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Temporal flux in the morphological and molecular diversity of UK barley

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Abstract Genetic-diversity assessments, using both phenotypic and molecular-marker data, were made on a collection of 134 barley varieties (both winter and spring types), chosen on the basis of their representation on the NIAB 'Recommended List' over the period 1925–1995. Genotypic (AFLP and SSR) and phenotypic (UPOV characters) data were analysed to determine short- and long-term temporal trends in diversity over the period. A consistent pattern emerged demonstrating that only a minor proportion of the overall variance appears to be the result of any temporal drift, although there were strong indications of qualitative shifts in diversity, probably related to the changing relative acreage of winter and spring barleys over the study period. Our overall conclusions are that systematic plant breeding does not inevitably lead to a reduction in the genetic diversity of agricultural crops, and that diverse breeding programmes and the variety delivery systems in place in the UK have generally been successful in maintaining sufficient genetic diversity to allow the steady rise in genetic potential that has been a feature of 20th century crop breeding. The concentration of breeding effort into a smaller number of independent programmes is likely to be prejudicial to the maintenance of the genetic diversity of a crop.

Keywords Genetic diversity · Barley · AFLP · SSR · Morphological markers

Introduction

Genetic diversity among currently cultivated major arable crops is an issue of much current interest. A popular impression is that intensive plant breeding over a sustained period has exerted a downward pressure on diversity, narrowing the germplasm-base available for future breeding advance under potentially changed environmental and agronomic conditions, and raising the risk of genetic vulnerability. In the small-grain cereals, this impression is strengthened by the near-universal adoption of varieties with a semi-dwarf habit, whose uniform height gives the illusion of genetic uniformity. While the domestication of a wild species into a crop, and the change from cultivation of landrace mixtures to pure line varieties both clearly represent genetic bottlenecks (Tanksley and McCouch 1997), it is less obvious that the scientific breeding process itself produces a further downward pressure on genetic variability. Objective evidence for this latter claim is conspicuously absent from the literature, partly because, until the advent of widespread DNA profiling, it has not been possible to generate sufficient genetic data to test it. However, using a range of both morphological characters and DNA- and protein-based markers, we have recently been able to show that there is no *prima facie* evidence for any diminution in the genetic diversity present in the portfolio of varieties that have dominated the UK winter wheat crop at a series of time points over the last 60 years (Donini et al. 2000). In contrast, Russell et al. (2000) showed that the spectrum of microsatellite alleles across 28 loci present in modern UK spring barley varieties is largely a subset of that present in a group of 19 landraces and key progenitors that were released in a period up to the 1930s (apart from four entries, released in the 1950s). This analysis therefore suggested that the breeding and selection process in UK spring barley has resulted in a considerable restriction in the genetic diversity of the crop, at least as assessed by microsatellite allele distribution.

In the UK, and in most other countries with Plant Breeders' Rights and National Listing systems, new varieties are effectively only released when proven to be both distinct from, and agronomically superior to those currently available. In barley this distinctness is assessed

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Table 1 Barley entries profiled, and arranged according to decade and type

1990s

Spring: (20 entries) Alexis, Blenheim, Brewster, Camargue, Chad, Chariot, Cooper, Dandy, Delibes, Derkado, Felicie, Forester, Hart, Heron-1, Heron-2, Nomad, Nugget, Prisma, Triumph, Tyne
Winter: (17 entries) Bronze, Clarine, Epic, Fighter, Firefly, Gypsy, Halcyon, Kira, Linnet, Marinka, Pastoral, Pipkin, Posaune, Puffin, Sprite, Target, Willow

1980s

Spring: (16 entries) Apex, Atem, Corniche, Delta, Digger, Doublet, Egmont, Golf, Joline, Klaxon, Koru, Kym, Natasha, Patty, Regatta, Tasman
Winter: (13 entries) Frolic, Gerbel, Igri, Libra, Magie, Maris-Otter, Melusine, Panda, Pirate, Plaisant, Sonja, Tipper, Torrent

1970s

Spring: (29 entries) Abacus, Aramir, Ark-Royal, Armelle, Athos, Berac, Deba-Abed, Georgie, Gerkra, Goldmarker, Hassan, Imber, Julia, Jupiter, Keg, Lofa-Abed, Magnum, Maris-Mink, Mazurka, Midas, Porthos, Proctor, Simon, Sundance, Tyra, Universe, Vada, Wing, Zephyr
Winter: (6 entries) Astrix, Athene, Hoppel, Maris-Trojan, Mirra, Senta

1960s

Spring: (11 entries) Cambrinus, Freja, Impala, Inis, Maris-Badger, Maris-Baldric, Mosane, Pallas, Sultan, Rika, Union
Winter: (3 entries) Dea, Maris-Puma, Pioneer

1950s

(6 entries) Spratt-Archer, Carlsberg-2, Earl, Herta, Maythorpe, Provost

1940s

(6 entries) Carlsberg, Golden-Archer, Camton, Bere, Plumage-Archer, Prefect
1920s/1930s
(7 entries) Golden-Pheasant, Maltster, Standwell, Kenia, Maja, Victory, Webbs-Sunrise

using largely quantitative (and partially subjective) measures of a modest number of morphological characters. The extent to which variation in these characters reflects levels of genetic diversity is unknown, since the genetic basis of many of the characters has not been elucidated. In contrast, DNA-based polymorphisms are both qualitative and numerous, and some (in particular RFLP and SSR, but not AFLP or RAPD) are genetically defined. Thus, in principle, marker-derived assessments are better suited than are morphological descriptors to characterise patterns of genetic diversity, provided that any such assay of genetic variation is not biased.

We report here a retrospective analysis of the genetic diversity in the UK barley crop, exploiting the tools and methodologies developed for a similar analysis of UK winter wheat (Donini et al. 2000). The UK barley crop area is second only to that sown to wheat, averaging around 1.2 Mha in the 1990s, representing about 10% of the total arable area; while its relative yield level is about 80% that of wheat. Areas sown to both barley and wheat have doubled since 1950. The barley crop remains therefore a major component of UK agricultural production. Analogous to our study on wheat, we have used inclusion in the NIAB (National Institute of Agricultural Botany) 'Recommended Lists' as the basis for assembling representative germplasm for analysis, since these lists have been highly effective in guiding varietal choice by the farmer. Unlike the situation with wheat, however, where winter-sown materials have dominated the crop for many decades, in barley there has been a major change in the balance between winter- and spring-sown varieties. Prior to the 1960s, most of the winter-sown varieties were non-vernalisation requiring, winter-hardy types, and were thus genetically 'spring', rather than 'winter', genotypes. However, since this period, winter-sown varieties have been strictly vernalisation-requiring. From the late 1980s, the area of winter-sown barley has exceeded that of spring-sown, whereas as recently as 1970, spring-sown barley acreage was more than ten-times that of winter-sown (Lupton 1992). There has been relatively little intermixing of the gene pools of winter and spring barleys in breeding programmes. Thus, in our analysis of the temporal trends in diversity, we have treated these as separate crops, an approach which we show to be justifiable on the basis of molecular fingerprinting.

Materials and methods

Plant materials and the generation of molecular fingerprints

The 134 barley entries (listed in Table 1) were chosen on the basis that they were represented on the NIAB Recommended List in the 4th year of each of the decades since 1920 (i.e., 1924, 1934,…,1994). The earlier materials have all been treated as spring types, since they do not require vernalisation for early flowering. However for the later materials, spring-sown and winter-sown materials can be distinguished on the basis of their requirement for vernalisation. Molecular genotyping was carried out using AFLP and SSR, following established methods (Donini et al. 1997, 1998). For AFLP, eight primer combinations were used to generate DNA fingerprints: S12-M16, -M20, -M21, -M22 and S24-M17, -M20, -M21, -M24. Relevant AFLP adapters and oligonucleotides are described in Donini et al. (1997), with the addition of three Mse +2 primers: M16 +CC, M22 +GT and M24 +TC. The Sse adapter was not biotinylated, as it has been shown that

Table 2 Morphological traits included in diversity analysis

Character	State 1	State 2	State 3	State 4	State 5	State 6	State 7	State 8	State 9
Lower leaves: hairiness of sheaths	Absent								Present
Leaf width			Narrow	Narrow to medium	Medium	Medium to wide	Wide		
Ear: number of rows	Two	Six							
Ear: density	Very lax	Very lax to lax	Lax	Lax to medium	Medium	Medium to dense	Dense	Dense to very dense	Very dense
Flag leaf: attitude at ear emergence	Erect	Erect to semi-erect	Semi-erect	Semi-erect to horizontal	Horizontal	Semi-recurved	Recurved	Recurved to deflexed	Deflexed
Flag leaf: anthocyanin colouration of auricles	Absent	Very weak	Weak	Weak to medium	Medium	Medium to strong	Strong	Strong to very strong	Very strong
Ear: glaucosity	Absent or very weak	Very weak to week	Weak	Weak to medium	Medium	Medium to strong	Strong	Strong to very strong	Very strong
Flag leaf: glaucosity of sheath	Absent or very weak	Very weak to week	Weak	Weak to medium	Medium	Medium to strong	Strong	Strong to very strong	Very strong
Awn: anthocyanin colouration of tips	Absent	Very weak	Weak	Weak to medium	Medium	Medium to strong	Strong	Strong to very strong	Very strong
Awn: length compared to ear	Shorter	Equal	Longer						
Awn: spread			Weak	Weak to medium	Medium	Medium to strong	Strong		
Collar type	Recurrent		Platform	Platform to shallow cup	Shallow cup		Cup		
Rachis: length of first segment	Very short	Very short to short	Short	Short to medium	Medium	Medium to long	Long	Long to very long	Very long
Grain: shape in dorsal view	Plump	Average	Elongated						
Grain: lemma base type	Plain, angular depres. or nick	Level							
Grain: rachilla hair type	Short and woolly	Long and straight	Short and straight ("G" type)						
Grain: spiculation of inner lateral nerves	Absent/v. weak (0–2 per nerve)		Weak (1–2 per nerve)		Medium (3–5 per nerve)		Strong (5–10 per nerve)		Very strong (>10 per nerve)
Grain: ventral furrow – presence of hairs	Absent								Present
Grain: length of lodicules ("collar" type only)	Very short		Short	Short to Intermediate	Intermediate	Intermediate to Long	Long		Very long
Reaction to DDT	Susceptible	Resistant							
Seasonal type	Winter type	Alternative type	Spring type						

*Mse*I–*Mse*I fragments are not generally amplified under comparable reaction conditions (Vos et al. 1995; Law et al. 1998). Pre-amplifications for primer combinations S12-M22, S24-M17, S24-M24, and S12-M16 were carried out using primer combination *Sse*+0/*Mse*+0 (Donini et al. 1997); but for the remaining four primer combinations, *Mse*+0 was replaced by the *Mse*+1 primer M03 +G. For SSR, 21 primer pairs were used to amplify 106 alleles at 22 loci (HVM-11 amplifies two independent loci) (Donini et al. 1998). Morphological data were based on a set of 21 characters, corresponding to a subset of standard UPOV 'notes' (listed in Table 2), and were obtained from existing historical records held at NIAB.

Data analysis

The data analysis procedures were as previously published (Law et al. 1997; Donini et al. 2000). SSR data were converted to 'pat-

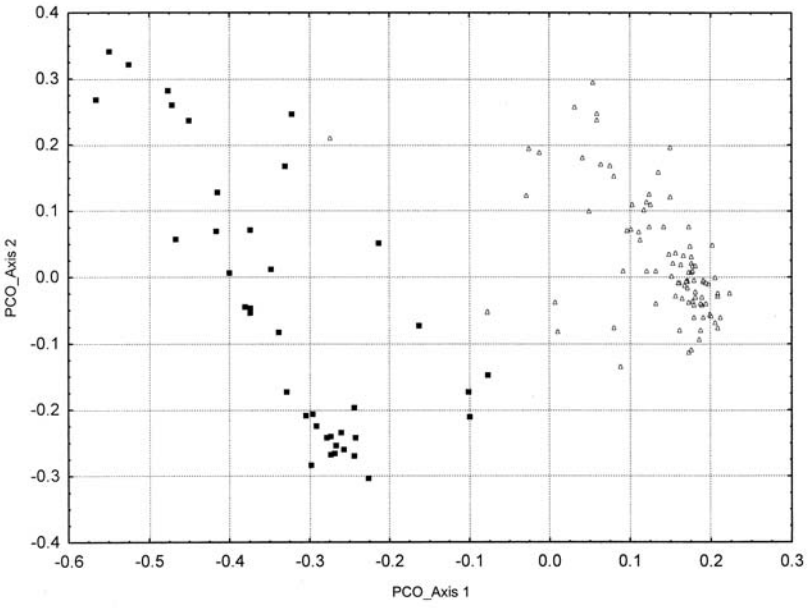

Fig. 1 Principal coordinate (PCO) analysis of winter (*filled squares*) and spring (*open triangles*) barleys using morphological characteristics. The first axis accounts for 23.0% and the second axis 8.5% of the total variation

tern scores' in Excel (Law et al. 1997). The data from AFLP, SSR and morphology were singly, and in combination, analysed using Arlequin (Schneider et al. 1996) software to compute the analysis of molecular variance (AMOVA), partitioning the observed variation hierarchically. Principal co-ordinate (PCO) analyses were computed by optimising the construction of similarity matrices (Jaccard's method for AFLP and the 'city-block' approach for both SSR patterns and morphology) in the general statistical software package Genstat (Genstat 5 Committee, Statistics Department, Rothamsted Experimental Station, Harpenden, Herts, UK).

Results

AFLP and SSR profiles

Across the varietal set, 144 polymorphic AFLP bands were identified. The primer combinations (PCs) varied widely in their efficiency in uncovering variation. For example, S24-M20 generated 36 polymorphic bands, as against just three polymorphisms using S24-M24. The informativeness of each PC, as described by its polymorphic information content (PIC) value (Botstein et al. 1980), was not correlated with the number of polymorphic bands that it identified – PIC values ranged from 0.24 to 0.38 for the six PCs which generated more than five polymorphisms. Some of the variable AFLP bands were monomorphic within one or other of the barley types, 19 among the winter, and 3 among the spring barleys. Across the whole data set, the 22 SSR loci all showed allelic variation, but the loci defined by HVM-7, -9 and -26 were monomorphic with respect to the winter entries; the former two show very little variation across the whole data set, while the latter detects two alleles, one of which is present in 91% of all the entries.

Separation of winter and spring types

To justify the separate treatment of the spring and winter barleys, we applied an AMOVA. With respect to all three data types, the within-seasonal type variance represented 80–84% (dependent on variate type) of the whole, the remainder being attributed to the between-seasonal type variance (Table 3). In order to demonstrate this separation further, we conducted PCO analyses, using data from morphology, SSRs and AFLPs, alone or in combination. When based on the morphology alone, the first two PCO axes accounted for approximately 33% (winter barleys) and 19% (spring barleys) of the variance; for SSRs and AFLPs, these totals were, respectively, 37%/26% and 16%/11%, in line with similar studies described elsewhere (Law et al. 1997). The PCO plot (Fig. 1) shows the spring entries forming a well-defined cluster, despite being represented by a higher number of entries (95 as against 39 winter varieties). This cluster appeared to be largely distinct from the more dispersed winter grouping. Similar analyses based on molecular markers supported the separation (data not shown). Taken together, these results justify the treatment of spring and winter barley as separate crop types in the subsequent analyses.

Table 3 Analysis of molecular variance (AMOVA): winter barleys versus spring barleys

Source	df	Variance components	% Variation accounted for
SSR			
Between	1	1.20 Va	20.2
Within	132	4.76 Vb	79.8
AFLP			
Between	1	3.55 Va	15.9
Within	132	18.82 Vb	84.1
Morphology			
Between	1	1.16 Va	18.4
Within	132	5.12 Vb	81.6

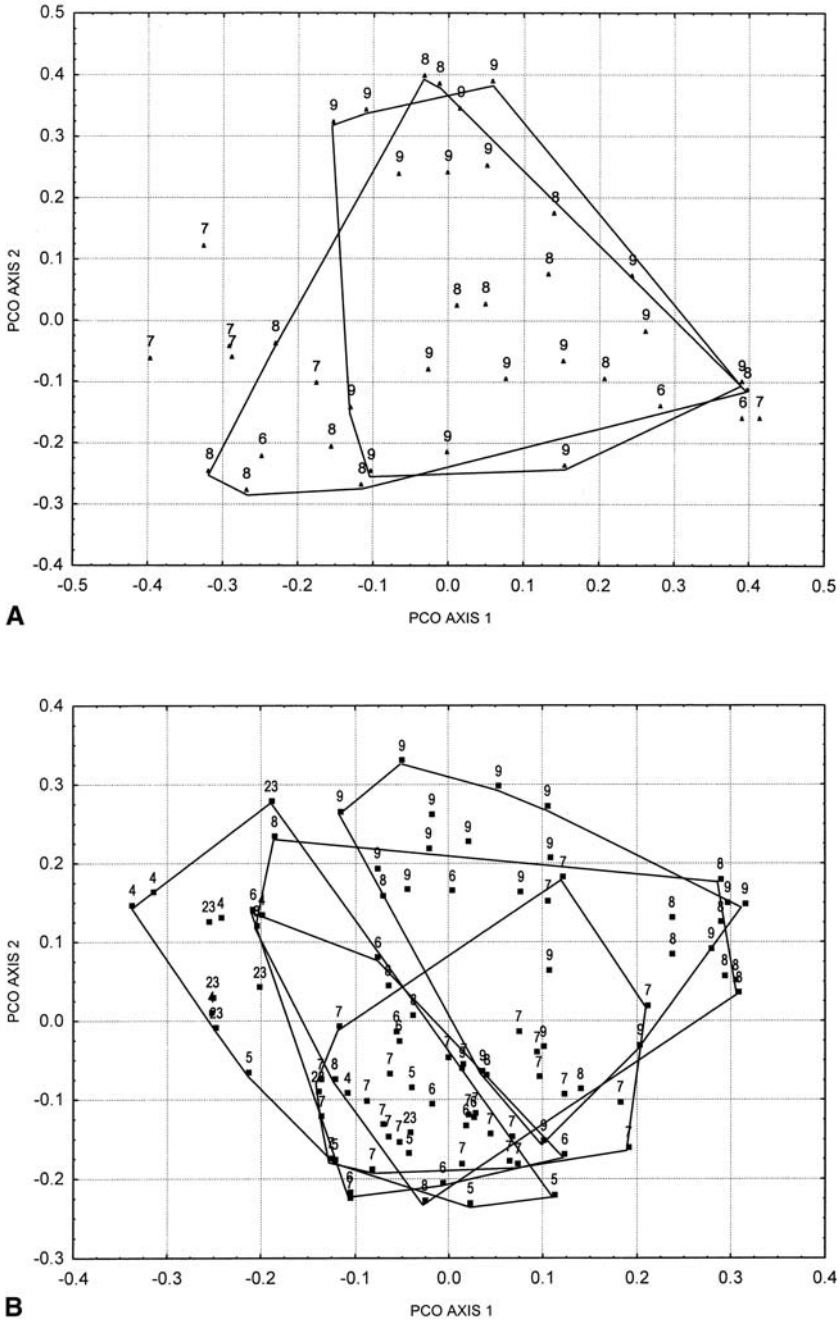

Fig. 2 **A** Winter barley PCO-based (AFLP, SSR and morphology) convex hulls for the 1980s and 1990s materials. Entries from the decades 1960s and 1970s (6 and 7, respectively) are too few to construct meaningful convex hulls. Entries marked 6, 7, 8 and 9 belong, respectively, to the decadal groups 1960s, 70s, 80s and 90s. Proportion of the variance accounted for by the two PC axes are, respectively, 9.7% and 8.7%. **B** Spring barley PCO-based (AFLP, SSR and Morphology) convex hulls for 1920–1950s, 1960s, 1970s, 1980s and 1990s materials. Entries marked 2, 3, 4, 5, 6, 7, 8 and 9 belong to the decadal groups 1920s, 30s, 40s etc. The proportion of the variance accounted for by the two PC axes are, respectively, 5.8% and 5.4%

Long-term temporal trends in diversity

AMOVA allows an examination of the pattern of variance between and within groups. To maintain sufficient group sizes, an AMOVA was conducted on three groupings for spring types, early (1920s to 1950s), intermediate (1960s to 1970s) and modern (post 1980), and the latter two only for the winter types. The results showed clearly that the within-group component (Vc) of the molecular variance was overwhelmingly dominant for all of the data types alone and in combination (Table 4). For the spring barleys, only the morphological data set gave an estimate of below 90% for this component, while for the winter barleys, the SSR set delivered the lowest estimate of Vc (80%), with the other variates and combinations of variates delivering a Vc of above 85%. Thus in all cases, only a minor proportion of the overall variance appears to be the result of any temporal drift between decadal groups. The genetic distance between groups, F(st) values (Reynolds et al. 1983; Weir and Cockerham 1984), exceeded both the degree of in-breeding within groups [F(sc) values] and the degree of relatedness between markers/traits within varieties [F(ct) values]. Thus, mirroring the situation in winter wheat (Donini et al. 2000), there is no indication of any consistent, quantitative, temporal loss in the overall level of genetic diversity in either spring or winter UK barley over the past 60 years.

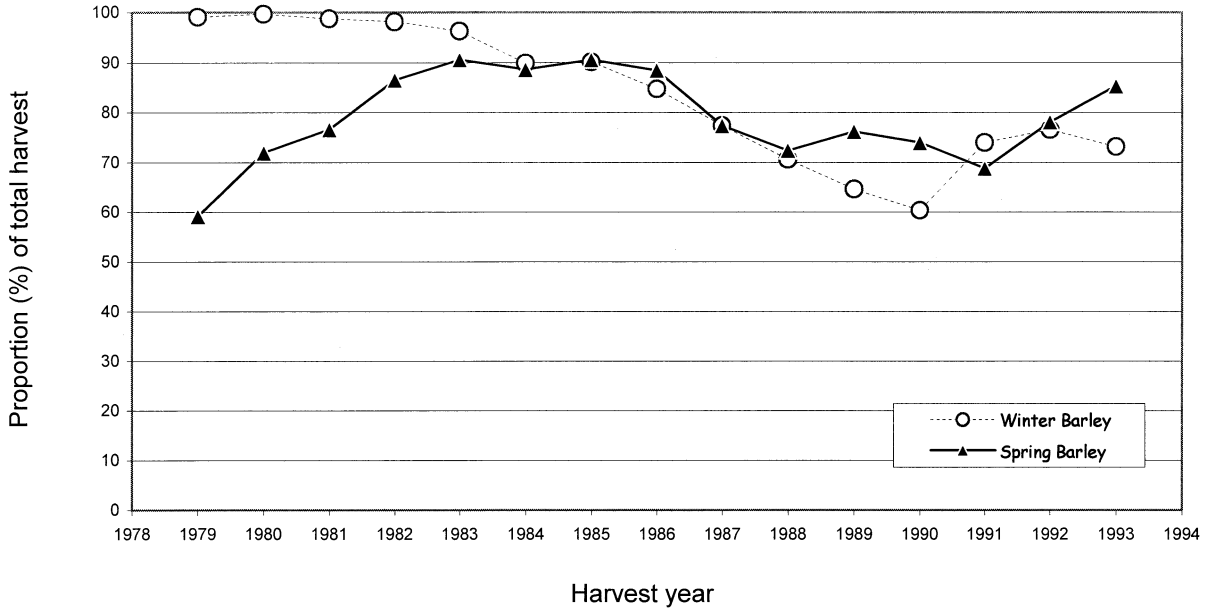

Fig. 3 Percentage of the annual harvest (1978–1994) represented by the five widest grown varieties of winter (*dotted line*) and spring (*solid line*) barley

Table 4 AMOVAs: spring barleys early (1920/30/40/50) vs intermediate (1960/70) vs late (1980/90); winter barleys intermediate (1960/70) vs late (1980/90)

Variate	Spring barley[a]						Winter barley[b]					
	Va(%)	Vb(%)	Vc(%)	F_{sc}	F_{st}	F_{ct}	Va(%)	Vb(%)	Vc(%)	F_{sc}	F_{st}	F_{ct}
SSR	6.2	3.1	90.7	0.03	0.09	0.06	5.6	14.6	80.0	0.15	0.20	0.06
Morphology	0.8	14.2	84.9	0.14	0.15	0.01	12.1	2.7	85.1	0.03	0.15	0.12
S+M	3.4	8.9	87.8	0.09	0.12	0.03	8.6	4.1	87.3	0.05	0.13	0.09
AFLP	4.0	3.3	92.7	0.03	0.07	0.04	4.4	1.4	94.3	0.01	0.06	0.04
A+S	4.5	3.2	93.3	0.03	0.08	0.04	4.4	2.2	93.4	0.02	0.07	0.04
A+M	3.3	5.6	91.0	0.06	0.09	0.03	6.1	1.7	92.3	0.02	0.08	0.06
A+S+M	3.8	5.2	91.0	0.05	0.09	0.04	5.8	2.3	91.9	0.02	0.08	0.06

[a] Grouped by decades: 1920/30/40/50; 1960/70; 1980/90
[b] Grouped by decades: 1960/70; 1980/90

As demonstrated for winter wheat (Donini et al. 2000), we used PCO analysis to reduce the multi-dimensionality of the data to a form where qualitative shifts in diversity over-time can be visualised (Fig. 2). The polygon defined by the position, in 2D-PCO space, of the outermost entries within each decade, which we have referred to as a 'hull' or convex 'hull', allows a graphical picture of the diversity represented by the crop in each time period. In the winter wheat analysis, it was clear that the later decades included much of the genetic diversity present in the earlier materials, and that there was little evidence of any temporal shift in the position of the centroid of the temporal polygons (Donini et al. 2000), and the latter phenomenon is certainly recognisable for the later winter barleys (Fig. 2A). For the spring barleys, a similar pattern emerged, but with the later material appearing to be shifted in PCO-space from the early and intermediate varieties (Fig. 2B). In this analysis we also examined the higher dimensionality of PCO space. This supported the interpretation of the 2D – PCO space which remains the simplest representation of the data.

Recent temporal trends in diversity (1970–1996)

Because of the large numbers of entries within each of the decades contributing to the modern grouping, we were able to visualise temporal variation in diversity in more detail over the period 1970–1996. NIAB Recommended List status varieties form the overwhelming bulk of the crop, as illustrated by plots of the proportion of overall production from the top five varieties of spring and winter barley in the period 1979–1993 (Fig. 3). However, to gain the most complete picture, we included in this analysis all entries on the Recommended List for each year, which consists additionally of varieties that were either provisionally recommended, or classified as outclassed (i.e. no longer among the top

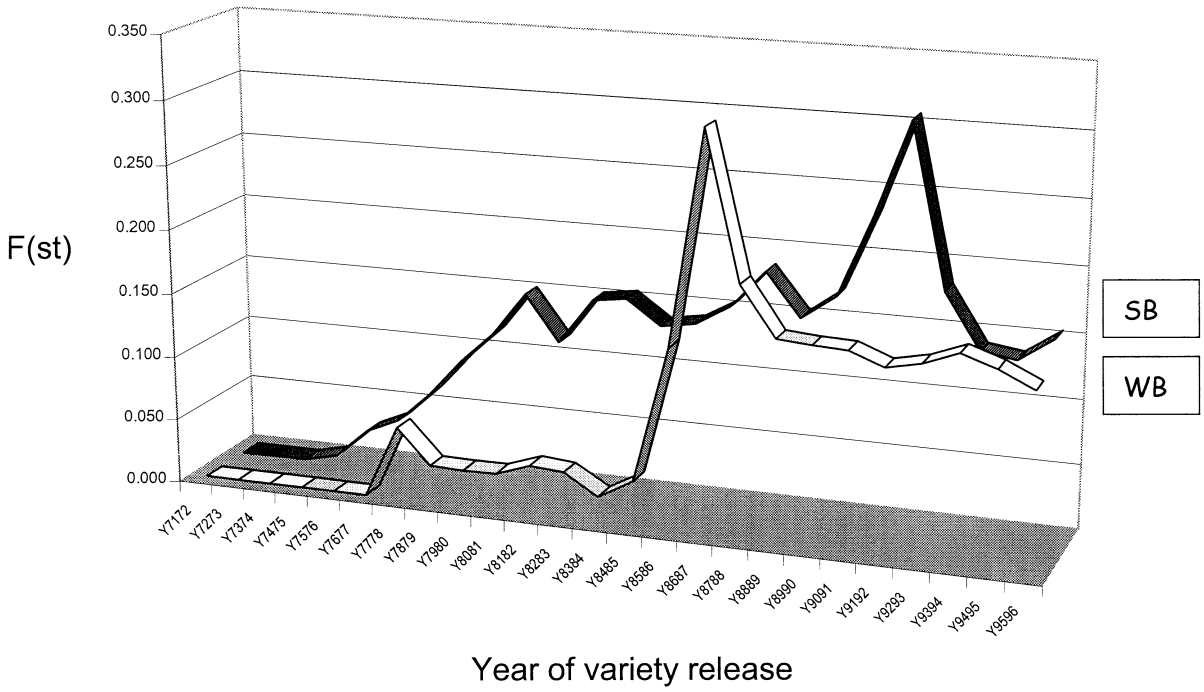

Fig. 4 Moving mean annual F(st) 1970–1996, compared to 1970, based on AFLP for winter (*light ribbon*) and spring (*dark ribbon*) barley varieties on the NIAB Recommended List

performing varieties on the Recommended List). Detailed movements in the F(st) over the period were analysed by combining contiguous pairs of years (1970+1971, 1971+1972 etc.), an approach which was designed to smooth out sudden changes caused by the introduction or the removal of a number of varieties in any particular year. When this metric was calculated on the basis of either AFLP or SSR data, the resulting plots were both highly correlated between these data sets in spring (0.92) and winter (0.92) entries. Similarly high correlations were found between the SSR- and morphological-based data sets (0.82, 0.83), and between AFLP and morphology in the winter set (0.72); the latter correlation for the spring set was only moderate (0.42), a probable reflection of the weaker level of discrimination that was achievable using the morphological traits in the spring barley varietal set. Representative plots are shown in Fig. 4. The molecular marker-based plots showed a consistent pattern, in which F(st) was maintained at a rather constant level, either side of a peak for winter barley in the mid 1980s and similarly for the spring barleys in the early 1990s, although this latter peak was not so evident in the SSR-based plot (data not shown). The morphology based plots behaved rather differently (data not shown), with the winter materials oscillating unstably, while the spring materials showed a single peak in the early 1980s with a monotonic falling-away on either side of this period.

Discussion

Temporal trends in diversity 1930–1990

The major goal of our study was to test the assertion that the genetic diversity of the UK barley crop has narrowed as a result of 60 years of scientifically based pedigree selection. All the indicators that we have derived show that, just as was the case for UK winter wheat (Donini et al. 2000), there is little objective evidence for this having occurred. Thus the diversity encompassed by the later materials is no less than that represented by the earlier ones. Ellis et al. (1997) analysed AFLP profiles of a sample of spring barleys largely congruent with (although smaller) than ours, and interpreted their data as demonstrating a significant narrowing of the genetic base in recent years. However the basis of this suggestion has not been elaborated, and we have failed to observe any such trends in our analyses, which extend the data set beyond AFLPs to SSRs and morphology. The recent study of spring-barley diversity reported by Russell et al. (2000) convincingly demonstrated greater allelic diversity among foundation genotypes (mainly early 20th century landrace selections) compared to modern (post-1985) varieties. A small number of the 22 SSR loci described in this study were shown to have evidently lost a substantial number of alleles (one losing 8 out of 13 alleles, one 5 out of 8, one 4 out of 7), with the result that the modern varieties were much more homogeneous; this pattern of abrupt loss of diversity is compatible with erosion via purifying selection, whereby deleterious alleles (at agronomically linked loci) are eliminated. In contrast, the remaining 19 SSR loci showed a net loss of 22 alleles from 87 (on average, about one allele per locus over a period in excess of half a century); such a level is

Table 5 Correlation between F(st) and number of breeding programmes represented in the NIAB Recommended List

Period	A_{sb}[a]	B_{sb}[b]	$F(st)_{sb}$	A_{wb}[a]	B_{wb}[b]	$F(st)_{wb}$[c]
80–85	14	9	0.143			
81–86	12	8	0.141			
82–87	12	8	0.149	7	5	0.108
83–88	12	7	0.156	7	7	0.139
84–89	12	8	0.156	11	10	0.166
85–90	12	8	0.163	13	9	0.189
86–91	12	7	0.181	13	8	0.192
87–92	11	7	0.211	13	6	0.159
88–93	14	10	0.208	14	7	0.150
89–94	17	11	0.201	14	6	0.150
90–95	15	10	0.185			

[a] $A_{sb,\ wb}$: number of spring (sb) and winter (wb) entries (provisional+general+outclassed) appearing on the NIAB Recommended List for the first time in the period
[b] $B_{sb,\ wb}$: number of independent spring (sb) and winter (wb) breeding programmes, represented by cvs. listed in A
[c] $F(st)_{sb,\ wb}$: mean F(st) in the period for spring (sb) and winter (wb) entries

hardly sufficient to support a hypothesis that there has been a sustained and continuing loss of genetic diversity over time. Moreover, the authors also highlighted a significant number of alleles unique to modern varieties, presumably arising from a combination of introgression and *de novo* generation. Thus the situation is far from simple. Although there is a loss of alleles at some SSR loci, there is a gain at others. We have also noted such a dynamic situation in more detailed analysis of our SSR data (Donini et al. 2001). Furthermore, we would argue that the higher level of genomic sampling in our study is likely to generate a more reliable picture of genetic flux over time.

In our present work, we have noted some evidence of movement over time of the centroids of the spring barley PCO convex hulls (Fig. 2), which represent the diversity present in the various time periods we have sampled, a phenomenon which was not noted in winter wheat, and which can only be suggested for the winter barley, since the latter's representation is low in years before 1980. Such a movement would imply a qualitative, rather than a quantitative, shift in diversity. A major qualitative shift can be rationalised in terms of the expansion over the time period of winter barleys, and the concomitant contraction of spring barleys. Thus, as the change-over to winter barleys occurred, it was clearly necessary to introduce novel germplasm into breeding programmes; similarly the spring barley programmes also had to adapt to the move away from a general purpose to a more-specialised crop (specifically for malting end use), once again stimulating the introgression of new genetic variation.

'Functional' versus 'silent' genetic diversity

An important question surrounding the use of molecular markers to determine genetic diversity concerns the relationship between DNA sequence variation and allelic variation at genes. Neither AFLPs nor SSRs discriminate between an expressed and a non-expressed sequence; indeed, despite the use of methylation-sensitive restriction enzymes for AFLP profiling in order to bias the sampling of genomic DNA against the large repetitive component, genetic analyses have shown that many, if not most, AFLP bands do not represent a single-copy sequence (Huang et al. 2000), while the frequency of SSRs within EST sequences is demonstrably lower than in random genomic sequence (Cardle et al. 2000). Thus, it is likely that the qualitative difference we have observed between the patterns drawn from marker- versus the morphology-based data sets probably reflects the fact that the former are effectively invisible and therefore unselectable by the breeders, whereas the latter can be deliberately targeted for selection, and thus are subject to prevailing ideas surrounding the optimal plant ideotype. In this sense, the molecular marker-based F(st)s are more likely to generate an unbiased picture of diversity trends than the morphology based ones. In this context it is significant that there was a high correlation between F(st) values calculated on the basis of AFLPs and those on the basis of SSRs. This underlines our earlier conclusion (Donini et al. 2000) concerning the appropriateness of AFLP as a measure of genetic diversity, which has been criticised on the grounds that AFLP markers may not be randomly distributed across the genome. Interestingly, Ellis et al. (1997) have shown that a sample of the AFLP bands used for their analysis are dispersed across six out of the seven barley chromosomes.

Trends in diversity 1970–1996

We have attempted to visualise short-term changes in diversity by analogy to a moving-mean analysis (Fig. 4). In principle the 'bulking' of years can be extended from a 2 year, as used here (i.e. 1970+1971; 1971+1972 etc.) to a 3 year (1970+1971+1972; 1971+1972+1973 etc.) or higher model, with the effect of an increased flattening of the profile as the number of years included in each sample increases. Significantly, the trends in F(st) are largely interpretable by a simple correlation between the

mean F(st) and the number of distinct breeding programmes represented by these entries; in contrast, there is no evidence of any correlation between mean F(st) and the number of actual new entries (Table 5). A similar correlation has been observed when patterns of SSR alleles are followed over time (Donini et al. 2001).

The similarity of the temporal trends in genetic diversity between barley and wheat is suggestive of a generalised pattern of genetic flux in response to breeding. It is unarguably the case that the shift from wild populations to cultivated landraces is associated with a major reduction in the genetic variability present in the 'crop'. It is also axiomatic that for specific simply inherited traits of clear value for crop productivity, breeding programmes would be failing if they were unable fix the optimal allele(s) at the relevant genes. However, few production traits fall into this category, leaving open the question as to whether systematic and intensive breeding leads to a genome-wide loss of genetic diversity. Current hypotheses suggesting that such a narrowing is the inevitable result of modern plant breeding rest on the assumption that the process excludes genetically variable, but less productive, primitive ancestors (Tanksley and McCouch 1997). Our analyses do not support this contention, at least in the case of the development of wheat and barley, two of the most intensely selected crops, over a prolonged period of breeding. The overall conclusion is that diverse breeding programmes have generally been successful in maintaining sufficient genetic diversity to allow the steady rise in genetic potential that has been a feature of 20th century crop breeding. It follows that concentration of breeding effort into fewer independent programmes is more likely to be prejudicial to the maintenance of the genetic diversity of a crop.

Given that Plant Breeders' Rights, as determined under the UPOV system, are designed to encourage plant breeding by reward and intellectual property protection through the establishment of differences between varieties, their effect on genetic diversity could be expected, on the basis of the results above, to be beneficial and not, as has been asserted, the other way around. In addition, the associated processes (National and Recommended List testing) involved in delivering to the farmer the output of plant breeding, i.e. new varieties, appear from our analyses to have had no detrimental effect on the availability of genetic diversity to UK agriculture.

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