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ORIGINAL PAPER

Indicators to assess temporal genetic diversity in the French Catalogue: no losses for maize and peas

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Abstract The aim of this study, led by the GEVES (Research and Control Group for Varieties and Seeds), was to suggest indicators to assess the diversity available to farmers since the French Official Catalogue for Plant Varieties and Species was initiated. The largest datasets of 1990 inbred maize lines and 578 pea lines from the last 50 years were analysed using morphological and enzymatic parameters. Lines were grouped into three to five periods. Genetic diversity was estimated in each period from morphological and enzymatic markers by computing numerous indices, such as the number of classes of scores for each characteristic, allelic richness or genetic diversity index (H_e). Population differentiation parameters (G_{ST}, G_{ST}', F_{ST}, Q_{ST}) were also estimated between periods. While genetic diversity computed from distinction, uniformity, stability traits was more marked for maize (0.66) than for garden peas (0.35) or feed peas (0.29), the opposite trend was observed with enzymes, resulting in a genetic diversity of 0.43, 0.35 and 0.22 for garden peas, feed peas and maize, respectively. However, no significant changes in genetic diversity were observed over time, and genetic differentiation was slight between periods. All our results demonstrated that no significant reduction in the diversity available to farmers had been observed since initiation of the French Catalogue. The H_e was a good indicator providing a quantitative estimate of genetic diversity, but it should be interpreted alongside a more precise indicator such as allelic richness or the number of classes for morphological characteristics.

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Introduction

Before it can be placed on the market in France or Europe, a variety must be registered in the French Official Catalogue for Plant Varieties and Species. Candidate varieties are submitted to DUS (distinctness, uniformity and stability) and VCU (value for cultivation and use) tests for agricultural crops. This procedure, based on recording a number of phenotypic characteristics, has often been accused of reducing the diversity available to farmers. As pointed out by Srinivasan et al. (2003), because of strong commercial pressures, breeders are forced to develop new varieties from a narrow range of tried and tested 'elite' germplasms. However, although a loss of diversity due to the substitution of landraces by elite cultivars is generally admitted and has already been demonstrated by Roussel et al. (2004) or Dubreuil and Charcosset

(1999), it is still necessary to prove that plant breeding leads inevitably to the loss of genetic diversity in newly registered varieties. Previous studies had already assessed temporal changes to the genetic diversity of crops such as wheat (Khlestkina et al. 2004; Maccaferri et al. 2003; Srinivasan et al. 2003; Manifesto et al. 2001; Donini et al. 2000), barley (Koebner et al. 2003) or maize (Lu and Bernardo 2001), to name but a few. If only registered varieties are considered, the general conclusion has been that breeding mainly leads to qualitative rather than quantitative shifts. A few studies have been undertaken in France to determine whether a loss of diversity has occurred in agricultural crops since the French Catalogue was initiated (bread wheat, Roussel et al. 2004; maize, Le Clerc et al. 2005; peas, Baranger et al. 2004).

The objective of the present study was thus to suggest indicators to assess the diversity available to farmers since the French Official Catalogue for Plant Varieties and Species was first set up. The putative loss or gain of diversity was also examined over the same period. The choice of these indicators is discussed relative to their pertinence, their applicability to diversity analysis and in the light of the datasets currently available. For this study, we chose maize and peas as examples of an agricultural species and a vegetable species, respectively, because of the large quantity of data available over a long period and the large number of maize varieties registered. For instance, in 2004, 1,117 cultivars of maize (including 147 new cultivars) were registered in the National List, i.e. 25% of new varieties registered, including not only agricultural species but also vegetable and fruit trees species.

Before discussing potential applications to other species of the indicators selected, we analysed the genetic diversity of morphological and enzymatic characteristics collected with respect to 1,990 maize inbred lines and 578 pea inbred lines from the last 50 years, with regard to their breeding history. This is probably the first diversity study to have considered such a large number of lines. Indeed, in each species, the largest available dataset was used so that it would be representative of all genetic diversity available since the French Catalogue was set up.

Materials and methods

Plant material

Morphological and enzymatic data were available from the GEVES (Research and Control Group for Varieties and Seeds) ''Thalie'' database. Of the 5,832 maize inbred lines described morphologically in this database between 1957 and 2004, 1,990 lines leading to registered hybrids were retained for further analyses. The other 3,842 were mainly lines whose hybrids had not been retained for registration (34%), protected lines (19%) or lines in test (12%). Isozyme data collected on 1,921 inbred lines were also recovered (i.e. 97% of the morphologically described inbred lines). For peas, garden peas and feed peas were analysed separately. 384 garden peas registered between 1952 (date when the catalogue opened) and 2003 were analysed using morphological data. A classification for feed peas in the catalogue only started in 1976, so 193 varieties were analysed over the period 1976 and 2003. Isozyme and storage protein data from 163 and 142 varieties of garden and feed peas, respectively, were also included in the analysis (i.e. 53% of the morphologically described inbred lines). It should be noted that commercial varieties of peas were analysed but descriptions for maize hybrids were not always available so that parental inbred lines of the registered hybrids were analysed.

With respect to maize, lines were organised into decades according to the date of registration of the first hybrid using the line and the date of deletion of the last hybrid using the line. Thus lines whose hybrids were registered during 1 decade and not deleted during the next decade were included in the accounts of both decades. The same procedure was adopted for commercial pea lines, taking account for each decade of all cultivars present in the French Catalogue. In this way, we were able to analyse all the diversity available (diversity index and allelic richness) during a decade in the French Catalogue (Table 1). In order to analyse the diversity created during each decade and hence the putative gain, genetic differentiation parameters were calculated, taking account for each decade only of those lines newly employed in a commercial hybrid for maize, and of newly registered cultivars for peas. With respect to peas, decades were divided as a function of the date of opening of the French catalogue. However, because so few maize hybrids were registered during the period 1957 to 1967, it was decided to create an extended period running from 1957 to 1983 (instead of a decade). Notwithstanding this adjustment, reference shall always be made in this article to decades.

Morphological and enzymatic data

For maize, 34 characteristics (leaf, stem, tassel, ear and grain), were retained, based on CPVO-TP/2/2 and national characteristics (see table in SEM). For peas, 61 characteristics (flower, seed, leaflet, node, disease

Table 1 Number of lines analysed using morphological traits per decade for each species

Species	Type		Decade 1	Decade 2	Decade 3	Decade 4	Decade 5
Maize[a]		Period of the study	1957–1983	1984–1993	1994–2004		
		No. of lines	178	767	1,847		
Pea[b]		Period of the study	1952–1962	1963–1972	1973–1982	1983–1992	1993–2003
	Garden pea	No. of lines	91	129	150	221	197
	Feed pea	No. of lines	0	0	10	83	181

[a]Parents of hybrids present in the French Catalogue during this period

[b]Commercial cultivars present in the French Catalogue during this period

resistances, varietal type, etc.), were retained, based on CPVO-TP/7/1 guidelines. The list of traits and expression levels for both species are accessible at http://www.cpvo.eu.int/documents/TP/. With respect to quantitative traits (10 out of 34 for maize and 9 out of 61 for peas), phenotypic data were determined by measurements or counting; for qualitative traits, they were determined by visual observation. Quantitative traits were transformed into ranked classes to enable comparisons between all lines characterised throughout the evaluation period. Morphological data resulted from two cycles of study with two to four repetitions/trials. UPOV reference lines were systematically included in each trial to ensure the accurate ranking of varieties or lines. For maize, quantitative information was classified on the basis of reference lines included in the test. The centre of each class was calculated by averaging all reference lines (up to 100 reference lines/class). Consequently, all data were recorded according to qualitative scales and thus smoothed to take account of environmental factors such as years or sites. In addition, it should be noted that scores had been allocated in each species by the same person for the last 30 years, thus ensuring a high level of consistency.

Enzyme variations, previously investigated by Bio-GEVES, were observed on 17 polymorphic maize loci, including four malate dehydrogenase loci (Mdh1, 2, 3, and 5), two isocitrate dehydrogenase loci (Idh1, and 2), two 6-phosphogluconate dehydrogenase loci (Pgd1, and 2), two phosphoglucomutase loci (Pgm1, and 2), one phosphoglucose isomerase locus (Pgi1), one acid phosphatase locus (Acp1), one diaphorase locus (Dia1), one alcohol dehydrogenase locus (Adh1), two glutamate–oxaloacetate transaminase loci (Got1, and 2) and one catalase locus (Cat3). All these loci were distributed on nine out of the ten maize chromosomes.

Eight enzyme loci were recovered for pea, including Pgm1, Pgm2, Got, Pgd1, Pgd2, Idh, α-amylase locus (Amy) and shikimate dehydrogenase locus (Shdh). Banding patterns of storage proteins, scored as profiles in four zones of acrylamide gels (A, B, C and D) as described by Bourgoin-Grenèche and Lallemand (1993), were also retrieved and added to the data on enzymes, considering that each zone presented different loci.

Morphological analysis

Genetic diversity in the two species was estimated for each decade by computing different parameters. For each morphological characteristic and by decade, a diversity index was calculated according to Nei's unbiased genetic diversity system (Nei 1978), as follows:

$$H_e^c = \frac{{}^2n_c}{{}^2n_c - 1}\left(1 - \sum_{a=c}^{a=A_c}(P_{ac})^2\right)$$

Where P_{ac} is the frequency of inbred lines with score a for trait c in 1 decade, A_c is the number of classes scored for this trait, and n_c, the number of inbred lines analysed for this characteristic.

Mean genetic diversity was estimated for each decade by averaging H_e^c for the decade and for all characteristics as $H_e = (1/C)\cdot\sum_{c=1}^{C} H_e^c$, with C being the total number of characteristics (table in SEM).

As explained by Hennink and Zeven (1991), the Nei variation index reaches a maximum when inbred lines are uniformly distributed over classes for one characteristic. The number of classes for each trait was also examined for each decade.

Diversity was also compared between decades by calculating population differentiation parameters, as performed previously by Dubreuil and Charcosset (1998) on enzyme and DNA markers. For each combination of 2 decades, the total genetic diversity (H_T) was partitioned into within-decade diversity (H_S) and between-decade diversity (D_{ST}). The coefficient of genetic differentiation was also evaluated using $G_{ST} = (D_{ST}/H_T)$ (Nei 1973). These population differentiation parameters were computed for each characteristic and for all characteristics taken together. When estimating G_{ST}, only new lines were taken into account

in each decade, so that differentiations between decades would be highlighted.

Statistical tests were performed using version five of Statgraphics plus software in order to determine the significance of our results with 95% confidence intervals. For each trait, statistical analysis was performed on scores highlighting significant qualitative shifts between decades ($P = 0.05$). The Kruskal–Wallis nonparametric test was performed on the mean genetic diversity index H_e and on allelic richness in order to assess quantitative shifts in diversity between decades. As shown by Roussel et al. (2004) with respect to allelic richness, a sign test was performed to detect significant differences between decades in the number of classes.

For maize, another differentiation parameter was used for quantitative and qualitative ordinal characteristics (termed Q_{ST} by Spitze 1993). For each characteristic, this parameter is analogous to that of a single locus, G_{ST}, such as:

$$Q_{ST} = \frac{\sigma^2_{GB}}{2\sigma^2_{GW} + \sigma^2_{GB}}$$

with σ^2_{GB} being the variance between decades and σ^2_{GW}, the variance within a decade.

Calculations were made from ANOVA analysis under version five of Statgraphics plus software for each combination of 2 decades. Of the 34 maize characteristics, 21 were deemed to offer a normal or pseudo-normal distribution (Table 4).

So as to estimate graphically the genetic diversity maintained during each decade, relationships between lines were depicted using Uniwin software version Plus 5.11, through principal component analysis (PCA). Only quantitative and qualitative ordinal traits presenting nine classes were used for this analysis, i.e. 21 traits out of 31. Tolerance ellipses were drawn, containing 95% of the lines per decade. PCA results are presented for maize and garden peas.

Enzymatic analysis

Allele frequencies were deemed to verify the appearance or disappearance of certain alleles between decades. Nei's unbiased genetic diversity index (Nei 1987), the number of alleles and allelic richness were estimated for each locus and each decade using FSTAT software version 2.9.3.2 (Goudet 2001). Allelic richness is a measure of the number of alleles independent of sample size, which enables this quantity to be compared in different sample sizes. Nei population differentiation parameters (1987) were estimated for each locus and each decade. In this case, H_T', H_S', D_{ST}' and G_{ST}' were computed; G_{ST}' being an equivalent estimator of Gst but taking account of the number of compared samples (in the present case, the number of decades compared). The Weir and Cockerham (1984) F_{ST} estimator was also calculated and compared with G_{ST}'. Unlike the G_{ST}', Weir and Cockerham's F_{ST} weights allele frequencies according to the number of lines per decade.

Significant differences between decades were tested with 95% confidence intervals for Nei's index and allelic richness under Statgraphics software, using a Kruskal–Wallis test. For differentiation parameters, pairwise tests between decades were performed using FSTAT with a level of significance at 5%.

Roger's genetic distances between inbred lines were calculated with LCDMV software developed by the GEVES using SAS tools (Dubreuil et al. 2003). Because of the large size of the resulting files, the mean genetic distance between all inbred lines during each decade was calculated under Microsoft Access software version 2002.

Only new lines were taken into account for each decade when estimating G_{ST}, G_{ST}' and F_{ST} parameters.

Results

Morphological diversity in maize

In order to avoid redundant information when estimating diversity, correlations between characteristics were examined using PCA analysis. A strong correlation (0.71) was detected between anthocyanin coloration of the sheath and anthocyanin coloration of internodes, and the latter trait was deleted when calculating the diversity index. Diversity indices ranged from 0.023 for the ''shape of ear'' characteristic to 0.838 for the ''length of main axis above lowest side branch'' tassel characteristic (Fig. 1). On the whole, these indices were high and few changes were observed from one decade to another. Although the H_e index declined slightly from decade 1 to decade 3, the difference was not significant (Table 2). On the contrary, differences in scores were significant with respect to some specific traits. Diagrams are only presented for the 'time of anthesis' and 'type of grain' characteristics (Fig. 2).

The appearance of a class within the scoring the scale for a characteristic also reflected new diversity. Examination, characteristic by characteristic, revealed a constant or increased number of classes between decades 1 and 3, except for the number of tassel

branches. In fact, the mean number of classes rose slightly between decades 1 and 3, ranging respectively from 6.69 to 7.54 (Table 2), although only the difference between decades 1 and 2 was significant. It is important to note that the appearance of a class was sometimes only due to the appearance of one new inbred line.

When looking at a differentiation between 2 decades (Table 3), G_{ST} values were very low and no significant differences were observed. The total differentiation between the 3 decades represented only 1%, and most of the total diversity (H_T) was due to diversity within decades (H_S). Mean G_{ST} values were slightly lower than Q_{ST} values, whichever decades were compared (Table 4). The highest Q_{ST} values were mainly observed for traits subjected to breeding such as plant length, ear diameter, length of ear, etc., and they were higher than G_{ST} values. For example, when comparing decades 1 and 3, the Q_{ST} of plant length was 0.014, against 0.004 for G_{ST}.

The first three PCA components explained about 36% of total variation, with 18.1% for the first, 9.9% for the second and 8.3% for the third component, respectively. Whereas the first axis was mainly explained by characteristics of earliness and yield such as time of anthesis, length of ear and length of plant (and more surprisingly by the anthocyanin coloration of internodes), the second axis was mainly explained by the anthocyanin coloration of glumes, brace roots, anthers and silk colour. Tolerance ellipses for each decade were almost of the same size but a slight shift between decades was observed on the first axis (Fig. 3).

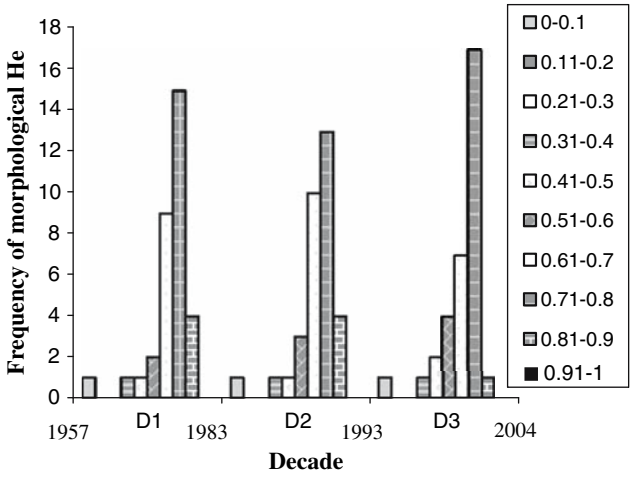

Fig. 1 Distribution of the mean morphological diversity index in maize, for each decade

Enzymatic diversity in maize

A total of 44 alleles were found among the 17 loci. Four alleles appeared during decades 2 and 3 at the pgd1, pgd2, pgm1 and cat3 loci but were only found in one to four inbred lines. Only one rare allele disappeared during decade 3, at the pgi1 locus. Apart from these first five alleles, five, six and seven rare alleles were counted in decade 1, decade 2 and decade 3, respectively. Their frequencies declined between decades 1 and 3. While the average number of alleles ranged from 2.35 to 2.53, allelic richness decreased slightly from 2.39 to 2.26, although there was no significant difference (Table 5). In the same way, the mean genetic diversity index slightly decreased between decades 1 and 3, but again, this difference was not statistically significant. The mean genetic distance between lines in a decade slightly decreased between decades 1 and 3, while the greatest distance between two lines (0.660) was observed in the second decade. During the same period, the proportion of lines not differentiated by enzymes inevitably increased from 9.3 to 14.6% throughout the decades, because of their growing number (Table 6).

Genetic differentiation between all decades, estimated using the F_{ST} or G_{ST}' value, was very slight ($G_{ST}' = 1.1\%$) and comparable to that observed with morphological data. Though it was slight, it was significant between 2 decades, the strongest differentiation being seen between periods 1 and 3 (Table 3).

Table 2 Indicators estimated on 33 and 57 morphological traits in maize and peas, respectively

Species	Type	Indicator	1957–1983	1984–1993	1994–2004
Maize		$H_e \pm$ SD	0.67 ± 0.16	0.66 ± 0.16	0.65 ± 0.17
		Mean number of classes	6.69	7.37	7.54

		Indicator	1952–1962	1963–1972	1973–1982	1983–1992	1993–2003
Pea	Garden pea	$H_e \pm$ SD	0.36 ± 0.28	0.37 ± 0.28	0.34 ± 0.30	0.35 ± 0.31	0.37 ± 0.31
		Mean number of classes	3.18	3.51	3.32	3.56	3.67
	Feed pea	$H_e \pm$ SD			0.27 ± 0.30	0.30 ± 0.29	0.30 ± 0.29
		Mean number of classes			1.96	2.93	3.02

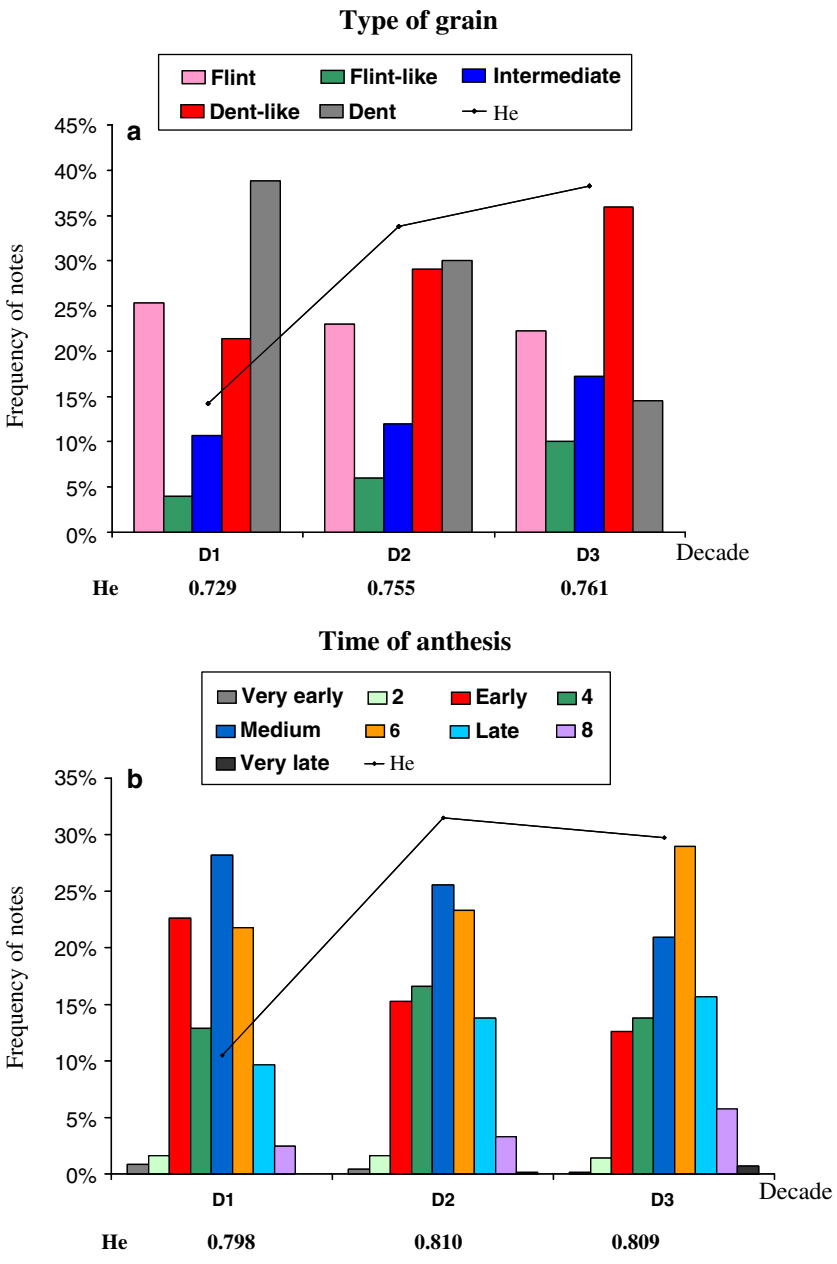

Fig. 2 Distribution of scores and diversity indices (H_e) for the 'type of grain' trait (**a**) and 'time of anthesis' characteristic (**b**), by decade

Table 3 Differentiation parameters estimated on 33 morphological and 17 enzymatic traits of maize

Data	Decades	Total H_T'	Intra H_S'	Inter D_{ST}'	G_{ST}'	$F_{ST(W\&C)}$
Morphological characters	1–2	0.6661	0.657	0.0004	0.005	
	1–3	0.659	0.655	0.004	0.006	
	2–3	0.655	0.650	0.005	0.007	
	All the decades	0.660	0.653	0.007	0.010	
Enzymes	1–2	0.230	0.227	0.003	0.013	0.013*
	1–3	0.229	0.222	0.007	0.030	0.032*
	2–3	0.212	0.210	0.002	0.007	0.008*
	All the decades	0.224	0.220	0.004	0.017	0.013

*Significant difference between decades ($P < 0.05$)

Table 4 Comparative estimates of Q_{ST} and G_{ST} values for 21 morphological traits in maize

National code	Character	G_{ST} 1-2-3	Q_{ST} 1-2-3	G_{ST} 1-2	Q_{ST} 1-2	G_{ST} 2-3	Q_{ST} 2-3	G_{ST} 1-3	Q_{ST} 1-3
C947	First leaf: anthocyanin coloration of sheath	0.001	0.000	0.001	0.000	0.001	0.000	0.001	0.000
C975	Tassel: length of main axis above lowest side branch	*0.001*	*0.002*	*0.001*	*0.002*	*0.001*	*0.002*	0.000	0.000
C814	**Ear: length of peduncle**	*0.001*	*0.005*	*0.001*	*0.003*	*0.004*	*0.005*	0.000	0.000
C933	Stem: anthocyanin coloration of secondary roots	0.003	0.000	0.003	0.000	0.002	0.000	0.004	0.000
C831	Tassel: density of spikelets	0.004	0.000	0.004	0.000	0.000	0.000	0.002	0.000
C905	flo_femelle	0.001	0.001	0.001	0.001	0.000	0.000	0.001	0.001
C921	Ear: number of rows	*0.001*	*0.005*	0.001	0.000	*0.003*	*0.004*	0.000	0.001
C928	Leaf: width of blade	*0.001*	*0.002*	0.001	0.001	0.000	0.001	0.001	0.001
C843	Ear: diameter of cob	*0.000*	*0.001*	*0.000*	*0.002*	0.000	0.000	*0.001*	*0.002*
C974	Tassel: angle between main axis and lateral branches	*0.002*	*0.007*	0.002	0.000	*0.003*	*0.007*	*0.001*	*0.003*
C920	Ear: length of husks	*0.001*	*0.003*	*0.001*	*0.005*	0.000	0.000	*0.001*	*0.004*
C899	**Tassel: time of male flowering**	*0.001*	*0.006*	*0.001*	*0.002*	*0.002*	*0.004*	*0.002*	*0.004*
C810	Tassel: anthocyanin coloration of anthers	0.008	0.006	0.008	0.002	*0.002*	*0.004*	0.008	0.005
C937	**Tassel: number of lateral branches**	*0.000*	*0.011*	0.000	0.000	*0.006*	*0.010*	*0.005*	*0.006*
C838	**Ear: length**	*0.001*	*0.008*	*0.001*	*0.004*	*0.001*	*0.004*	*0.001*	*0.006*
C869	Leaf: attitude of leaf	*0.003*	*0.008*	*0.003*	*0.005*	*0.002*	*0.004*	*0.003*	*0.006*
C976	Tassel: length of main axis above highest side branch	*0.003*	*0.010*	*0.003*	*0.005*	*0.001*	*0.006*	*0.002*	*0.008*
C866	Leaf: angle between blade and stem	*0.002*	*0.041*	0.002	0.000	0.038	0.038	*0.005*	*0.010*
C839	**Ear: diameter of ear**	*0.001*	*0.014*	*0.001*	*0.006*	*0.003*	*0.009*	*0.002*	*0.010*
C978	**Plant: ratio height of insertion of upper ear to plant length**	*0.004*	*0.011*	*0.004*	*0.008*	*0.002*	*0.005*	*0.004*	*0.010*
C914	**Plant: length**	*0.004*	*0.017*	*0.004*	*0.010*	*0.002*	*0.010*	*0.004*	*0.014*
	Mean	0.002	0.008	0.002	0.003	0.003	0.005	0.002	0.004

Bold text refers consciously selected characteristics, italic values refer $Q_{ST} > G_{ST}$

Morphological and enzymatic diversity in peas

Numerous traits were strongly correlated: in all the peas, ''time of flowering'' was correlated with ''number of nodes'', and ''weight of pod'' with ''weight of 1,000 seeds''; in garden peas, ''number of flowers'' with ''number of pods per stage''; and for feed peas, numerous leaflet characteristics such as size, width and number. Fifty-seven out of the 61 characteristics were thus used to estimate the phenotypic diversity of garden peas and feed peas. The mean diversity index was slightly higher in garden peas (0.35) than in feed peas

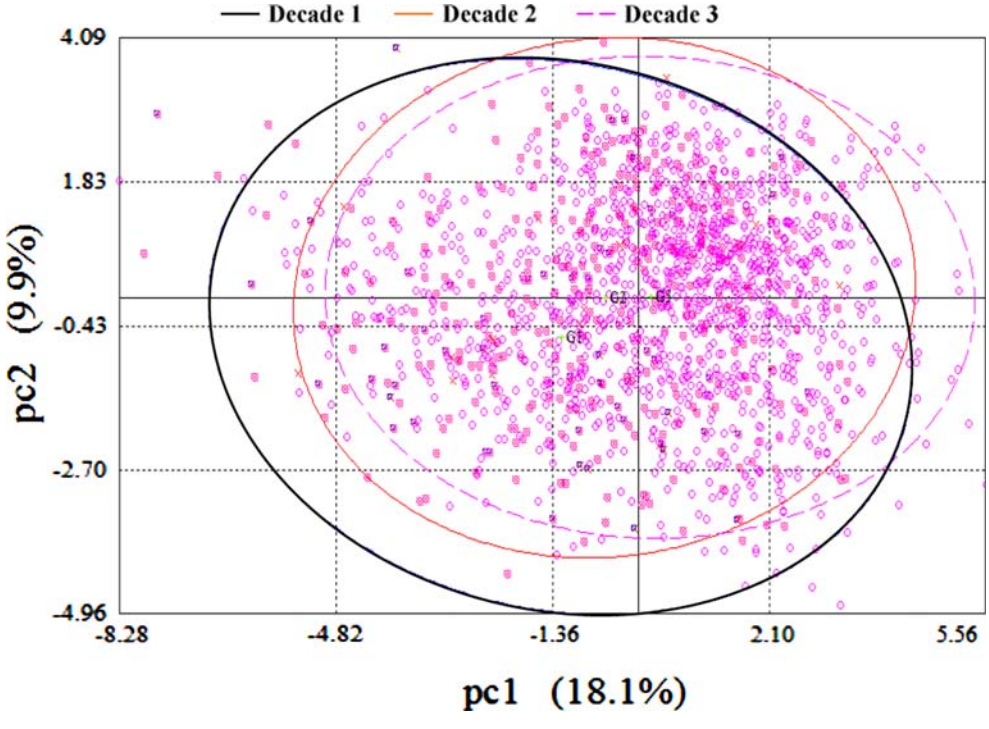

Fig. 3 Associations among inbred maize lines during 3 decades as revealed by principal coordinate analysis. Ellipses of tolerance represent 95% of lines in each decade

Table 5 Genetic diversity, number of alleles and allelic richness per locus and per decade for maize, estimated on 17 loci

Locus	Gene diversity per locus*			No. of alleles			Allelic richness per locus*		
	Dec 1	Dec 2	Dec 3	Dec 1	Dec 2	Dec 3	Dec 1	Dec 2	Dec 3
mdh1	0.10	0.18	0.18	2	2	2	2.00	2.00	2.00
mdh2	0.52	0.51	0.50	3	3	3	3.00	2.87	2.52
mdh3	0.10	0.05	0.08	2	2	2	2.00	2.00	2.00
mdh5	0.14	0.12	0.08	2	2	2	2.00	2.00	2.00
idh1	0.13	.010	0.11	2	2	2	2.00	2.00	2.00
idh2	0.49	0.46	0.42	2	2	2	2.00	2.00	2.00
pgd1	0.34	0.28	0.30	2	3	3	2.00	2.62	2.30
pgd2	0.19	0.20	0.13	2	3	3	2.00	2.62	2.17
pgm1	0.00	0.00	0.00	1	1	2	1.00	1.00	1.16
pgm2	0.44	0.36	0.28	4	4	4	4.00	4.00	4.00
pgi1	0.27	0.21	0.16	3	3	2	3.00	2.38	2.00
acp1	0.62	0.61	0.58	4	4	4	4.00	3.91	3.60
dia1	0.26	0.20	0.14	2	2	2	2.00	2.00	2.00
adh1	0.14	0.20	0.22	2	2	2	2.00	2.00	2.00
got1	0.07	0.03	0.01	2	2	2	2.00	1.99	1.84
got2	0.04	0.08	0.22	2	2	2	2.00	2.00	2.00
cat3	0.24	0.18	0.17	3	4	4	3.00	3.29	2.76
Mean	0.24	0.22	0.21	2.35	2.53	2.53	2.35	2.39	2.26

*No significant difference between decades ($P < 0.05$)

Table 6 Mean genetic distances within each decade, minimum and maximum genetic distances between two lines in a decade and percentage of non-distinguishable maize lines

Decade	Mean genetic distance	Distance		Percentage of non-distinguishable lines
		Min	Max	
1	0.238	0	0.588	9.3
2	0.218	0	0.660	12.8
3	0.209	0	0.647	14.6

(0.29), but no significant differences were observed between decades for the two types (Table 7). Eight characteristics were monomorphic for garden peas and 14 for feed peas, which could be explained by the fact that the same list of traits was used for both types of peas. Differentiation between all decades was also more marked for garden peas (8.5%) than for feed peas (5.7%). In garden peas, the smallest genetic differentiation was observed between decades 1 and 2 (4.5%), whereas the greatest differentiation was observed between decades 1 and 5 (10.7%, data not shown). In garden peas, the first two PCA components explained 39.1% of the total variation, 25.8% regarding the first component (Fig. 4). Whereas the first axis was explained mainly by seed size characteristics such as the weight of 1,000 seeds or the width and length of pods, the second axis was explained by the time of flowering and the number of nodes. As for maize, tolerance ellipses for each decade were almost the same size and were shifted to the left on the first axis, towards smaller seeds.

Table 7 Summary of the statistics calculated for pea and maize

Data	Indicator	Garden pea	Feed pea	Maize
Morphological characters	Diversity index	0.35	0.29	0.66
	G_{ST} (%)	8.5	5.7	0.5
Enzymes	Diversity index	0.43	0.35	0.22
	Allelic richness (min–max)	2.8–3.1	2.8–2.9	2.2–2.3
	G_{ST}'/F_{ST} (%)	1.3/1.6	0.4/0.4	1.3/1.7

As regards enzymatic diversity in garden peas, three alleles appeared during decade 3, two in decade 4 and one in decade 5, but no alleles were lost (Table 8). In terms of morphological traits, allelic richness and the genetic diversity index were also higher in garden peas than in feed peas. In both types of pea, no significant differences between decades were observed with respect to allelic richness and the genetic diversity index. Genetic differentiation between all decades was similar in both garden peas and maize (1.3%), whereas a very slight differentiation of 0.4% was observed for feed peas (Table 7). The most marked differentiation for garden peas was logically observed between the first and the last decades (5.2%), with a significant difference in decades 4 and 5 compared to decades 1 and 2.

Discussion

During the past 50 years, intensive plant breeding has led to undeniable genetic advances based on using the

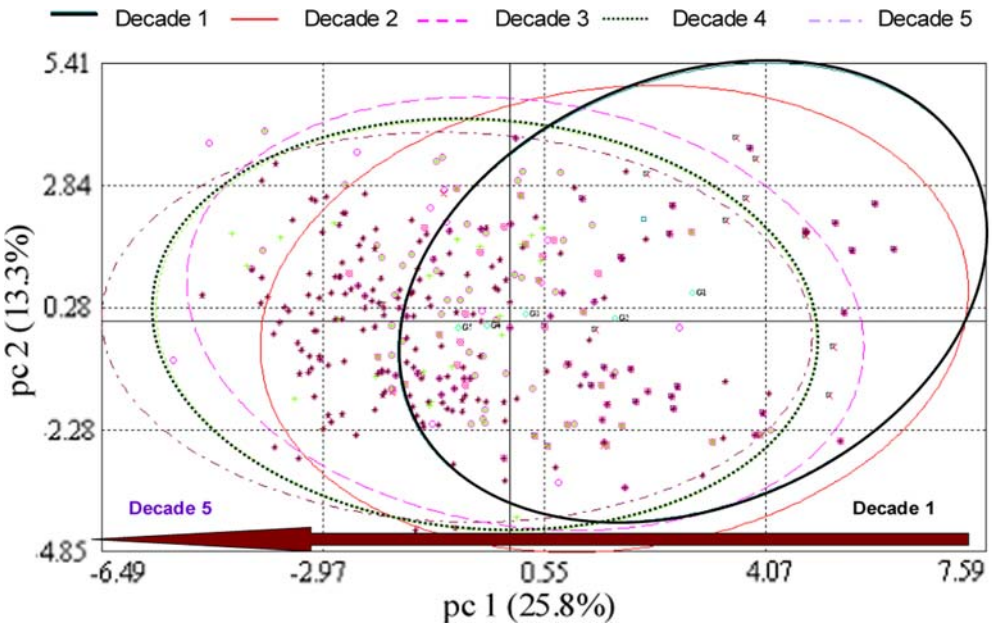

Fig. 4 Associations among garden peas during 5 decades as revealed by principal coordinate analysis. Ellipses of tolerance represent 95% of lines in each decade

Table 8 Genetic diversity, number of alleles and allelic richness per locus and per decade for garden peas

Locus	Gene diversity per locus*					No. of alleles					Allelic richness per locus*				
	Dec 1	Dec 2	Dec 3	Dec 4	Dec 5	Dec 1	Dec 2	Dec 3	Dec 4	Dec 5	Dec 1	Dec 2	Dec 3	Dec 4	Dec 5
Pgm 1	0.20	0.28	0.24	0.16	0.17	2	2	2	2	2	2.00	2.00	2.00	2.00	2.00
Pgm 2	0.52	0.51	0.51	0.50	0.50	2	2	2	2	2	2.00	2.00	2.00	2.00	2.00
Got	0.35	0.41	0.37	0.34	0.35	2	2	2	2	2	2.00	2.00	2.00	2.00	2.00
Shdh	0.52	0.51	0.51	0.48	0.47	2	2	2	2	2	2.00	2.00	2.00	2.00	2.00
Pgd 1	0.50	0.51	0.51	0.47	0.45	2	2	2	2	2	2.00	2.00	2.00	2.00	2.00
Pgd2	0.25	0.20	0.31	0.40	0.42	2	2	2	2	2	2.00	2.00	2.00	2.00	2.00
Idh	0.00	0.00	0.07	0.05	0.09	1	1	2	2	2	1.00	1.00	1.93	1.82	1.95
amy	0.30	0.28	0.31	0.37	0.38	2	2	2	3	3	2.00	2.00	2.00	2.39	2.31
Zone A	0.30	0.28	0.27	0.28	0.31	2	2	2	2	3	2.00	2.00	2.00	2.00	2.31
Zone B	0.78	0.78	0.79	0.75	0.76	8	8	8	9	9	8.00	7.64	7.35	7.54	7.98
Zone C	0.71	0.70	0.71	0.64	0.63	5	5	5	5	5	5.00	5.00	4.98	4.90	4.83
Zone D	0.68	0.67	0.69	0.68	0.68	4	4	6	6	6	4.00	3.76	4.95	5.12	5.36
Mean	0.43	0.43	0.44	0.43	0.43	2.83	2.83	3.08	3.25	3.33	2.83	2.78	2.93	2.98	3.06

*No significant difference between decades ($P < 0.05$)

genetic diversity available. For instance, the evaluation of genetic advances in eight agricultural species revealed improvements in yield, quality and resistance in most of them (Luciani 2004). In contrast, there is a general impression that plant breeding has contributed to reducing genetic diversity, even if this may appear less obvious in vegetable crops because of the different morphologically distinct types dedicated to different end-uses and users (amateurs or professionals). Statistical data collected on numerous species have tended to reinforce this impression. For instance, some of the genetic advances achieved in maize could probably be explained by the standardization of varietal types. While in 1970, the proportions of single, double and three-way hybrid seeds sold in France were, respectively, 4, 73 and 21%, by 2002 the figures had changed completely, with 80, 0.003 and 16%, respectively (Anonymous 2004). In 1990, the well-known F2 maize inbred line was still being used as a parental line in 85% of all seed for early and medium-early hybrids (Anglade et al. 1992). According to Le Buanec (1998), there has been a general trend towards limiting genetic variability to varieties used in the field. These views only refer to the diversity that farmers choose to use, which differs from the diversity available in the French Catalogue. The aim of this paper was to examine the

overall diversity existing in the French Catalogue for two species, and to put forward some proposals for pertinent indicators to assess genetic diversity.

Based on the mean H_e indices estimated from morphological characteristics, we conclude that the same degree of diversity in maize was conserved in each decade. This stage of the analysis only provided quantitative information and we had no information about putative qualitative shifts. As a general rule, this index took account of the number and distribution of frequencies in each class. For example, for the ''cob colour'' trait, the increase in the H_e index between decades 1 and 3 was due to an increase in the number of classes from 6 to 8. When the number of classes was the same between decades, such as for the ''type of grain'' characteristic, the H_e increase was due to changes in the note frequencies and, more usually, to an equiprobable distribution of the note frequencies. While the mean diversity index per decade decreased slightly between decades 1 and 3, the mean number of classes increased. This increase was mainly due to the appearance of extreme classes for a trait rather than intermediate classes, suggesting that this new diversity may have been introduced from the original germplasm. Although quantitative shifts in diversity were not observed, qualitative changes were obvious when each characteristic was considered separately. They reflected current trends in maize breeding, where the emphasis was on yield, earliness and lodging resistance. In Western Europe, and particularly in France, most hybrids had one American dent parent and one European flint parent (Gay 1984). One of the first selection objectives in France has been to obtain hybrids combining the earliness of European flint types with the higher yield of American dent lines. With regard to CPVO trait no. 29: '' type of grain'' (Fig. 2a), the frequency histogram underlines the increase in intermediates at the expense of extreme types of flints and dents. An important innovation in France has been a focusing on early hybrids to allow the extension of maize growing areas in northern France (Gallais 2002). The histogram of note frequencies for the 'time of anthesis' trait (Fig. 2b) highlights increasing proportions of 'medium to late' to 'very late'inbred lines. Several hypotheses could explain this trend: firstly, the introgression of dent lines into flint lines [dents lines being associated with late flowering but early grain maturity leading to rapid grain filling (Barrière, personal communication)], and secondly, meteorological data collected since 1946 which have indicated a global warming over the past 30 years. Earlier sowing dates combined with technological advances in production and an improvement in farming expertise has notably led to producers choosing slightly later maturing varieties (Lorgeou and Souverain 2003). Moreover, with the recent breeding of inbred lines dedicated to maize silage, harvest maturity is not a priority and genotypes with a higher plant yield (such as dent inbred lines) are required because the whole plant is harvested.

In terms of diversity, general changes between decades, estimated by the G_{ST}' or Q_{ST} differentiation parameters, were very small. During the past 2 decades, the number of inbred lines leading to hybrids has considerably increased, but inbred lines have tended to become more and more similar from a phenotypic point of view. Principal component analysis provided a graphic transcription of these results. Tolerance ellipses mostly overlapped, which was not very surprising considering that a high proportion of inbred lines leading to hybrids registered before 1994 were still being used in hybrids during the last decade. From a qualitative point of view, a shift was observed (throughout all decades) from early flint lines towards late dent lines, with an increasing proportion of intermediate genotypes. Analysis of these tolerance ellipses revealed that the 1847 inbred lines of the last decade represented the same degree of diversity as the 178 inbred lines of the first decade, suggesting that no significant changes in maize diversity had occurred during the last 2 decades. Similar conclusions could be drawn from enzymatic data. While the genetic diversity index was lower than that estimated from morphological data, no significant differences were observed between the 3 decades. The low level of genetic diversity within each decade was mainly due to the low level of polymorphism detected by isozymes. Indeed, when studying the genetic diversity of ten populations of maize, Dubreuil and Charcosset (1998) reported a similar average diversity of 0.23 when analysing the same enzyme loci, whereas genetic diversity calculated from RFLP loci was more marked (0.60). Le Clerc et al. (2005) also reported a higher degree of genetic diversity (0.59) calculated from 51 SSR on maize hybrids. As expected, we found allelic richness to be low but similar within each decade. However, enzymes are becoming increasingly less efficient in distinguishing between the increasing number of inbred maize lines.

Compared with maize, the large number of non-discriminating characteristics used for DUS (distinction, uniformity, stability) tests in peas was probably responsible for the lower level of phenotypic diversity found in garden peas and feed peas, and also for the high standard deviations. Nevertheless, greater genetic diversity was detected in garden peas than in feed peas. This could easily be explained by the recent opening of the French Catalogue for feed peas and by the various uses of garden peas as green immature seeds, dry split

peas or immature pods. The study by Baranger et al. (2004) also suggested a loss of diversity during recent spring feed pea selection. During the present study, we did not separate winter-sown and spring-sown feed peas and this probably explains why we did not observe as significant a loss of alleles in feed pea as they did. We demonstrated no loss of diversity in peas based on morphological or enzymatic data for the past 50 years, but some morphological qualitative shifts were highlighted. We also showed a higher degree genetic differentiation in peas than in maize. Based on enzymes, genetic differentiation was slight in both species (Table 7). The very slight genetic differentiation in feed peas confirmed the high degree of genetic redundancy found in this recent gene pool (Baranger et al. 2004), while morphological differentiation reflected constant breeding for yield, mainly explained by resistance to lodging, a reduction in foliar area due to the Afila mutation and breeding for cold resistance (Pitrat and Foury 2003). In garden peas, breeding goals have been numerous and dependent on the type of use, leading, for example, to earlier varieties for canned peas or the breeding of varieties with small grain size enabling the production of extra-fine peas, as shown by the shift of tolerance ellipses between decades.

Proposals for pertinent indicators of diversity

Our general conclusions are similar for both maize and peas. Whatever the type of data analysed, no significant differences were observed in the degree of genetic diversity available to farmers from the French Catalogue, or to breeders, during recent decades, and phenotypic qualitative shifts (even slight) were highlighted. However, it is crucial to examine our results carefully, depending on the choice of markers and indicators analysed.

Indeed, nowadays, a wide variety of markers are available to analyse the genetic diversity of a species (morphological, enzymatic or molecular markers), but the conclusions may differ. The consideration of morphological markers may lead to an inaccurate analysis of genetic diversity. Indeed, visible diversity may markedly increase while genetic diversity does not. Moreover, morphological markers may be more or less strongly correlated, leading to redundant information. To improve the assessment of genetic diversity through the use of phenotypic diversity, it may be possible to optimize the choice of morphological characteristics by preferring those with known genetic determinism. Among morphological characteristics, it may be useful to distinguish neutral and selected traits and study their impact on estimates of genetic diversity. However, it should be noted that all DUS characteristics are selected by breeders for Uniformity. In the light of current knowledge, and as was suggested by Koebner et al. (2003), it can be said that morphological characteristics reflect the genetic diversity targeted for selection, whereas molecular markers, invisible and unselected by breeders, are more likely to generate an unbiased picture of diversity trends. Maize isozymes, because of their claimed selective neutrality and their location on the genome, may offer a better analysis of genetic diversity. However, their discriminative power is not very strong. With the advent of numerous molecular markers, new alternatives are now available to analyse genetic diversity. For example, genetic diversity among maize cultivars, representative of the maize grown in France over the past 5 decades, was previously analysed using microsatellites (Le Clerc et al. 2005). They were indeed more polymorphic than enzymes when comparing inbred lines in order to deduce historical hybrids. However, the general conclusions are in line with the present results. While some alleles present in the cultivars released before 1976 were lost during subsequent decades (mainly because populations were replaced by hybrids), genetic diversity has been conserved in the most recent cultivars.

It is not only the choice of markers, but also that of the indicators used to analyse genetic diversity which plays a major role in the interpretation of results. One of our aims was to suggest indicators which could be used regardless of the species and type of markers, in order to facilitate comparison and allow the periodic updating of databases. The H_e meets all the above requirements and could therefore be proposed. It provides a quantitative estimate of genetic diversity and can easily be compiled under Excel or a free population genetic software such as FSTAT. However, it should not be interpreted on its own, because of its poor sensitivity to rare alleles or low score frequencies in the case of morphological markers. It should therefore be supplemented by another, finer, indicator such as allelic richness or the number of classes per morphological characteristic. For genetic markers, an investigation of allelic frequencies and more particularly, the proportion of rare alleles, provides information about trends in breeding. While the appearance of new alleles may be due to the introduction of exotic germplasm, a marked reduction in allele frequencies may be a signal for breeders to broaden their genetic bases. As emphasised by Fu et al. (2003), allelic diversity in specific loci, rather than average genetic diversity, is sensitive to plant breeding practices. The G_{ST} differentiation parameter provides more general information about the shifts which occur in genetic

diversity and is also easily computable under Excel or free software.

What about the choice between G_{ST} and Q_{ST} parameters? Recent theoretical and simulation studies have been published on this subject, but their conclusions were contradictory (Merila and Crnokrak 2001; McKay and Latta 2002; Crnokrak and Merilä 2002).

The Q_{ST} parameter takes account of the scoring scale, whereas G_{ST} gives the same weighting to each class, resulting in a loss of information about existing differentiation. Moreover, Q_{ST} highlights the effects of breeding. However, during the present study, only small differences were observed between G_{ST} and Q_{ST} values, even if the highest Q_{ST} values were observed for traits subjected to breeding, such as plant length. It therefore seemed more judicious to compute G_{ST} rather than Q_{ST} because G_{ST} takes account of all quantitative and qualitative characteristics in a single parameter. It would probably have produced similar results for peas because a large number of qualitative traits are considered. Theoretical studies have suggested that $Q_{ST} = F_{ST}$ for neutral traits. Moreover, most DUS characteristics are more neutral regarding selection than VCU (value for cultivation and utilization) characteristics which are obviously of agronomic interest. It therefore seems preferable to use F_{ST} or G_{ST} rather than Q_{ST} indices when estimating the genetic differentiation of DUS traits.

In conclusion, although genetic diversity has been maintained over time in the French Catalogue, it is probable that a relatively similar genetic base has been used by the breeders. Even though the appearance of new alleles is encouraging, the introduction of new germplasm from genetic resources needs to be reinforced, suggesting that the preservation of genetic diversity in gene banks constitutes a crucial guarantee for future breeding.

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