Theor Appl Genet (1998) 96: 577–587 

**P. Dubreuil · A. Charcosset**

# Genetic diversity within and among maize populations: a comparison between isozyme and nuclear RFLP loci

Received: 1 June 1997 / Accepted: 3 November 1997

**Abstract** In order to compare the potential of enzyme and DNA markers to investigate genetic diversity within and among populations, ten maize populations were characterized for (1) 20 isozyme loci and (2) restriction fragment length polymorphism (RFLP) for 35 probe-enzyme combinations. Each population was represented by a sample of at least 30 individuals. The average number of alleles detected per locus was clearly higher for RFLPs (6.3) than for isozymes (2.4). Similarly, total diversity was higher for RFLPs (0.60) than for isozymes (0.23). This difference is consistent with observations on inbred-line collections and can be related to the fact that many variations at the DNA level do not change the amino-acid composition or the global charge of proteins. By contrast, the magnitude of population differentiation, relative to the total diversity, was similar for isozymes (23%) and RFLPs (22%). This suggests that the isozyme and RFLP loci considered in this study are subject to similar evolutionary forces, and that both are mostly neutral. However, RFLPs proved clearly superior to isozymes both to (1) identify the origin of a given individual and (2) reveal a relevant genetic structure among populations. The higher polymorphism observed for RFLP loci and the greater number of these loci contributed to the superior discriminative ability of the RFLP data.

**Key words** RFLP · Isozymes · Diversity · Populations · *Zea mays* L.

Communicated by A. L. Kahler

P. Dubreuil (✉) · A. Charcosset
INRA-UPS-INAPG, Station de Génétique Végétale,
Ferme du Moulon, F-91190 Gif-sur-Yvette, France
Fax: +33(0)169332340
E-mail: dubreuil@moulon.inra.fr

## Introduction

A primary concern of population geneticists and conservation biologists has long been to gain insight into the level and distribution of genetic diversity within species. Since the advent of protein electrophoresis in the 1960s, isozyme markers have been extensively used to assess the amounts of genetic diversity within and among plant populations (Brown 1979). The starch- gel electrophoresis technique is still widely used for several reasons. First, isozyme surveys represent a basic, but fruitful, level of investigation for species that are poorly documented. Secondly, isozymes are universal in the sense that estimates of extent and distribution of genetic diversity can be directly compared between populations and species. Third, isozyme methods are appropriate to investigate genetic variation from large samples of individuals because they are usually relatively simple and inexpensive.

Alternative sources of markers detecting variation directly at the DNA level have been available for two decades and present several advantages over isozymes (i.e. a larger number of potential loci and better resolution among individuals). Nevertheless, it has to be stressed that the number of studies that have addressed genetic diversity within and among populations with DNA markers (Huff et al., 1993; Kangfu and Pauls 1993; Zhang et al., 1993; Bark and Havey 1995; Pogson et al., 1995; Vicario et al., 1995) is still limited. Moreover, to our knowledge, only two papers (Zhang et al., 1993; Pogson et al. 1995) have presented a comparison between isozyme and nuclear RFLP variation within and among populations, although such comparisons can provide useful information for geneticists who work on species for which DNA markers are not yet available.

In maize, genetic-diversity analysis can benefit from the large sets of public DNA probes, originally developed for gene mapping (Helentjaris et al. 1986; Burr

et al. 1988) and thus distributed widely through the genome. As a result, probes are readily available to be used in studies pertaining to genetic diversity. Many papers have reported on the RFLP structure among inbred lines (Melchinger et al. 1991; Livini et al. 1992; Messmer et al. 1992; Mumm and Dudley 1994; Dubreuil et al. 1996) and comparisons have been made with isozymes (Messmer et al. 1991; Burstin et al., 1994 and Gerdes and Tracy 1994) but, to our knowledge, no one has yet provided such results within and among maize populations.

In the present study, we report on examination of genetic diversity based on enzyme and RFLP loci within and among maize populations which represent significant sources of variability. The purpose of this study was: (1) to compare estimates of genetic diversity within, and its partition among, maize populations using isozymes and RFLPs, and (2) to evaluate the usefulness of both classes of markers in genetically characterizing similar or distinct germplasms.

## Materials and methods

### Genetic material

Ten populations of maize (*Zea mays* L.) were sampled among European and North U.S. germplasm. All populations were provided by the INRA-PROMAIS maize gene bank. One or two multiplication phases were conducted using a standard procedure designed to limit genetic drift and homogamy (i.e. populations were renewed from 100 pair-crosses by sampling on average five kernels per ear).

Populations from Europe included four open-pollinated varieties from southwest France (Lacaune, Roux de Chalosse, Gazost and Moncassin) and one from North Italy (Va 84 cinquantino rosso). Moncassin, Gazost and Roux de Chalosse originated from a rather restricted area (these populations were less than 100 km apart), whereas the Lacaune population was more distant from the others (170–270 km). In particular, this population was successfully used to develop early inbred lines for breeding programs in Northern Europe.

Northern U.S. populations consisted of three open-pollinated varieties (Minnesota 13, Golden Glow, Compton's Early) and two synthetic varieties (BS13-S-C4 and BSL-S-C4). Minnesota 13 is one of the most important varieties in the Northern cornbelt (Baker 1984). It may have originated from Pride of the North variety developed in Iowa (Gerdes et al. 1993). The Golden Glow population was derived from a cross between the former variety and Toole's North Star population (Gerdes et al. 1993). Compton's Early is a Northern flint variety that was cultivated in Canada and New England. Its origin may trace back to Sioux or Iroquois indians' strains (Gerdes et al. 1993). BS13-S-C4 derives from the BSSS population. Originally designated Iowa Stiff Stalk, BSSS is a synthetic population developed from a polycross of 16 lines mostly derived from Reid Yellow Dent germplasm (Baker 1984). BSL-S-C4 was derived from BSL, which traces back to the Lancaster Sure Crop variety. It was developed by four cycles of recurrent $S_1$ selection for stalk-rot resistance and root quality (Eberhart et al. 1972).

### Isozyme survey

Thirty nine to fifty genotypes per population (mean of 44.5 genotypes) were assayed for enzyme variation. Analysis was conducted by GEVES (Groupement d'Etude et de Contrôle des Variétés et des Semences), following the method reported by Stuber et al. (1988). Each population was investigated for variation at 20 isozyme loci. These included five malate dehydrogenase loci (Mdh*1*, *2*, *3*, *4*, and *5*), two isocitrate dehydrogenase loci (*Idh1*, and *2*), two 6-phosphogluconate dehydrogenase loci (*Pgd1*, and *2*), two phosphoglucomutase loci (*Pgm1*, and *2*), three glutamate-oxaloacetate transaminase loci (*Got 1*, *2*, and *3*), one phosphohexose isomerase locus (*Phi1* or *Pgi1*), one acid phosphatase locus (*Acp1*), one diaphorase locus (*Dia1*), one alcohol dehydrogenase locus (*Adh1*), and one catalase locus (*Cat3*). The Chromosomal locations of loci that displayed variation among populations are listed in Table 1.

### RFLP assays

For technical reasons, another sample from the same seed stock was used for RFLP analysis. Thirty genotypes per population were first sampled and then selfed to derive $S_1$ progenies. Balanced bulks of adult leaf pieces were taken from ten plants within each $S_1$ family and used for RFLP assays. Based on the results of Michelmore et al. (1991), we assumed that an allelic variant was always detected when at least four copies occured in the mixture (e.g. two-fifth heterozygous genotypes or one-fifth homozygous genotypes). Following this assumption, the risk of not detecting heterozygozity at a given locus within a mixture of ten $S_1$ plants does not exceed 0.3%.

With few modifications, the procedure used in this study was similar to that employed by Dubreuil et al. (1996) for inbred lines of maize. Genomic DNA was extracted according to Tai and Tanksley (1990), purified through cesium-chloride gradients (Murray and Thompson 1980), and hydrolysed separately with E*co*RI and *Hin*dIII restriction enzymes. DNA fragments were electrophoresed overnight on 0.7% w/v neutral agarose gels using a 0.09 M Tris-phosphate, 0.002 M EDTA TPE pH 7.7 running buffer (Sambrook et al., 1989). Then, separated fragments were vacuum transferred from gels to charged nylon filters using alkalin buffer. Probes were radiolabelled by random priming according to Feinberg and Volgestein (1983) and hybridizations were made following the procedure described by Church and Gilbert (1984). Before autoradiographic exposure, filters were washed three times (15–20 min per wash) at 68°C in 1% SDS, 40 mM $NaHPO_4$ buffer.

To account for possible migration distortions, molecular-weight markers of 24-kb obtained from the *Xba*I restriction of Lambda DNA, and of 1.5 kb from specific priming PCR amplification, were added to each genomic DNA sample just before loading onto gels. Molecular-weight markers of 23.1, 9.4, 6.5, 4.4, 2.3, and 2.2 from a *Hin*dIII digest of Lambda DNA were also run in two lanes per comb. Further, three other lanes per comb were loaded with mixtures of digested DNA from six inbred lines of maize which were previously observed to be polymorphic for 45 RFLP loci (61 probe-enzyme combinations). These genomic standards provided a partial "band dictionary" (mean of 3.24 alleles per locus), which ensured the identification of banding patterns on autoradiograms tracing back to different gels.

DNA probes included 31 genomic clones from the BNL (Brookhaven National Laboratory, Upton, N.Y.) and UMC (University of Missouri, Columbia, Mo.) public banks of maize probes, and three cDNA clones (Keith et al. 1993). Their chromosomal location was determined from one $F_2$ and three recombinant inbred-line populations (Causse et al. 1996). Even if some probes selected for this study revealed minor bands, all of them enabled us to identify major bands corresponding to alleles at a single locus. Thus, the 35 probe-enzyme combinations assayed (probe B5.09 was interpreted in combination with E*co*RI and *Hin*dIII used separately) were scored in terms of a single RFLP locus. Heterozygote genotypes have been recognized as displaying two different banding patterns recorded among homozygous genotypes or inbred lines previously assayed for RFLPs (Dubreuil et al., 1996). Alleles present in a given genotype were visually identified by a number ranging from 1 to *n* (*n* being the

total number of alleles per locus among all profiled genotypes). In rare cases where two alleles were not clearly distinguishable over autoradiograms tracing back to different gels, although different, they were grouped.

### Statistical methods

Nei's (1978) unbiased estimation of genetic diversity (or heterozygote frequency under Hardy-Weinberg expectations) was computed for each polymorphic enzyme and RFLP locus as follows

$$H_e^l = (2n_l/2n_l - 1)\cdot\left(1 - \sum_{al=1}^{A_l} P_{al}^2\right),$$

where $P_{al}$ is the frequency of allele $a$ at locus $l$ within the total population, and $n_l$ the effective number of individuals characterized at locus $l$. Genetic diversity was estimated in a similar fashion within each population; $P_{al}$ and $n_l$ referring then to a given population. Mean genetic diversity that occured within a population was computed by averaging $H_e^l$ estimates from this population over all loci. Departures from Hardy-Weinberg expectations were derived for each population as

$$(F_{is})_s = 1 - \frac{(\bar{H}_o)_s}{(\bar{H}_e)_s},$$

where $(\overline{H_e})_s$ and $(\overline{H_o})_s$ are the mean expected and actual heterozygote frequencies within the population $s$ considered. Estimates of 95% confidence intervals for $F_{is}$ were determined from the distribution of $F_{is}$ estimates obtained by bootstrapping the original set of polymorphic loci within each population (5000 bootstrap samples) (Michalakis, personal communication, 1996). Assuming statistical independance between loci within each population, departure from Hardy-Weinberg expectations was tested across loci by Fisher's combined probability test incorporated in the GENEPOP software version 1.2 (Raymond and Rousset 1995). Heterozygote excess or deficiency was tested using the exact test proposed by Rousset and Raymond (1995).

Additionally, single-locus genetic differentiation among populations was evaluated following Nei (1973) as $G_{st}^l = D_{st}^l/H_e^l$ where $D_{st}^l = H_e^l - \overline{H_w^l}$ is the absolute difference between the total genetic diversity ($H_e^l$) and the weighted mean of within-population diversity at locus $l$ (i.e. $\overline{H_w^l} = \sum_{p=1}^{P} n_p^l/N^l\ H_p^l$ where $n_p^l$ is the number of individuals characterised at the locus $l$ for the population $p$; $N^l$ the number of individuals characterised at locus $l$ within the overall population, and $H_p^l$ the estimated diversity of the population $p$ at the locus $l$). Individual locus differentiation, estimated by $G_{st}^l$, represents the fraction of genetic diversity which occurs among populations at a given locus relative to the total diversity. It ranges theoretically from 0 to 1. It becomes 0 if all populations have the same allele frequencies; it is 1 only if all populations are genetically monomorphic, even if some, but not all, of them are fixed for the same allele. Finally, multilocus $G_{st}$ estimation was computed over all the $L$ loci analysed as, $G_{st} = D_{st}/H_e$, where

$$D_{st} = (1/L)\cdot\sum_{l=1}^{L} D_{st}^l, \quad \text{and} \quad H_e = (1/L)\cdot\sum_{l=1}^{L} H_e^l.$$

Genetic distances were estimated through isozyme and RFLP data between each pair of populations by the Modified Rogers' Distance (Rogers 1972; Wright 1978) as follows

$$MRD_{XY}^2 = \frac{1}{L}\sum_{l=1}^{L}\sum_{a=1}^{A_l}\frac{1}{2}(P_{al}^X - P_{al}^Y)^2,$$

where $P_{al}^X$ and $P_{al}^Y$ are the frequencies of allele $a$ at locus $l$ within populations $X$ and $Y$ respectively. Because molecular distances were not all independant, correlation was tested as described by Mantel (1967) using the GENEPOP software version 1.2 (Raymond and Rousset 1995). Dendrograms were constructed by UPGMA (unweighted pair-group method using arithmetic averages) clustering analyses (Sneath and Sokal 1973) from distance matrices based on isozyme and RFLP data. Furthermore, associations between individuals were graphically depicted through Principal Component Analysis (PCA) from correlation matrices of allele frequencies. When not specified, statistical computations were performed using SAS-STAT and SAS-IML software (SAS Institute Inc. 1990).

## Results

In this study, a total of 445 genotypes were analysed at 20 enzyme loci and the average number of genotypes profiled for a RFLP locus was 285.3. All RFLP loci exhibited polymorphism among populations, whereas no polymorphism was found at five enzyme loci. These loci were *Mdh3*, *Mdh4*, *Mmm*, *Pgm1* and *Got3*, which were fixed for alleles 16, 12, Mmm-M, 9, and 4, respectively.

### Comparison of the total genetic diversity revealed at isozyme and RFLP loci

A total of 221 alleles was observed among the 35 RFLP loci, while only 36 alleles (including a null allele at the *Pgd2* locus) were found among the 15 polymorphic enzyme loci. Tables 1 and 2 show individual locus statistics for enzyme and RFLP analyses respectively. Among polymorphic enzyme loci, the number of alleles ranged from 2 to 4 and averaged 2.40. When monomorphic loci were considered, enzyme variation did not exceed 1.80 alleles per locus. The diversity index exhibited a wide range of variation (from 0.040 at the *Pgd2* locus to 0.499 at the *Idh2* locus) and averaged 0.232. Allelic diversity at RFLP loci varied greatly from one locus to another but was high on average (6.31 alleles per locus). The least polymorphic locus (CSU81-*Hin*dIII) exhibited two alleles whereas the most polymorphic (B6.25-*Hin*dIII and H68-*Hin*dIII) displayed 11 alleles across all populations. The mean diversity value (0.607) was almost three-times higher than that of isozymes.

### Comparison between isozyme and RFLP diversity within populations

Consistent with previous results, there was a sharp discrepancy between RFLP and enzyme variation within populations (Table 3). The average number of alleles per locus and per population was quite larger when estimated using RFLPs (3.46) as opposed to isozymes (1.69). Regardless of the type of marker employed, populations from North U.S. were on average slightly more diverse than those from Europe. Nonetheless, the mean number of alleles per RFLP locus

**Table 1** Genetic variation within and among ten maize populations at enzyme loci

| Enzyme locus | Chromosome location | No. of alleles | $H_e$ | $\overline{H_w}$ | $D_{st}$ | $G_{st}$ |
|---|---|---|---|---|---|---|
| *Adh1* | 1L | 2 | 0.42 | 0.34 | 0.08 | 0.19 |
| *Pgi1* | 1L | 3 | 0.07 | 0.06 | 0.01 | 0.15 |
| *Dia1* | 2L | 2 | 0.08 | 0.06 | 0.01 | 0.17 |
| *Got1* | 3L | 2 | 0.03 | 0.03 | ~ 0.00 | 0.08 |
| *Pgd2* | 3L | 3 | 0.04 | 0.04 | ~ 0.00 | 0.06 |
| *Cat3* | 4L | 2 | 0.18 | 0.16 | 0.02 | 0.13 |
| *Got2* | 5L | 2 | 0.08 | 0.07 | ~ 0.00 | 0.06 |
| *Mdh5* | 5S | 2 | 0.07 | 0.05 | 0.01 | 0.17 |
| *Pgm2* | 5S | 4 | 0.19 | 0.17 | 0.01 | 0.08 |
| *Idh2* | 6L | 2 | 0.50 | 0.31 | 0.18 | 0.37 |
| *Mdh2* | 6L | 2 | 0.50 | 0.30 | 0.20 | 0.40 |
| *Pgd1* | 6L | 2 | 0.50 | 0.40 | 0.09 | 0.19 |
| *Idh1* | 8L | 2 | 0.08 | 0.07 | 0.01 | 0.18 |
| *Mdh1* | 8L | 2 | 0.13 | 0.11 | 0.02 | 0.13 |
| *Acp1* | 9L | 4 | 0.62 | 0.49 | 0.13 | 0.20 |
| *Mean* | – | 2.40 | 0.23 | 0.18 | 0.05 | 0.18 |

$H_e$: genetic diversity within the overall population; $\overline{H_w}$: mean diversity within populations; $D_{st}$: absolute differentiation between populations; $G_{st}$: relative differentiation between populations

**Table 2** Genetic variation within and among ten maize populations at RFLP loci

| Locus | | Chromosome location | No. of alleles | $H_e$ | $\overline{H_w}$ | $D_{st}$ | $G_{st}$ |
|---|---|---|---|---|---|---|---|
| Probe | Enzyme | | | | | | |
| B 5.62 | *Eco*RI | 1 | 8 | 0.76 | 0.60 | 0.15 | 0.20 |
| B 8.29 | *Eco*RI | 1 | 5 | 0.32 | 0.27 | 0.05 | 0.17 |
| H 107 | *Eco*RI | 1 | 4 | 0.42 | 0.35 | 0.07 | 0.16 |
| H 140 | *Eco*RI | 1 | 8 | 0.45 | 0.42 | 0.03 | 0.07 |
| H 84 | *Eco*RI | 1 | 9 | 0.69 | 0.55 | 0.14 | 0.20 |
| B 5.59 | *Hin*dIII | 1 | 9 | 0.76 | 0.62 | 0.14 | 0.19 |
| B 6.32 | *Hin*dIII | 1 | 8 | 0.83 | 0.67 | 0.16 | 0.19 |
| H 161 | *Eco*RI | 1 | 3 | 0.43 | 0.38 | 0.05 | 0.12 |
| GSY 40 | *Eco*RI | 2 | 7 | 0.80 | 0.59 | 0.21 | 0.26 |
| GSY 199 | *Eco*RI | 2 | 4 | 0.57 | 0.40 | 0.18 | 0.31 |
| H 4 | *Hin*dIII | 2 | 5 | 0.73 | 0.50 | 0.23 | 0.31 |
| H 10 | *Eco*RI | 3 | 8 | 0.77 | 0.54 | 0.23 | 0.30 |
| H 102 | *Eco*RI | 3 | 5 | 0.46 | 0.39 | 0.07 | 0.15 |
| H 60 | *Eco*RI | 3 | 5 | 0.56 | 0.44 | 0.12 | 0.21 |
| H 47 | *Eco*RI | 4 | 4 | 0.27 | 0.21 | 0.06 | 0.22 |
| H 66 | *Eco*RI | 4 | 8 | 0.72 | 0.56 | 0.16 | 0.22 |
| H 15 | *Hin*dIII | 4 | 6 | 0.47 | 0.39 | 0.08 | 0.17 |
| H 19 | *Hin*dIII | 4 | 4 | 0.52 | 0.39 | 0.12 | 0.24 |
| H 51 | *Eco*RI | 5 | 10 | 0.78 | 0.69 | 0.08 | 0.10 |
| H 54 | *Eco*RI | 5 | 4 | 0.58 | 0.50 | 0.08 | 0.14 |
| B 6.25 | *Hin*dIII | 5 | 11 | 0.79 | 0.59 | 0.19 | 0.25 |
| H 68 | *Hin*dIII | 5 | 11 | 0.83 | 0.65 | 0.18 | 0.22 |
| H 134 | *Eco*RI | 6 | 4 | 0.65 | 0.43 | 0.21 | 0.33 |
| H 21 | *Eco*RI | 6 | 7 | 0.78 | 0.67 | 0.11 | 0.14 |
| H 62 | *Eco*RI | 6 | 4 | 0.47 | 0.34 | 0.13 | 0.27 |
| H 65 | *Eco*RI | 6 | 8 | 0.72 | 0.52 | 0.20 | 0.28 |
| H 85 | *Hin*dIII | 6 | 7 | 0.75 | 0.52 | 0.23 | 0.31 |
| CSU 81 | *Hin*dIII | 7 | 2 | 0.50 | 0.41 | 0.09 | 0.18 |
| H 35 | *Hin*dIII | 7 | 7 | 0.43 | 0.32 | 0.12 | 0.27 |
| H 103 | *Eco*RI | 8 | 5 | 0.45 | 0.34 | 0.11 | 0.25 |
| B 9.11 | *Hin*dIII | 8 | 6 | 0.55 | 0.44 | 0.11 | 0.20 |
| B 5.09 | *Eco*RI | 9 | 3 | 0.48 | 0.37 | 0.11 | 0.22 |
| B 5.10 | *Eco*RI | 9 | 8 | 0.61 | 0.50 | 0.11 | 0.18 |
| B 14.28 | *Hin*dIII | 9 | 7 | 0.54 | 0.28 | 0.26 | 0.48 |
| B 5.09 | *Hin*dIII | 9 | 7 | 0.75 | 0.63 | 0.12 | 0.16 |
| | *Mean* | – | 6.31 | 0.61 | 0.47 | 0.13 | 0.22 |

B refers to BNL probes; H refers to UMC probes. $H_e$: genetic diversity within the overall population; $\overline{H_w}$: mean diversity within populations; $D_{st}$: absolute differentiation between populations; $G_{st}$: relative differentiation between populations

**Table 3** Genetic diversity within ten maize populations as revealed by isozymes and RFLPs

| Populations | Mean sample size per locus | No. of alleles | | Mean allele number per locus | $H_0$ | $H_e$ | $F_{is}$ | 95% confidence intervals for $F_{ts}$ | Departure from HW[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Unique | Total | | | | | | All | Def. | Exc. |
| Isozymes | | | | | | | | | | | |
| Lacaune | 46 | 0 | 25 | 1.67 | 0.11 | 0.14 | +0.21 | ( +0.05; +0.47) | *** | *** | ns |
| Gazost | 46 | 0 | 25 | 1.67 | 0.15 | 0.16 | +0.08 | ( −0.13; +0.30) | ** | * | ns |
| Va 84 | 50 | 0 | 24 | 1.60 | 0.16 | 0.17 | +0.08 | ( −0.08; +0.29) | ** | *** | ns |
| Moncassin | 40 | 0 | 22 | 1.47 | 0.14 | 0.14 | 0.00 | ( −0.21; +0.16) | ns | ns | ns |
| Roux de C. | 46 | 0 | 25 | 1.67 | 0.14 | 0.16 | +0.17 | ( +0.02; +0.34) | ** | *** | ns |
| Golden Glow | 48 | 0 | 28 | 1.87 | 0.22 | 0.20 | −0.11 | ( −0.21; −0.02) | ns | ns | ** |
| Minnesota 13 | 42 | 0 | 27 | 1.80 | 0.22 | 0.25 | +0.11 | ( −0.07; +0.29) | *** | *** | ns |
| Compton's E. | 42 | 2 | 28 | 1.87 | 0.20 | 0.21 | +0.02 | ( −0.14; +0.19) | * | ns | ns |
| BSL-S-C4 | 39 | 0 | 24 | 1.60 | 0.17 | 0.20 | +0.18 | ( −0.07; +0.43) | *** | ** | ns |
| BS13-S-C4 | 46 | 0 | 26 | 1.73 | 0.13 | 0.16 | +0.16 | ( −0.03; +0.45) | *** | *** | ns |
| Europe | 45.4[a] | 0[b] | 24.2 | 1.62 | 0.14 | 0.16 | +0.11 | | | | |
| North U.S | 43.2[a] | 0.02[b] | 26.6 | 1.77 | 0.19 | 0.20 | +0.07 | | | | |
| RFLP | | | | | | | | | | | |
| Lacaune | 27.6 | 1 | 104 | 2.97 | 0.36 | 0.40 | +0.09 | ( +0.02; +0.18) | * | ** | ns |
| Gazost | 26.6 | 5 | 148 | 4.23 | 0.50 | 0.56 | +0.10 | ( +0.06; +0.15) | ** | *** | ns |
| Va 84 | 27.8 | 3 | 101 | 2.88 | 0.37 | 0.37 | 0.00 | ( −0.07; +0.07) | ns | * | ns |
| Moncassin | 29.8 | 2 | 116 | 3.31 | 0.40 | 0.41 | +0.01 | ( −0.03; +0.05) | ns | ns | ns |
| Roux de C. | 28.5 | 2 | 130 | 3.71 | 0.46 | 0.51 | +0.10 | ( +0.04; +0.16) | * | *** | ns |
| Golden Glow | 29.3 | 5 | 132 | 3.77 | 0.53 | 0.56 | +0.05 | (0.00; +0.11) | *** | *** | ns |
| Minnesota 13 | 29.8 | 5 | 120 | 3.43 | 0.49 | 0.55 | +0.10 | ( +0.03; +0.16) | *** | *** | ns |
| Compton's E. | 28.0 | 2 | 123 | 3.51 | 0.42 | 0.46 | +0.08 | ( +0.01; +0.15) | *** | *** | ns |
| BSL-S-C4 | 29.9 | 4 | 113 | 3.23 | 0.47 | 0.48 | +0.01 | ( −0.06; +0.08) | *** | ns | ns |
| BS13-S-C4 | 28.0 | 6 | 126 | 3.60 | 0.34 | 0.43 | +0.21 | ( +0.15; +0.27) | *** | *** | ns |
| Europe | 28.0[a] | 0.07[b] | 119.8 | 3.42 | 0.42 | 0.45 | +0.07 | | | | |
| North U.S | 29.0[a] | 0.13[b] | 122.8 | 3.51 | 0.45 | 0.49 | +0.09 | | | | |
| Isozymes | 44.2[a] | 0.01[b] | 25.4 | 1.69 | 0.16 | 0.18 | +0.08 | | | | |
| RFLP | 28.5[a] | 0.10[b] | 97.3 | 3.46 | 0.43 | 0.47 | +0.08 | | | | |

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns = $P > 0.05$

[a] Values are harmonic means

[b] Values are mean number of unique alleles per locus

[c] Significance levels of rejecting Hardy-Weinberg proportions for all possible reasons (All), for heterozygote deficiency only (Def.), and for heterozygote excess only (Exc.)

was more variable among European populations (2.88–4.23) than among North American populations (3.23–3.77).

Comparisons between the actual and expected frequencies of heterozygotes within populations showed significant deviations from panmixia for both sets of markers (Table 3). As previously observed at enzyme loci within maize populations (Brown and Allard 1970; Kahler et al., 1986; Salanoubat and Pernes 1986; Lefort-Buson et al., 1991; Garnier-Géré 1992), most deviations resulted from significant heterozygote deficiencies for both isozyme and RFLP loci. The only significant excess of heterozygous genotypes was for the isozyme loci from the Golden Glow population. Surprisingly, this result was not supported by the data obtained from RFLP loci. This may be explained by the scoring problems that are sometimes met with enzyme loci (Lefort-Buson et al. 1991).

## Comparison of structures revealed by isozymes and RFLPs

### *Differentiation between populations*

Unlike RFLPs, enzyme loci exhibited very few alleles that were specific to one population. Only two enzyme alleles, both specific to Compton's Early population, were detected, while 35 RFLP alleles were specific to a population. The Lacaune population displayed only one unique allele among a total of 104 RFLP alleles. In contrast, BS13-S-C4 was the most distinct population with six unique alleles among a total of 126. Overall, the mean number of unique alleles per locus was ten-times lower for enzyme loci than for RFLP loci. Additionally, all populations showed the same most common allele for 67% of enzyme loci, whereas only 11% of RFLP loci displayed the same most common allele in all populations.

**Table 4** Modified Rogers' distances between ten maize populations based on isozyme (above diagonal) and RFLP data (below diagonal)

| Populations | Origins | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Europe | | | | | North U.S. | | | | |
| | Lac | Gaz | Va84 | MC | RC | GG | Min13 | CE | BSL | BS13 |
| Lac | – | 0.06 | 0.07 | 0.05 | 0.05 | 0.11 | 0.09 | 0.08 | 0.08 | 0.13 |
| Gaz | 0.13 | – | 0.03 | 0.04 | 0.03 | 0.07 | 0.05 | 0.09 | 0.03 | 0.05 |
| Va84 | 0.18 | 0.15 | – | 0.05 | 0.03 | 0.04 | 0.07 | 0.10 | 0.06 | 0.06 |
| MC | 0.14 | 0.06 | 0.17 | – | 0.01 | 0.05 | 0.06 | 0.10 | 0.07 | 0.06 |
| RC | 0.13 | 0.05 | 0.14 | 0.06 | – | 0.04 | 0.05 | 0.12 | 0.06 | 0.05 |
| GG | 0.18 | 0.10 | 0.16 | 0.16 | 0.13 | – | 0.04 | 0.09 | 0.06 | 0.03 |
| Min13 | 0.20 | 0.16 | 0.20 | 0.21 | 0.17 | 0.09 | – | 0.06 | 0.03 | 0.04 |
| CE | 0.16 | 0.13 | 0.18 | 0.18 | 0.15 | 0.12 | 0.14 | – | 0.05 | 0.10 |
| BSL | 0.22 | 0.18 | 0.19 | 0.20 | 0.16 | 0.15 | 0.14 | 0.16 | – | 0.03 |
| BS13 | 0.24 | 0.18 | 0.23 | 0.22 | 0.18 | 0.16 | 0.18 | 0.15 | 0.15 | – |

Estimates of relative genetic differentiation ($G_{st}$) were variable between loci, either at enzyme or RFLP loci (see Tables 1 and 2). They ranged from 0.07 at the H140-*Eco*RI locus to 0.48 at the B14.28-*Hin*dIII locus. A slightly smaller range (from 0.06 at the *Pgd2* locus to 0.40 at the *Mdh2* locus) was observed among enzyme loci. Although the average $G_{st}$ over loci was higher for RFLPs than for isozymes (0.22 vs 0.18), the $G_{st}$ value computed from the average values of diversity did not differ significantly between both classes of loci (0.22 vs 0.23). Similar estimates based on enzyme loci have been reported by Geric et al. (1989) among 18 groups of 277 Yugoslavian maize populations from different geographic areas, and by Lefort-Buson et al. (1991) and Garnier-Géré (1992) among 115 and 59 maize populations from very diverse origins, respectively. These levels of genetic differentiation are intermediate between those estimated for outcrossing wind (0.099) and selfing (0.510) populations from different taxa (Hamrick and Godt 1990).

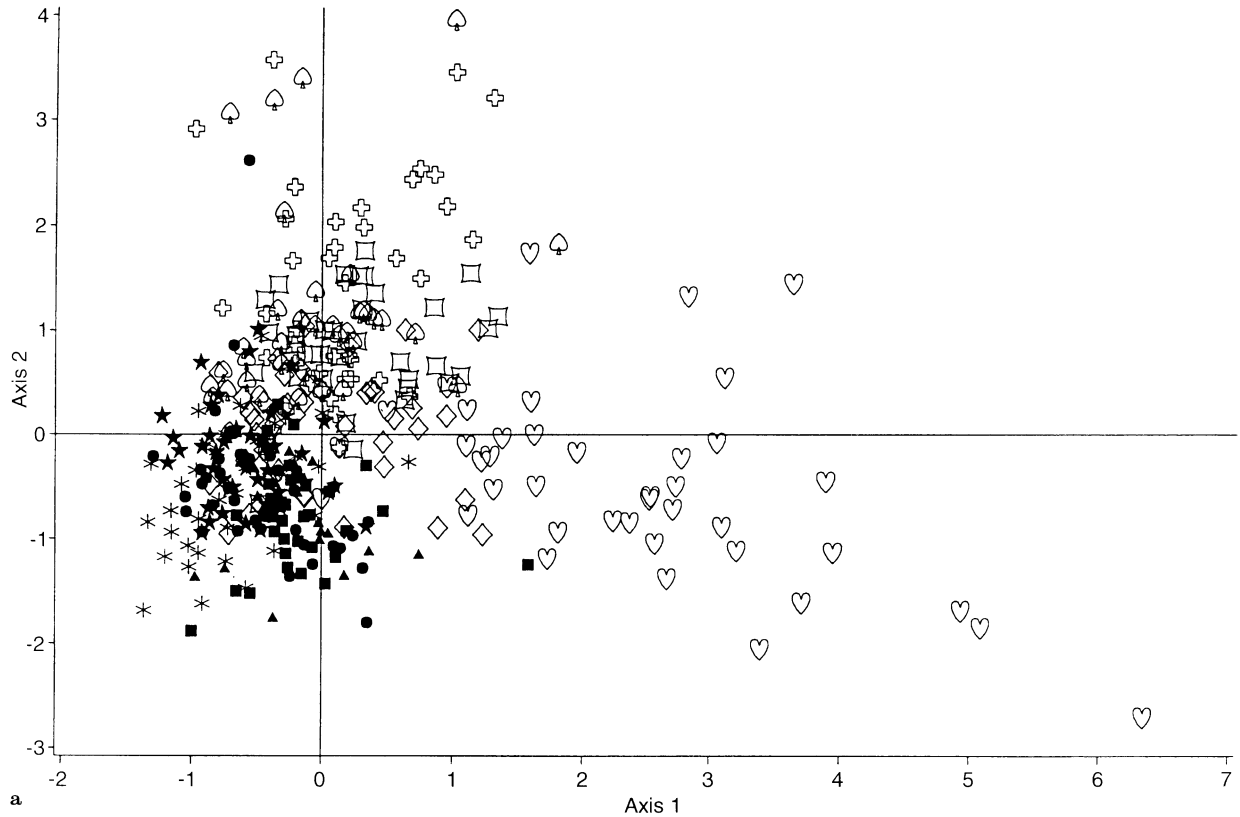
Table 4 presents the genetic distances between populations. As expected, for any kind of marker the mean $MRD^2$ between populations from different continents was greater than that between populations from the same continent. For any given pair of populations, the $MRD^2$ values estimated on enzyme markers were less than those estimated on RFLPs. Consistent with the discrepancy between $D_{st}$ estimates from both kinds of markers, the difference between mean $MRD^2$ values within and among origins was two-times greater when computed on RFLPs (0.047) than on isozymes (0.022). The rank correlation between isozyme and RFLPs distances was low ($r = 0.430$) but highly significant ($P = 0.007$) according to Mantel's test.

## Associations among individuals

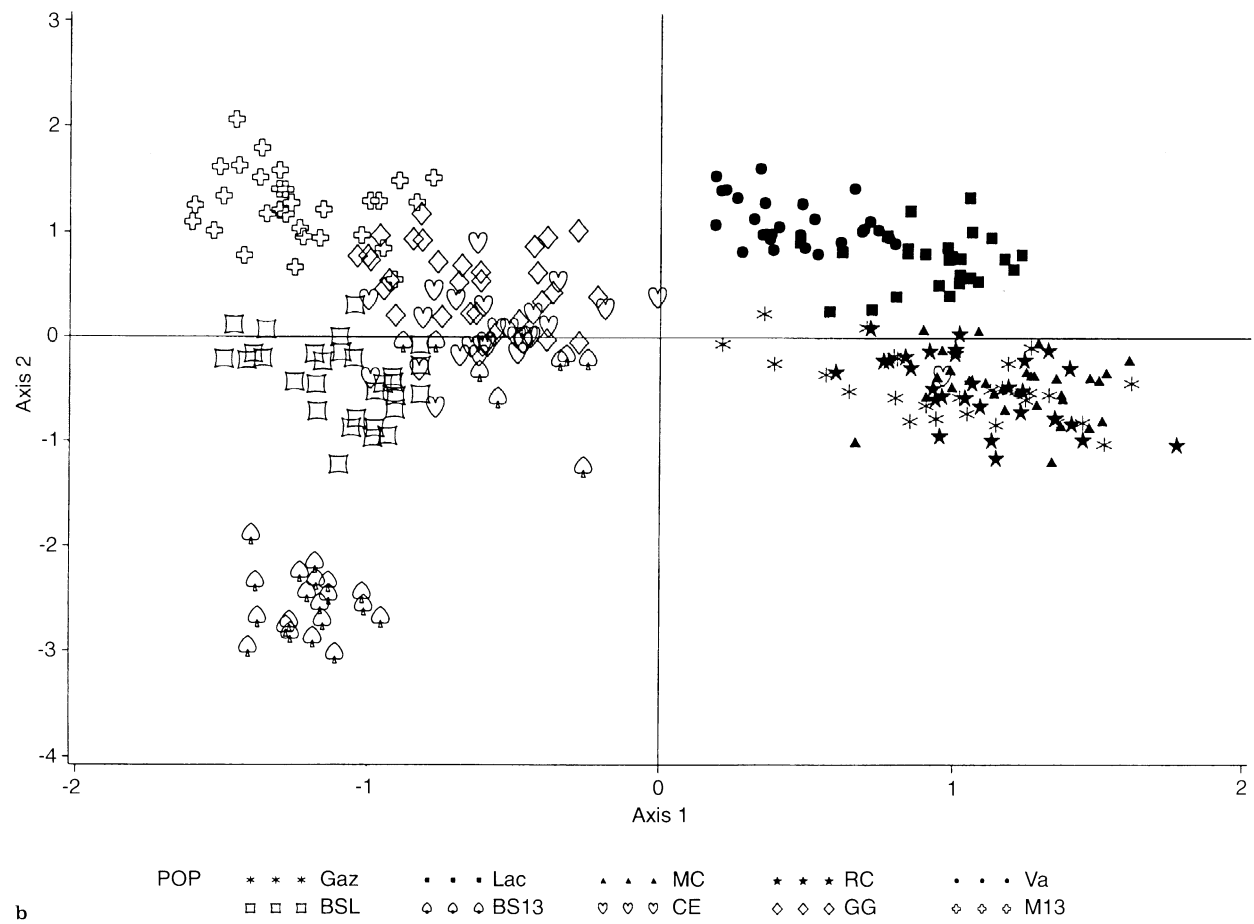
Associations among individuals sampled from populations were investigated by PCA. For the RFLP data, this analysis was restricted to the best-characterised set of individuals; this included 286 genotypes from a total of 300. In cases where some locus genotypes could not be determined, the resulting missing data (less than 1.8% of the total data points) were replaced by allele frequencies within the population to which the individual concerned belonged. Analysis from isozyme data was performed on the entire set of individuals assayed at 15 polymorphic loci. Figure 1 a and b compare PCA plots obtained from the two sets of polymorphisms. The location of individuals was defined by the two first principal components. These components explained 7.52% and 4.58% of the total variation at the DNA level, and 11.51% and 8.63% of the total variation at the enzyme level.

Comparisons between the genetic structures of populations displayed significant discrepancies. Whereas PCA based on RFLPs yielded a good separation between European and U.S. germplasms, PCA based on enzyme polymorphism exhibited a weak separation. Moreover, RFLP markers grouped individuals from the same population more closely. Most genotypes from BS13-S-C4 formed a group which was located quite distant from the remaining U.S. populations, while, Va 84 and Lacaune mapped apart from the other European populations. The Moncassin, Roux de Chalosse and Gazost populations completely overlapped, as did Golden Glow and Compton's Early within the American germplasm. PCAs performed using 15 randomly sampled RFLP loci proved that differences for the number of loci alone could not explain the discrepancies observed between both sets of markers. RFLP loci, particularly, remained more powerful in distinguishing both geographical origins (Europe and North U.S.) even if populations were less clearly outlined.

**Fig. 1 a** Associations among individuals as revealed by PCA from isozyme data (axis1 = 11.51%; axis2 = 8.63%). **b** Associations among individuals as revealed by PCA from RFLP data (axis1 = 7.52%; axis2 = 4.58%)


Axis 2
Axis 1
4
3
2
1
0
-1
-2
-3
-2
-1
0
1
2
3
4
5
6
7

a


Axis 2
Axis 1
3
2
1
0
-1
-2
-3
-4
-2
-1
0
1
2
POP
Gaz
Lac
MC
RC
Va
BSL
BS13
CE
GG
M13

b

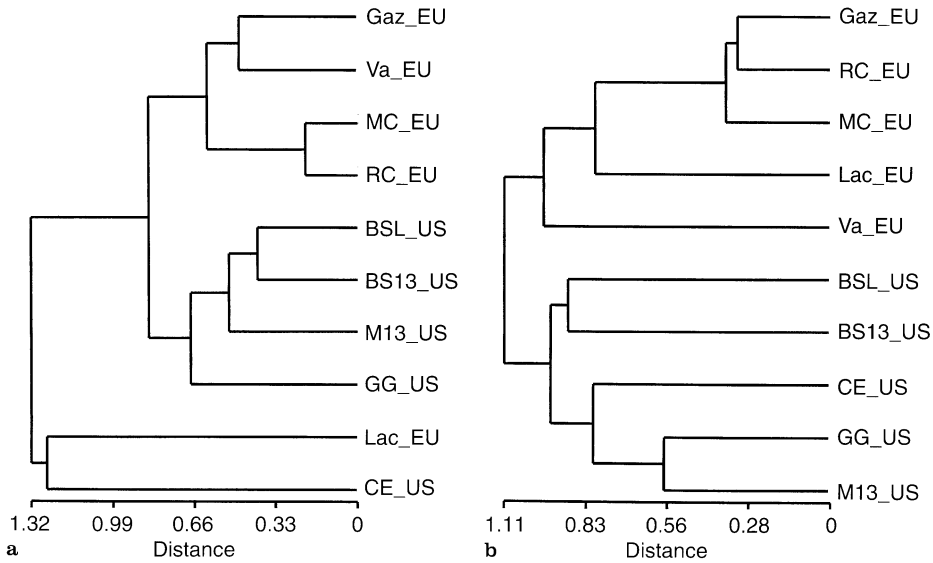



**Fig. 2** **a** Dendrogram representing genetic relationships between populations as revealed by UPGMA on enzyme data-based distances **b** Dendrogram representing genetic relationships between populations as revealed by UPGMA on RFLP data-based distances

Comparisons between groupings of all individuals based on RFLP and isozyme data using UPGMA confirmed that RFLPs more closely grouped genotypes from the same population than did isozymes. Remarkably, the dendrogram (data not shown) based on RFLPs displayed clusters that corresponded exactly to populations (all individuals from one population within a unique class and no individuals from other populations in this class). Populations that were identified in this way were Lacaune, Va 84, and Minnesota 13.

### Relationships between populations

Figures 2a and b show dendrograms representing genetic distances among populations as estimated from isozymes and RFLPs, respectively. Co-phenetic correlations displayed a good agreement between original distances and those given by the UPGMA clustering process ($r = 0.78$ and 0.80 respectively at the 0.001 level of significance), indicating that the trees gave a reliable representation of the genetic distances between populations.

The classification based on RFLPs exhibited a break between both geographical origins, whereas the classification based on enzyme polymorphism brought out the distinctiveness of the Lacaune and Compton's Early populations. These were distantly clustered together away from the others. The enzyme distinctiveness of Compton's Early was supported by: (1) the existence of alleles specific to this population at the *Pgi1* and *Acp1* loci, and (2) the outlying locations of most individuals as revealed by PCA analysis (see Fig. 2 a). In agreement with investigations on the genetic divergence between Northern flints and races from Mexico and Southwestern U.S. (Doebley et al., 1986), it was noted that *Pgm2* locus showed a strong discontinuity in allele frequencies between Compton's Early and other U.S. populations used in this study. Whereas Doebley et al. (1986) reported a clinal variation in frequency for the *Idh2-4* allele, we observed a strong discontinuity for the same allele. These authors also identified the *Adh1*, *Got2*, *Pgd2* and *Mdh1* loci as displaying strong heterogeneous frequency patterns which could not be observed for the smaller sample of populations employed here.

## Discussion

Significant differences in polymorphism were shown between both types of markers. Enzyme loci were almost three-times less diverse on average than RFLP loci. A difference of the same magnitude was previously observed among Atlantic cod *Gadus morhua* individuals (Pogson et al. 1995). Conversely, Zhang et al. (1993) did not find significant difference for the diversity between isozymes and RFLPs among populations of barley. Nonetheless, they admitted that the observed isozyme diversity may have been inflated by the use of four esterase loci, which are usually more polymorphic than other enzyme loci. Within accessions of *Brassica campestris*, McGrath and Quiros (1992) also did not report large differences between estimates of genetic diversity from cDNA and enzyme markers but, as in our study, found three-times more alleles at the DNA level than at the protein level. It is well known that these two electrophoretic techniques do not have the same capability for detecting genetic polymorphisms. Many variations at the DNA level can remain hidden at the protein level because several single mutations do

not change the amino-acid composition or the global charge of proteins. Moreover, RFLPs are able to detect differences up to several kilobases within regions that flank the genomic loci surveyed.

## Genetic diversity within populations

Estimates from isozymes and RFLPs showed that populations displayed a small range of genetic diversity (from 0.136 to 0.247 as estimated using enzymes, and from 0.372 to 0.559 as estimated using RFLPs). Based on isozyme polymorphism, a similar range was found within a large sample of Northern Spanish maize populations (Llauradó et al. 1993). Nonetheless, other studies reported larger intervals of variation for isozyme diversity within populations. Salanoubat and Pernes (1986) demonstrated that Czech and Hungrian populations exhibited low levels of genetic diversity (mean of 0.048) in comparison with Spanish, French or Yugoslavian populations (mean of 0.151). On the other hand, Kahler et al. (1986) showed that maize populations could retain larger amounts of genetic diversity than those reported herein. Among Corn-belt open-pollinated and adapted exotic varieties of maize, the estimates of Kahler et al. (1986) varied from 0.150 to 0.422. It has to be noted that the Minnesota 13 and Golden Glow populations were almost two-times more diverse in the study of Kahler et al. (1986) than in ours. This discrepancy can be partly related to the diverse sets of loci used. In particular, the survey of Kahler et al. (1986) included highly polymorphic enzyme loci (*Acp4*, *Est1*, and *Glu1*), which were not used here.

Most populations showed a deficit of heterozygous individuals. This can be related to various causes: positive assortative matings between individuals (homogamy), selection favoring homozygotes and the Wahlund effect (artificial grouping of individuals from different populations). Positive assortative mating between individuals is probably the major cause of heterozygote deficiency within populations. Although multiplication procedures are designed to prevent non-random mating between individuals, it is likely that late plants are preferentially crossed to late ones, and early plants to early ones. The high level of heterozygote deficiency observed within the BS13-S-C4 population could also be related to the Wahlund effect. This was supported by PCA from the RFLP data, which showed two distinct groups of individuals within this population.

A multiplication phase can lead to the rapid loss of genetic diversity if too few individuals are employed. This questions the efficiency of the procedures used to maintain the genetic diversity available within populations in the long term. However, the results presented here and those previously mentioned demonstrate that very few maize open-pollinated varieties exhibit low levels of genetic diversity. This finding is unexpected since farmers used to renew these populations from year to year with seeds taken from a small number of ears. Consequently, mass selection, which was practiced in the U.S. before the development of hybrids in the late thirties, could have led to a rapid loss of internal genetic diversity. Nevertheless, since inbreeding noticeably affects ear traits, such as length and diameter (see Hallauer and Miranda 1981 for a review), it is likely that farmers unconsciously selected ears from the most heterozygous plants, so reducing the effects of genetic drift in small populations.

QTL mapping studies in maize have provided evidence for overdominance effects at many loci for grain yield, ear height and plant height (Stuber et al. 1992; Moreau and Charcosset, personal communication 1996). These effects may result from the aggregation of significant dominance effects of linked QTLs, although true overdominance effects cannot be ruled out [Cockerham and Zeng (1996) from the data of Stuber et al. (1992)]. However, heterozygous genotypes are favored under both hypotheses, so that maize mass selection should have contributed to maintaining a high level of genetic diversity within populations. Low levels of genetic diversity might be found in populations derived from commercial varieties and maintained for many years in isolated locations without significant breeding efforts (populations cultivated for domestic uses).

## Genetic differentiation between populations and origins

The relative magnitudes of genetic differentiation were very similar between both kinds of markers. Surveys conducted by Zhang et al. (1993) and Pogson et al. (1995) demonstrated a discrepancy between enzyme and RFLP estimates of genetic differentiation among barley and Atlantic cod populations, respectively. In particular, the weak genetic differentiation observed at enzyme loci in comparison with RFLPs led the last authors to question the selective neutrality of isozymes. By contrast, our results suggest that the same neutral evolutionary forces drive the divergence between populations at both enzyme and RFLP loci.

Although estimates of genetic differentiation among populations were quite similar between the two kinds of markers, RFLP loci proved superior to isozymes for revealing a relevant genetic structure in terms of origin. This was illustrated by the comparison of PCAs (see Fig. 1 a and b) and consistent results from hierarchical analyses (see Fig. 2 a and b). As expected, PCAs performed from 15 randomly chosen RFLP loci did not yield a genetic structure as good as that obtained using all loci. Nevertheless, as previously noted, the separation between individuals from Europe and North U.S. was not strongly affected, indicating that the difference in the number of loci between both sets of markers did not solely account for the discrepancy observed. Thus,

the higher number of alleles found at RFLP loci also contributed to the better distinction between origins. RFLP alleles specific to a given genetic origin play a significant role in the distinction between germplasms, as observed using PCA.

Striking differences were evident between trees constructed from isozyme and RFLP data. Whereas isozymes exhibited the distinctiveness of the Lacaune and Compton's Early populations, RFLPs clearly differentiated between both origins. Among U.S. populations, genetic relationships based on RFLPs appeared more relevant than those obtained through isozymes. The parentage relationship between the Golden Glow and Minnesota 13 populations was not reflected by isozymes but was by RFLPs. The Northern flint variety (Compton's Early) did not exhibit distinct RFLP patterns, as compared with our enzyme results and those from Doebley et al. (1986). Concerning European populations, both geographical origins and molecular results indicate that the Moncassin, Gazost and Roux de Chalosse populations trace back to the same genetic origin. It was not clear whether or not the Lacaune and Va 84 Cinquantino Rosso populations derive from the same genetic origin. As illustrated by PCA on RFLP markers, these populations appeared to be rather close. Nevertheless, they were not classified together by clustering analysis. Although no reliable historical record is available, it seems that the Lacaune population would trace back to an Italian population (Monod, personal communication 1996). This information would re-inforce the PCA results. Further investigations using a larger sample of populations would be useful to gain an insight into the genetic structure of European germplasm.

**Acknowledgements** This study was supported by a grant from the french Ministère de l'Agriculture et de la Forêt and the PROMAIS association members: Caussade Semences, Ciba-Geigy, Cargill, Coop de Pau, Limagrain, Maïsadour, Nickerson, Pioneer France, RAGT, Rustica Prograin, SDME-KWS, SES-ICI, and Verneuil Semences. We are also grateful to A. Gallais for helpful discussions and encouragement throughout this research program. We are also grateful to M. Grenèche (GEVES) for isozyme analyses, to M. Causse and S. Santoni for helpful discussions concerning RFLP analyses, to F. Dumas for expert technical assistance, and to B. Gouesnard and J. Dallard for providing the populations analysed in this study and helpful discussions concerning maize germplasm. We finally, we express our gratitude to D. Manicacci and an anonymous referee for helpful comments on the early draft of this paper.

## References

Baker R (1984) Some of the open-pollinated varieties that contributed the most to modern hybrid corn. Proc 20th Annu Illinois Corn Breeder's School, Champaign 6–8 May 1984, University of Illinois, Urbana-Champaign, book 1147 : 1–19

Bark OH, Havey MJ (1995) Similarities and relationships among populations of the bulb onion as estimated by nuclear RFLPs. Theor Appl Genet 90 : 407–414

Brown AHD (1979) Enzyme polymorphism in plant population. Theor Pop Biol 15 : 1–42

Brown AHD, Allard RW (1970) Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. Genetics 66 : 133–145

Burr B, Burr FA, Thompson KH, Albertsen MC, Stuber W (1988) Gene mapping with recombinant inbreds in maize. Genetics 118 : 519–526

Burstin J, de Vienne D, Dubreuil P, Damerval C (1994) Molecular markers and protein quantities as genetic descriptors in maize. I. Genetic diversity among 21 inbred lines. Theor Appl Genet 89 : 943–950

Causse M, Santoni S, Damerval C, Maurice A, Charcosset A, Deatrick J, de Vienne D (1996) A composite map of expressed sequences in maize. Genome 39 : 418–432

Church MG, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81 : 1991–1995

Cockerham CC, Zeng ZB (1996) Design III with marker loci. Genetics 143 : 1437–1456

Doebley JF, Goodman MM, Stuber CW (1986) Exceptional genetic divergence of Northern flint corn. Am J Bot 73 : 64–69

Dubreuil P, Dufour P, Krejci E, Causse M, de Vienne D, Gallais A, Charcosset A (1996) Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Sci 36 : 790–799

Eberhart SA, Hallauer AR, Russel WA (1972) Registration of four maize germplasm synthetics. Crop Sci 12 : 132

Feinberg A, Volgenstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132 : 6–13

Garnier-Géré P (1992) Contribution à l'étude de la variabilité inter- et intra-population chez le maïs (*Zea mays* L.): valorisation d'informations agromorphologiques et enzymatiques. Thèse de Doctorat, Institut National Agronomique Paris-Grignon

Gerdes JT, Tracy WF (1994) Diversity of historically important sweet corn inbreds as estimated by RFLPs, morphology, isozymes, and pedigree. Crop Sci 34 : 26–33

Gerdes JT, Behr CF, Coors JG, Tracy WF (1993) Compilation of North American maize breeding germplasm. Tracy WF, Coors JG, Geadelmann JL (eds.). Published by CSSA, Madison, Wisconsin USA

Géric I, Zlokolica M, Géric C, Stuber CW (1989) Races and populations of maize in Yugoslavia. Isozyme variation and genetic diversity. Report from IBPGR

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, USA

Hamrick JL, Godt MJW (1990) Allozyme diversity in plant species. In: Brown AHD, MT Clegg, Kahler AL, Weir BS (eds.) Plant population genetics, breeding, and genetic resources. Sinauer Associates Inc., Sunderland, Massachusetts, USA

Helentjaris T, Slocum M, Wright S, Schaeffer A, Nenhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72 : 761–769

Huff DR, Peakall R, Smouse PE (1993) RAPD variation within and among natural populations of outcrossing buffalograss [*Buchlo dactyloides* (Nutt.) Engelm.]. Theor Appl Genet 86 : 927–934

Kahler AL, Hallauer AR, Gardner CO (1986) Allozyme polymorphisms within and among open-pollinated and adapted exotic populations of maize. Theor Appl Genet 72 : 592–601

Kangfu Y, Pauls KP (1993) Rapid estimation of genetic relatedness among heterogeneous populations of alfalfa by random amplification of bulked genomic DNA samples. Theor Appl Genet 86 : 788–794

Keith CS, Hoang DO, Barrett BM, Feigelman B, Nelson MC, Thai H, Baysdorfer C (1993) Partial sequence analysis of 130 randomly selected maize cDNA clones. Plant Physiol 52 : 221–228

Lefort-Buson M, Lavergne V, Daudin JJ, Charcosset A, Sampoux JP, Gallais A (1991) Genetic variability among populations of maize germplasm. 2. Enzymatic polymorphism and its relationship to quantitative trait diversity. Maydica 36 : 237–246

Livini C, Ajmone-Marsan P, Melchinger AE, Messmer MM, Motto M (1992) Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLPs. Theor Appl Genet 84 : 17–25

Llauradó M, Moreno-Gonzalez J, Arús P (1993) Classification of Northern Spanish populations of maize by methods of numerical taxonomy. II. Isozyme variation. Maydica 38 : 249–258

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27 : 209–220

McGrath JM, Quiros CF (1992) Genetic diversity at isozyme and RFLP loci in *Brassica campestris* as related to crop type and geographical origin. Theor Appl Genet 83 : 783–790

Melchinger AE, Messmer MM, Lee M, Woodman WL, Lamkey KR (1991) Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms. Crop Sci 31 : 669–678

Messmer MM, Melchinger AE, Lee M, Woodman WL, Lee EA, Lamkey KR (1991) Genetic diversity among progenitors and elite lines from the Iowa Stiff Stalk Synthetic (BSSS) maize population : comparison of allozyme and RFLP data. Theor Appl Genet 83 : 97–107

Messmer MM, Melchinger AE, Boppenmaier J, Herrmann RG, Brunklaus Jung E (1992) RFLP analyses of early-maturing European maize germ plasm. I. Genetic diversity among flint and dent inbreds. Theor Appl Genet 83 : 1003–1012

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis : a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88 : 9828–9832

Mumm RT, Dudley JW (1994) A classification of 148 U.S. maize inbreds: I. Cluster analysis based on RFLPs. Crop Sci 34 : 842–851

Murray M, Thompson W (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8 : 4231–4325

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

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

Pogson GH, Mesa KA, Boutilier RG (1995) Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozymes and nuclear RFLP loci. Genetics 139 : 375–385

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. The Journal of Heredity 86 : 248–249

Rousset F, Raymond M (1995) Testing heterozygote excess and deficiency. Genetics 140 : 1413–1419

Rogers JS (1972) Measures of similarities and genetic distances. Studies in genetics VII. Univ Texas Publ 7213 : 145–153

Salanoubat M, Pernes J (1986) Enzyme polymorphisms within and between European maize populations. Maydica 31 : 269–278

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, 2nd edn. Cold Spring Harbor Laboratory, Gold Spring Harbor, New York

SAS/IML User's Guide, Release 6.03 Edition (1990) SAS Institute Inc., Cary, North Carolina

Sneath PHA, Sokal RR (1973) Numerical taxonomy. WH Freeman, San Franscisco

Stuber CW, Wendel JF, Goodman MM, Smith JSC (1988) Techniques and scoring procedures for starch-gel electrophoresis of enzymes from maize (*Zea mays* L.). Tech Bull 286 Res Service, North Carolina State University, USA

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132 : 823–839

Tai TH, Tanksley SD (1990) A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol Biol Rep 8 : 297–303

Vicario F, Vendramin GG, Rossi P, Liò P, Giannini R (1995) Allozyme, chloraplast DNA and RAPD markers for determining genetic relationships between *Abies alba* and the relic population of *Abies nebrodensis*. Theor Appl Genet 90 : 1012–1018

Wright S (1978) Evolution and the genetics of populations, Vol.4 : variability within and among natural populations. University of Chicago Press, Chicago

Zhang Q, Saghai Maroof MA, Kleinhofs A (1993) Comparative diversity analysis of RFLPs and Isozymes within and among populations of *Hordeum vulgare* ssp. *spontaneum*. Genetics 134 : 909–916