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ORIGINAL PAPER

Broadening the genetic base of European maize heterotic pools with US Cornbelt germplasm using field and molecular marker data

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Abstract Maize (*Zea mays* L.) breeders are concerned about the narrowing of the genetic base of elite germplasm. To reverse this trend, elite germplasm from other geographic regions can be introgressed, but due to lack of adaptation it is difficult to assess their breeding potential in the targeted environment. The objectives of this study were to (1) investigate the relationship between European and US maize germplasm, (2) examine the suitability of different mega-environments and measures of performance to assess the breeding potential of exotics, and (3) study the relationship of genetic distance with mid-parent heterosis (MPH). Eight European inbreds from the Dent and Flint heterotic groups, 11 US inbreds belonging to Stiff Stalk (SS), non-Stiff Stalk (NSS), and CIMMYT Pool 41, and their 88 factorial crosses in F_1 and F_2 generations were evaluated for grain yield and dry matter concentration. The experiments were conducted in three mega-environments: Central Europe (target mega-environment), US Cornbelt (mega-environment where donor lines were developed), and Southeast Europe (an intermediate mega-environment). The inbreds were also fingerprinted with 266 SSR markers. Suitable criteria to identify promising exotic germplasm were F_1 hybrid performance in the targeted mega-environment and F_1 and parental performance in the intermediate mega-environment. Marker-based genetic distances reflected relatedness among the inbreds, but showed no association with MPH. Based on genetic distance, MPH, and F_1 performance, we suggest to introgress SS germplasm into European Dents and NSS into European Flints, in order to exploit the specific adaptation of European flint germplasm and the excellent combining ability of US germplasm in European maize breeding programs.

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Introduction

In hybrid maize breeding, inbred lines are primarily developed by pedigree breeding, i.e., crossing elite lines within heterotic groups followed by inbreeding and selection. In the long run, this implies (a) reduced genetic variation within breeding pools (Duvick et al. 2004), (b) lower-selection gains in the long term for yield and other traits, and (c) potentially increased susceptibility and vulnerability to biotic and abiotic stresses (Smith et al. 2004).

Exotic germplasm is regarded as a valuable source of new desirable alleles to broaden the genetic base of the elite maize breeding germplasm (cf. Ron Parra and Hallauer 1997; Goodman et al. 2000). For maize breeding in Central Europe, promising exotic sources are tropical, subtropical, and unadapted temperate germplasm.

Introgression of elite US Cornbelt inbreds as donors into the elite recipient European maize germplasm is considered a promising approach (Šimić et al. 2003) and is commonly practised in private and public breeding programs. This flow of germplasm could enhance the genetic distance between heterotic pools and, with a targeted approach, potentially increase the heterotic response and hybrid performance. Consequently, an understanding of the genetic and heterotic relationships between the widely used US and European heterotic groups is warranted.

Heterosis for grain yield (GY) of hybrids between Central European and US germplasm has been studied by several authors (for review see Soengas et al. 2003). The focus in these studies was on the targeted mega-environment, i.e., the environment to which the presumed recipient germplasm was adapted. Therefore, crosses between adapted and unadapted parents were tested only in the targeted mega-environment. In this approach, expression of the favorable alleles in the donor germplasm may be masked by other genes responsible for the lack of adaptation. Mega-environments having intermediate climatic conditions may be deployed to reduce the adaptation-related problems in assessing the true breeding potential of the unadapted germplasm.

Reif et al. (2003) reported that genetic distance, determined from suitable molecular markers, is a helpful complementation to field trials for identifying groups of genetically similar germplasm and heterotic patterns. This approach may be used for systematic introgression of exotic germplasm to broaden the genetic base of heterotic pools and enhance heterotic response. The suggestion is based on the assumption of a positive association between genetic distance and heterosis (Falconer and Mackay 1996). Numerous experimental studies have been reported in maize on this relation (for review see Melchinger 1999), but there is to the best of our knowledge, little published information on crosses involving rather diverse parents.

We studied maize inbred lines of different European and US heterotic groups and their factorial crosses in F_1 and F_2 generations in field trials conducted in three mega-environments: (a) Central Europe (CEM), the target mega-environment of the presumed recipient parents, (b) the source mega-environment US Cornbelt (USM), where the donor lines were developed, and (c) Southeast Europe (SEM) having intermediate climatic conditions. We also fingerprinted the inbred parents to estimate marker-based genetic distances. Our objectives were to (1) study the heterotic relationships between the European and US heterotic groups, (2) examine the suitability of the different mega-environments, different generations (parents, F_1, F_2), and different measures [per se performance and mid-parent heterosis (MPH)] to reliably assess the breeding potential of unadapted exotic germplasm, and (3) investigate the relationship of genetic distance based on molecular markers with MPH. The overall goal was to develop a strategy for targeted introgression of US maize germplasm into currently used European heterotic groups.

Materials and methods

Plant materials

Eighty-eight factorial crosses between eight European inbreds and 11 US inbreds were made and advanced to the F_2 generation. The 19 inbred parents are a representative sample of modern public elite maize inbreds developed during the 1990s in Germany at the University of Hohenheim, Stuttgart, and in the USA at Iowa State University, Ames (Table 1). Four of the European lines were dent (D23, D51, P006, UH200), with A632, Co125 and an Iodent line of unknown pedigree being the main ancestors. A632 was derived from (Mt42 × B14(^{4}) and inbred B14 was developed from Iowa Stiff Stalk Syn. (BSSS) (see Smith et al. 1985). Co125 was developed from an unknown source population at the Ottawa Research Station, Canada (see Messmer et al. 1992). Other North American lines occurring in the pedigree of the European dents include V3, WD (see Mumm and Dudley 1994), W401 (see Smith et al. 1985), and Co158 (see Cardy and Kannenberg 1982). The Illinois High Protein population contributed very limited germplasm to the pedigree of the four European Dent lines. The ancestries of the four European Flint lines (D152, F011, F016, UH001) trace back mainly to three European open-pollinated populations: Lacaune from France, Lizargarote from Spain, and Gelber Badischer Landmais from Germany. Four US lines (B98, B101, B105, B109) belong to the Stiff Stalk (SS) heterotic group (Hallauer and Wright 1995, Hallauer et al. 1994, 1997, 1998). B101 and B105 were directly derived from BSSS, B109 was developed from a backcross of a BS20 (Iowa Late Rootworm Syn.) inbred with B73 (recipient parent), and B98 was derived from BS11 (Pioneer Two-ear Syn.). B98 is considered NSS, but it combines well with B73 and based on its plant type in relation to Central European heterotic groups and earlier observations on its performance in our experiments and on genetic distances we considered it as SS. Five lines (B97, B99, B100, B102, B106) belong to the NSS heterotic group (Hallauer et al. 1994, 1995, 1997). B97 and B99 were derived from BSCB1 (Iowa Corn Borer Syn. 1), B100 and B102 were developed from a backcross (B85 × H99) × H99, and B106 was derived from Lancaster Sure Crop. Two lines (B107, B108) were derived from CIMMYT Pool 41 (P41) (Hallauer et al. 1998), which had been developed by utilizing primarily US germplasm and some materials from China, Korea, and Lebanon (CIMMYT 1988).

Table 1 An overview of the inbred lines, their source germplasm, and heterotic group

Inbred	Source germplasm	Heterotic group
D23	A632, Co125, and an Iodent line of unknown pedigree	European Dent
D51	A632, Co125, and an Iodent line of unknown pedigree	European Dent
P006	A632, Co125, and an Iodent line of unknown pedigree	European Dent
UH200	A632, Co125, and an Iodent line of unknown pedigree	European Dent
D152	Lacaune, Lizargarote, and Gelber Badischer Landmais	European Flint
F011	Lacaune, Lizargarote, and Gelber Badischer Landmais	European Flint
F016	Lacaune, Lizargarote, and Gelber Badischer Landmais	European Flint
UH001	Lacaune, Lizargarote, and Gelber Badischer Landmais	European Flint
B98	BS11(FR)C5	US_Stiff Stalk
B101	BSSS-53	US_Stiff Stalk
B105	BSSS(R)C9	US_Stiff Stalk
B109	(B73 X BS20 sel.)	US_Stiff Stalk
B97	[BSCB1(R)C9]	US_non-Stiff Stalk
B99	[BSCB1(R)C10]	US_non-Stiff Stalk
B100	[(B85 × H99)H99]	US_non-Stiff Stalk
B102	[(B85 × H99)H99]	US_non-Stiff Stalk
B106	Lancaster Sure Crop	US_non-Stiff Stalk
B107	(CIMMYT POOL41-C15)	CIMMYT Pool 41
B108	[CIMMYT POOL 41(IA)-C15]	CIMMYT Pool 41

Field trials

All three groups of materials (19 inbred parents, 88 F_1 hybrids, 88 F_2 hybrids) were grown in 13 location-year combinations (referred to as environments) in three mega-environments: (1) four in CEM (Eckartsweier and Bad Krotzingen in Southwest Germany for 2 years), (2) five in the USM (Ames, Carroll, Crawfordsville, Fairfield, and Rippey in Iowa for 1 year), and (3) four in SEM (Pachfurth in Austria and Boly in Hungary for 2 years). At each location within each mega-environment, the three groups of materials were evaluated in separate but adjacent α-designs with two replications. In the trial for evaluation of the 88 F_1 hybrids, seven hybrids from Europe (Clarica, Monalisa, 38P05, Matea, Dunia, Florencia 34G81) were included as checks in all three mega-environments. In addition, five hybrids from the US Cornbelt (B73 × Mo17, B73 × B97, DK626, Pioneer3335, Pioneer33A14) were included as checks in the trials conducted in the USM. We used two-row plots with a row-to-row distance of 0.76 m. The row length was 6.00 m in CEM, 5.49 m in USM, and 9.00 m in SEM. Plant density was 86,667, 72,202, and 70,000 plants ha^{-1} in CEM, USM, and SEM, respectively. Plots were machine-planted and combine-harvested. Data were collected on number of plants, dry matter concentration (DMC %), and GY (Mg ha^{-1}) adjusted to a moisture concentration of 155 g H_2O kg^{-1}. In CEM and SEM, DMC was estimated by drying a grain sample of each plot in an oven to 0%. The sample was weighed before and after drying. In USM, DMC was electronically measured from each plot sample on the harvesting machine.

Molecular data

The 19 parental inbreds were fingerprinted following standard protocols with 266 simple sequence repeat (SSR) markers uniformly distributed across the entire maize genome. The resulting amplification products were resolved by electrophoresis in polyacrylamide gels using an ALF Express (Amersham Biosciences Europe GmbH, Freiburg, Germany) automated sequencer. The data on fragment size were recorded by software ALFwin version 2 (Amersham Biosciences Europe GmbH, Freiburg, Germany) and manually checked. Schrag et al. (2009) provides more for details.

Statistical analyses

For each of the 13 environments, ordinary lattice analyses of variance (ANOVA) were performed separately for the parental inbreds, F_1 hybrids, and F_2 hybrids using the number of plants per plot as a covariable (Cochran and Cox 1957). Based on adjusted entry means, MPH and inbreeding depression (ID) were calculated for each cross as $MPH = F_1 - (P1 + P2)/2$ and $ID = 2(F_1 - F_2)$, where F_1 and F_2 refer to the performance of the hybrids in the F_1 and F_2 generations, respectively, and P1 and P2 refer to the performance of the two parental inbreds. Adjusted means

of each entry in all three groups of materials, MPH and ID of each hybrid, and error variance for each experiment in an environment were used in the combined analyses across environments within each mega-environment. Best linear unbiased prediction (BLUP) of per se performance in various generations, MPH, ID, and variance component of genotypes (σ_G^2), genotype × environment interactions (σ_{GE}^2), and pooled error (σ^2) were estimated with PROC MIXED in SAS (SAS Institute 1999) with the following linear model:

$$G + E + GE,$$

where G and E denote genotype and environment, respectively. Genotypes, environments, and all interactions were treated as random effects. A Wald's F test was used to test whether variances were significantly greater than zero. Heritability (h^2) on an entry-mean basis was estimated for all three types of materials in each mega-environment as the ratio of genotypic to phenotypic variance:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{e} + \frac{\sigma^2}{er}},$$

where e and r denote the number of environments and replications, respectively.

Non-linear response to increased homozygosity by inbreeding was examined by the contrast NL = (P1 + P2)/2 + F_1 − 2F_2 using an appropriate two-tailed t test (Snedecor and Cochran 1980). Simple correlation coefficients (r) among different mega-environments were calculated for BLUP values of parental, F_1, and F_2 performance, and MPH of GY and DMC. In addition, r between GY and DMC using BLUP values of the parental lines, F_1 and F_2 hybrids, and MPH were computed for each environment and across mega-environments. Significance of r was tested by using tabulated values based on Fisher's z transformation.

The average number of alleles per locus was determined for different germplasm groups. Principle coordinate analysis (PCoA) was performed following Gower (1966), based on the modified Rogers' distance (MRD) of Wright (1978). Pearson correlations (r_p) and Spearman Rank correlations (r_s) were calculated between MRD^2 versus MPH and F_1 hybrid performance. All analyses of the molecular data were performed with software Plabsoft (Maurer et al. 2008).

Results

The ANOVA for each mega-environment revealed significant variances for genotypes (σ_G^2) and genotype × environment interactions (σ_{GE}^2) in parents and F_1 and F_2 generations for both GY and DMC, except for σ_{GE}^2 for GY of inbreds in USM (Table 2). Estimates of entry mean h^2 were high for both traits. For GY, they were similar in F_1 and F_2 generations, but slightly higher for inbreds. The range of $\hat{h}^2$ among the three mega-environments was smaller for GY than for DMC.

Mean GY in both F_1 and F_2 generations and MPH of all groups of hybrids were highest in CEM and lowest in USM (Table 3). The values in SEM closely followed those in CEM for GY of F_1 and F_2 generations, and were intermediate between CEM and USM for MPH. For GY,

Table 2 Estimates of variance components (genotypes, σ_G^2; genotype × environment interactions, σ_{GE}^2; pooled error, σ^2) and heritability (h^2) on entry mean basis for 19 inbred parents and their 88 factorial crosses in F_1 and F_2 generations evaluated in four environments (location-year combinations) in Central Europe mega-environment (CEM), five environments in US Cornbelt mega-environment (USM), and four environments in Southeast Europe mega-environment (SEM) for grain yield (GY) and dry matter concentration (DMC)

*, **, *** Variance component significant at the 0.05, 0.01, and 0.001 probability level, respectively

Statistic	GY (MG ha^{-1})			DMC (%)		
	CEM	USM	SEM	CEM	USM	SEM
Parents						
σ_G^2	1.52*	0.76**	1.18**	74.19**	9.47***	29.74*
σ_{GE}^2	1.17***	0.07	0.48**	13.59***	7.39***	25.87**
σ^2	0.20	0.48	0.44	3.72	2.89	16.69
h^2	0.83	0.92	0.87	0.95	0.84	0.78
F_1 hybrids						
σ_G^2	0.81***	0.37***	0.42***	3.54***	0.43***	2.53***
σ_{GE}^2	0.43***	0.22***	0.16**	1.02***	0.21***	2.91***
σ^2	0.55	0.46	0.80	0.60	0.56	2.11
h^2	0.82	0.81	0.75	0.91	0.81	0.72
F_2 hybrids						
σ_G^2	0.61**	0.29***	0.28***	3.39***	0.35***	2.17***
σ_{GE}^2	0.39**	0.16***	0.16***	0.69***	0.23***	1.81***
σ^2	0.47	0.32	0.42	0.65	0.46	2.18
h^2	0.79	0.82	0.75	0.93	0.79	0.75

Table 3 Mean performance of European and US Cornbelt inbreds from different heterotic pools, and mean performance in F_1 and F_2 generations and mid-parent heterosis (MPH) in F_1 generation of different groups of inter-pool factorial hybrids for GY and DMC in three mega-environments (CEM, USM, SEM)

Material	GY (MG ha^{-1})			DMC (%)		
	CEM	USM	SEM	CEM	USM	SEM
Parents						
All parents	3.67	2.11	4.11	64.34	82.08	74.26
European dent (D)	4.61 ± 0.38	2.34 ± 0.15	4.63 ± 0.35	71.88 ± 2.08	84.34 ± 0.19	79.39 ± 0.35
European flint (F)	3.66 ± 0.22	1.25 ± 0.16	3.33 ± 0.30	73.20 ± 1.09	84.89 ± 0.45	78.73 ± 1.20
US Stiff Stalk (SS)	2.82 ± 0.57	2.07 ± 0.47	3.86 ± 0.63	54.06 ± 3.42	78.47 ± 0.94	68.07 ± 1.05
US non-SS (NSS)	3.43 ± 0.46	2.51 ± 0.38	4.26 ± 0.53	60.69 ± 0.57	80.67 ± 0.59	71.81 ± 0.76
US Pool41 (P41)	4.06 ± 1.45	2.43 ± 1.03	4.77 ± 0.64	61.22 ± 0.44	82.69 ± 1.42	73.54 ± 1.32
F_1 hybrids						
All F_1 hybrids	11.83	6.81	10.63	67.47	83.89	77.09
D × SS	12.22 ± 0.26	7.13 ± 0.16	10.81 ± 0.15	65.51 ± 0.30	84.01 ± 0.12	76.60 ± 0.25
F × SS	12.00 ± 0.17	6.64 ± 0.14	10.78 ± 0.13	66.58 ± 0.57	83.27 ± 0.18	75.61 ± 0.34
D × NSS	12.10 ± 0.18	6.99 ± 0.13	10.84 ± 0.12	67.93 ± 0.35	83.97 ± 0.09	77.81 ± 0.18
F × NSS	11.32 ± 0.11	6.69 ± 0.08	10.34 ± 0.11	68.68 ± 0.30	83.84 ± 0.10	77.44 ± 0.28
D × P41	11.63 ± 0.29	6.56 ± 0.21	10.26 ± 0.22	67.83 ± 0.57	84.44 ± 0.13	78.11 ± 0.40
F × P41	11.51 ± 0.14	6.55 ± 0.10	10.59 ± 0.08	68.62 ± 0.39	84.30 ± 0.09	77.38 ± 0.30
Checks	14.02	10.04[a]	12.30	67.61	80.57	77.37
F_2 hybrids						
All F_2 hybrids	8.31	4.57	7.83	67.15	83.93	77.42
D × SS	8.69 ± 0.16	4.83 ± 0.11	8.05 ± 0.12	65.16 ± 0.32	83.83 ± 0.13	77.00 ± 0.28
F × SS	8.32 ± 0.14	4.33 ± 0.12	7.78 ± 0.10	66.54 ± 0.35	83.39 ± 0.15	76.27 ± 0.29
D × NSS	8.72 ± 0.16	4.92 ± 0.09	8.04 ± 0.11	67.39 ± 0.38	84.01 ± 0.08	77.87 ± 0.32
F × NSS	7.77 ± 0.10	4.44 ± 0.06	7.63 ± 0.09	68.33 ± 0.28	83.94 ± 0.07	77.62 ± 0.18
D × P41	8.29 ± 0.19	4.41 ± 0.11	7.61 ± 0.15	67.52 ± 0.47	84.56 ± 0.12	78.46 ± 0.36
F × P41	7.86 ± 0.19	4.13 ± 0.20	7.67 ± 0.14	68.48 ± 0.34	84.31 ± 0.08	77.86 ± 0.28
MPH						
All hybrids	8.11	4.69	6.54	1.66	1.39	2.14
D × SS	8.55 ± 0.14	4.92 ± 0.10	6.59 ± 0.10	1.96 ± 0.50	2.26 ± 0.17	2.55 ± 0.27
F × SS	8.71 ± 0.08	4.87 ± 0.09	7.08 ± 0.11	2.25 ± 0.38	1.34 ± 0.23	2.07 ± 0.39
D × NSS	8.09 ± 0.13	4.59 ± 0.08	6.44 ± 0.08	1.48 ± 0.15	1.40 ± 0.15	2.23 ± 0.21
F × NSS	7.76 ± 0.11	4.70 ± 0.04	6.51 ± 0.12	1.40 ± 0.21	1.11 ± 0.16	2.18 ± 0.37
D × P41	7.42 ± 0.47	4.19 ± 0.28	5.69 ± 0.26	1.27 ± 0.23	1.09 ± 0.18	1.81 ± 0.33
F × P41	7.65 ± 0.14	4.64 ± 0.12	6.52 ± 0.07	1.41 ± 0.14	0.70 ± 0.14	1.50 ± 0.50

CEM Central Europe mega-environment, *USM* US Cornbelt mega-environment, *SEM* Southeast Europe mega-environment, *GY* grain yield, *DMC* dry matter concentration

[a] Mean GY of US commercial check hybrids only

European Dent × SS, European Flint × SS, and European Dent × NSS hybrids performed better than European Flint × NSS, European Dent × P41, and European Flint × P41 hybrids in CEM. Similar trends were observed in SEM and USM, except that European Flint × NSS (6.69 Mg ha^{-1}) performed similar to the European Flint × SS (6.64 Mg ha^{-1}) in USM. European commercial checks outyielded the F_1 hybrids in CEM and SEM by 16–18%; the GY superiority of US commercial hybrids checks was significantly higher in USM. Inbred lines yielded highest in SEM and lowest in USM. European Dent lines outyielded European Flint lines in all three mega-environments, whereas among US lines, NSS and P41 lines yielded higher than SS lines.

The US lines generally showed higher MPH in combination with European Flints than with European Dents (Table 3). Among US inbred lines, MPH was generally highest for hybrids of lines from SS, lowest for hybrids of P41 lines and intermediate for hybrids of NSS lines. Mean DMC of all germplasm groups was lowest in CEM and

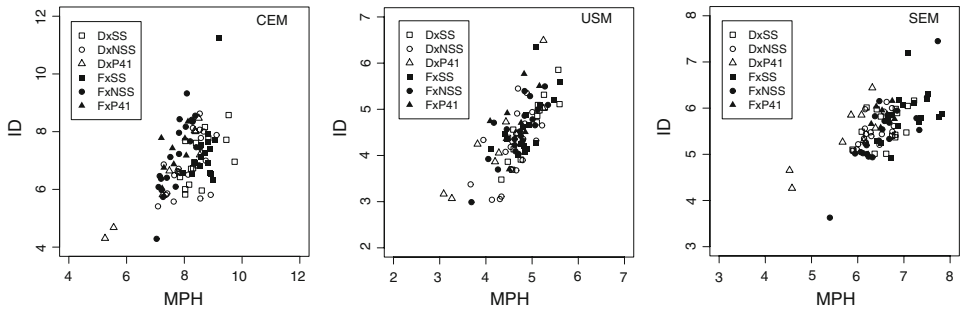

Fig. 1 Relationship between mid-parent heterosis (*MPH*) and ID = 2(F_1–F_2) for grain yield of hybrids of inbred lines belonging to the European Dent (*D*) and European Flint (*F*) heterotic groups with those of US Cornbelt Stiff Stalk (*SS*) and non-Stiff Stalk (*NSS*) heterotic groups, and CIMMYT's Pool 41 (*P41*) in Central Europe (*CEM*), US Cornbelt (*USM*), and Southeast Europe (*SEM*) mega-environments

highest in USM. Contrast NL was significantly negative for both traits in each mega-environment (Fig. 1). Correlations between MPH and ID for GY were significant with intermediate values ($r = 0.57$–0.72) in all three mega-environments.

Correlations among mega-environments for parental, F_1, and F_2 performance, and MPH were significantly positive in all cases but one (Table 4). Estimates of *r* were generally (a) highest between CEM and SEM, (b) higher for parents, F_1, and F_2 than MPH, and (c) higher for GY than DMC except for the parents. Correlations between GY and DMC in each mega-environment and across mega-environments were negative and mostly significant in F_1 and F_2 hybrids, with highest values being observed in CEM and lowest in USM (Table 5). For parental performance, the correlation between both traits was significant only in CEM.

The total number of alleles detected for the 266 SSR loci was 1381. Among various inter-pool comparisons, estimates of MRD were the largest for European Dents versus European Flints (Supplementary Table 1). In the PCoA based on MRD estimates for all inbred lines, the first three principal coordinates (PC) explained 32.4% of the molecular variance (Fig. 2). European Flint lines were placed in a clearly separated group with respect to PC1. Further, two NSS lines (B100, B102) were distinctly separated from the rest with respect to PC2. Inbred B107 from P41 was positioned within the European Dents for PC1 and PC3.

Table 5 Simple correlation coefficients (*r*) between GY and DMC within each mega-environment (CEM, SEM, USM) for per se performance of inbred parents, F_1 hybrids and F_2 hybrids, and mid-parent heterosis (MPH)

	r (GY, DMC)			
	CEM	USM	SEM	Overall
Parents	0.50*	−0.26	0.15	0.21
F_1 hybrids	−0.45***	−0.16	−0.30**	−0.40***
F_2 hybrids	−0.45***	−0.22*	−0.26*	−0.38***
MPH	0.18	0.16	0.21*	0.21*

CEM Central Europe mega-environment, *USM* US Cornbelt mega-environment, *SEM* Southeast Europe mega-environment, *GY* grain yield, *DMC* dry matter concentration

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

For GY, MRD^2 was more strongly correlated with MPH than with F_1 performance in all mega-environments (Fig. 3). The r_p values were significant for MRD^2 versus MPH in all mega-environments, and MRD^2 versus F_1 performance in SEM. However, when two outlier crosses of lines P006 and D51 with B107 were excluded, r_p values

Table 4 Simple correlation coefficients (*r*), based on GY and DMC, among three mega-environments (CEM, SEM, USM) for the best linear unbiased predictions of inbreds, F_1 hybrids, F_2 hybrids, and mid-parent heterosis (MPH)

	GY			DMC		
	CEM, USM	CEM, SEM	USM, SEM	CEM, USM	CEM, SEM	USM, SEM
Parents	0.54*	0.77***	0.70***	0.74***	0.89***	0.85***
F_1 hybrids	0.79***	0.78***	0.72***	0.25*	0.76***	0.53***
F_2 hybrids	0.73***	0.82***	0.72***	0.24*	0.77***	0.39***
MPH	0.61***	0.68***	0.49***	0.11	0.40***	0.45***

CEM Central Europe mega-environment, *USM* US Cornbelt mega-environment, *SEM* Southeast Europe mega-environment, *GY* grain yield, *DMC* dry matter concentration

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

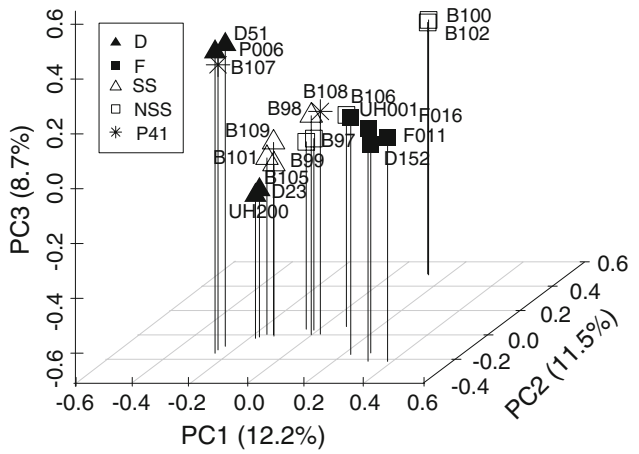

Fig. 2 Principal coordinate analysis (PCoA) of maize inbreds belonging to European and US Cornbelt heterotic groups, based on 266 SSR markers. Values in parentheses refer to the proportion of variance explained by the principle coordinates (*PC1*, *PC2*, *PC3*). *D* European Dent, *F* European Flint, *SS* Stiff Stalk, *NSS* non-Stiff Stalk, *P41* CIMMYT's Pool 41

were not significant with a single exception ($r = 0.21$), and the absolute value of r was generally <0.1. The r_S value was only significant for MRD^2 versus MPH in SEM.

Discussion

The potential usefulness of exotic germplasm can be assessed on the basis of several criteria: (1) performance of F_1 hybrids of adapted × exotic inbreds, (2) per se performance of the exotic inbreds, (3) heterotic response in adapted × exotic hybrids, and (4) contribution to genetic variability. The first criterion is the most important, because the main objective of a breeder is to develop superior hybrids disregarding whether the superiority is due to increased per se performance of parents or heterosis. The second criterion is of interest for an economical seed production of hybrids and parents. Genetic variability and heterotic response have been responsible for the success of hybrid breeding and have potential long-term implications in hybrid breeding. Under a biallelic genetic model and no epistasis, MPH is expected to have linear correlation with genetic distance between parents (Falconer and Mackay 1996). Consequently, increasing the genetic distance between heterotic groups should be beneficial in hybrid breeding. Broadening the genetic variation within heterotic groups will enhance the selection gain and hybrid performance in future breeding cycles.

Hybrid performance in different mega-environments

The targeted CEM was more favorable for GY than USM, the mega-environment of the donor inbreds (Table 3). The lower GY in USM may be explained by an unusually low average GY during the year of testing in Iowa. It may also be partly due to field losses, which can increase after genotypes have reached physiological maturity of about 70% DMC, because of a higher incidence of dropped ears, stalk lodging, ear rots, and losses during harvest (Olson and Sander 1988). However, similar h^2 estimates in the USM and other mega-environments suggest that this was presumably only a minor reason for the lower GY. On the whole, the level of GY in CEM, h^2 estimates comparable to other mega-environments, and the highest h^2 for DMC, indicate that CEM should be preferred over SEM and USM to evaluate hybrids.

Per se performance of adapted and exotic inbreds

The relatively poor yield of SS lines in CEM and European Flints in USM may be due to poor adaptation. The European Flints were originally developed in Central Europe from open-pollinated populations adapted to the region and, therefore, have specific adaptation to the agro-climatic conditions in the targeted environment. In spite of their very early maturity, these lines showed good GY per se performance in CEM.

The late maturity of US inbreds caused problems in the joint evaluation of inbreds in CEM, as these did not reach physiological maturity. However, all lines matured in SEM, indicating the utility of deployment of an intermediate mega-environment for the evaluation when the germplasm is highly diverse for adaptation traits like maturity. The number of heat degree days in SEM is intermediate between CEM and USM. This is corroborated by higher r values of SEM with CEM and USM than of CEM with USM for both traits.

Heterosis and inbreeding depression

The higher estimates of MPH for GY of all groups of hybrids in CEM than SEM were because of higher GY of hybrids and partly due to poor adaptation of US inbreds in CEM. The generally smaller MPH in hybrids of European Dents with US inbreds relative to MPH in hybrids of European Flints was expected in view of the genetic distance of European Dents and Flints with US parental lines determined by SSR markers (Supplementary Table 1).

Quantitative genetic theory suggests that MPH equals ID in the absence of epistasis (Lynch and Walsh 1997). However, in our study NL was significantly negative for both traits in all mega-environments, indicating the presence of epistasis (Crow and Kimura 1970). This is in agreement with many studies in which epistasis for GY was reported to be present; it had a small magnitude in contrast to others in maize wherein epistasis was not

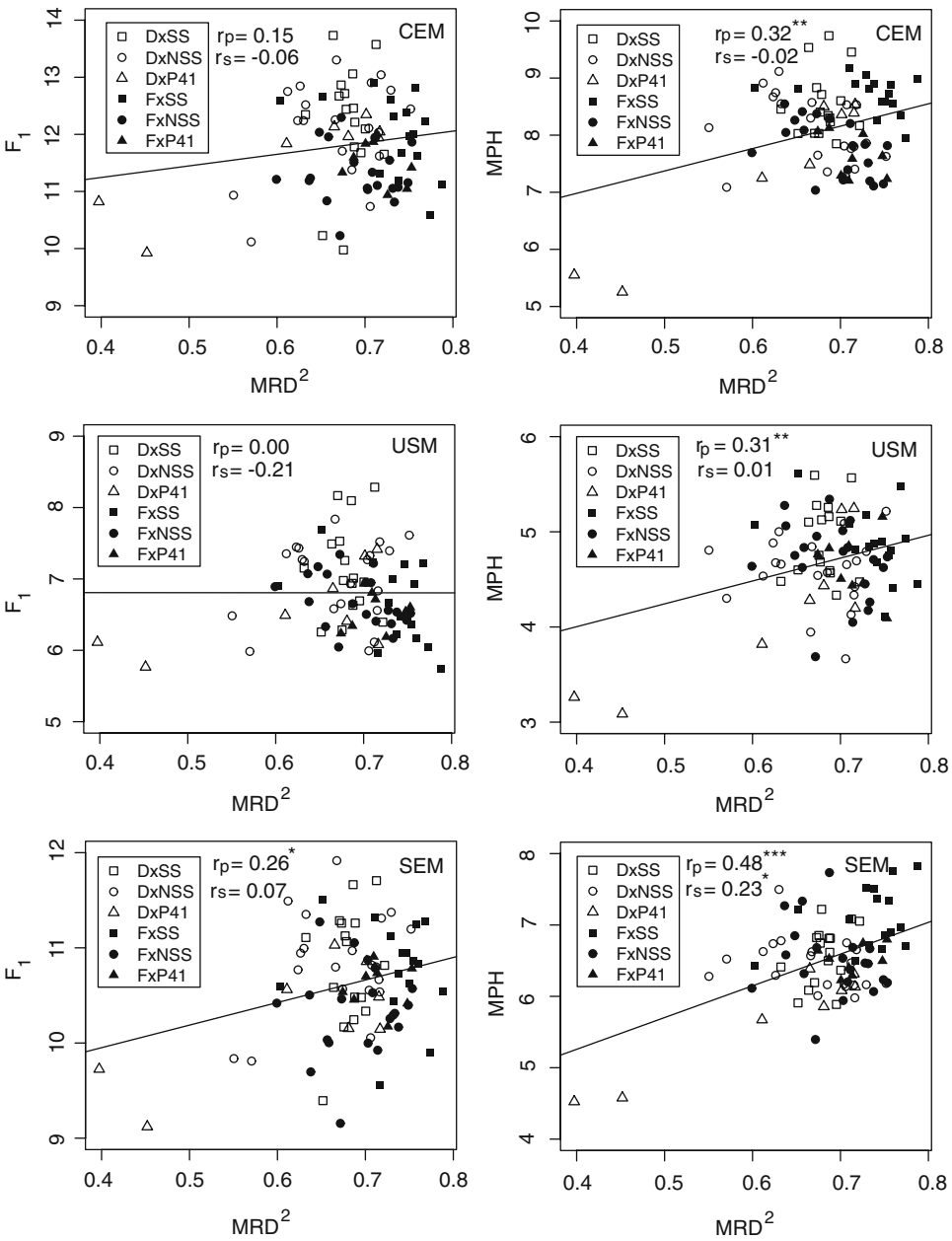

Fig. 3 Relationship of the squared Modified Rogers' distance (MRD^2) with mid-parent heterosis (*MPH*) and F_1 hybrid performance for grain yield in different mega-environments (CEM, USM, SEM). *D* European Dent, *F* European Flint, *SS* Stiff Stalk, *NSS* non-Stiff Stalk, *P41* CIMMYT's Pool 41, *CEM* Central Europe, *USM* US Cornbelt, *SEM* Southeast Europe

present (for review, see Edwards and Lamkey 2002). In addition to epistasis for GY per se, in the present study the genes responsible for adaptation are most likely to contribute to epistasis for GY in CEM and USM. Inbreds are known to be more prone to adaptation-related problems than hybrids. The US lines did not mature in CEM; conversely, the European Flint inbreds reached physiological maturity too early to make use of the entire growing season in USM. Thus, there may have been an overestimation of MPH as compared with ID. In contrast to CEM and USM, SEM represents a more balanced mega-environment in which adaptation-related problems are expected to play a less important role. The significant NL contrast in SEM, therefore, reinforces the presence of epistasis and also indicates epistatic interactions of genes other than those directly related to adaptation. Furthermore, our results demonstrate that ID has limited predictive value for MPH in the case of wide crosses.

Evaluation parameters and environments

The present study indicated that the focus should be on hybrid evaluation in the target CEM, because there was limited additional advantage of hybrid evaluation in the intermediate SEM. If the germplasm is highly diverse for adaptation traits like maturity and response to stresses, it

may be desirable to evaluate parental inbreds in an intermediate mega-environment. If resources permit, the F_1 hybrids may also be evaluated in the intermediate mega-environment to obtain more reliable estimates of their performance and MPH, and information on the stability of performance. In this case, F_1 performance in the targeted and intermediate mega-environment should get highest weight followed by MPH in the intermediate mega-environment, and the parental performance could be used as a supplement. Germplasm showing high GY potential and MPH could be subjected to selection for adaptation traits if required. Determination of ID would require production and evaluation F_2 hybrids and, thus, be less cost effective.

Molecular marker based broadening of heterotic groups

In contrast to the expectation of a linear relationship between MPH and MRD^2 under certain assumptions (Falconer and Mackay 1996), we observed no significant correlation between MPH for GY and MRD^2 in any mega-environment. Melchinger (1999) reviewed a number of studies in maize on this relationship and distinguished three scenarios: (a) strong positive *r* in crosses among related lines, (b) moderate positive *r* in crosses of unrelated lines from the same heterotic group, and (c) at best a weak positive *r*, when parents originated from different heterotic groups. As the parents used in our study belong to the third category, our results are in agreement with earlier findings. The lack of association may be due to (a) low *r* between heterozygosity estimated from marker data and heterozygosity at QTL affecting GY (Charcosset and Esioux 1994), (b) low *r* between heterozygosity and heterosis at QTL in the crosses studied (Charcosset et al. 1991), (c) presence of multiple alleles per locus (Cress 1966), and (d) epistasis (Moll et al. 1965; Falconer and Mackay 1996). In crosses of maize landraces from different geographic regions, Moll et al. (1965) reported that MPH increased with genetic divergence of parents, but there was an optimum level of parental divergence beyond which MPH decreased. This decrease is attributed to epistatic gene action associated with different coadapted gene complexes present in highly divergent parents (Falconer and Mackay 1996). The materials evaluated by us have been bred over a long time for performance in and adaptation to CEM or USM and are highly diverse. Consequently, each of the four reasons mentioned above may have contributed to the low *r* values.

While the present study revealed no significant correlation between molecular marker-based genetic distances and MPH, it demonstrated the separation of the European Flint and two NSS lines (B100, B102) from the rest. European Dents showed closer relationship with SS than with NSS inbreds as expected from their ancestry, which is dominated by US Dents, most notably SS lines. The SSR data revealed also a close, previously unknown relationship between line B107 from P41 and the European Dents. The estimates of MRD^2 confirm the greater relatedness between European Dent and SS lines than European Flint and SS lines (Supplementary Table 1). These results reinforce the notion that new elite SS germplasm may be introgressed into the European Dent pool and elite NSS (particularly germplasm like B100 and B102) into the European Flint pool. While this introgression is expected to broaden the genetic base within the European Dent and European Flint heterotic groups, it will not necessarily increase the genetic distance (and consequently MPH) between them, because the average MRD between European Flint and European Dent lines was larger than between SS and NSS lines.

Strategy for a targeted broadening of the European heterotic pattern

Strategies have been proposed and applied in European maize breeding programs to broaden the genetic base of heterotic pools. One strategy is to enrich the European Flint pool by introgressing adapted European open-pollinated varieties and the European Dent pool by making use of elite US inbreds. This provides an avenue to fully exploit the genes for specific adaptation in European Flints, which have been assembled through natural and artificial selection over years, such as imparting cold tolerance. However, it disregards the improvement in combining ability and per se performance of the US heterotic groups during the past decades.

Alternatively, elite US germplasm may be also introgressed into the European Flint pool, thus, making use of dent germplasm in both European heterotic groups in maize (Moreno-Gonzalez et al. 1997). This is practised in the warmer regions of Europe where Dent × Dent hybrids are replacing Dent × Flint hybrids. But this does not make full use of the specific adaptation of European Flint germplasm, which is very important in cooler regions such as CEM. The higher yield potential of Dent × Dent hybrids and the possible effects of climate change with increasing temperatures, European Flint pool in CEM also may be gradually enriched with US and other Dent germplasms as a long-term strategy. This will facilitate a steady shift towards development of Dent × Dent maize hybrids to exploit the greater yield potential of dent germplasm.

Based on the results from marker-based genetic distances, MPH and F_1 performance in the present study, our recommendation is to introgress SS into the European Dent and NSS into the European Flint pools. This strategy exploits the specific adaptation of the European Flint populations as well as excellent combining ability and high per se performance of the US germplasm. It is expected to

broaden the genetic base within European heterotic pools and improve the productivity of their hybrids.

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