosing a combination which delivered the lowest proportion of the molecular variance attributable to ‘between decades within decadal groupings’ – V(b). The chosen groupings represent in broad terms ‘old’ (1930 s, 1940 s, 1950 s), ‘intermediate’ (1960 s, 1970 s, corresponding broadly to the dominance of var ‘Cappelle-Desprez’ and its descendants) and ‘modern’ (1980 s, 1990 s) material. Table 2 presents separate AMOVAs for the various datasets, along with combined analyses. Since plant height changed substantially with the introduction of semi-dwarf varieties in the 1970 s, the morphological data were re-analysed to highlight the influence of plant height.

The AMOVA showed that for all individual types of data the within-decadal groups component [V(c)] of variance overwhelmingly dominates, accounting for between 79% and 93% of the total variance. The genetic distance between decadal groups [F(st) values, Weir and Cockerham 1984; Reynolds et al. 1983] in all cases exceeded the degree of in-breeding within decades [F(sc) values] and the degree of relatedness between genes within varieties [F(ct) values]. The morphology and AFLP datasets were quite consistent with one another, while the SSR and protein datasets generated somewhat lower but still substantial values of V(c). The likelihood is that this reflects an effect of sampling, since combining the protein and SSR data (and hence increasing the number of loci) produced a high value of V(c) (Table 2). Changing the decadal groupings used had no significant effect (data not shown). The influence of plant height can be clearly seen from the rise in V(a) when plant height is analysed in isolation; its contribution to the overall morphology AMOVA is still noticeable in causing an increase in V(a) from 7.4% to 10.0%. When all of the molecular data (AFLP, SSR, protein) were combined, 94% of the variance was contained within the decadal groups. This fell slightly to 92% when the morphology data were included. The F(ct)values, although generally small, reflect the fact that there is some heterogeneity within the varieties for the characters measured, which has been demonstrated in these and other varieties by the analysis of individual seeds (Cooke and Law 1998; Donini et al. 1998).

Multidimensional scaling (MDS) complements the AMOVA by providing a spatial representation of the relative genetic distances [F(st) values] between decadal groups of varieties. Scatterplots in three dimensions, both for the individual data sets (not shown) and for the combined data from morphology, storage protein, AFLP and SSR analysis (Fig. 1), showed no clear directional patterns of change in the distances between the decadal groups, other than random step-wise perturbations.

Temporal trends in diversity were further assessed by computing the Theta (Pi) population statistic from the different data types and the average diversity per character (Cooke and Law 1998). Theta (Pi) – the product of the mutation rate and the effective number of genes under study – represents the mean number of paired differences (Tajima 1989a,b) and for all datasets, the values did not

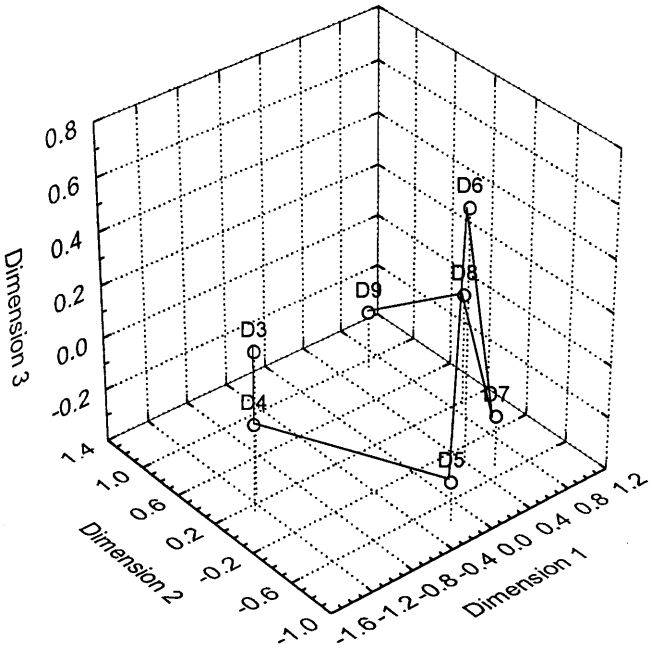

Fig. 1 MDS scatterplot of linearised F(st) values for 55 wheat varieties grown in the UK between 1934 and 1994. *D3* Varieties from the 1930 s, *D4* varieties from the 1940 s, etc

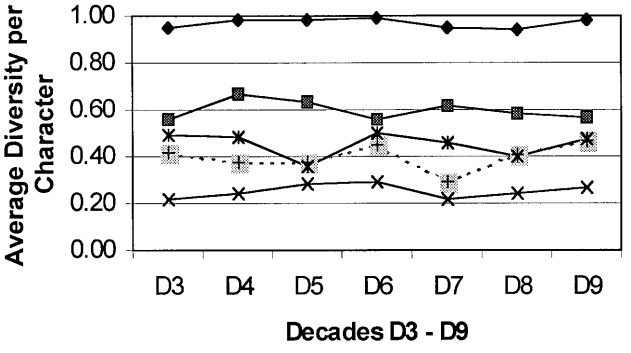

Fig. 2 Changes in the diversity per character for various types of data from 55 wheat varieties grown in the UK between 1934 and 1994. *From top to bottom* – AFLPs scored as characters per primer pair (◆), morphological characters (■), storage proteins (✳), SSRs (⊞) and individual AFLP loci (×)

vary significantly between decades (data not shown). The diversity per character (Fig. 2) values also showed no significant variations over time. The AFLP data were analysed both as individual bands (≡84 loci) and by considering each primer pair as a character (≡6 'loci').

Principal co-ordinate analysis (PCO)

PCO analysis of the various datasets allowed the visual representation of the diversity present in a decade by drawing convex hulls around the extremes of each decade. This approach highlights the ranges of diversity of the varieties within each time period (Fig. 3, only 1990 s

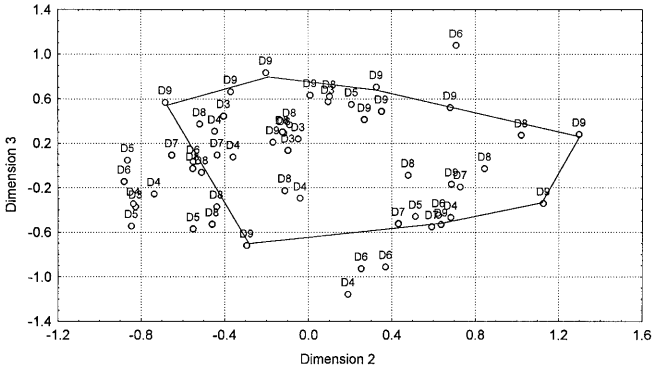

Fig. 3 PCO plots of the distribution of diversity for 55 wheat varieties grown in the UK from 1934 to 1994, as analysed by a combination of AFLPs, SSRs, storage proteins and morphological characters. *D3* Varieties from 1930, etc. The lines (*convex hulls*) join together the extremes of the variation from each decade; for clarity, only the 1990 s hull is shown

hull shown, for clarity). The first two axes of the PCO analysis for all data types accounted for 20–30% of the total variation (e.g. 21% for AFLPs, 24% for morphology), which is in line with other reported analyses of this kind (Law et al. 1997) and indicates that these are high-dimension systems. The PCO plots, both of the individual (not shown) and combined datasets (Fig. 3), clearly showed the overlapping nature of the diversity revealed by molecular and morphological analysis of UK wheat varieties over the past 60 years, with only a minor shift in the centroid of the decadal groups (cf. Fig. 1). The shapes of the convex hulls are of some interest, e.g. the 1940 s and 1970 s varieties tended in all cases to produce a long thin hull, especially noticeable for the molecular markers. This may be indicative of a potentially undesirable 'bottleneck' situation in those periods, since the omission of only 1 or 2 varieties could lead to a considerable reduction in diversity. Nonetheless this 'narrowing' (if this is what is indicated) is clearly only a temporary situation, since the convex hulls for the modern (1980 s and 1990 s) varieties were extensive and encompassed a considerable proportion of the varieties from the previous decades in all cases.

Discussion

Our analyses consistently indicate that the levels of overall diversity present in UK winter wheat, as measured in a number of ways, have been maintained substantially unchanged since the 1930 s. The AMOVA showed that the great majority of the observed variance occurs within decades rather than between them, a fact confirmed by the PCO analysis with clearly overlapping diversity for all decades and no sustained diminution over time. As shown by the MDS analysis, any temporal changes in diversity that have taken place have no consistent directional effect imposed by breeding activities. Hence, our data offer no support to the notion that modern plant

breeding inevitably reduces diversity. However, there has perhaps been a certain narrowing at various times, particularly in the 1970 s at a time when the UK wheat crop was dominated by output from a single breeding programme. There have clearly also been qualitative shifts in the type of diversity that breeders utilise in their programmes and consequently, in the varieties that become commercially successful, largely following changes in husbandry techniques and plant ideotype that have occurred over this period. A particular example of this is the move to the semi-dwarf habit, which generates high harvest indices and permits the use of higher fertility conditions, without the risk of lodging. The necessity of introducing novel disease resistance genes/alleles will also have been an important driver of diversity turnover. Breeders have clearly been able to maintain diverse sources of germplasm within their nurseries and collections, and as different parts of this are used in breeding programmes, so different sub-sets of the overall diversity are released in the form of commercial varieties. At the same time, it seems that although the breeding process has fixed the allelic state at a number of agronomically important loci (such as those determining vernalisation requirement or semi-dwarf habit), it has had little impact on allele frequency at loci not affected by selection. More generally, since only that portion of genetic variation directly affected by breeders' selection pressure thus far is likely to have been subject to erosion, the remainder probably remains intact. This portion may include allelic variation of relevance to prospective changes in husbandry and/or environment, which will be of critical importance to the success of future breeding.

It is interesting to note (Fig. 2) that the morphological characters apparently show more diversity per character than the molecular markers. This is probably because the individual morphological characters are generally multigenic, and hence variation at more than one locus is being analysed. This is supported by the difference in the diversity/character indices observed when the AFLP data are scored as 84 individual loci or as six primer pair characters. In addition, the morphological characters have been chosen on the basis of their usefulness for distinctness testing in wheat, whereas there is no such selection for the other markers.

An anticipated criticism of the use of AFLPs for genetic diversity analysis relates to their status as unmapped markers whose genomic distribution is unknown. It has been proposed that if AFLPs have a tendency to be genetically clustered, they will not represent independent variables nor provide the genome coverage that is said to be necessary for diversity analyses (Karp et al. 1997). We have argued previously that there is little indication of extensive linkage among the AFLPs in this dataset (Law et al. 1998). The analysis here of mapped markers (SSRs and storage proteins), as well as morphological characters (whose genetic control is assumed to be dispersed across the genome), has given rise to similar conclusions that could have been drawn from the use of AFLPs alone. The evidence is, therefore, that AFLPs, at least those assayed using a methylation-sensitive enzyme as the rare cutter as in the present work (Donini et al. 1997), provide a satisfactory sample of genetic diversity in the wheat genome. There would thus not seem to be any particular merit in choosing one type of marker in preference to another in this instance.

Clearly the kind of temporal trends in diversity that we have investigated do not represent a complete picture of agricultural genetic diversity on UK farms. The varieties analysed were selected on the basis of their 'Recommended List' status and commercial success but represent only a proportion of those available for cultivation at the various times. However, analysis of the seed certification statistics for the 1970 s onwards showed that the varieties chosen for this study, with two exceptions, achieved a 5% market share at some stage during the decade (Law, unpublished) and so do comprise the major varieties grown. In addition, no account has been taken of the relative area of land occupied by each variety, a factor which would be relevant to the overall quantification of the diversity of the UK crop. Interestingly, our data seem to indicate no significant effect of Plant Breeders' Rights (introduced in the UK in 1964) on levels of diversity, since there appears to be no overall or sustained alteration in diversity post-1964. This would need to be examined more closely, both with more varieties and with different species. Comparisons of diversity levels within as well as between varieties and in countries that do not have PBR would also be instructive in this respect. Finally, it is apparent that the 'Recommended Lists' in the UK are not only delivering wheat varieties to farmers that are agronomically superior, but also that the system is not adversely affecting the overall levels of diversity.

We believe that the approaches used in this work will make a valuable contribution to the discussions related to the conservation of crop genetic resources, since in order to be able to decide what needs to be conserved, it is first necessary to be able to measure the diversity that exists. We suggest that the use of molecular markers such as AFLPs and SSRs, in conjunction with other approaches and the appropriate statistical tools, allows such objective measurements of diversity to be made. These in turn provide a firm basis for important and informed decisions concerning the effects of modern plant breeding and agricultural practices and the role of diversity in the future.

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