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HETEROTIC PATTERNS AMONG FRENCH AND SPANISH MAIZE POPULATIONS

P. Revilla[1,*], A. Boyat[2], Á. Álvarez[3], B. Gouesnard[2], P. Soengas[1], A. Ordás[1], R.A. Malvar[1]

[1] *Misión Biológica de Galicia, Spanish Council for Scientific Research, Apartado 28, 36080 Pontevedra, Spain*
[2] *Institut National de la Recherche Agronomique, Centre de Montpellier, Unité Mixte de Recherches "Diversité et Génômes des Plantes Cultivées", Domaine de Melgueil, 34130 Mauguio, France*
[3] *Estación Experimental de Aula Dei, Spanish Council for Scientific Research, Apartado 202, 50080 Zaragoza, Spain*

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ABSTRACT - The objective of this work was to identify heterotic patterns among maize (*Zea mays* L.) populations representing the variability of Southwestern Europe. Six Spanish and six French maize populations were crossed in a complete diallel without reciprocals. The average yield of hybrids and populations was 6.1 t ha^{-1}; mid-parent heterosis for yield was 22.0%, ranging from 12.2% to 51.9%. The population Lazcano produced the hybrids with highest yield, followed by Tuy, Rastrojero and Millette du Lauragais (ML). The environment × population interaction was significant for yield, therefore each environment was analyzed separately in order to determine the best heterotic pattern for each environment. The heterotic patterns suggested were Tuy × Rastrojero (Pontevedra), Rastrojero × ML (Zaragoza), Bade × Millette Montagne Noire (Mauguio), and Tuy × Lazcano (Saint Martin de Hinx). The most promising early heterotic pattern was Bade × Esterre. The most promising combination across sites was Lazcano × ML with the highest yield (7.7 t ha^{-1}) and a mean heterosis of 30.7%. The cluster analysis based on mid-parent heterosis showed three clusters, corresponding to the dry areas of Spain, northern Spain, and southern France. These clusters are consistent with previous knowledge based on history, isozymes, and RFLP. The potential heterotic patterns are dry Spain × humid Spain or dry Spain × south France, although within these groups there were also some favorable combinations.

KEY WORDS: *Zea mays*; Heterosis; Heterotic patterns.

INTRODUCTION

European maize has better adaptation to European conditions but generally lower yield than American germplasm. Currently the best hybrid combinations follow the well-known heterotic pattern European flint × U.S. dent (MORENO-GONZÁLEZ, 1988; MISEVIC, 1989; ORDÁS, 1991; GARAY *et al.*, 1996a,b; SINOBAS and MONTEAGUDO, 1996). U.S. hybrids are adapted to the southern Europe and 'dent × dent' hybrids selected for early maturity could replace the 'flint × dent' hybrids in more northern regions (MORENO-GONZÁLEZ *et al.*, 1997). However, European maize can be used as a source of adaptation.

European maize is not homogeneous, based on the heterotic patterns found within countries (MISEVIC, 1989; ORDÁS, 1991; RADOVIC and JELOVAC, 1995), combining abilities among inbreds from different countries (REVILLA *et al.*, 2002), isozymes (REVILLA *et al.*, 1998, 2003), and RFLP markers (MESSMER *et al.*, 1992, 1993; REBOURG *et al.*, 2001; GAUTHIER *et al.*, 2002). European flint inbreds have revealed some differences based on their combining ability in hybrids to inbreds from U.S. heterotic groups (MORENO-GONZÁLEZ, 1988; CARTEA *et al.*, 1999). MISEVIC (1989) and RADOVIC and JELOVAC (1995) studied heterotic patterns among Yugoslavian populations. ORDÁS (1991) found a heterotic pattern among northern and southern Spanish populations. REVILLA *et al.* (2002) suggested a 'north-central Europe × southern Europe' heterotic pattern from a diallel cross among nine inbred lines from several European countries.

Based on isozymes (REVILLA *et al.*, 1998, 2003) and RFLPs (MESSMER *et al.*, 1992, 1993; REBOURG *et al.*, 2001; GAUTHIER *et al.*, 2002), European maize populations have been classified into two main groups. The smaller group contains germplasm from northern Europe while the largest one groups together populations from southwest and southeast Europe and has a higher allelic richness than the smaller group. Allelic richness and the number of unique alleles are largest in the south: Portugal, Spain, and Italy (GAUTHIER *et al.*, 2002), where sev-

* For correspondence (fax: 34 986 841362; e.mail: previlla@mbg.cesga.es).

eral navigators are known to have introduced maize populations from America during the 16th century. Spain was one of the main doorways for the entrance of maize to Europe. Mediterranean maize likely came from Central America, Mexico, Guatemala, and the Caribbean Islands, while maize in northern and western Europe maize could have been introduced from North America, mainly the USA, via the Atlantic coast. The former introductions started at the end of the 15th century, while the later ones are documented around the beginning of the 17th century (REVILLA *et al.*, 1998, 2003).

RFLP studies of a southwestern European collection of maize populations (REBOURG *et al.*, 2001; GAUTHIER *et al.*, 2002) revealed that the French germplasm is distributed in different clusters. The northeast European cluster includes all populations from Alsace. The southwest European cluster includes populations from the Pyrenees. The southeast European cluster also includes French populations. The fact that populations from France – except those from Alsace – tend to be distributed among the three main clusters suggests mixed origins and possibly hybridization in these regions. Although maize is not a major crop in Spain, its variability is comparable to that in the main maize producing countries of Europe because most of the earlier historical introductions of maize into Europe came through Spain (REVILLA *et al.*, 2003). France is the largest maize producer in Europe, ranking fifth in world maize supply.

MALVAR *et al.* (2005) studied the genotype × environment interaction for crosses among Spanish and French maize populations and concluded that different varieties or crosses should be used as base breeding germplasm for each of the French and Spanish breeding stations included in the study. The objective of this work was to identify heterotic patterns among maize populations representing the variability of Southwestern Europe.

MATERIALS AND METHODS

Six Spanish and six French maize populations (Table 1) were crossed in a complete diallel without reciprocals in 1999, using paired rows with 15 plants per row. Five sets of paired rows were used for each cross, using each plant once, as male or female. Also, each variety was increased to obtain seeds in the same environmental conditions.

The 12 parental varieties and their 66 hybrids were planted in Pontevedra (northwestern Spain; lat. 42°24'N, long. 8°38'W, 20 m above sea level), Zaragoza (eastern Spain; lat. 41°44'N, long. 0°47'W, 250 m above sea level), Mauguio (southeastern France; lat. 43°36'N, long. 3°51'W, 13 m above sea level), and Saint Martin de Hinx (southwestern France; lat. 43°34' N, long. 1°18'W, 40 m above sea level) during 2000 and 2001. Three commercial va-

TABLE 1 - *Accession name and number and origin of the open-pollinated maize varieties crossed in a diallel fashion in 1999.*

Accession Name	Accession number	Origin	
		Country	Region (Latitude[1], Longitude, Elevation)
Tuy	ESP0090205	Spain	Galicia (N4205, W00865, 30 m a.s.l.)
Viana	ESP0090214	Spain	Galicia (N4218, W00710, 700 m a.s.l.)
Lazcano	ESP0070892	Spain	Basque Country (N4303, W00210, 630 m a.s.l.)
BR[23]	ESP0090338	Spain	Andalucia
Rastrojero[3]	ESP0090032	Spain	Ebro river valley
ElH[23]	ESP0090025	Spain	South and East
Bade	FRA0410006	France	Alsace
Lacaune	FRA0410015	France	Midi-Pyrenees (N4333, E00235, 813 m a.s.l.)
Esterre	FRA0410022	France	Midi-Pyrenees (N4252, E00000, 1087 m a.s.l.
Ain	FRA0410474	France	Rhone-Alpes
ML[2]	FRA0410639	France	Languedoc Roussillon
MMN[2]	FRA0410668	France	Languedoc Roussillon (N4323, E00216, 741 m a.s.l.)

[1] Latitude and longitude are abbreviated, the first two digits correspond to the degrees and the next two digits to the minutes.
[2] Abbreviations correspond to: BR for Basto/Rastrojero, ElH for Enano levantino/Hembrilla, ML for Millette du Lauragais, and MMN for Millette Montagne Noire.
[3] These populations were synthesized by SÁNCHEZ–MONGE (1962) from crosses among two or more open-pollinated populations. Two of them, Basto/Rastrojero and Rastrojero are the only semi-dent populations, while the other ten populations are flint.

TABLE 2 - *Mean squares from the analysis of variance of yield and yield components for a diallel of twelve French and Spanish populations grown in two Spanish and two French locations during 2000 and 2001.*

Source of variation	Degrees of freedom[1]	Grain yield	Grain moisture	100-kernel weight	Degrees of freedom[2]	Ear length	Kernel rows
Environments	7 (6)	127.41*	2184.36*	116298.12*	3	390110.01*	6.97*
Populations	77	6.74*	48.33*	10635.54*	77	134.49*	7.30*
Varieties (v_j)	11	28.83*	324.01*	66586.9*	11	747.28	47.04*
Heterosis (h_{ij})	66	3.05*	2.39*	1310.31*	66	32.35*	0.67
Average (h)	1	146.21*	7.69*	43288.91*	1	1218.95	0.94
Variety (h_j)	11	0.58*	3.15*	1041.90*	11	19.76	1.02
Specific (s_{ij})	54	0.91*	2.13*	587.61*	54	12.94	0.60*
Environments × populations	539 (462)	0.61*	1.14*	256.64	231	84.58*	1.23*
Environments × v_j	77 (66)	2.52*	3.78*	672.85	33	462.72*	4.64*
Environments × h_{ij}	462 (396)	0.29*	0.70*	187.27	198	21.55*	0.66*
Environments × h	7 (6)	1.08*	1.69*	618.59	3	715.15*	0.58*
Environments × h_j	77 (66)	0.26*	0.85*	230.68	33	17.99*	1.99*
Environments × s_{ij}	378 (324)	0.28*	0.65	170.44	162	9.43*	0.39*
Error	[3]	0.17	0.49	203.55	[3]	6.39	0.13

* Significant at P = 0.05.

[1] Degrees of freedom for 100-kernel weight are between parenthesis. The difference was due to lack of data from one of the locations in one year.

[2] Degrees of freedom for ear length and number of kernel rows were different because these traits were recorded only in the two Spanish locations.

[3] Degrees of freedom for the error term were 1207 for yield, 1170 for grain moisture content, 1045 for 100-kernel weight, 592 for ear length, and 640 for kernel rows.

rieties were included as checks to complete a 9 × 9 triple partially balanced lattice design. However, due to lack of seed for the second year, the commercial varieties changed for the different stations and only the commercial check Dunia was common to all environments.

Each experimental plot consisted of two rows spaced 0.80 m apart, with 25 two-plant hills spaced 0.21 m apart. Plots were overplanted and thinned, obtaining a final density of approximately 60,000 plants ha^{-1}. Data taken for each plot were days to silking, grain yield (t ha^{-1} at 140 g kg^{-1} of moisture content), grain moisture content (g kg^{-1}), 100 kernel weight (g), ear length (cm), and number of kernel rows.

Individual analyses of variance were carried out for each environment (COCHRAN and COX, 1957). If the relative effectiveness of the lattice design was smaller than 105% for a trait, the data were analyzed as a randomized complete block design. Analyses were performed with PROC LATTICE (SAS, 2000). Combined analyses of lattices were made with adjusted treatment means, using PROC GLM (SAS, 2000). Treatment mean squares were orthogonally partitioned into diallel populations (parental varieties and hybrids), check varieties, and among groups. Diallel populations were divided according to the Analysis II of GARDNER and EBERHART (1966). The standard errors were calculated following GRIFFING (1956) and MORENO-GONZÁLEZ *et al.* (1997), and the standard error of heterosis was calculated as the square root of 1.5 times the variance of the combined error (KEERATINIJAKAL and LAMKEY, 1993). A cluster analysis was conducted to determine the relationships among parental varieties, with the unweighted pair-group method using arithmetic averages (UPGMA) (ROMESBURG, 1984) method and the mid-parent heterosis for yield as a measure of the dissimilarity between any two populations. Cluster analyses were performed with the NTSYS-PC (1997) program.

RESULTS AND DISCUSSION

Combined analysis of variance

The combined analysis of variance for grain yield showed significant differences for all sources of variation, including environment × population interaction (Table 2). For grain moisture content, all sources of variation were significant except the specific heterosis × environment interaction. For 100-kernel weight, all sources of variation were significant except for the genotype × environment interactions.

Yield of populations and hybrids averaged 6.1 t ha^{-1}, which was half of the commercial check Dunia (12.2 t ha^{-1}). The populations Rastrojero (6.0 t ha^{-1}), Enano levantino/Hembrilla (ElH) (5.9 t ha^{-1}), Lazcano (5.9 t ha^{-1}), Tuy (5.8 t ha^{-1}), and Millette du Lauragais (ML) (5.7 t ha^{-1}) had a significantly higher yield than each of the other populations. While Rastrojero was also the population with the highest moisture content, the other high yielding populations had low grain moisture content, particularly Lazcano. Among the 14 hybrids having the highest yield, Lazcano was involved in six hybrids, and Tuy, Rastrojero and ML in four hybrids each. Four of the five hybrids with a high moisture content involved Rastrojero. Viana and Lacaune yielded low and produced low yielding hybrids, and Lacaune had low grain moisture content (Table 3).

The average mid-parent heterosis for yield was 22.0%, and ranged from 12.2% for a hybrid between two southern Spanish populations Rastrojero × ElH, to 51.9% for Ain × Basto/Rastrojero. PÉREZ-VELÁSQUEZ *et al.* (1995) found similar mean heterosis (20.0%) among Colombian populations, and ORDÁS (1991) among Spanish populations (21.1%). ORDÁS (1991) also found average heterosis of 15.2% for 'Northern Spain × Southern Spain', 16.3% for 'Northern Spain × US dent', and 15.2% for 'Southern Spain × US dent'. SOENGAS *et al.* (2003) found larger average heterosis (30.0%) among flint populations, which ranged from 2.5 to 64.7%. OYERVIDES-GARCÍA *et al.* (1985) reported values of 34.8% between BSSS and Lancaster populations.

Individual analysis of variance: variety and heterosis effects

The environment × population interaction was significant for several traits, including grain yield, for which the interactions affected the rank of populations crosses (Table 2). MALVAR *et al.* (2005) had already concluded that specific heterotic patterns should be defined for each breeding station, although some of them could have a broader stability. Therefore, the discussion on heterotic patterns will be based on the individual analyses of variance for each environment. And general conclusions will be made from the individual results. Specific heterosis was significant in all environments, except in both years in Pontevedra (data not shown).

The population × year interaction was not significant and the variety effects were significant both years in Pontevedra. The population Tuy had the largest variety effects, followed by Millette Montagne Noire (MMN), and Lazcano in 2000 and by Rastrojero, ML and ElH in 2001 (Table 4). On average, Tuy and ML had the highest variety effects. Also, many hybrids involving Tuy were among the highest yielders (data not shown), particularly Tuy × Rastrojero with 8.0 and 7.5 t ha^{-1} in 2000 and 2001, respectively, and Tuy × ML with 7.3 and 7.5 t ha^{-1}. Tuy × Rastrojero was included in the heterotic pattern 'northern Spain × southern Spain' previously defined by ORDÁS (1991), but Tuy × ML would be a new heterotic pattern, northern Spain × southern France. Variety heterosis was significant in 2000 and only Rastrojero had a positive significant value (Table 5).

All sources of variation were significant in

TABLE 3 - *Mean yield (on the right and above the diagonal, t ha^{-1}) and grain moisture content (on the left and below the diagonal, g kg^{-1}) for a diallel of twelve maize populations[1] evaluated during 2000 and 2001 in two locations of Spain and two locations of France.*

	Tuy	Viana	Lazcano	BR	Rastrojero	ElH	Bade	Lacaune	Esterre	Ain	ML	MMN
Tuy	273-5.8	5.1	7.3	6.6	7.4	7.2	6.0	6.2	6.4	6.4	7.1	6.5
Viana	254	236-3.2	5.4	5.4	6.3	6.1	4.6	4.4	4.8	4.8	5.7	5.5
Lazcano	258	248	252-5.9	7.2	7.5	7.3	6.5	6.4	6.5	6.3	7.6	7.1
BR	274	247	264	255-5.0	6.3	6.6	6.5	6.5	6.3	6.8	6.8	6.7
Rastrojero	300	285	294	294	320-6.0	6.7	6.7	6.7	6.8	6.9	7.0	6.8
ElH	288	275	280	274	308	283-5.9	6.9	6.6	6.6	6.9	7.0	6.8
Bade	246	223	233	246	264	252	205-4.4	5.2	5.7	5.2	6.2	6.3
Lacaune	245	228	235	253	274	262	218	220-3.7	4.9	5.3	5.4	5.4
Esterre	246	232	239	244	258	262	216	220	222-4.8	5.8	6.4	6.2
Ain	265	240	251	259	271	274	228	238	230	239-4.0	6.2	6.2
ML	277	255	274	269	309	301	240	256	257	260	288-5.7	5.9
MMN	272	253	262	274	303	289	232	251	239	251	283	280-4.9

LSD (0.05) = 0.8 for yield and 10 for grain moisture content

[1] BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.
Mean yield and grain moisture content of Dunia, a commercial check, were 12.2 t ha^{-1} and 279, respectively.

TABLE 4 - *Variety effects for yield (t ha^{-1}) for a diallel cross of twelve maize populations[1] evaluated in eight environments, 2000 and 2001 in two locations of Spain and two locations of France.*

	Pontevedra		Zaragoza		Mauguio		Saint Martin de Hinx	
Population	2000	2001	2000	2001	2000	2001	2000	2001
Tuy	1.64*	1.70*	-0.45*	-0.05	-0.91	0.19	1.99*	2.39*
Viana	-1.06*	-1.31*	-1.53*	-1.95*	-0.60	-3.40*	-1.97*	-1.96*
Lazcano	1.22*	-0.00	0.44*	0.40*	1.33*	1.02*	2.07*	1.39*
BR	-0.08	0.05	1.19*	0.49*	-0.64	0.55	-0.93*	-0.29
Rastrojero	-0.04	1.44*	1.69*	1.92*	-0.97	2.10*	0.70	1.63*
ElH	0.49	0.45	0.89*	1.57*	1.21*	0.14	2.10*	1.06*
Bade	-0.55	-1.00	-0.99*	-0.92*	0.29	0.20	-1.28*	-0.33
Lacaune	-1.61*	-0.15	-0.98*	-1.21*	-0.07	-1.71*	-1.79*	-2.05*
Esterre	0.11	-1.08*	-0.44*	-0.73*	-0.23	-0.54	0.93*	0.69
Ain	-2.18*	-1.56*	-1.00*	-0.89*	0.43	-0.51	-0.93*	-1.18*
ML	0.79	1.36*	0.54*	0.78*	0.00	2.07*	0.31	0.31
MMN	1.28*	0.09	0.65*	0.61*	0.15	-0.12	-1.17*	-1.68*
LSD (0.05) [2]	1.31	1.49	0.38	0.25	1.47	1.18	1.10	1.40

* Exceeded twice the standard error.
[1] BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.
[2] LSD is shown only when populations are significantly different.

TABLE 5 - *Variety heterosis for yield (t ha^{-1}) for a diallel cross of twelve maize populations[1] evaluated in eight environments, 2000 and 2001 in two locations of Spain and two locations of France.*

	Pontevedra		Zaragoza		Mauguio		Saint Martin de Hinx	
Population	2000	2001	2000	2001	2000	2001	2000	2001
Tuy	-0.01	0.12	0.21*	-0.22*	0.01	-0.78*	0.04	-0.27
Viana	-0.66*	-0.06	-0.50*	-0.53*	-0.23	0.40	-0.39	0.02
Lazcano	-0.01	0.14	0.20*	0.09	-0.19	0.20	0.12	0.33
BR	0.42	0.27	0.09	0.47*	0.14	-0.54*	0.70*	0.28
Rastrojero	0.70*	-0.36	0.34*	0.41*	-0.08	-0.38	0.13	-0.22
ElH	-0.14	-0.34	0.11	-0.23*	-0.08	0.80*	-0.22	0.52
Bade	-0.19	0.13	-0.01	0.16*	-0.02	0.09	0.06	-0.53
Lacaune	0.12	-0.16	-0.02	-0.17*	0.10	-0.08	0.04	0.16
Esterre	-0.57*	0.26	-0.40*	-0.23*	0.24	-0.22	-0.35	-0.30
Ain	0.46	0.13	0.04	0.18*	0.46	0.58*	0.11	0.09
ML	-0.14	-0.50	-0.03	0.10	0.11	-0.37	-0.24	-0.18
MMN	0.03	0.38	-0.05	-0.03	-0.45	0.31	0.01	0.10
LSD (0.05) [2]	0.74		0.21	0.14		0.67	0.62	

* Exceeded twice the standard error.
[1] BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.
[2] LSD is shown only when populations are significantly different.

TABLE 6 - *Specific heterosis for yield (t ha^{-1}) for a diallel cross of twelve maize populations*[1] *evaluated during 2000 (above the diagonal, t ha^{-1}) and 2001 (below the diagonal, t kg^{-1}) in Zaragoza (Spain).*

	Tuy	Viana	Lazcano	BR	Rastrojero	ElH	Bade	Lacaune	Esterre	Ain	ML	MMN
Tuy		-0.19	-0.32*	0.18	0.25*	0.41*	-0.29*	0.08	0.21	-0.33*	-0.22	0.22
Viana	-0.47*		-0.10	-0.40*	-0.04	-0.07	-0.17	0.38*	0.39*	0.20	0.08	-0.07
Lazcano	-0.05	-0.59*		-0.20	0.34*	0.06	-0.14	-0.19	-0.34*	0.16	1.01*	-0.28*
BR	0.05	-0.23*	0.41*		-0.99*	-0.21	0.10	0.05	0.38*	0.29*	0.44*	0.35*
Rastrojero	0.38*	0.82*	0.10	-0.76*		-0.69*	-0.17	0.49*	-0.80*	-0.07	0.67*	1.01*
ElH	0.46*	0.64*	0.00	-0.54*	-0.96*		0.36*	0.30*	-0.14	-0.01	-0.01	0.00
Bade	-0.73*	-0.39*	-0.48*	0.75*	-0.50*	0.54*		-0.01	0.71*	-0.41*	-0.01	0.03
Lacaune	0.41*	0.23*	0.18*	0.51*	0.02	0.40*	0.04		0.08	0.28*	-0.99*	-0.48*
Esterre	0.00	-0.33*	-0.22*	0.02	0.23*	0.01	0.05	-0.41*		0.31*	-0.63*	-0.92*
Ain	-0.25*	-0.20*	-0.10	0.41*	0.19*	0.29*	-0.17*	-0.37*	-0.45*		-0.45*	0.03
ML	0.62*	0.63*	0.85*	-0.54*	0.73*	-0.85*	0.63*	-1.20*	0.10	0.02		-0.27*
MMN	-0.43*	-0.11	-0.10	-0.08	-0.26*	0.01	0.26*	0.18*	0.88*	0.63*	-1.04*	

LSD (0.05) = 0.36 for hybrids sharing a common parent and 0.34 for unrelated hybrids in 2000

LSD (0.05) = 0.24 for hybrids sharing a common parent and 0.22 for unrelated hybrids in 2001

* Exceeded twice the standard error.

[1] BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.

TABLE 7 - *Specific heterosis for yield (t ha^{-1}) for a diallel cross of twelve maize populations*[1] *evaluated during 2000 (above the diagonal, t ha^{-1}) and 2001 (below the diagonal, t kg^{-1}) in Mauguio (France).*

	Tuy	Viana	Lazcano	BR	Rastrojero	ElH	Bade	Lacaune	Esterre	Ain	ML	MMN
Tuy		0.71	0.22	-1.30*	-0.51	-0.37	0.27	-0.62	0.06	1.26*	0.40	-0.12
Viana	-0.29		-0.54	0.31	0.21	0.16	0.15	-0.82	-0.43	-0.31	0.27	0.29
Lazcano	-0.34	-0.39		-1.39*	-0.58	-0.38	0.23	1.01*	-0.08	-0.35	1.62*	0.25
BR	-0.28	0.81*	0.88*		0.50	-0.28	-0.34	0.74	0.53	-0.25	0.88	0.60
Rastrojero	-0.17	0.31	0.45	-1.84*		0.41	-0.35	1.02*	0.91	0.14	-1.13	-0.60
ElH	-0.12	0.45	0.23	-1.33*	-0.21		0.18	0.21	0.11	0.29	-0.05	-0.26
Bade	-1.22*	-1.10*	-1.06*	0.79*	0.60	0.73		-0.64	-0.26	-0.72	-0.01	1.50*
Lacaune	1.17*	-0.78*	0.12	1.41*	0.49	-0.17	0.45		-0.34	-0.05	-0.19	-0.31
Esterre	0.81*	0.71	0.93*	-0.89*	-0.45	-0.32	0.29	-0.75*		-0.29	0.06	0.01
Ain	-0.09	-0.84*	-1.14*	1.49*	0.45	0.32	-0.03	-0.59	0.37		-0.11	0.37
ML	0.96*	0.20	-0.42	-0.68	0.55	0.66	0.02	-1.05*	-0.66	0.41		-1.59*
MMN	-0.42	0.94*	0.75*	-0.38	-0.18	-0.24	0.52	-0.31	-0.33	-0.36	-0.13	

LSD (0.05) = 1.39 for hybrids sharing a common parent and 1.31 for unrelated hybrids in 2000

LSD (0.05) = 1.11 for hybrids sharing a common parent and 1.05 for unrelated hybrids in 2001

* Exceeded twice the standard error.

[1] BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.

Zaragoza in both years (data not shown). The population Rastrojero had the largest variety effect (Table 4) and one of the highest variety heterosis (Tables 5). Specific heterosis was highest in both years for Rastrojero × ML and Lazcano × ML (Table 6). Given that Rastrojero × ML has also one of the highest yields (6.0 t ha^{-1}), this combination would be the best heterotic pattern for Zaragoza.

All sources of variation were significant for Mauguio except variety heterosis in 2000 (data not shown). Lazcano had one of the largest variety effects in both years (Table 4). Other populations

TABLE 8 - *Specific heterosis for yield (t ha^{-1}) for a diallel cross of twelve maize populations*[1] *evaluated during 2000 (above the diagonal, t ha^{-1}) and 2001 (below the diagonal, t kg^{-1}) in Saint Martin de Hinx (France).*

	Tuy	Viana	Lazcano	BR	Rastrojero	ElH	Bade	Lacaune	Esterre	Ain	ML	MMN
Tuy		-1.12	0.34	-0.64	0.62	-0.11	-0.11	0.49	-0.91*	0.27	0.74*	0.43
Viana	-0.68		-0.14	-0.54	0.27	0.44	0.50	-0.40	-0.18	-0.09	0.65	0.59
Lazcano	0.56	-0.48		0.27	-0.07	-0.37	-0.10	0.11	-0.67	-0.81*	0.85*	0.59
BR	0.05	0.13	0.39		-0.18	0.70	0.29	0.63	0.33	0.54	-0.04	0.05
Rastrojero	0.75	0.98*	-0.29	-1.46*		-1.42*	1.07*	0.19	0.63	0.51	-1.34*	-0.27
ElH	-0.10	0.70	-0.48	-0.31	-0.92		0.48	0.45	0.47	0.46	0.04	0.25
Bade	-0.40	-0.11	0.90*	-0.39	-0.11	0.48		0.14	-0.43	-0.85*	-0.58	-0.42
Lacaune	0.23	0.02	-0.31	0.99*	0.41	0.17	-0.63		-0.58	-0.52	-0.21	-0.30
Esterre	-0.31	-0.95*	0.37	0.13	0.33	-0.16	0.33	-0.79		0.18	0.11	0.26
Ain	0.32	-0.08	-0.49	0.91*	-0.12	0.56	-0.87	0.36	-0.35		0.62	-0.31
ML	-0.22	0.36	-0.17	-0.75	0.52	0.01	-0.40	0.13	0.81	-0.03		-1.25*
MMN	-0.20	0.10	-0.01	0.31	-0.10	0.06	1.23*	-0.58	-0.33	-0.22	-0.73	

LSD (0.05) = 1.04 for hybrids sharing a common parent and 0.99 for unrelated hybrids in 2000

LSD (0.05) = 1.33 for hybrids sharing a common parent and 1.26 for unrelated hybrids in 2001

* Exceeded twice the standard error.

1 BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.

with high variety effects in one of the years were ElH, Rastrojero, and ML. Only Ain and ElH had positive significant variety heterosis in 2001 (Table 5). Hybrid Lazcano × ML had the highest specific heterosis in 2000, but in 2001 the highest specific heterosis was for Ain × Basto/Rastrojero. None of these two hybrids had favorable specific heterosis in both years (Table 7). Although the year × population interaction was significant and clearly affected the ranking of populations, Lazcano × ML could be the best heterotic pattern, considering that this was the highest yielder.

Finally, all sources of variation were significant for Saint Martin de Hinx in both years, except variety heterosis in 2001 (data not shown). Tuy, Lazcano, and ElH had positive significant variety effects both years while Rastrojero had only a positive significant variety heterosis effect in 2000 (Table 5). The highest specific heterosis was for Bade × Rastrojero in 2000 and for Bade × MMN in 2001, but considering both years, the most favorable specific heterosis was for Lacaune × Basto/Rastrojero and Ain × Basto/Rastrojero (Table 8). Considering yield and variety effects, the best across both years were Tuy × Lazcano and Tuy × Rastrojero whose yields averaged across years were 9.7 and 9.4, respectively. Therefore, the most adequate heterotic pattern for Saint Martin de Hinx could be Tuy × Lazcano.

Growing cycle

Given the wide ranges of kernel moisture content (Table 3) and silking (data not shown), the previous comparisons for yield would be more accurate if differences of growing cycle among populations are considered. The earliest population was Bade, followed by Lacaune and Esterre, of which Bade had the lowest variety effect for grain moisture content, followed by Lacaune and Esterre (Table 9). The earliest was Bade × Esterre, which did not differ significantly from Bade × Lacune and Esterre × Lacaune. None of the early populations had a significant variety heterosis and hybrids among early populations had no significant specific heterosis. The early population Esterre had significantly larger yield per se than Viana and Lacaune. The highest yield among the early populations was for Bade × Esterre (5.7 t ha^{-1}), which also had the lowest moisture content (216 g kg^{-1}), below the commercial check Dunia (279), though not differing significantly from other hybrids (Table 3). Troyer and Hallauer (1968) detected a heterosis of 72.0% among early flint populations, while the mid-parent heterosis of the early heterotic pattern Bade × Esterre was 25.1%. Viana was the only early population that did not show any significant specific heterosis effect in crosses to other populations, but the number of significant specific heterosis effects

TABLE 9 - *Variety effects and variety heterosis for four traits for a diallel cross of twelve maize populations[1] evaluated during 2000 and 2001 in two locations of Spain and two locations of France.*

	Variety effects				Variety heterosis			
Population	Grain moisture content	100-kernel weight	Ear length	Number of kernel rows	Grain moisture content	100-kernel weight	Ear length	Number of kernel rows
	$g\ kg^{-1}$	g	cm		$g\ kg^{-1}$	g	cm	
Tuy	1.67*	36.30*	2.75	-0.06	-0.10	10.54*	-0.69	0.11
Viana	-2.05*	28.51*	-11.34*	-0.57	-0.07	-12.77*	-1.13	0.17
Lazcano	-0.39	29.40*	2.18	0.289	0.09	-0.71	-0.77	0.24
BR	-0.09	33.62*	-0.61	-0.56	0.52*	3.98	1.80	0.42
Rastrojero	6.42*	38.73*	8.30	-2.31*	-0.11	7.16	0.03	0.05
ElH	2.70*	-12.93	-1.09	-1.16*	0.78*	-7.28	-0.06	-0.37
Bade	-5.15*	38.14*	3.75	-2.81*	0.05	7.42*	1.12	-0.16
Lacaune	-3.56*	-39.73*	-2.52	-0.19	0.09	0.84	1.46	0.04
Esterre	-3.38*	50.34*	-4.60	0.06	-0.39	-1.65	-1.06	-0.12
Ain	-1.75*	-81.85*	-8.25	2.86*	0.04	3.51	2.15	0.15
ML	3.19*	-69.29*	-5.29	2.84*	-0.29	10.33*	-1.08	0.17
MMN	2.38*	-51.23*	16.70*	1.61*	-0.60*	-0.71	-1.77	-0.68*
LSD (0.05)	1.07	18.49		1.57	0.60	10.48		

* Exceeded twice the standard error.

[1] BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.

TABLE 10 - *Specific heterosis for 100-kernel weight (g) (above the diagonal) and grain moisture content ($g\ kg^{-1}$) (below the diagonal) for a diallel of twelve maize populations[1] evaluated during 2000 and 2001 in two locations of Spain and two locations of France.*

	Tuy	Viana	Lazcano	BR	Rastrojero	ElH	Bade	Lacaune	Esterre	Ain	ML	MMN
Tuy		-20.06*	1.46	1.42	1.41	-15.42*	7.88	1.87	13.09*	-1.54	1.80	8.08
Viana	-0.18		-0.40	3.69	2.13	-1.41	2.17	-3.78	-9.59	7.95	11.02	8.28
Lazcano	-0.79*	0.13		2.93	-8.16	-0.16	-10.76	0.98	4.94	6.09	1.09	1.99
BR	0.24	-0.56	0.09		-13.08*	-13.08*	6.30	21.15*	8.92	-3.02	-7.68	-7.54
Rastrojero	0.29	0.60	0.53	-0.13		-3.61	11.90*	-1.93	0.43	6.88	-7.82	11.86*
ElH	-0.01	0.52	0.10	-1.09*	-0.37		9.05	13.73*	-12.02*	8.43	5.27	9.21
Bade	0.49	0.04	0.06	0.75*	-0.07	-0.35		-4.23	3.95	-8.28	-10.90	-7.09
Lacaune	-0.45	-0.31	-0.58	0.60	0.08	-0.15	0.09		-4.92	-12.07*	2.13	-12.94*
Esterre	0.05	0.45	0.22	0.06	-1.15*	0.21	0.25	-0.13		-6.24	-3.53	-0.02
Ain	0.65	0.01	0.15	0.38	-1.04*	0.18	0.23	0.45	-0.04		11.14	-9.34
ML	-0.22	-0.61	0.30	-0.81*	0.58	0.71*	-0.72*	0.11	0.13	-0.42		-4.99
MMN	-0.07	-0.08	-0.22	0.47	0.68	0.25	-0.77*	0.30	-0.98*	-0.54	0.48	

LSD (0.05) = 17.55 for hybrids sharing a common parent and 16.55 for unrelated hybrids for 100-kernel weight

LSD (0.05) = 1.01 for hybrids sharing a common parent and 0.95 for unrelated hybrids for kernel moisture content

* Exceeded twice the standard error.

[1] BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.

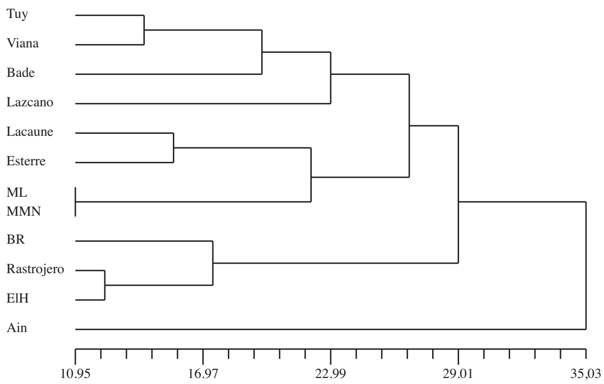

FIGURE 1 - Phenogram of six French and six Spanish populations[1] using UPGMA with mid-parent heterosis for yield as the measure of dissimilarity. [1] BR=Basto/Rastrojero, ElH=Enano levantino/Hembrilla, ML=Millette du Lauragais, and MMN=Millette Montagne Noire.

among the other populations was low and most of them were negative (Table 10).

Kernel weight

The analysis of 100-kernel weight was peculiar because the four populations with significant negative varietal effects were French, while all the Spanish populations except ElH had significant positive varietal effects (Table 9). Variety heterosis was significant and positive only for Tuy, Bade, and ML, and negative for Viana (Table 9). Significant specific heterosis effects were negative among Spanish populations as well as among French populations, while significant heterosis effects of hybrids between Spanish and French populations were positive except Esterre × ElH. Two populations, Lazcano and ML, had no significant specific heterosis effect for 100-kernel weight with any other population. Based on 100-kernel weight, these populations could be classified into two main groups, one comprising most Spanish populations and the other containing most French populations. The relationship among populations based on 100-kernel weight had no relationship with the previous heterotic patterns identified based on yield.

Heterotic patterns

Summarizing by locations, the heterotic patterns were Tuy × ML for Pontevedra, Rastrojero × ML for Zaragoza, Lazcano × ML for Mauguio, and Tuy × Lazcano for Saint Martin de Hinx. Although the performance of hybrids in different environments was highly variable, breeders might be interested in developing a heterotic pattern with wide adaptation. Among the heterotic patterns suggested above, the most promising combination for hot and dry climatic conditions of growth was Lazcano × ML, which was adequate for Zaragoza and Mauguio and, in the combined analysis across locations had the largest yield (7.7 t ha^{-1}) and a mean heterosis of 30.7%. Additionally, this heterotic pattern includes the best performing population per se, Lazcano, and the population that produces the best heterotic patterns for most locations, ML. Accordingly, MALVAR *et al.* (2005) identified Lazcano × ML as the most stable population hybrid across environments.

Cluster analysis

The cluster analysis (Fig. 1) revealed a close relationship between ML and MMN, both from the French region Languedoc-Roussillon and with a mean heterosis of 11.0%. These two populations were not closely related in clusters based on RFLPs (REBOURG *et al.*, 2001; GAUTHIER *et al.*, 2002), or in the isozyme classification (REVILLA *et al.*, 2003), though the RFLP cluster places them both in the same cluster. The other closely related pair of populations was Rastrojero and ElH from the dry Spain and with a mean heterosis of 12.2%. These populations were similar based on isozyme data (REVILLA *et al.*, 1998, 2003) and were in the same cluster based on RFLP data (GAUTHIER *et al.*, 2002). Tuy and Viana from the northwest of Spain had 14.3% average heterosis and were also similar based on RFLP and isozyme data (GAUTHIER *et al.*, 2002; REVILLA *et al.*, 2003). Finally, Lacaune and Esterre, from the French Midi-Pyrenees, with a mean heterosis of 15.9%, were neither together in the RFLP nor in the isozyme clusters. We postulate that the explanation for the similarity between the populations of these four groups may be common ancestry or geographical proximity that would have caused a similar adaptation to climatic conditions.

The three populations from the dry Spain, Basto/Rastrojero, Rastrojero, and ElH constitute a homogeneous cluster clearly distinct from other populations. The relative positions of these three populations fit perfectly the expectations based on isozyme distances (REVILLA *et al.*, 1998) and RFLP data in the same South Eastern cluster (GAUTHIER *et al.*, 2002). The similarity among these populations and their distance with the other populations was previously explained by their origin, possibly in Central America (REVILLA *et al.*, 1998).

The southern French group includes Lacaune, Esterre, ML, and MMN. These populations are from southern France, Midi-Pyrenees and Languedoc Roussillon, and are separated from Bade, which originated in the northeast, in Alsace, and from Ain, which originated from Rhône-Alpes. This cluster is not consistent with the previous observation, because in the RFLP classification, Esterre was in the South Western cluster while the three other populations were in the North Eastern cluster (GAUTHIER *et al.*, 2002), and in the isozyme classification these French populations were dispersed among a wide miscellaneous group of European populations (REVILLA *et al.*, 2003).

The Northern Spanish cluster includes Tuy, Viana, Bade, and Lazcano. The relationship among Tuy, Viana, and Lazcano fits expectations based on RFLP and isozyme data, but Bade was separate in such classifications (GAUTHIER *et al.*, 2002; REVILLA *et al.*, 1998, 2003). Bade comes from Alsace and, in isozymes and RFLP classifications, was closer to other French populations. Bade is an early population with a modest yield (4.4 t ha^{-1}). Tuy and Viana are populations from northwestern Spain, presumably introduced from North America about three centuries ago, and Lazcano is a northern Spanish population introduced into Spain also from North America, presumably from a different area (REVILLA *et al.*, 1998).

Northern Spanish and southern French populations constitute a group of germplasm clearly different from the maize of the dry Spain. The main exception was Ain, which had the highest heterosis with Basto/Rastrojero (51.9%) and a large heterosis with other populations. Ain had an isozyme pattern similar to Esterre, and a RFLP composition similar to MMN, ML, and Lacaune.

As conclusion, the clusters based on mid-parent heterosis generally conform to the clusters previously identified based on isozyme data: a Mediterranean cluster including the currently defined dry Spanish maize and a large miscellaneous group that includes most other maize populations. Most of the potential heterotic patterns previously defined agree with the combination humid Spain × Southern France.

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