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Large scale molecular analysis of traditional European maize populations. Relationships with morphological variation

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A representative sample of 130 European traditional maize populations was analysed for both their morphological and molecular variation. The morphological analysis of 19 variables revealed a significant variability. Correlation analysis allowed us to distinguish between traits affected by earliness (plant and ear height) and structural traits (plant architecture, grain structure). Two main morphological types could be distinguished. Molecular analyses were performed for 29 RFLP loci on DNA bulks. The number of alleles detected was high when compared to previous studies (9.59 alleles per locus). Genetic diversity was also high (0.55), with a strong differentiation between populations (G_{ST} value of 35.6%). A clear relationship between the genetic diversity of the populations and their agronomic performances was highlighted. Morphological and molecular distances showed a tendency towards a triangular relationship. We therefore considered a two-phase process to be the most efficient approach for the classification of genetic resources: firstly, a molecular study to define groups of genetically close populations, and secondly a morphological description of populations from each group. In our European collection, this approach allowed us to separate the populations from Northern and Southern Europe and to define six groups of genetically close populations, comparable to European races. This study opens new prospects concerning the molecular analysis of very large collections of genetic resources, hitherto limited by the necessity of individual analyses, and proposes a first molecular classification of European maize germplasm.

Keywords: classification, European populations, genetic diversity, morphological variation, RFLP, *Zea mays* L.

Introduction

Starting from early domestication in Mexico, maize was introduced into many regions worldwide, becoming adapted to highly contrasting climates and agronomic conditions. Its is now clearly established that it was first introduced into Europe following the discovery of the New World in 1492. Contrasting variety types were then progressively cultivated in several European regions. After the Second World War, maize hybrids rapidly replaced traditional populations in most European regions. In order to avoid loss of genetic variability, many maize collections were established in different countries. It is important to characterize the genetic diversity of these collections in order to optimize conservation and facilitate their use. Furthermore, this characterization is necessary for the historical understanding of the introduction of maize in Europe.

Morphological descriptions of some of these collections were carried out in the 1960s in Spain, Italy, Yugoslavia and Romania (see for review Brandolini, 1970). Thereafter, other authors reported descriptions of populations from Portugal (Costa-Rodrigues, 1971), France (Gouesnard *et al*., 1997) and northern Spain (Llauradó & Moreno-Gonzalez, 1993). Several authors were also interested in classification of populations on a European scale. Nevertheless, the relevance of morphological characters for the classification of populations appears limited, particularly because of the effect of environment on the expression of characters. The evaluation of diversity at individual loci overcomes these problems. Historically, isoenzymatic markers were first used, and allowed the analysis of different samples of European populations (Salabounat & Pernes, 1986;

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Geric *et al*., 1989; Lefort-Buson *et al*., 1991; Garnier-Géré, 1992; Llauradó *et al*., 1993; Revilla *et al*., 1998). Subsequently, DNA markers, particularly RFLPs, were used. This allowed the classification of maize inbred lines within heterotic groups (Melchinger *et al*., 1991; Livini *et al*., 1992; Messmer *et al*., 1992; Dubreuil *et al*., 1996). RFLP was more effective than isoenzymes for the analysis of maize populations (Dubreuil & Charcosset, 1998). In order to use RFLPs for the analysis of large samples of populations, a DNA pooled-sampling strategy was developed (Dubreuil *et al*., 1999). This method was tested and proved efficient for the analysis of maize populations (Rebourg *et al*., 1999), therefore providing a convenient tool for the molecular analysis of large collections of populations.

The aim of this study was to evaluate the potential of this approach for obtaining a better understanding of European maize diversity. We analysed 131 European populations, carefully chosen to represent European origins, in order to define the most important European types. Besides the molecular analysis, these populations were evaluated for morphological and agronomic traits in order to compare molecular and phenotypic descriptions. This comparison allowed us to evaluate the best way to combine information in a comprehensive approach.

Materials and methods

Genetic material

We analysed a sample of 131 European maize populations. The INRA-PROMAÏS maize gene bank (Groupe Maïs DGAP-INRA & PROMAÏS, 1994) supplied the majority, while other European institutes provided the others (Table 1). We tried to obtain a representative sample of populations for each country. Unfortunately, the sample was limited in some cases. The analysis was carried out on 37 South-Western European populations (36 from Spain and one from Portugal), 32 French populations, 16 Italian populations, 32 North-Eastern populations (13 from Germany, one from Switzerland, five from Czechoslovakia, eight from Poland, two from Austria and three from Ukraine) and 14 South-Eastern European populations (four from Hungary, three from Romania, four from Yugoslavia and three from Bulgaria). For some countries, we classified populations according to the region of origin (see 'origin' in Table 1).

Morphological analyses

In 1998, all the populations were sown at two INRA maize stations, Mauguio (Southern France near Montpellier) and Le Moulon (Northern France near Paris). German population 23 did not sprout, so that 130 European populations were finally analysed. The populations were distributed into four earliness groups (based on preliminary observations performed in 1997) and randomized within groups. The experimental design had two replicates in each location, with a one-row plot of 25 plants each. The sowing density was 74 000 plants per ha at Le Moulon and 58 000 plants per ha at Mauguio.

We measured for each plot 26 morphological traits taken from 10 competitive plants, and from two kernels per ear. The kernel texture was noted on initial seeds with a scale from 1 for flint to 4 for dent. These primary traits were used to define 19 descriptors (Table 2). The flowering date was converted into accumulative degree-days as: $TT = \sum_{d=1}^{n}[(TX_d + TN_d)/2 \quad Tb]$, where n is the number of days from sowing to flowering, TX_d and TN_d are, respectively, the maximum and minimum temperatures (°C) of day d, Tb (6°C) is the critical temperature under which development is stopped (Bonhomme *et al*., 1994). The ear shape was estimated using the conicity index defined by Ordas & De Ron (1988).

We performed analyses of variance in order to test the significance of variation between populations. These analyses allowed us to estimate genotypic and environmental variances, as well as the heritability of each morphological trait. A Principal Component Analysis was carried out on the phenotypic correlation matrix of the adjusted means of the populations for the 19 descriptors (Table 2), using the FACTOR procedure from SAS (SAS Institute, 1990). The matrix of distances between populations was calculated upon the standardized principal components with eigenvalue higher than one. We used the Euclidean distance called 'Mahalanobis generalized distance' defined by Goodman (1972) as:

$$D_{ij} = \left[\sum_{k=1}^{k=K}\left(\frac{Y_{ik} \quad Y_{jk})^2}{\lambda_k}\right)\right]^{1/2}$$

where Y_{ik} and Y_{jk} are the values of principal component k for populations i and j, respectively, and λ_k is the eigenvalue of principal component k. K is the total number of principal components considered.

Molecular analyses

RFLP assays were carried out using a DNA pooled-sampling strategy described more fully in Dubreuil *et al*. (1999) and Rebourg *et al*. (1999). Each population was represented by 30 plants, using two DNA bulks each extracted from leaf disks of 15 individuals. DNA was extracted according to Tai & Tanksley (1990). DNA samples were digested separately with three restriction

Table 1 List of the 131 European maize populations analysed

No.	Name	Code†	Origin	No.	Name	Code	Origin	No.	Name	Code	Origin
1	Schindelmeiser	PPS 58	Austria	54	Lalin	PPS 982	Spain, Galicia	102	Sarrancolin	PPS 1039	France, Pyrenees
3	Voralberger Weisser	IPK 60	Austria	55	Orense	PPS 983	Spain, Galicia	103	Lourdes	PPS 1051	France, Pyrenees
4	Karavelovo.2	PPS 368	Bulgaria	56	Inia Galicia 1	PPS 991	Spain, Galicia	104	St Panteleon	PPS 631	France
5	Stara Zagora	PPS 520	Bulgaria	57	Inia Galicia 2	PPS 993	Spain, Galicia	105	Lacaune	PPS 15	France
6	Manolovo	PPS 525	Bulgaria	58	Norteno	PPS 444	Spain, Galicia	106	Foldeaki Corne	PPS 140	Hungary
7	Reinthaler	PPS 94	Switzerland	59	Basto	EPZMV 23	Spain, South	107	Martonvasari	PPS 151	Hungary
9	Kocovska Skora	PPS 43	Czechoslovakia	61	Amarillo de Utrera	EPZMV 156	Spain, South	108	Iregszemscei 12 hetes	PPS 458	Hungary
10	Partizenka	PPS 48	Czechoslovakia	62	Pabililo de Granada	EPZMV 159	Spain, South	109	Magyarovari	PPS 534	Hungary
12	Stupicka Rana	PPS 462	Czechoslovakia	63	Blanco de Ricote	EPZMV 173	Spain, South	110	Nostrano di Bolzano	VA 136	Italy
15	Slovenska Velkozrna	RYS 1	Czechoslovakia	66	Andaluz	EPZMV 310	Spain, South	111	Nostrano Quarantino	VA 145	Italy
16	Slovenska Zlta	RYS 2	Czechoslovakia	68	Conil	EPZMV 333	Spain, South	113	Locale di Macerata	VA 563	Italy
17	Chiemgauer	IPK 1	Germany	69	Medina	EPZMV 334	Spain, South	114	VA204 Spezia	PPS 116	Italy
20	Rottaler Weihen-stephan	PPS 386	Germany	70	Vejer	EPZMV 336	Spain, South	115	Quarantino Scuro di Benevento	VA 437	Italy
21	Jaune de Bade	PIO	Germany	71	Villamartin	EPZMV 337	Spain, South	116	Trecchinese di Lagonegro	VA 469	Italy
22	Mahndorfer	IPK 158	Germany	72	Andaluz ABC	PPS 770	Spain, South	117	Locale di Chiaromonte	VA 481	Italy
23	Janetzkis Astra	IPK 14	Germany	73	Trimesino ABC	PPS 774	Spain, South	118	Zemenzella di Reggio Calabria	PPS 487	Italy
24	Mecklenburger	IPK 242	Germany	74	Weckelsheim	PPS 3	France, Alsace	119	VA1 Ott Tortonese	PPS 187	Italy
25	Caspersmeyer II	IPK 15	Germany	75	Wantzenau	PPS 5	France, Alsace	120	Nostrano di Parma	VA 224	Italy
26	Strenzfelder	IPK 239	Germany	76	Bade	PPS 6	France, Alsace	121	VA20 Pignoletto	PPS 687	Italy
27	Pautzfelder	IPK 9	Germany	77	Colmar	PPS 28	France, Alsace	125	VA46 Quarantino Famiglia	PPS 381	Italy
28	Terras Binder	IPK 6	Germany	78	Seltz	PPS 571	France, Alsace	126	Cinquantino di Montagnana	VA 565	Italy
29	Dr Delliles Körnermais	IPK 25	Germany	79	Ain	PPS 474	France	127	VA5 Nostrano dell Isola	PPS 686	Italy
30	Rimpaus Binder	IPK 26	Germany	80	Passins	PPS 606	France	128	Marano	PPS 115	Italy
31	Braunes Schin-delmeiser	IPK 152	Germany	81	Ratenelle	PPS 1012	France	129	VA84 Cinquan-tino Rosso	PPS 735	Italy
33	Millariega Asturies	PPS 423	Spain, Galicia	82	Ruffec	PPS 36	France	130	Malopolenka	PPS 37	Poland

34	Vegadeo Asturias	PPS 985	Spain, Galicia	83	Grand Roux Basque	PPS 641	France, Pyrenees	131	Wanrzenczycka	PPS 40	Poland
35	Vasco 1	EPZMV 70	Spain, Pyrenees	84	Landes 1	PPS 98	France	132	Wielkopolenka	PPS 448	Poland
36	Amarillo de Maranon	EPZMV 155	Spain, Pyrenees	85	Landes 2	PPS 363	France	133	Angliezka	PPS 451	Poland
37	Rojo Grande de Bernedo	EPZMV 161	Spain, Pyrenees	86	Dubos	PPS 628	France	134	Muszynski	PPS 453	Poland
38	Aduna	EPZMV 168	Spain, Pyrenees	87	Millette du Lauragai	PPS 639	France, Pyrenees	135	Szyldeca	KRO 1	Poland
39	Rojo de Tolosa	EPZMV 171	Spain, Pyrenees	88	Millette Montagne Noire	PPS 668	France, Pyrenees	136	Oksonska	KRO 2	Poland
40	Amarillon Pequeno de Bernedo	EPZMV 232	Spain, Pyrenees	89	Barisis	PPS 17	France	137	Rozyllo	KRO 3	Poland
41	Bilbao	PPS 434	Spain, Pyrenees	90	Liesse	PPS 19	France	139	Portugal	PPS 1234	Portugal
43	Orendain	PPS 986	Spain, Pyrenees	91	Estarvielle	PPS 10	France, Pyrenees	140	Ariesan	PPS 160	Romania
44	Ormaiztegui	PPS 987	Spain, Pyrenees	92	Sost	PPS 11	France, Pyrenees	143	Portocaliua	PPS 547	Romania
45	Bidasoa	PPS 990	Spain, Pyrenees	93	Massat	PPS 14	France, Pyrenees	145	Suceava 1b	PPS 551	Romania
46	Arzua	PPS 876	Spain, Galicia	94	Campan Galade	PPS 20	France, Pyrenees	149	Zuti Osmak	MRIZP 832	Yugoslavia
47	Gomesende	PPS 877	Spain, Galicia	95	Esterre	PPS 22	France, Pyrenees	151	Niska Sermija	MRIZP 586	Yugoslavia
48	La Estrada	PPS 878	Spain, Galicia	96	Laruns	PPS 23	France, Pyrenees	152	Zuti Rani	MRIZP 47	Yugoslavia
49	Pueblo de Brollon	PPS 880	Spain, Galicia	97	Estaut	PPS 488	France, Pyrenees	154	Zuti Murt-Optak	MRIZP 676	Yugoslavia
50	Tamallancos	PPS 882	Spain, Galicia	98	St Laurent de Neste	PPS 602	France, Pyrenees	155	Ukraine 1	UKR 37	Ukraine
51	Gallice	PPS 883	Spain, Galicia	99	Argeles	PPS 964	France, Pyrenees	156	Ukraine 2	UKR 38	Ukraine
52	Agraar la Coruna 1	PPS 979	Spain, Galicia	100	Pontacq	PPS 967	France, Pyrenees	157	Ukraine 3	UKR 39	Ukraine
53	Agraar la Coruna 2	PPS 980	Spain, Galicia	101	Monein	PPS 969	France, Pyrenees				

†The codes for populations reflect the gene bank that stores them.
PPS, INRA-PROMAÏS gene bank, France.
IPK, Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany.
MRIZP, Maize Research Institute, Yugoslavia.
KRO, Plant Breeding and Acclimatization Institute, Poland.
RYS, Zeamvent A.S., Slovakia.
UKR, Limagrain Genetics Grandes Cultures, France.
VA, Instituto Sperimentale per la Cerealicoltura, Italy.
EPZMV, Consejo Superior de Investigaciones Cientificas, Spain.
PIO, Pioneer Génétique France.

Table 2 List of the 19 morphological descriptors for maize, with range, mean values and heritability

Abbreviation	Morphological descriptor	Range	Mean	h^2
DDS	Accumulative degree-days to silking	(563–1200.9)	815.6	0.80
PHT	Plant height (cm)	(82.4–206.7)	164.2	0.71
EHT	Ear height (cm)	(20.9–129.3)	72.9	0.83
ELG	Ear length (mm)	(86.8–179)	138.4	0.75
ERN	Ear row number	(8–21.1)	11.8	0.87
ECI	Ear conicity index	(1.4–9)	4.6	0.61
EDI	Ear diameter (mm)	(23–49.8)	38	0.85
TLG	Tassel length (mm)	(355.3–675.1)	560.2	0.77
BTL	Branched part/tassel length	(14.9–31.8)	21.9	0.65
CTL	Central spike/tassel length	(27–49.9)	38.2	0.58
PBN	Number of tassel primary branches	(6.5–30.4)	16.5	0.77
KLG	Kernel length (mm)	(6.4–11.8)	9.2	0.82
KWI	Kernel width (mm)	(6.1–12.0)	9.3	0.91
KWE	1000 kernels weight (g)	(105–425.4)	287.6	0.79
EKN	Ear kernel number	(131.3–596.8)	261	0.76
TIL	Tillering percentage	(0–136)	30.5	0.59
HLL	Husk-leaves length	(0–25.6)	8.6	0.62
COB	Percentage of red cob	(0–95)	4.1	—
TEX	Kernel texture (scale 1–4)	(1–4)	3.9	—

enzymes (*Eco*RI, *Hin*dIII and *Eco*RV) and submitted to electrophoresis according to the Southern Blot procedure described by Sambrook *et al.* (1989). Separate DNA fragments were then vacuum transferred from gels to nylon membranes.

We used 15 UMC (University of Missouri, Colombia, MO) genomic probes, eight BNL (Brookhaven National Laboratory, Upton, NY) genomic probes, two NPI (Native Plants Inc., Pioneer Hi-Bred International) genomic probes and two cDNA clones. Seven probes were assayed with *Eco*RI, 13 with *Hin*dIII, five with *Eco*RV and two with both *Eco*RI and *Hin*dIII, so that we finally analysed 29 probe–enzyme combinations. DNA probes were radiolabelled with ^{32}P-dCTP by random priming synthesis (Feinberg & Volgenstein, 1983). Hybridization was performed as described by Church & Gilbert (1984). After washing, nylon membranes were exposed to autoradiographic films.

All autoradiographic films were scanned. The ratio of the optical density of each band to total optical density of bands from the same lane was estimated using image analysis software (RFLPscan, Scanalytics). Probes were chosen that detected single loci and yielded a single band pattern, so that the ratio estimated for a band could be interpreted as the allelic frequency of an allele. For each population, we estimated allelic frequencies by the average frequencies of the two DNA pools representing this population (Fig. 1). The accuracy of this approach was established in preliminary experiments (Dubreuil *et al.*, 1999).

Nei's unbiased genetic diversity (Nei, 1978) was computed for each locus (H_{el}) and for all the loci (H_e) as

$$H_e = \frac{1}{L}\sum_{l=1}^{l=L} H_{el} \text{ and } H_{el} = \frac{2n_l}{2n_l \quad 1}\cdot\left(1 \quad \sum_{a=1}^{a=A_l}(p_{al})^2\right)$$

where p_{al} is the frequency of allele a at locus l in the whole sample, A_l is the number of alleles detected at this locus, L is the total number of loci analysed and n_l is the number of individuals characterized for locus l. Genetic diversity within a given population i was estimated similarly at each locus (H^i_{wl}) and for all loci (H^i_w). In this case, p_{al} is the frequency of allele a at locus l within the population i considered and A_l is the number of alleles detected at this locus within this population. The mean of within-population diversity among the total sample was then estimated by $\bar{H}_w = \frac{1}{P}\sum_{i=1}^{i=P} H^i_w$ with P the total number of populations. We evaluated genetic differentiation between populations as $G_{ST} = D_{ST}/H_e$ (Nei, 1973) where $D_{ST} = H_e \quad \bar{H}_w$.

Genetic distances between populations were evaluated by the Modified Rogers' Distance (Rogers, 1972; Wright, 1978) defined as

$$MRD^2_{ij} = \frac{1}{L}\sum_{l=1}^{l=L}\sum_{a=1}^{a=A_l}\frac{1}{2}\ p^i_{al} \quad p^j_{al}\Big)^2$$

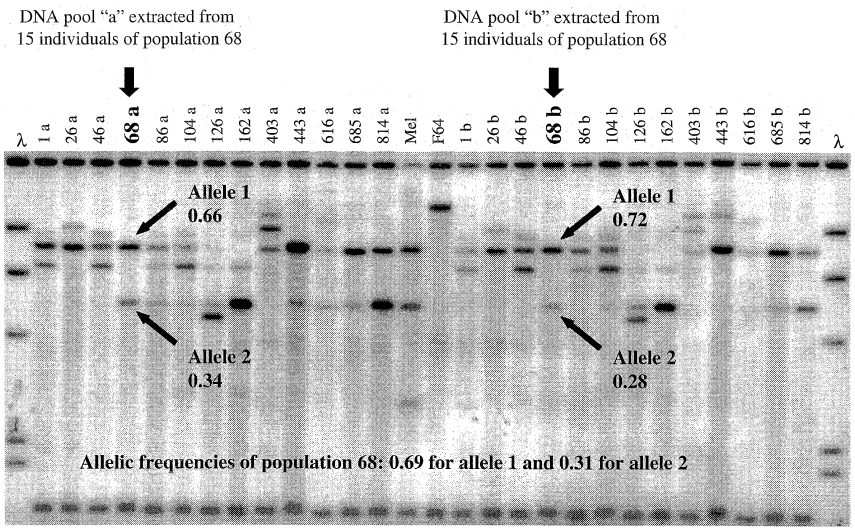

Fig. 1 Image of autoradiography resulting from hybridization of maize DNA digested by *Eco*RI with probe BNL5.09. Image analysis software allowed us to estimate allelic frequencies in each DNA pool extracted from one population (see example of population 68). The average frequencies in the two DNA pools are computed to estimate allelic frequencies within the population.

where p_{al}^{i} and p_{al}^{j} are the frequencies of allele a at locus l within populations i and j, respectively, A_l is the number of alleles detected at this locus l and L is the total number of loci analysed. We carried out a Ward's hierarchical ascendant classification (Ward, 1963) using the CLUSTER procedure from SAS (SAS Institute, 1990).

Results

Morphological variation: distribution of variables

Populations displayed dramatic variation for earliness, plant architecture traits, tassel traits, and ear and kernel characteristics, with the exception of kernel texture and cob colour that were, respectively, flint and white for most accessions. Consequently, high heritability values were observed for most traits (Table 2). Relationships between traits were investigated using graphs, correlation coefficient estimation and principal component analysis. The first four principal components (PC) accounted for 70.6% of the total variation (Table 3). In the first PC (28.2%), the most important traits were earliness and correlated traits (plant height and ear height, number of tassel primary branches). Ear diameter and kernel length also appeared important. In the second PC (19.2%), predominant traits were the ear length, correlated with the tassel length, the kernel width and associated traits (kernel weight and number of ear rows). The third PC (14%) described variation in the traits relating to the shape of ear (ear length and conicity), plant architecture (husk-leaf length and tillering) and ear kernel number. In the fourth PC (9.2%), predominant traits were the proportions of central spike and branched part in total tassel length.

Table 3 Eigenvectors, eigenvalues and accumulated variation of the first four principal components (PC) from the correlation matrix based on maize population means

	PC1	PC2	PC3	PC4
DDS	−0.73	0.05	−0.32	−0.26
PHT	−0.77	0.34	−0.21	−0.06
EHT	−0.88	0.06	−0.06	−0.17
ELG	−0.17	0.58	−0.62	−0.06
ERN	−0.51	−0.66	−0.19	0.29
ECI	−0.37	−0.35	0.66	0.15
EDI	−0.69	0.18	0.30	0.40
TLG	−0.20	0.69	−0.46	0.19
BTL	−0.47	0.04	−0.04	−0.78
CTL	−0.33	−0.06	−0.26	0.60
PBN	−0.73	−0.27	−0.20	−0.38
KLG	−0.73	0.44	0.25	0.18
KWI	−0.03	0.90	0.37	0.02
KWE	−0.23	0.81	0.49	0.08
EKN	−0.55	−0.42	−0.52	0.27
TIL	0.48	0.26	−0.48	0.03
HLL	0.46	0.32	−0.55	0.20
COB	−0.32	−0.07	−0.04	0.23
TEX	0.48	0.12	−0.10	−0.14
Eigenvalue	5.37	3.64	2.66	1.74
Accumulated variation (%)	28.2	47.4	61.4	70.6

Morphological variation: variation among populations and geographical origins

The 130 populations were plotted in a plane defined by the first two PC which accounted for 47.4% of the total variation (Fig. 2). This representation distinguished different groups with particular morphological characteristics and highlighted a relation between geographical

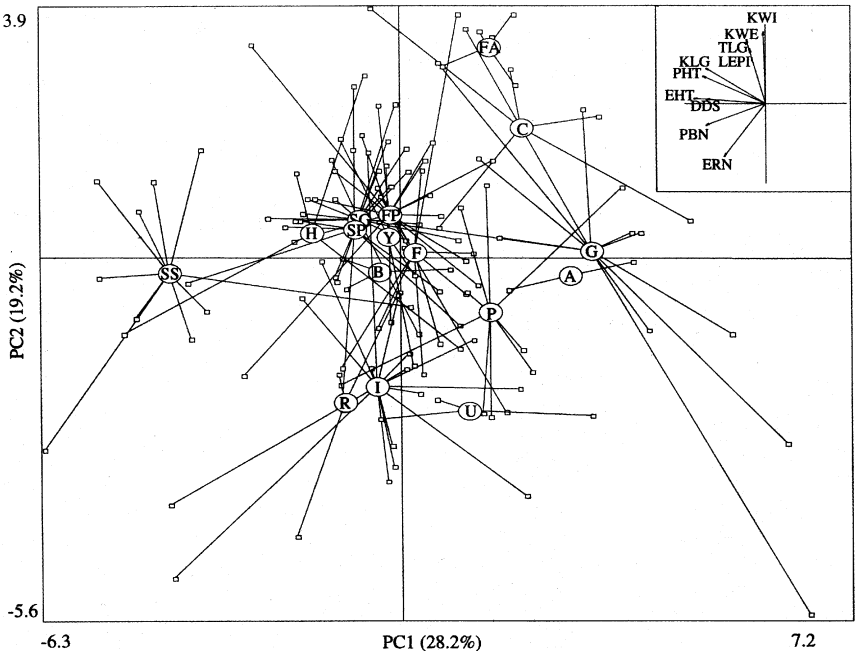

Fig. 2 Distribution of 130 European maize populations on the first two principal components PC1 and PC2 of the PCA performed for morphological data. Lines link each population to the barycentre of its geographical group, represented by letters: SS (Spain South), SP (Spain Pyrenees), SG (Spain Galicia), F (France, excepted Pyrenees and Alsace), FA (France Alsace), FP (France Pyrenees), I (Italy), G (Germany), A (Austria), P (Poland), H (Hungary), C (Czechoslovakia), B (Bulgaria), R (Romania), Y (Yugoslavia) and U (Ukraine). The most important variables for each Principal Component are represented.

origins and morphological characteristics. Axis 1 separated populations according to precocity, plant height and ear height. The populations originating from the south of Spain (group SS, Fig. 2) were tall and late whereas the populations originating from North-Eastern Europe, mainly from Germany (group G), Austria (A) and Czechoslovakia (C) were shorter and earlier. Polish populations also appeared rather early. Axis 2 separated the populations according to kernel size and number of ear rows. It distinguished in particular the Italian populations (group I), as well as the Ukrainian (U) and Rumanian populations (R), which were characterized by many ear rows and very small kernels. The plot also illustrated the distinctiveness of French Alsatian populations (group FA) when compared to other French populations (groups F and FP).

Molecular analysis: structure of polymorphism

Number of alleles A total of 278 alleles was recorded within the collection for the 29 probe–enzyme combinations. The number of alleles appeared highly dependent on the locus and varied from three (UMC47/*Eco*RI and UMC132/*Eco*RV) to 18 (SC322/*Eco*RI and BNL6.06/*Hin*dIII) with an average value of 9.59 alleles per locus (Table 4). Within-population mean number of alleles varied between populations from 1.17 (German population 29) to 3.52 (Polish population 137) with an average value of 2.50. Within-population number of alleles represented 26% of total number of alleles and therefore appeared restricted when compared to total variation. Hierarchical analysis of the number of alleles showed that differentiation (i) between geographical groups (i.e. South-Western Europe, France, Italy, North-Eastern Europe and South-Eastern Europe) (ii) between countries within groups and (iii) between populations within countries represent, respectively, 28%, 10% and 36% of total number of alleles. Those observations illustrate a clear structure for the number of alleles at these different levels.

Diversity Total diversity varied highly with the locus (Table 4) from 0.106 (BNL8.29/*Eco*RI) to 0.793 (SC322/*Eco*RI). For all loci, within-population diversity varied with the population from 0.051 (German population 29) to 0.489 (Polish population 137). Average values for total and within-population diversity were 0.550 and 0.354, respectively. The corresponding G_{ST} value of 0.356 illustrated a high relative differentiation between populations, within-population variation representing 64% of total diversity.

Differentiation (i) between geographical groups (i.e. South-Western Europe, France, Italy, North-Eastern Europe and South-Eastern Europe) (ii) between countries within groups and (iii) between populations within countries represent, respectively, 6%, 3% and 27% of diversity.

Molecular analysis: relationships between populations

Cluster analysis of the molecular data first underlined a major differentiation between populations from Northern and Southern Europe (groups A and B in Fig. 3).

Table 4 Number of alleles and diversity estimated at 29 RFLP loci in maize populations

Probe–enzyme combination	Chromosome location	Total no. of alleles	Average no. alleles per population	H_e	$\bar{H}_w$	G_{ST}
BNL5.09–*Eco*RI	9	4	1.92	0.524	0.314	0.402
BNL5.09–*Hin*dIII	9	14	3.15	0.671	0.464	0.309
BNL8.29–*Eco*RI	1	6	1.37	0.106	0.084	0.207
UMC10–*Eco*RI	3	12	3.40	0.742	0.499	0.327
UMC103–*Hin*dIII	8	8	1.88	0.291	0.233	0.199
UMC55–*Eco*RV	2	4	1.76	0.519	0.303	0.415
UMC47–*Eco*RI	4	3	1.72	0.233	0.162	0.305
UMC89–*Eco*RV	8	5	2.11	0.653	0.465	0.288
BNL5.10–*Eco*RI	9	11	3.40	0.486	0.258	0.468
UMC4–*Hin*dIII	2	8	2.79	0.772	0.526	0.319
NPI270–*Eco*RI	4	12	3.52	0.756	0.482	0.362
UMC15–*Hin*dIII	4	15	3.05	0.645	0.466	0.278
BNL7.71–*Hin*dIII	5	10	2.24	0.551	0.394	0.284
UMC19–*Hin*dIII	4	9	1.82	0.304	0.193	0.365
BNL14.28–*Hin*dIII	9	13	2.77	0.523	0.335	0.359
UMC107–*Hin*dIII	1	5	1.99	0.505	0.314	0.377
BNL7.56–*Hin*dIII	5	4	2.19	0.453	0.298	0.343
UMC161–*Eco*RI	1	5	1.82	0.465	0.269	0.422
CSU81–*Hin*dIII	7	8	2.04	0.508	0.337	0.336
BNL5.71–*Hin*dIII	5	12	2.73	0.574	0.387	0.326
UMC132–*Eco*RV	6	3	2.37	0.618	0.328	0.470
NPI406–*Hin*dIII	1	4	1.73	0.343	0.184	0.464
SC322–*Eco*RI	5	18	3.5	0.793	0.523	0.341
BNL5.10–*Hin*dIII	9	14	2.88	0.581	0.389	0.331
UMC60–*Eco*RV	3	14	2.41	0.507	0.310	0.388
UMC85–*Hin*dIII	6	11	2.52	0.689	0.417	0.395
BNL6.06–*Hin*dIII	3	18	3.85	0.784	0.524	0.332
UMC168–*Eco*RV	7	13	3.00	0.668	0.408	0.389
UMC106–*Eco*RI	1	15	2.46	0.673	0.401	0.405
All loci		9.59	2.50	0.550	0.354	0.356

H_e, total genetic diversity at each locus *l*.
$\bar{H}_w$, average within-population genetic diversity for locus *l*.
G_{ST}, relative differentiation between populations for locus *l*.

Group A included primarily populations of the North of Europe, i.e. the populations from France (centre) and Alsace, from Germany, Austria, Switzerland, Poland, Ukraine, Czechoslovakia, Hungary and Rumania. It was divided into two groups A1 and A2. The A1 group included mainly the German and Alsatian populations, referred to as 'German Flint'. The group A2 was called 'North-Eastern European Flint'. Group B consisted of populations originating from lower latitudes, i.e. populations from Spain, from the Pyrenees, from Italy or from Bulgaria. It was separated into two subgroups B1 and B2. The group B1 itself regrouped two sets: the 'Italian Orange Flint' (B1b) and various 'Southern European Flint' (group B1a, constituted of populations from southern Spain, Italy and Bulgaria). The B2 group is a very homogeneous group made up of the populations from Galicia and the Pyrenees, the 'Pyrenees-Galicia Flint', except for a small subset (B2a) which we call the 'Czechoslovakian type'. This classification into six groups explained 42% of the variation observed.

Comparison between molecular and morphological variation

Relationship between heterozygosity and performance was investigated through the comparison of diversity and morphological traits or agronomic characteristics. Significant positive correlations were observed between diversity and the height of the plants or between diversity and the weight of the kernels per ear (Fig. 4).

The relationship between morphological and molecular distances was significant ($r = 0.34$***). Analysis of the graph (Fig. 5) illustrates that low distances tend to

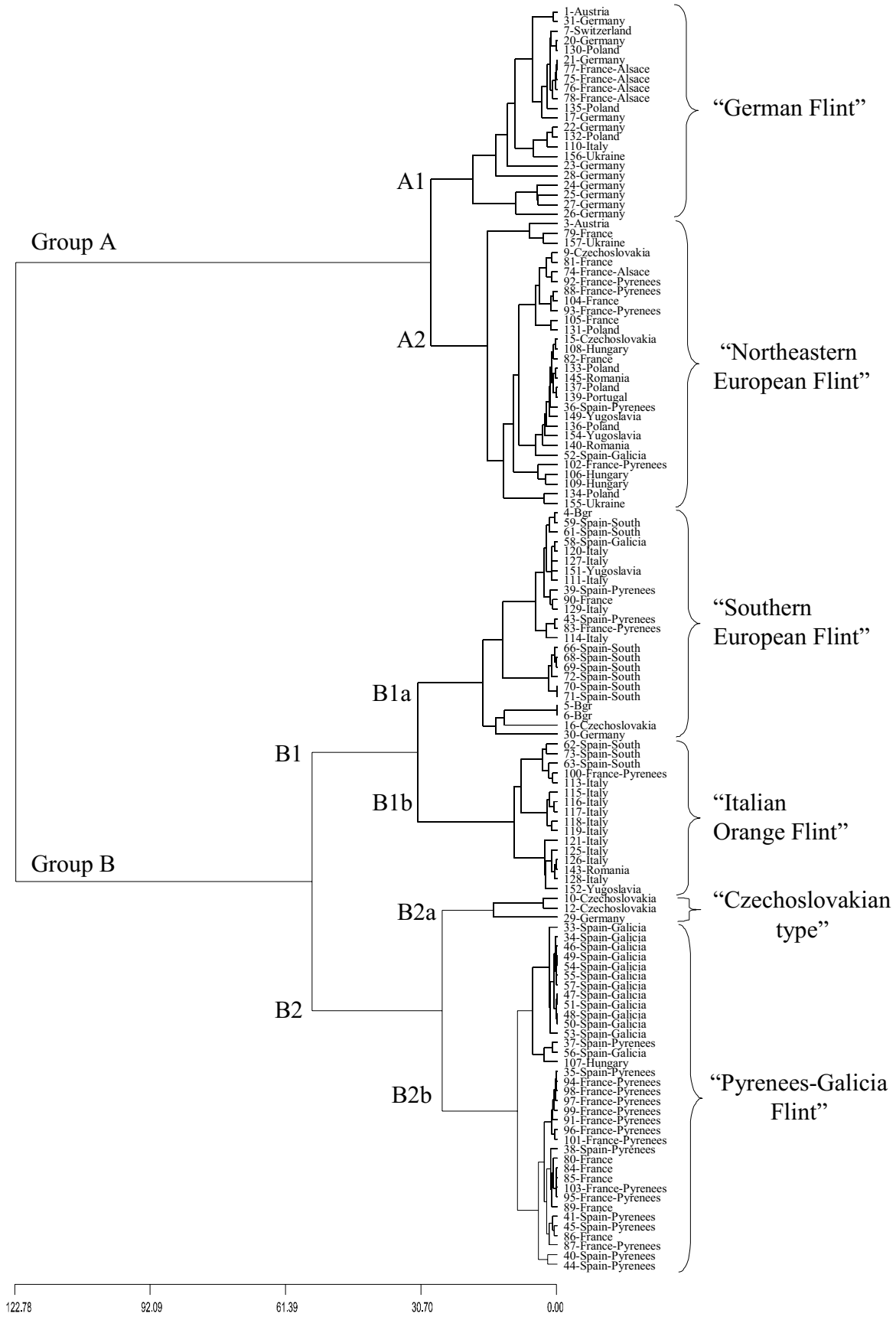
1-Austria
31-Germany
7-Switzerland
20-Germany
130-Poland
21-Germany
77-France-Alsace
75-France-Alsace
76-France-Alsace
78-France-Alsace
135-Poland
17-Germany
22-Germany
132-Poland
110-Italy
156-Ukraine
23-Germany
28-Germany
24-Germany
25-Germany
27-Germany
26-Germany
3-Austria
79-France
157-Ukraine
9-Czechoslovakia
81-France
74-France-Alsace
92-France-Pyrenees
88-France-Pyrenees
104-France
93-France-Pyrenees
105-France
131-Poland
15-Czechoslovakia
108-Hungary
82-France
133-Poland
145-Romania
137-Poland
139-Portugal
36-Spain-Pyrenees
149-Yugoslavia
136-Poland
154-Yugoslavia
140-Romania
52-Spain-Galicia
102-France-Pyrenees
106-Hungary
109-Hungary
134-Poland
155-Ukraine
4-Bgr
59-Spain-South
61-Spain-South
58-Spain-Galicia
120-Italy
127-Italy
151-Yugoslavia
111-Italy
39-Spain-Pyrenees
90-France
129-Italy
43-Spain-Pyrenees
83-France-Pyrenees
114-Italy
66-Spain-South
68-Spain-South
69-Spain-South
72-Spain-South
70-Spain-South
71-Spain-South
5-Bgr
6-Bgr
16-Czechoslovakia
30-Germany
62-Spain-South
73-Spain-South
63-Spain-South
100-France-Pyrenees
113-Italy
115-Italy
116-Italy
117-Italy
118-Italy
119-Italy
121-Italy
125-Italy
126-Italy
143-Romania
128-Italy
152-Yugoslavia
10-Czechoslovakia
12-Czechoslovakia
29-Germany
33-Spain-Galicia
34-Spain-Galicia
46-Spain-Galicia
49-Spain-Galicia
54-Spain-Galicia
55-Spain-Galicia
57-Spain-Galicia
47-Spain-Galicia
51-Spain-Galicia
48-Spain-Galicia
50-Spain-Galicia
53-Spain-Galicia
37-Spain-Pyrenees
56-Spain-Galicia
107-Hungary
35-Spain-Pyrenees
94-France-Pyrenees
98-France-Pyrenees
97-France-Pyrenees
99-France-Pyrenees
91-France-Pyrenees
96-France-Pyrenees
101-France-Pyrenees
38-Spain-Pyrenees
80-France
84-France
85-France
103-France-Pyrenees
95-France-Pyrenees
89-France
41-Spain-Pyrenees
45-Spain-Pyrenees
86-France
87-France-Pyrenees
40-Spain-Pyrenees
44-Spain-Pyrenees
Group A
Group B
A1
A2
B1
B1a
B1b
B2
B2a
B2b
"German Flint"
"Northeastern European Flint"
"Southern European Flint"
"Italian Orange Flint"
"Czechoslovakian type"
"Pyrenees-Galicia Flint"
122.78
92.09
61.39
30.70
0.00

Fig. 3 Cluster analysis of 131 European maize populations based on RFLP allele frequencies. Ward's (1963) classification based on Rogers' (1972) distance.

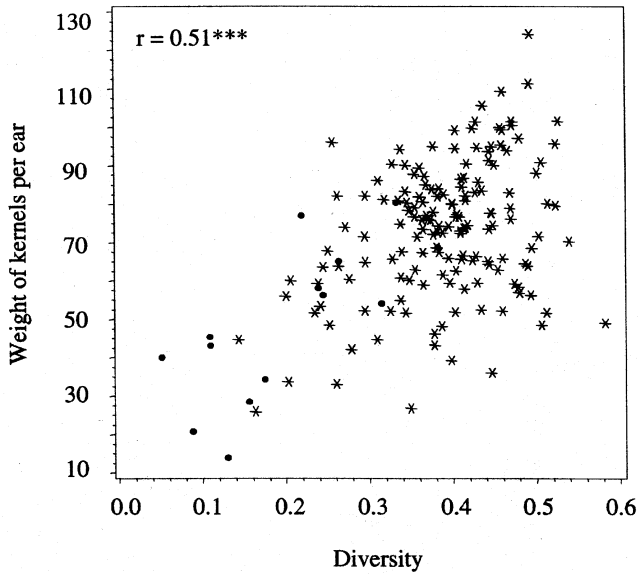

Fig. 4 Correlation between genetic diversity in maize and the weight of kernels per ear. The dots represent German and Austrian populations, the stars represent the other populations.

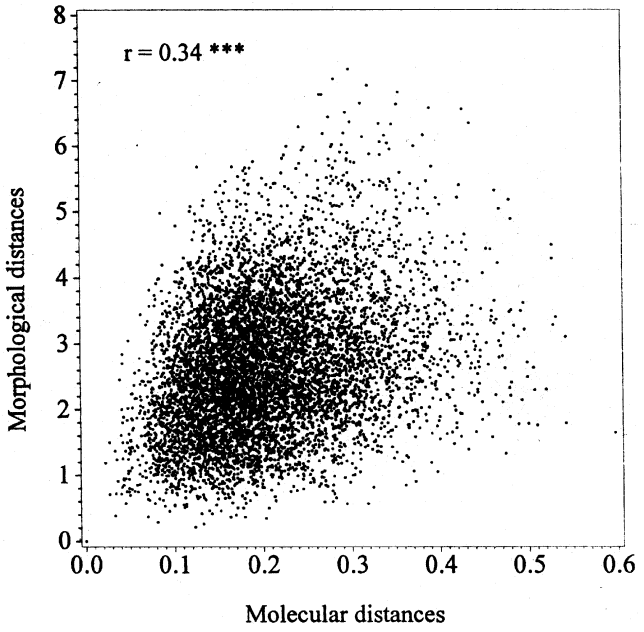

Fig. 5 Relationship between morphological and molecular distances in maize.

be associated whereas the relationship decreases as the distance increases. Some asymmetry can be observed in the sense that morphological similarity can be associated with rather high molecular divergence whereas the reverse was not clearly observed.

Discussion

Morphological variability of European germplasm

This study first confirmed that traditional European maize populations display a large range of morphological variation with the remarkable exception of kernel texture (flint) and cob colour (white). High heritability values (higher than 0.6) were observed for most traits. The traits with the highest heritability were kernel width and number of ear rows (Table 2), as previously observed by Gouesnard *et al.* (1997) for French populations. The lowest heritability values were observed for tillering, husk leaf length and ear conicity index. The Principal Components Analysis (Table 3) revealed on the first PC a very strong effect of precocity on several morphological traits: plant height, ear height and number of tassel primary branches. This major role of earliness in phenotypic variation is consistent with observations in different samples of Spanish and French populations (Llauradó & Moreno-Gonzalez, 1993; Gouesnard *et al.*, 1997). Further components were determined by traits related to ear shape and kernel shape, plant structure (i.e. tillering and husk leaf length), and tassel traits. The second PC was mostly determined by ear length and tassel length (Table 3). These two traits were highly correlated, as were number of ear rows and number of tassel primary branches. These observations are consistent with an homology between ear and tassel, consequent to the origin of the ear from the feminization of a tillering tassel (Iltis, 1983).

The PCA allowed us to distinguish groups with different morphological characteristics. In particular, two morphological types were clearly distinct: (i) Southern Spain populations (group SS, Fig. 2) consisting of tall and late plants, with few tillers and short husk leaves, and (ii) German and Austrian populations (groups G and A) consisting of shorter and early plants, with many tillers and long husk leaves. Among French populations, we could separate Alsatian populations, consisting of early plants with cylindrical ears, from the others, as was previously observed by Gouesnard *et al.* (1997).

The importance of various variables in distance computation was balanced by standardizing the Principal Components. Nevertheless, earliness still played an important role in the classification that was obtained. This is questionable because this trait is highly heritable and can

be easily modified by several cycles of selection (Hallauer, 1987, personal com.), so that maize populations with the same genetic background may have different earliness. As a consequence, morphological analysis does not seem fully appropriate for the classification of maize populations according to their genetic origin.

Molecular diversity of European germplasm

Despite being restricted to European germplasm, this study showed a high number of molecular alleles (9.59 alleles per locus) when compared to previous studies performed on either lines or populations. Dubreuil *et al.* (1996) found 5.9 alleles per locus in a large sample of 116 inbred lines and Rebourg *et al.* (1999) observed 6.6 alleles per locus in a smaller sample of 65 populations. Within-population number of alleles represented only 26% of the total number, illustrating a great contrast between populations. This was confirmed by a G_{ST} value of 36%, which is very high when compared to the work of Hamrick & Godt (1997), who observed values of G_{ST} from 23% in allogamous cereals to 59% in autogamous cereals. It also can be noted that our G_{ST} value is higher than that previously found in smaller sets of populations by Dubreuil & Charcosset (1998) and Rebourg *et al.* (1999). The significant differentiation between populations results from the choice of the sample, representative of a great geographical area. An important part of diversity was also maintained between populations within countries (27% of total diversity), which implies limited gene flow.

Variation in the number of alleles and diversity within populations was examined in the light of genetic origins (Table 5). We observed a higher number of alleles in South-Western Europe (7.90 alleles per locus) than in North-Eastern (6.24) and South-Eastern Europe (5.72). Furthermore, populations from North-Eastern Europe encompassed a smaller average within-population number of alleles (2.02 allele per locus) than populations from South-Western Europe (2.78) and South-Eastern Europe (2.53). This variation of number of alleles between North and South of Europe was previously observed by Rebourg *et al.* (1999). It is consistent with the facts (i) that South-Western Europe is considered as being the site of most introductions of maize (Gay, 1984) and (ii) that adaptation of maize to Northern and Eastern Europe probably induced a loss of genetic variability, as a result of selection for tolerance to lower temperatures.

Populations with the lowest average number of alleles or diversity originated from Germany. This may be a consequence of an intrinsic narrow genetic basis of German germplasm. However, certain populations

Table 5 Partition of allele number and diversity among geographical groups of maize populations

Group	No. of populations	Total allele number†	Average allele number‡	H_e	G_{ST}
Total sample	131	9.59	2.49	0.550	0.356
North-Eastern Europe	32	6.24	2.02	0.501	0.477
Austria	2	2.14	1.55	0.314	0.411
Switzerland	1	2.28	2.28	0.367	0.000
Czechoslovakia	5	4.21	2.14	0.490	0.435
Germany	13	4.31	1.76	0.458	0.570
Poland	8	4.86	2.56	0.492	0.258
Ukraine	3	2.69	1.74	0.461	0.438
South-Eastern Europe	14	5.72	2.53	0.542	0.310
Hungary	3	4.59	2.67	0.549	0.246
Rumania	3	4.10	2.71	0.499	0.224
Yugoslavia	4	3.93	2.47	0.491	0.261
Bulgaria	3	3.21	2.25	0.413	0.213
Italy	16	5.45	2.30	0.485	0.322
France	32	6.66	2.72	0.512	0.248
South-Western Europe	37	7.90	2.78	0.545	0.251
Spain	36	7.86	2.77	0.544	0.254
Portugal	1	3.07	3.07	0.468	0.000

H_e, total genetic diversity within the group.
G_{ST}, relative differentiation between populations in the group.
†Average per locus.
‡Within-population allele number.

appeared close to fixation for RFLP alleles (number of alleles down to 1.17 and diversity down to 0.051) and it seems more probable that they were multiplied in a manner that favoured inbreeding. Salabounat & Pernes (1986) arrived to the same conclusion concerning some populations originating from Hungary or Czechoslovakia.

Investigation of the relationship between diversity and morphological traits shows that the performance of these populations is clearly affected by inbreeding depression (Fig. 4). On the other hand, no population displayed a very high number of alleles (maximum 3.5). As a whole, Fig. 4 suggests that the diversity of European maize populations is determined, at least partly, by an equilibrium between genetic drift on the one hand and selection of heterozygotes or allopollen advantage on the other. It clearly illustrates that drift generates in some cases a strong inbreeding depression, that strongly diminishes agronomic performance.

Molecular classification of European populations

We observed a tendency towards a triangular relationship between morphological distances and molecular distances (Fig. 5), showing that similar phenotypes can be produced in genetically distant populations. This observation is consistent with results on inbred lines and theoretical analyses (Burstin & Charcosset, 1997; Dillmann *et al*., 1997). It supports the superiority of molecular data for defining groups of populations with similar origins. The best way to classify and describe genetic resources such as traditional maize populations, therefore appears to be a two-step process: first a classification based on molecular data, and secondly a morphological description of each group.

Following this method, we elaborated a classification that distinguishes six major groups that are consistent with the origins of the populations and their morphological characteristics described below.

'German Flint' These 22 populations (group A1, Fig. 3) are mainly from Germany or Alsace. They are early flowering and small. Tassels have little ramification. They have many tillers and long husk leaves. Their ears are long, fine and cylindrical, presenting 10 rows on average (from 8 to 14). These populations correspond to the 8–10 row types highlighted by Leng *et al*. (1962) during the analysis of populations originating from Italy, Yugoslavia, Romania and Hungary. They present a morphological type near to that of Northern Flint described by Brown & Anderson (1947). This group also includes some populations of the type 'broad ears with 8–12 rows' characterized by shorter and broader ears, but strongly resembling type '8–10 rows' (Edwards & Leng, 1965).

'North-Eastern European Flint' These 30 populations (group A2, Fig. 3) are from very diverse origins, mainly from France, but also Spain, Portugal and several Eastern European countries (Poland, Hungary, Yugoslavia, Czechoslovakia, Ukraine). They are of intermediate earliness and have many tillers. Their ears are long, cylindrical and consist of a variable number of rows (8–18).

'Southern European Flint' These 24 populations (group B1a, Fig. 3) originate from various countries principally of Southern Europe (southern Spain, Italy, Bulgaria). They are late, tall, and have ramified tassels. They have few tillers and short husk leaves. The ears are large, conical, with 12 rows on average. The kernels are much lengthened but not very broad.

'Italian Orange Flint' This is a rather homogeneous group (group B1b, Fig. 3), made up of 16 populations mostly originating from Italy, with some others from southern Spain. The populations are late, with ramified tassels. They have few tillers and short husk leaves. The ears are short, conical, with many rows (from 8 to 22, on average 13) and very small kernels. This type of population seems to correspond to the type 'flint with small kernels' defined by Leng *et al*. (1962) during the study of South-Eastern European corn.

'Czechoslovakian type' This group (group B2a, Fig. 3) includes only two Czechoslovakian and one German population. The populations are early, small, with poorly ramified tassels. They have many tillers and long husk leaves. The ears are fine, cylindrical and consist of eight rows with few kernels. The kernels are broad, but short. This is a rather peculiar group, as the populations present morphological characteristics close to the populations of the groups 'Germany' and 'North-Eastern Europe', but they are distantly related at the molecular level.

'Pyrenees-Galicia Flint' This group (group B2b, Fig. 3) consists of 36 populations. It can be divided into two very homogeneous subgroups, one comprising the populations from Galicia, the other populations from the Pyrenees (French and Spanish) and other regions from France. The populations are early, but tall, and have short husk leaves. Their ears are short, conical, and very broad. They have an average number of 12 rows (from 10 to 18) with very large kernels. During the morphological analysis of the collection of French populations, Gouesnard *et al*. (1997) also highlighted a 'Pyrenean type' made up of early populations with broad kernels. During a morphological analysis of

Spanish populations, Llauradó & Moreno-Gonzalez (1993) classified the populations of northern Spain into only one race. By isoenzyme analysis, Llauradó *et al.* (1993) observed differences between Galicia and Cantabria. The populations analysed here originate from Galicia and Asturia, and are close at the molecular level. Similar populations were described in Portugal under the name of 'Conico' (Costa-Rodrigues, 1971).

Conclusion

This study illustrates the potential of molecular markers for the large scale analysis of genetic resource collections, in addition to morphological descriptors. The molecular analyses were performed by RFLP analysis of DNA bulks. This technique enabled us to highlight in this European collection a great genetic diversity and a strong differentiation between populations. The analysis of bulks does not allow the estimation of heterozygote frequency, which can be prejudicial for population genetics approaches. However, it remains to date the most effective tool for the RFLP diversity analysis of a significant number of populations.

The main limitation of this technique is the number of suitable RFLP probes. It would therefore be interesting to develop other markers suitable for the analysis of DNA bulks. In particular, the use of the microsatellite markers could be interesting because of their higher polymorphism, lower cost and potential for automation. These markers should also facilitate the definition of an international 'standard'. This would help to compare results from analyses performed in different laboratories. Ultimately, it would also be interesting to develop markers within genes of known function.

The methodology which appeared as the most effective to us for the analysis and the description of large collections of genetic resources, was a two-phase process: firstly, a molecular study leading to the definition of closely related groups at the DNA level; secondly a morphological study and description of the populations from the various genetic groups. We could define six genetic groups for European maize populations. These different groups can be referred as European races. Nevertheless, some questions remain, in particular for countries which were under-represented, or not represented at all in our study. In particular, it would be interesting to gain information concerning the Portuguese material, and to study other populations from Eastern Europe to better characterize the material here defined here by only three populations. A project associating various European laboratories and coordinated by INRA at Montpellier is currently under way (web page http://meleze.ensam.inra.fr/gap/resgen88). In addition, the various European races defined here, lead to questions about their origins. These races could result from a common origin and have diverged during the adaptation of maize in Europe. However, it seems more probable that they have different origins. The joint analysis of European and American germplasm should make it possible to identify American races closest to the various European races, and to make conclusions about the introduction of maize into Europe (Rebourg, 2000).

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References

BONHOMME, R., DERIEUX, M. AND EDMEADES, G. O. 1994. Flowering of diverse maize cultivars in relation to temperature and photoperiod in multilocation field trials. *Crop Sci.*, **34**, 156–164.

BRANDOLINI, A. 1970. Razze Europee di Mais. *Maydica*, **15**, 5–27.

BROWN, W. L. AND ANDERSON, E. 1947. The Northern flint corns. *Ann. Mo. Bot. Gard.*, **34**, 1–28.

BURSTIN, J. AND CHARCOSSET, A. 1997. Relationship between phenotypic and marker distances: theoretical and experimental investigations. *Heredity*, **79**, 477–483.

CHURCH, G. M. AND GILBERT, W. 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 1991–1995.

COSTA-RODRIGUES, L. 1971. Races of maize in Portugal. *Agron Lusit.*, **31**, 239–284.

DILLMANN, C., BAR-HEN, A., GUÉRIN, D., CHARCOSSET, A. *ET AL.* 1997. Comparison of RFLP and morphological distances between maize *Zea mays* L. inbred lines. Consequences for germplasm protection purposes. *Theor. Appl. Genet.*, **95**, 92–102.

DUBREUIL, P. AND CHARCOSSET, A. 1998. Genetic diversity within and among maize populations: a comparison between isozyme and nuclear RFLP loci. *Theor. Appl. Genet.*, **96**, 577–587.

DUBREUIL, P., DUFOUR, P., KREJCI, E., CAUSSE, M., DE VIENNE, D. *ET AL.* 1996. Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. *Crop Sci.*, **36**, 790–799.

DUBREUIL, P., REBOURG, C., MERLINO, M. AND CHARCOSSET, A. 1999. The DNA-pooled sampling strategy for estimating the RFLP diversity of maize populations. *Plant Mol. Biol. Report*, **17**, 123–138.

EDWARDS, R. J. AND LENG, E. R. 1965. Classification of some indigenous maize collections from southern and south-eastern Europe. *Euphytica*, **14**, 161–169.

FEINBERG, A. AND VOLGENSTEIN, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analyt. Biochem.*, **132**, 6–13.

GARNIER-GÉRÉ, P. 1992. *Contribution à l'étude de la variabilité génétique inter- et intra-population chez le ma* (Zea mays *L.*): *Valorisation d'informations agronomiques et enzymatiques*. Thèse de Doctorat, Institut National Agronomique Paris-Grignon, Paris.

GAY, J.-P. 1984. *Fabuleux maïs: histoire et avenir d'une plante*. Association Générale de Producteurs de Maïs, Pau.

GERIC, I., ZLOKOLICA, M., GERIC, C. AND STUBER, C. W. 1989. *Races and Populations of Maize in Yugoslavia. Isozyme Variation and Genetic Diversity*. I.B.P.G.R., Rome.

GOODMAN, M. M. 1972. Distance analysis in biology. *Syst. Zool.*, **21**, 174–186.

GOUESNARD, B., DALLARD, J., PANOUILLÉ, A. AND BOYAT, A. 1997. Classification of French maize populations based on morphological traits. *Agronomie*, **17**, 491–498.

GROUPE MAÏS DGAP–INRA AND PROMAÏS. 1994. Cooperative programme for management and utilization of maize genetic resources. In: Balfourier, F. and Perretant, M. R. (eds) *Evaluation and Exploitation of Genetic Resources Pre-breeding*. Proceedings of the Genetic Resources of EUCARPIA, 15th–18th March 1994. Clermont-Ferrand, France.

HAMRICK, J. L. AND GODT, M. J. W. 1997. Allozyme diversity in cultivated crops. *Crop Sci.*, **37**, 26–30.

ILTIS, H. H. 1983. From teosinte to maize: the catastrophic sexual transmutation. *Science*, **222**, 886–894.

LEFORT-BUSON, M., LAVERGNE, V., DAUDIN, J.-J., CHARCOSSET, A. *ET AL.* 1991. Genetic variability among populations of maize germplasm 2. Enzymatic polymorphism and its relationship to quantitative trait diversity. *Maydica*, **36**, 237–246.

LENG, E. R., TAVCAR, A. AND TRIFUNOVIC, V. 1962. Maize of Southeastern Europe and its potential value in breeding programmes elsewhere. *Euphytica*, **11**, 263–272.

LIVINI, C., AJMONE-MARSAN, P., MELCHINGER, A. E., MESSMER, M. M. *ET AL.* 1992. Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLPs. *Theor. Appl. Genet.*, **84**, 17–25.

LLAURADÓ, M. AND MORENO-GONZALEZ, J. 1993. Classification of Northern Spanish populations of maize by methods of numerical taxonomy. I. Morphological traits. *Maydica*, **38**, 15–21.

LLAURADÓ, M., MORENO-GONZALEZ, J. AND ARÚS, P. 1993. Classification of Northern Spanish populations of maize by methods of numerical taxonomy. II. Isozyme variation. *Maydica*, **38**, 249–258.

MELCHINGER, A. E., MESSMER, M. M., LEE, M., WOODMAN, W. L. *ET AL.* 1991. Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms. *Crop Sci.*, **31**, 669–678.

MESSMER, M. M., MELCHINGER, A. E., BOPPENMAIER, J., BRUNKLAUS-JUNG, E. *ET AL.* 1992. Relationships among early European maize inbreds. I: genetic diversity among flint and dent lines revealed by RFLPs. *Crop Sci.*, **32**, 1301–1309.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 3321–3323.

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

ORDAS, A. AND DE RON, A. M. 1988. A method to measure conicalness in maize. *Maydica*, **33**, 261–267.

REBOURG, C. 2000. *Diversité génétique de populations européennes et américaines de ma: Analyse moléculaire et morphologique*. Thèse de doctorat, Université Paris XI, Orsay.

REBOURG, C., DUBREUIL, P. AND CHARCOSSET, A. 1999. Genetic diversity among maize populations: bulk RFLP analysis of 65 accessions. *Maydica*, **44**, 237–249.

REVILLA, P., SENGAS, P., MALVAR, R. A., CARTEA, M. E. *ET AL.* 1998. Isozyme variation and historical relationships among the maize races of Spain. *Maydica*, **43**, 175–182.

ROGERS, J. S. 1972. Measures of similarities and genetic distances. In: Wheeler, M. R. (ed.) *Studies in Genetics VII*, vol. 7213, pp. 145–153. University of Texas Publications.

SALABOUNAT, M. AND PERNES, J. 1986. Enzyme polymorphisms within and between European maize populations. *Maydica*, **31**, 269–278.

SAMBROOK, J., FRITSCH, E. F. AND MANIATIS, T. 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SAS INSTITUTE. 1990. *SAS/STAT User's Guide*. SAS Institute, Cary, NC.

TAI, T. H. AND TANKSLEY, S. D. 1990. A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. *Plant Mol. Biol. Report*, **8**, 297–303.

WARD, J. H. 1963. Hierarchical grouping to optimize an objective function. *Am. Stat. Ass. J.*, **56**, 236–244.

WRIGHT, S. 1978. *Evolution and Genetics of Populations*. University Chicago Press, Chicago.