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Maize introduction into Europe: the history reviewed in the light of molecular data

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Abstract The resolution that can be obtained from molecular genetic markers affords new prospects for understanding the dispersion of agricultural species from their primary origin centres. In order to study the introduction and the dispersion of maize in Europe, we have characterised a large and representative set of maize populations of both American and European origins for their variation at 29 restriction fragment length polymorphism loci. Polymorphism was higher for American populations than for European populations (respectively, 12.3 and 9.6 alleles per locus, on average), and only a few alleles were specific to European populations. Investigation of genetic similarity between populations from both continents made it possible to identify various types of American maize introduced into Europe at different times or in different places and which have given rise to distinctive European races. Beyond confirming the importance of Caribbean germplasm, the first maize type to be introduced into Europe, this research revealed that introductions of Northern American flint populations have played a key role in the adaptation of maize to the European climate. According to a detailed historical investigation, the introduction of these populations must have occurred shortly after the discovery of the New World.

Keywords Molecular markers · Maize · Populations · Diversity

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Introduction

Maize (*Zea mays* ssp. *mays*) was domesticated from the wild grass teosinte in Central America about 9,000 years ago (Beadle 1939). It spread northwards and southwards and was particularly abundant in the Aztec and Inca empires at the time when the New World was discovered. Despite some controversy, it became clear that maize was first introduced into Europe by Colombus, who brought it back from the West Indies to southern Spain in 1493. While subsequent introductions of maize originating from different parts of America are documented (Brandolini 1970), their relative contributions to the establishment of European maize genetic diversity have remained largely unknown. The main discourse concerning the spread of maize in Europe still refers, in social sciences especially, to a pattern of dispersion starting from its first introduction in Spain.

The relationships between lines or populations can now be efficiently described by the use of molecular markers. In particular, RFLP (restriction fragment length polymorphism) markers were successfully used to classify inbred lines according to heterotic groups (Melchinger et al. 1991; Livini et al. 1992; Messmer et al. 1992; Dubreuil et al. 1996) and to investigate the relationships between elite inbred lines and traditional population varieties (Dubreuil and Charcosset 1999). Furthermore, a simplified Bulk-RFLP method (Dubreuil et al. 1999) was developed in order to analyse large sets of maize populations. The analysis of a representative sample of the French INRA-PROMAIS gene bank was performed first (Rebourg et al. 1999), followed by the analysis of more than 450 European maize populations (Rebourg et al. 2001; Gauthier et al. 2002). These studies lead to a clas-

sification of populations in genetic groups and showed a clear differentiation according to latitude, suggesting several independent introductions of maize in Europe.

The aim of present study reported here was to use the same approach and protocols to document genetic relationships between major European groups and several American groups considered as putative sources of European germplasm. We therefore characterised – using 29 RFLP loci – a large set of 217 maize populations representative of both groups in order to (1) compare the polymorphism of European populations with that observed in America and (2) identify the contribution of different American maize types to the establishment of the main European maize races. These data were interpreted in the light of a detailed historical analysis of New World discovery.

Materials and methods

Population origin

The classification of a sample of 131 European populations was previously described (Rebourg et al. 2001). The same sample was used for the present study, with the exception of two Czechoslovakian populations (numbers 10 and 12 in Rebourg et al. 2001), which, according to the classification, appeared to have been recently contaminated by dent American hybrids. This set of 129 European populations comprised 37 southwestern European populations, 32 French populations, 16 Italian populations, 30 northeastern European populations and 14 southeastern European populations. With few exceptions, all of these European populations display a flint kernel texture. Most were supplied by the INRA-PROMAIS gene bank and various European institutes (Rebourg et al. 2001).

We sampled 88 American maize populations, representative of the main American races, with an emphasis on those that could have provided the origins of European corn according to bibliographical sources (Kupzow 1968; Brandolini 1969, 1970; Gerrish 1982). Our choice of tropical populations also took into account their sensitivity to day-length (Gouesnard et al. 2002), low sensitivity being a key factor for adaptation to temperate climates. These American populations, listed Table 1, present different types of kernel texture, principally flint, dent and popcorn. They were provided by CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo, Mexico), USDA (United States Department of Agriculture, USA) or INRA (Institut National de la Recherche Agronomique, France).

Molecular analysis

This entire set of 217 American and European maize populations was analysed using 29 RFLP loci. RFLP assays were carried out using a DNA pooled-sampling strategy, the effectiveness of which was established previously (Dubreuil et al. 1999; Rebourg et al. 1999). Each population was represented by 30 plants using two DNA bulks each extracted from the leaf disks of 15 individuals. DNA extraction, Southern blot and hybridisation were done as previously described (Rebourg et al. 2001). We used 15 UMC genomic probes (UMC 10, 103, 55, 47, 89, 4, 15, 19, 107, 161, 132, 60, 85, 168 and 106; University of Missouri, Colombia, Mo.), 8 BNL genomic probes (BNL5.09, 8.29, 5.10, B7.71, 14.28, 7.56, 5.71 and 6.06; Brookhaven National Laboratory, Upton, N.Y.), 2 NPI genomic probes (NPI270 and 406; Native Plants, Pioneer Hi-Bred International) and 2 cDNA clones (SC322 and 155). 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 ultimately analysed 29 probe-enzyme combinations (see Table 2 for chromosome position). All autoradiographic films were scanned, and the relative density of bands was estimated using image analysis software (RFLPscan, Scanalytics, Fairfax, Va.). All probes were selected as single loci according to a preliminary screening on inbred lines. A lane per comb was loaded with a DNA sample that had been extracted from a bulk of six well-characterised inbred lines (F2, F252, F278, Io, F285 and F476) in order to verify locus specificity and facilitate allele scoring. The relative density of a band within a lane was then an estimate of the allele frequency. For each population, we estimated allele frequencies as the average allele frequencies within the two DNA pools representative of this population.

Statistical analyses

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

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

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 characterised 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

$$\overline{H_w} = \frac{1}{P} \cdot \sum_{i=1}^{i=P} H_w^i$$

with P the total number of populations. We evaluated the genetic differentiation among populations (Nei 1973) by

$$G_{st} = \frac{D_{st}}{H_e} \quad \text{and} \quad D_{st} = H_e - \overline{H_w}$$

Genetic distances between populations were evaluated by the Modified Rogers' Distance (Nei 1973; Wright 1978) defined as

$$MRD_{ij}^2 = \frac{1}{L}\sum_{l=1}^{l=L}\sum_{a=1}^{a=A_l} \frac{1}{2}\left(p_{al}^i - p_{al}^j\right)^2$$

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. A dendrogram was computed using the Ward's hierarchical ascendant classification (Ward 1963).

Results

Structure of polymorphism

Polymorphism within the total sample

The number of alleles varied greatly between loci from 4 (UMC132-*Eco*RV) to 24 (BNL6.06-*Hin*dIII), with an average of 13.07 alleles per locus (Table 2). The within-population allelic richness (number of alleles per locus per population) ranged from 1.41 (BNL8.29-*Eco*RI) to 4.12 (BNL6.06-*Hin*dIII), with an average value of 2.71. This low allelic richness, relative to that of the total col-

Table 1 List of the 88 American populations analysed

Number	Population's name or race	Code[a]	Origin[b]	Number	Population's name or race	Code	Origin
158	Balsa	PPS 920	Argentina	601	Palomero Toluqueño	CHIH 150	Mexico
162	Melipeuco	PPS 939	Chili	603	Palomero Toluqueño	MEXI 5 (1)	Mexico
163	Salta Lomocura	PPS 938	Chili	604	Conico	MEXI 3	Mexico
164	Puyehue Araucano	PPS 55	Chili	605	Conico	PUEB 70	Mexico
165	Conaripe Choshuenco	PPS 949	Chili	615	Gordo	CHIH 160	Mexico
166	Choshuernco.Aesa	PPS 961	Chili	616	Gordo	CHIH 131	Mexico
168	Curacalco	PPS 941	Chili	619	Apachito	CHIH 38	Mexico
169	Puranque	PPS 1066	Chili	620	Apachito	CHIH 207	Mexico
175	Cateto Amarillo	ARGE 564	Argentina	622	Nal Tel	YUCA GP2	Mexico
176	Cateto Sulino	URUG 8A	Uruguay	626	Oloton	GUAT 606	Guatemala
177	Cuarenton, Colorado Cateto	URUG 697	Uruguay	627	Oloton	GUAT 45	Guatemala
178	Cristal Sulino	ARGE GP8	Argentina	637	San Jeronimo	APUC 171	Peru
179	Cuarento Cateto	ARGE 486	Argentina	639	Confite Puneño	APUC 140	Peru
403	Palmer Rhode Island Flint	Ames 2749	North America – NF	640	Confite Morocho	PERU 1303	Peru
404	Wakefields Rhode Island Flint	Ames 2750	North America – NF	649	Canguil	ECUA 500	Ecuador
405	Grays Rhode Island Flint	Ames 2751	North America – NF	650	Canguil	ECUA 696	Ecuador
406	Quicks Rhode Island Flint	Ames 2752	North America – NF	658	Tuson	BARB GP2	Barbuda
409	Canada Yellow Flint	Ames 2755	North America – NF	666	Chandelle	CUBA 54	Cuba
412	Concho Brown	PI 213748	North America – NF	667	Chandelle	CUBA 85	Cuba
415	Mandan Blue Flour	PI 213763	North America – NF	673	Coastal Tropical Flint	ANTI GP2	Antigua
420	Assiniboine	PI 213793	North America – NF	674	Coastal Tropical Flint	BARB 5	Barbuda
427	King Philip	PI 217460	North America – NF	676	Coastal Tropical Flint	BRVI 104	British Virgin Island
430	Sioux Tribe	PI 401755	North America – NF	681	Coastal Tropical Flint	SVIN 5	Saint Vincent
431	Gaspe Flint	PI 401757	North America – NF	685	Early Caribbean	BRVI 139	British Virgin Island
433	Gehu Flint Synth.3	PPS 220	North America – NF	689	Early Caribbean	GUAD 6	Guadalupe
434	Longfellow	PPS 1156	North America – NF	691	Early Caribbean	MART 4	Martinique
435	Smut Nose	PPS 1132	North America – NF	693	Flint Cuba	BRVI 117	British Virgin Island
436	Krug Yellow Dent	PI 213699	North America – CBD	694	Flint Cuba	CUBA 12	Cuba
437	Midland Yellow Dent	PI 213712	North America – CBD	696	Flint Cuba	CUBA 44	Cuba
438	Reids Yellow Dent	PI 213719	North America – CBD	697	Flint Cuba	CUBA 63	Cuba
441	Falconer	PI 214189	North America – CBD	717	Harinoso de Ocho	NAYA 24	Mexico
443	Pride of Saline	PI 214295	North America – CBD	722	Tuxpeño	VERA 39	Mexico
444	Lancaster Surecrop	PI 280061	North America – CBD	723	Zapalote Grande	CHIS 104	Mexico
447	Minnesota 13	PPS 471	North America – CBD	724	Zapalote Chico	CHIS 662	Mexico
449	Gourdseed Dent	PI 213715	North America – SD	727	Chapalote	SINA 2	Mexico
451	Shoepeg	PI 269743	North America – SD	728	Chapalote	SINA 6	Mexico
452	White Dent	PI 311232	North America – SD	732	Zapalote Chico	PI 217413	Mexico
453	Hickory King	PI 311237	North America – SD	735	Bolita	OAXA 223	Mexico
454	Mexican June	PI 311243	North America – SD	801	Coastal Tropical Flint	GWA2-EGT014	Guadalupe
458	Papago Flour Corn	PI 217410	North America – SW	809	Coastal Tropical Flint	GWA10-GFO059	Guadalupe
459	Tesuque Pueblo	PI 218137	North America – SW	810	Chandelle	GWA11-STB073	Guadalupe
460	Santo Domingo Pueblo	PI 218143	North America – SW	814	Early Caribbean	GWA15-DES103	Guadalupe
462	Laguna Pueblo	PI 218169	North America – SW	815	Early Caribbean	GWA16-DES106	Guadalupe
465	Hopi Selection	PPS 559	North America – SW	819	Early Caribbean	GWA20-MGA235	Guadalupe

[a] Populations provided by: INRA, Montpellier, France, code PPS; INRA, Guadeloupe, French West Indies, code GWA; USDA, North America, code Ames or PI; CIMMYT, Mexico, all the other populations

[b] North American types: NF, Northern Flint; CBD, Corn Belt Dent; SD, Southern Dent; SW, South Western

lection, illustrates a high differentiation among populations.

The genetic diversity of the total sample also varied highly between loci, from 0.090 (BNL8.29-*Eco*RI) to 0.835 (SC322-*Eco*RI) and was high on average (0.58 according to Nei's index, Table 2). Within-population diversity ranged from 0.074 to 0.575 between loci and was correlated to their total diversity. Within-population diversity value was 0.381 on average, which revealed a large contribution of population differentiation (G_{st} value of 34%) in the total diversity.

Table 2 Number of alleles and diversity estimated at 29 RFLP loci. H_e is the total genetic diversity at each locus l, $\overline{H_w}$ is the average within-population genetic diversity for locus l, $G_{st}{}^l$ is the relative differentiation between populations for locus l

Probe-enzyme combinations	Chromosome location	Total number of alleles	Average number of alleles per population	H_e	$\overline{H_w}$	G_{st}
BNL8.29-*Eco*RI	1	8	1.41	0.090	0.074	0.178
NPI406-*Hin*dIII	1	10	1.76	0.304	0.171	0.438
UMC106-*Eco*RI	1	17	2.64	0.682	0.407	0.404
UMC107-*Hin*dIII	1	8	2.14	0.539	0.350	0.351
UMC161-*Eco*RI	1	6	1.72	0.403	0.230	0.430
UMC4-*Hin*dIII	2	11	2.69	0.772	0.532	0.311
UMC55-*Eco*RV	2	6	1.82	0.515	0.314	0.390
BNL6.06-*Hin*dIII	3	24	4.12	0.786	0.512	0.349
UMC10-*Eco*RI	3	14	3.63	0.783	0.527	0.327
UMC60-*Eco*RV	3	18	2.66	0.627	0.367	0.414
NPI270-*Eco*RI	4	15	3.64	0.781	0.500	0.360
UMC15-*Hin*dIII	4	16	3.42	0.694	0.509	0.266
UMC19-*Hin*dIII	4	14	2.06	0.346	0.225	0.350
UMC47-*Eco*RI	4	6	1.80	0.256	0.177	0.309
BNL5.71-*Hin*dIII	5	15	3.11	0.710	0.449	0.368
BNL7.56-*Hin*dIII	5	6	2.13	0.421	0.283	0.327
BNL7.71-*Hin*dIII	5	11	2.54	0.614	0.442	0.280
SC322-*Eco*RI	5	23	4.01	0.835	0.575	0.312
UMC85-*Hin*dIII	6	16	2.99	0.719	0.480	0.333
UMC132-*Eco*RV	6	4	2.40	0.578	0.337	0.417
CSU81-*Hin*dIII	7	11	2.23	0.516	0.369	0.285
UMC168-*Eco*RV	7	15	3.21	0.712	0.429	0.397
UMC89-*Eco*RV	8	12	2.57	0.611	0.412	0.325
UMC103-*Hin*dIII	8	12	2.20	0.371	0.295	0.204
BNL5.09-*Eco*RI	9	7	2.12	0.552	0.346	0.373
BNL5.09-*Hin*dIII	9	20	3.87	0.743	0.525	0.293
BNL5.10-*Eco*RI	9	18	3.52	0.616	0.359	0.418
BNL5.10-*Hin*dIII	9	19	2.99	0.646	0.439	0.320
BNL14.28-*Hin*dIII	9	17	3.26	0.612	0.421	0.313
All loci		13.07	2.71	0.580	0.381	0.343

Table 3 Partition of allelic richness and diversity among geographic groups. H_e is the total genetic diversity, $\overline{H_w}$ is the average within-population genetic diversity and G_{st} is the relative differentiation between populations

	Number of populations	Allelic richness (alleles per locus) continent/world	Number of unique alleles relative to populations	Mean allelic richness within	H_e	$\overline{H_w}$	G_{st}
Total sample	217	13.07	–/–	2.71	0.580	0.381	0.343
Europe	129	9.55	–/21	2.51	0.550	0.356	0.352
Northeastern Europe	30	6.10	10/3	2.05	0.498	0.268	0.462
Southeastern Europe	14	5.72	5/1	2.53	0.542	0.374	0.310
Italy	16	5.45	8/4	2.30	0.485	0.329	0.322
France	32	6.66	12/4	2.72	0.512	0.385	0.248
Southwestern Europe	37	7.90	26/4	2.78	0.545	0.408	0.251
America	88	12.34	–/102	3.01	0.587	0.417	0.290
Northern America	31	8.62	21/11	3.04	0.594	0.432	0.273
Central America	19	9.97	43/28	3.25	0.574	0.444	0.226
Caribbean Islands	20	6.66	8/2	2.67	0.467	0.369	0.210
North Andean region	5	5.28	6/5	2.70	0.499	0.372	0.255
Southern South America	13	6.93	7/5	3.25	0.548	0.433	0.210

Variation of polymorphism according to the geographic origin of populations

The number of alleles per locus and the within-population diversity varied greatly between populations according to their geographic origin. The less polymorphic populations displayed approximately 1.2 alleles per locus and a within-population diversity lower than 0.100 (German populations 28 and 29). The most polymorphic populations displayed more than four alleles per locus and a within-population diversity higher than 0.53 (Mexican populations 604 and 620, and North-American populations 452 and 462). American populations displayed a higher polymorphism (12.34 alleles per locus on average) than those from Europe (9.55 alleles per locus on average, Table 3). The number of continent-spe-

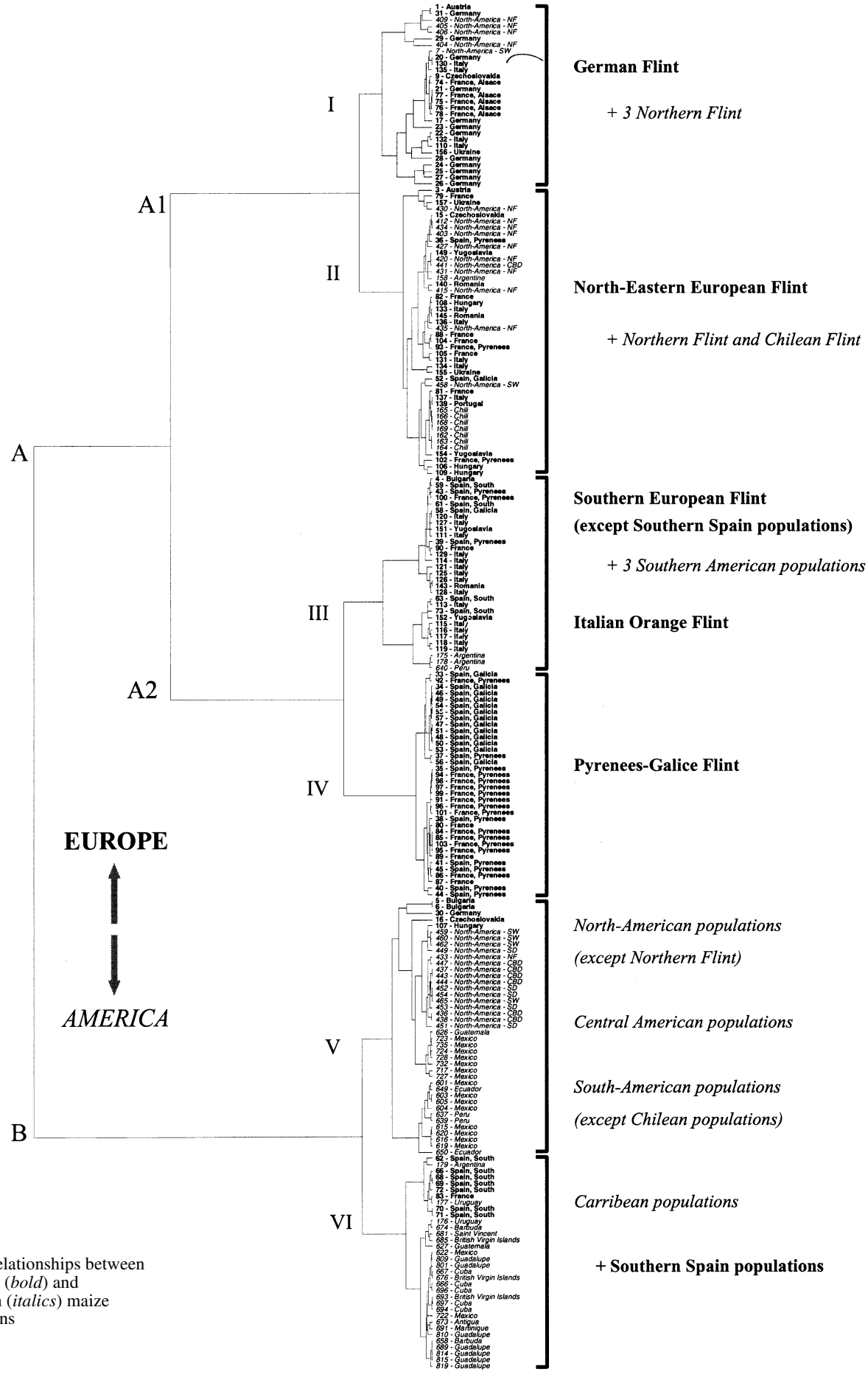

Fig. 1 Relationships between European (*bold*) and American (*italics*) maize populations

cific alleles was clearly lower for Europe (21) than for America (102). This discrepancy is mostly accounted for by Central American populations that showed a total number of 28 unique alleles (Table 3). Consistent with allelic richness, diversity was higher within the American groups than within European groups. The relative differentiation of populations was also higher for Europe (G_{st} = 0.352) than for America (G_{st} = 0.290), indicating a greater level of geneflow between populations.

Relationships between populations

Relationships between populations were investigated using hierarchical clustering. Coordinates of the populations on the first two axes of a principal component analysis were highly consistent with this classification and were not presented here for the sake of simplicity. Results (Fig. 1) illustrate the global specificity of European germplasm (Group A), compared to American germplasm (B). European germplasm is structured according to latitude (groups A1 vs. A2), as previously observed in European germplasm analysis, which classified the populations into five principal European races (Rebourg et al. 2001): The "German Flint", the "North-Eastern European Flint", the "Southern European Flint", the "Pyrenees-Galice Flint" and the "Italian orange Flint". Cluster B is mainly composed of American populations and comprises two main groups. Group V includes Central American populations, some Andean populations, and populations from North America, with the exception of Northern Flints. Group VI includes all of the Caribbean populations.

A very close proximity was observed between Northern Flints and Chilean populations, which is consistent with morphological and biological similarities (Rebourg 2000): precocity, many tillers and long husk leaves, cylindrical ears with few rows, and flint kernels.

Relationships between European and American populations

Six populations from southern Spain and one from the Pyrenees were closely related to the Caribbean populations (group VI). Some alleles specific to the Caribbean populations compared to other American samples were also found in Europe within the southern Spain and Pyrenean populations (probe-enzyme combination BNL5.09/*Hin*dIII, BNL8.29/*Eco*RI, SC155/*Hin*dIII, BNL5.10/*Hin*dIII and UMC60/*Eco*RV). A second suggestive relationship was the proximity of the Italian orange Flints with two Argentinean populations and one Peruvian population. These populations also share some ear characteristics, in particular small and hard orange kernels (Rebourg 2000).

Striking similarities were also apparent between American Northern Flint populations and North European populations. Northern Flint germplasm was close to German Flint and especially to North-Eastern European Flint. These similarities were also striking at a morphological level (Rebourg 2000), with very specific plant architecture (high tillering with long husk leaves) and ear characteristics (cylindrical with few rows).

Discussion

Molecular diversity within the collection

This study revealed a dramatic genetic diversity (13.07 alleles per locus on average), which was higher than that found in earlier RFLP studies of maize populations (Dubreuil and Charcosset 1998; Rebourg et al. 1999, 2001). This can be explained by the larger size of the sample and a higher diversity of geographical origins. We also found a low number of alleles within populations (2.71 alleles par locus on average) when compared to the total number of alleles. This strong difference may have been accentuated by the lower sensitivity of the bulk approach, which can not guarantee the detection of alleles with a frequency below 0.05 (Dubreuil et al. 1999). However, the G_{st} statistics is only slightly sensitive to the non-detection of rare alleles (which only have a very limited contribution to diversity estimation) and also showed strong differentiation between populations. According to this parameter, population differentiation accounted for 34.3% of the total diversity, which was higher than that previously estimated in maize (Lefort-Buson et al. 1991; Dubreuil and Charcosset 1998; Rebourg et al. 1999) and allogamous cereals (Hamrick and Godt 1997).

A higher polymorphism was observed for American populations than for European populations, which is consistent with studies carried out earlier with isozymes (Lefort-Buson et al. 1991) or RFLP molecular markers (Rebourg et al. 1999). This polymorphism is particularly high in Central America, suggesting a trend in a reduction of maize polymorphism as the geographic distance from the centre of domestication increases. It also suggests a "bottleneck" effect occurring during the introduction of maize in Europe and/or a possible loss of diversity due to a selective adaptation to European conditions.

Relationships within American populations

Despite a specific emphasis given to American populations representative of putative sources of European germplasm, our results confirmed the exceptional genetic divergence between Northern Flints and other North-American populations previously observed by allozyme analysis (Doebley et al. 1986). The genetic proximity between these Northern Flint populations and Chilean populations from the Chiloe Island, also observed at the morphological level (Rebourg 2000), suggests a common genetic origin, the dating of which remains to be determined.

Origin of European germplasm

Several associations between European and American germplasm were established, and these provide new insights into the genetic origin of European maize. Three associations appear particularly significant (Fig. 1).

1. The association between six southern Spain (and one Pyrenean) populations and Caribbean populations. This observation is consistent with historical data that mention the introduction into Spain of maize native to the Caribbean Islands shortly after the discovery of the New World. Our results show that these first introductions remained confined to southern Spain, which is consistent with (1) bibliographical data mentioning that this germplasm was poorly adapted to European conditions and did not spread widely (Brandolini 1969) and (2) isozyme results of Revilla et al. (1998).
2. The similarity between (1) the Italian orange Flint populations and (2) two Argentinean and a Peruvian population. The Italian orange Flint type seems to be derived from the South American *Cateto* type, which is characterised by ears with a high number of rows and small hard orange flint kernels. However, this putative *Cateto* origin of Italian populations requires further research and additional analyses of South American populations not covered in this study.
3. The similarity between American Northern Flint populations and North European populations. This association strongly supports the hypothesis that present-day North and Eastern European Flint germplasm is directly derived from American Northern Flint populations. Northern Flint populations are relatively insensitive to daylength and have low temperature requirements for flowering. Earliness is a key factor for adaptation to temperate climates and likely played a key role in the establishment of this germplasm in Europe. According to bibliographical sources, Northern Flints would have been introduced in Europe from the 17th century onwards (Brandolini 1969). The presence of maize in Germany was reliably attested to as early as 1539 in the work of the German herbalist Jerome Bock (Finan 1948). The plant became rapidly common in this country, since another German herbalist, Leonhard Fuchs, wrote in 1542 that "*it is now growing in all gardens, almost everywhere*" (Fuchs 1549, 1st edn 1542). This suggests either that (1) previous maize introductions in Northern Europe were replaced by Northern Flint introductions in the 17th century or (2) Northern Flint introductions occurred earlier.

The hypothesis of an introduction of Northern Flints in the first half of the 16th century: historical investigation

The hypothesis of an early introduction of Northern Flints into Europe was previously suggested by Finan (1948) in a detailed study on maize in the great herbals of the Renaissance but has not been re-examined since.

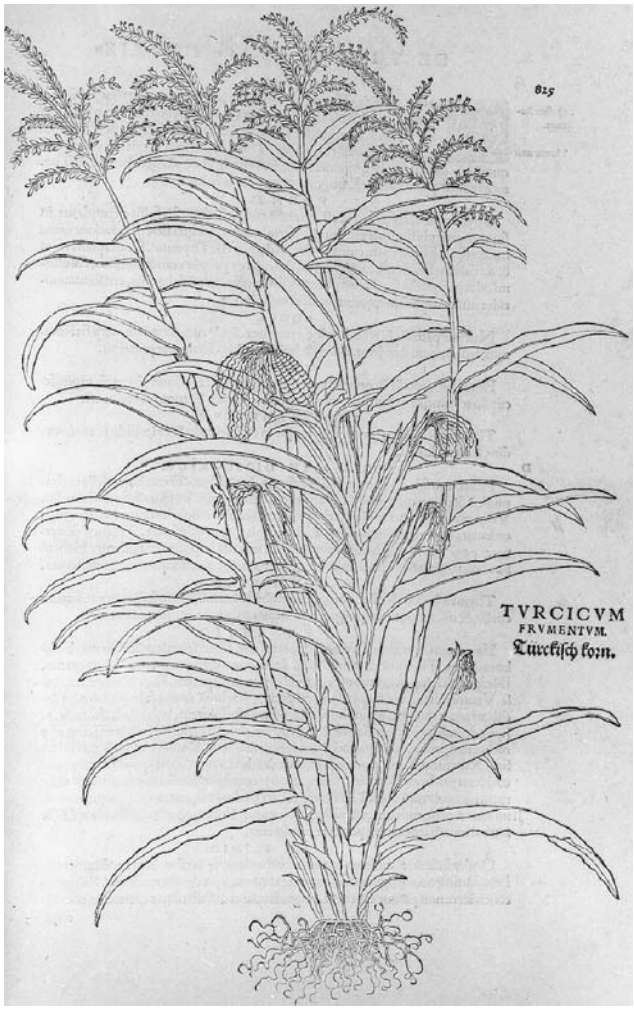

Fig. 2 The first illustration of maize in the Renaissance herbals (Fuchs, De historia stirpium, Bâle, Isingrin, 1542) © Bibliothéque centrale MNHN Paris 2002

Fuchs' description and the woodcut which illustrates it (Fig. 2), the first drawing of maize in a herbal, present a type of maize (ears of eight to ten rows, long husk leaves, tillering, absence of prop roots) that suggests an introduction of Northern Flints into Europe in the first half of the 16th century. In those days, many contacts were established between Europeans and Native Americans in the cultivation area of the Northern Flints which, according to archaeological and genetic studies, stretched from the Saint Laurent River to South Carolina (Brown and Anderson 1947; Feest 1978). Among several possible scenarios for the introduction of Northern Flint into Europe during the 16th century, the travels of Giovanni Verrazano and Jacques Cartier, both accomplished on behalf of the King François I of France, must be carefully investigated.

The "Relation" of Verrazano (1524, see Mollat du Jourdin and Habert 1982) is considered to be the first description of Native Americans on the East Coast of the United States, north of Florida. However, its contribution

to the history of maize introduction into Europe has remained unknown until now, due to a terminological problem. Verrazano wrote about the Indians of "Arcadia", near Chesapeake Bay (Virginia): "*Their food generally consists in legumes, abundant and different from ours in colour and size, but excellent and delectable*". He made the same observation at the "Refuge", in Narragansett bay (Rhode Island), where he also mentioned the sowing, which he may have attended. At that time in Europe, "*legumes*" were pod plants, hand-harvested, like peas or faba beans, as opposed to "*bleds*" (all sorts of cereals used for making bread), "*grasses*" and "*roots*". This view is also found in Furetière's Dictionnaire Universel (1978; 1st edn 1690): see the definitions of "*Blé*", "*Blé de Turquie*", "*Légume*" and "*Mays*". Verrazanno may have used "*legumes*" to describe beans, but we think that he also, and mostly, used this term for maize, a plant still unknown to him. In doing so, he implicitly compared the plant with "*peas*", a frequent occurrence among 16th century authors (see for example d'Anghiera 1907; 1st edn 1530). This hypothesis is supported by different records about the French expedition to Florida in the mid-16th century: maize, also named "*mil*" (a French term for pearl millet), was described as having "*a grain as big as a pea*" and was presented as "*one of the main legums* (*sic*) *of their food*" (Lussagnet 1958). Therefore, we can conclude that Verazzano saw – and ate – maize. Although we did not find any direct mention of it, it is likely that maize was transported on the return trip of "la Dauphine", since Verrazanno showed an interest in Native American resources and was faced with the necessity to gather food supplies, all initial stocks having been consumed during his crew's stay in America (Mollat du Jourdin and Habert 1982).

Jacques Cartier also mentioned maize several times during his first two voyages, in 1534 and in 1535–1536. Two events deserve special attention. On 8 September 1535, the Stadaconan Indians of "Orléans Island" (Quebec City) gave a feast to welcome the return of two men captured by Cartier during his first travel. They organised "*several ceremonies*" and brought to the French fish and "*two or three bulks of gros mil*" (a French term for sorghum, which refers here to maize) (Cartier 1992, 1st edn 1545). On 3 May 1536, returning to the same region, Cartier captured the chief of Stadacona and several of his companions. The following day, Native American women brought food to them, including maize, in preparation for the sea crossing. In neither case did Cartier mention whether this maize consisted in ears, grains or flour. It was indeed in flour form that Indians kept maize, putting aside the most beautiful ears for sowing (Heidenreich 1978; Simmons 1978). In May 1536, the food given by the Native Americans might have been flour, which would be easier to use during a return travel. In September 1535, the offerings of maize were part of a celebration that took place during the harvesting season, so it is likely they were then in the form of maize ears. Despite the fact that we have not found any direct record up to now, both Verrazano and Cartier were given several opportunities to introduce Northern Flint maize into France shortly after the New World's discovery.

Beyond their mere introduction and dispersion into northern Europe, Northern Flint populations also played a key role in the climatic adaptation and establishment of maize in Europe's middle latitudes. Maize has been a very common crop in northwestern Spain, in the Pyrenees and in southwestern France since the late 16th and early 17th centuries. Populations from these regions display no close relationship with any American types, whereas they share common alleles with both: (1) Caribbean and southern Spain late-maturing populations and (2) North American and European flints. This strongly supports the idea of hybridisation between northern and southern introductions.

Conclusion

Combining results from a large-scale molecular analysis of genetic diversity and a historical approach provides a new view of the processes involved in the geographic expansion and climatic adaptation of European maize. The introduction of early North-American flint populations (Northern Flints) into Europe occurred much sooner than previously assumed and made a major contribution to the germplasm of traditional varieties in most European regions. Maize adaptation to European conditions should therefore be viewed in terms of hybridisation between two germplasms, one related to northern introductions and the other coming from southern introductions, rather than as a slow northward dispersion accompanied by selection for earliness.

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