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Prediction of testcross means and variances among F_3 progenies of F_1 crosses from testcross means and genetic distances of their parents in maize

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Abstract Prediction of the means and genetic variances in segregating generations could help to assess the breeding potential of base populations. In this study, we investigated whether the testcross (TC) means and variances of F_3 progenies from F_1 crosses in European maize can be predicted from the TC means of their parents and F_1 crosses and four measures of parental genetic divergence: genetic distance (GD) determined by 194 RFLP or 691 AFLP™ [1] markers, mid-parent heterosis (MPH), and absolute difference between the TC means of parents ($|P_1 - P_2|$). The experimental materials comprised six sets of crosses; each set consisted of four elite inbreds from the flint or dent germplasm and the six possible F_1 crosses between them, which were evaluated for mid-parent heterosis. Testcross progenies of these materials and 20 random F_3 plants per F_1 cross were produced with a single-cross tester from the opposite heterotic group and evaluated in two environments. The characters studied were plant height, dry matter content and grain yield. The genetic distance between parent lines ranged between 0.17 and 0.70 for RFLPs and between 0.14 and 0.57 for AFLPs in the six sets. Testcross-means of parents, F_1 crosses, and F_3 populations averaged across the six crosses in a particular set generally agreed well for all three traits. Bartlett's test revealed heterogeneous TC variances among the six crosses in all sets for plant height, in four sets for grain yield and in five sets for dry matter content. Correlations among the TC means of the parents, F_1 crosses, and F_3 populations were highly significant and positive for all traits. Estimates of the TC variance among F_3 progenies for the 36 crosses showed only low correlations with the four measures of parental genetic divergence for all traits. The results demonstrated that for our material, the TC means of the parents or the parental F_1 cross can be used as predictors for the TC means of F_3 populations. However, the prediction of the TC variance remains an unsolved problem.

Key words *Zea mays* L · AFLP · RFLP · Testcross mean · Testcross variance · Genetic distance · Midparent heterosis

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[1] AFLP™ is a trade mark filed in the Benelux under number 825962 by Keygene N.V.

Introduction

Based on a survey among U.S. corn breeders, Bauman (1981) concluded that the main efforts in the development of new lines in hybrid maize breeding are devoted to the re-cycling of established inbreds. Most commonly, F_2 or backcross populations are used as base materials for re-cycling (Darrah and Zuber 1986). Out of a large number of base populations produced every year by public and private maize breeders, the majority are discarded after their preliminary evaluation for *per se* and testcross (TC) performance in an "early testing" program. If the breeding potential of such populations could be predicted in advance from the properties of their parental lines, this would increase the efficiency of breeding programs to a great extent because it would allow the concentration of resources on the most promising populations.

Schnell (1983) proposed the 'usefulness' criterion to assess the breeding prospects of base populations for

extracting superior lines. 'Usefulness' is defined as the sum of the population mean and the selection response. The latter is a function of the selection intensity, genetic variance (σ_g^2), and heritability of the trait in the respective population (Falconer and Mackay 1996). Obviously, 'usefulness' accounts for differences in both means and genetic variances in the base populations.

Melchinger (1987) presented a biometrical model for predicting the TC means of F_2 and backcross progenies and of their selfing generations derived from a F_1 cross between two pure breeding lines. Accordingly, in the absence of epistasis the TC means of various base populations (F_2 or backcross) are linear functions of the proportion of germplasm from each parent line. Experimental studies with individual crosses in maize indicated that the TC means of F_2 and backcross generations can be predicted from the TC performance of the parents (Melchinger et al. 1988; Lamkey et al. 1995). However, further research is needed to corroborate the general validity of this prediction approach.

In contrast, prediction of the TC genetic variance still remains an unsolved problem and forms the main focus of our present study. Theoretical results show that in the absence of epistasis, the TC variance among progenies of backcrosses, or later selfing generations of F_2 and backcross populations, can be predicted from the TC variance in F_2 populations (Melchinger 1987). However, prediction of the TC variance in the F_2 generation itself would still be required.

The genetic divergence between parents may be used as an indicator of the genetic variance in F_2 or later-segregating generations. Bhatt (1970, 1973) in wheat, and Cowen and Frey (1985, 1987) in oats, observed a close association of the genetic variance in segregating generations with the genetic distance between the parents of the initial cross. In all these studies, prediction was for the genetic variance for line *per se* performance. However, in contrast to the development of line cultivars, in hybrid breeding line *per se* performance is only of secondary importance compared to TC performance. In addition, correlation between line *per se* and TC performance is not very high for most traits of agronomic importance (Smith 1986). No experimental studies are available on the prediction of the TC genetic variance in segregating generations.

With the advent of molecular markers, such as RFLPs, AFLPs, RAPDs and SSRs, it has recently become possible to assess the genetic distance between different germplasms at the molecular level with any degree of precision desired at reasonable costs. In particular, RFLPs proved to be an extremely powerful tool for estimating the genetic similarity of lines and the grouping of germplasms (Melchinger 1993). Based on these findings, it was speculated that the parental genetic distance for molecular markers linked to quantitative trait loci (QTLs) may provide a clue for the range of genetic variance to be expected in the segregating generations of a specific cross (Melchinger et al. 1988).

The objectives of the present study were to investigate the possibilities in European maize of: (1) predicting the TC means of F_3 plants derived from the F_1 crosses by the TC means of their parent lines ($\bar{P}$) and parental F_1 cross, and (2) predicting the TC variance in segregating populations (F_3 plants) by four measures of parental genetic distance, viz. (i) the genetic distance determined from RFLPs, (ii) the genetic distance determined from AFLPs, (iii) the mid-parent heterosis in the F_1 cross, and (iv) the absolute value of the difference between the TC means of the parents.

Materials and methods

Plant materials

In each of six sets, four inbred lines were crossed in all possible combinations yielding a total of six F_1 crosses per set. Sets 1 to 4 consisted of lines from the flint germplasm, whereas sets 5 and 6 comprised lines from the dent germplasm (Table 1). Inbred KW4 was common to sets 1 and 4 and inbred DK105 was common to sets 3 and 4. All lines had been inbred for more than ten selfing generations and are representative of modern earlymaturing European breeding materials. Detailed pedigrees of these lines have been given by Messmer et al. (1992). F_1 plants from each cross were selfed to obtain F_2 populations. Ten randomly chosen F_2 plants were selfed in each cross. From each of the resulting ten F_3 lines, two random F_3 plants were testcrossed to genetically narrow-based single-cross testers from the opposite heterotic pool (flint plants with dent × dent single-cross testers and *vice versa*) to produce a total of 120 inter-pool TC progenies per set (6 crosses × 20 TC progenies from the 20 F_3 plants per F_1 cross).

Field experiments

The original data for estimating the TC means and variances were taken from the experiments reported by Leipert (1990). In this study, 120 TC progenies for each set, together with duplicate entries of TC progenies of the corresponding four parent lines and their six

Table 1 Maize inbred lines and single-cross testers used in the six sets of crosses examined in this study

Set	Designation	Type
Parental inbreds[a]		
1	KW4, F1772, F251, KW20	Flint
2	NS1, NS2, NS3, NS4	Flint
3	DK105, KW7, KW8, Co255	Flint
4	D503, DK105, KW4, F1444	Flint
5	D44, D405, D406, KW13	Dent
6	NS5, NS6, NS8, NS9	Dent
Single-cross tester[a]		
1	KW12 × F252	Dent
2	NS6 × NS7	Dent
3	KW12 × F252	Dent
4	D02 × F252	Dent
5	KW13 × KW20	Flint
6	DK105 × D140	Flint

[a] Pedigrees of the parental inbreds were given in detail by Messmer et al. (1992)

F_1 crosses as well as four check hybrids, were evaluated at two locations in a 12×12 lattice design with two replications. Set 1 was evaluated in 1985, sets 2 to 4 in 1986, and sets 5 and 6 in 1987. In each set, the research station at Hohenheim in Stuttgart was used as a common test location. As a second test site, we used Gondelsheim near Bruchsal (sets 1, 3, and 5), Kirschgartshausen near Mannheim (sets 2 and 6), both sites being located in the Upper Rhine Valley, and Compiègne near Paris (set 4).

A separate experiment described in detail by Boppenmaier et al. (1993) was conducted for estimating the mid-parent heterosis of each F_1 cross. Parental lines and their F_1 crosses were tested in separate, but adjacent, experiments in 1990 at Hohenheim and Emmendingen (near Freiburg) using a randomized complete block (RCB) design with three replications. In all the experiments designed for estimating the TC means and variances, as well as those for estimating heterosis, we used two-row plots with 0.75 m spacing between rows. The plot size varied from 2.6 to 4.5 m^2 in different sets. The plant density ranged from 7.8 to 10.0 plants m^{-2} at different sites. Data were recorded for plant height (height of lowest tassel branch above the ground level, in cm), dry matter content (of the grain at harvest, in%), and grain yield (q ha^{-1}, adjusted to 15.5% grain moisture). The dry matter content in the heterosis experiment of Boppenmaier et al. (1993) was determined only at Hohenheim.

RFLP analyses

RFLP data were taken from the study by Boppenmaier et al. (1993). Briefly, we used two restriction enzymes (*Eco*RI, *Hin*dIII) in combination with 101 single-copy genomic DNA clones, resulting in a total of 194 clone-enzyme combinations as listed by Boppenmaier et al. (1992). Clones were chosen to provide a fairly uniform coverage of the entire maize genome with at least seven clones per chromosome. RFLP profiles on autoradiographs for each clone-enzyme combination were scored visually. Scoring was done twice and independently by two persons. Each band or clone was assigned a number according to its migration distance in the gel. Only full-intensity bands were considered to obtain reliable data. Two bands were scored as different when they were clearly separated from each other across all lanes in which they appeared. If RFLP bands were absent on a good-quality autoradiograph, a null variant was assigned with the number zero. For subsequent statistical analyses, data were coded in binary form, i.e., the presence or absence of a band in a line was coded by 1 or 0, respectively.

AFLP analyses

AFLP analyses were performed as described by Vos et al. (1995). Genomic DNA of the maize inbreds was digested with the restriction enzymes *Eco*RI and *Mse*I. In a second step, the following adapter sequences were ligated to the restricted DNA fragments:

*Eco*RI: 5′-CTCGTAGACTGCGTACC
CATCTGACGCATGGTTAA-5′
*Mse*I: 5′-GACGATGAGTCCTGAG
TACTCAGGACTCAT-5′.

The primers used for pre-amplification and amplification were similar to those described by Vos et al. (1995) with the following extensions:

AAC/CAC, AAC/CAT, AAC/CTC, AAG/CAA, AAG/CAT, AAG/CTC, AAG/CTG, AAG/CTT, ACA/CAG, ACA/CAT, ACA/CTA, ACT/CAA and ACT/CTC,

where the sequence before the slash refers to the primer extension for the *Eco*RI primer and that after the slash refers to the primer extension for the *Mse*1 primer. The PCR products were separated by electrophoresis on a denaturing polyacrylamide gel. After drying, the gels were exposed to phospho-imager plates for 16 h. The imager plates were scanned with a phosphor-imager and polymorphic bands were coded in binary form by 1 and 0 for presence or absence in each inbred, respectively. AFLP data for inbred NS4 had to be discarded due to suspected seed contamination.

Estimation of genetic distances

Estimates of the genetic distance (GD) between the six possible line combinations within each set were computed for each marker system using the formula given by Nei and Li (1979):

$$GD_{ij} = (N_i + N_j - 2N_{ij})/(N_i + N_j).$$

Here, GD_{ij} is the genetic distance between two inbred lines i and j, N_{ij} is the number of common bands between lines i and j, and N_i and N_j are the total number of bands in line i and j, respectively, with regard to all clone-enzyme combinations considered in the case of RFLPs and with regard to all primer pairs considered in the case of AFLPs. Thus, GD reflects the proportion of bands in common between two inbred lines and may range from 0 (identical profiles for two lines) to 1 (no common bands).

Statistical analyses

Initially, individual lattice and RCB experiments were analysed separately. For combined analyses across locations, entry means from each experiment were used. Pooled error mean-squares were computed based on the error variance estimated in individual experiments according to Cochran and Cox (1957). Estimates of the variance components σ_g^2 (genotypic variance) and σ_{ge}^2 (genotype × environment interaction variance) were calculated as described by Searle (1971). Estimates of σ_g^2 for each cross ($1.5\ \sigma_A^2$) were obtained from the data of the 20 TC progenies of F_3 plants from each F_1 cross, using a least-square estimate of the "primary" variance (σ_A^2 among F_3 lines) and "secondary" variance ($0.5\ \sigma_A^2$ among F_3 plants within F_3 lines) (Melchinger 1987). Heterogeneity of σ_g^2 among the six F_1 crosses within a set was tested using Bartlett's criterion (Snedecor and Cochran 1980).

Estimation of heterosis

Mid-parent heterosis (MPH) was calculated separately for each of the 36 F_1 crosses from the difference between the F_1 performance and the means of parental inbreds, averaged across environments. Due to poor germination of line KW4, the number of plants was too small to obtain reliable heterosis estimates for the following four crosses: KW4 × F251, KW4 × KW20, D503 × KW4, and DK105 × KW4.

Estimation of correlation coefficients

For each trait, simple correlations (r) were calculated across the 36 crosses among the TC means of the parents ($x_{\bar{P}}$), the F_1 generation (x_{F_1}), and the F_3 progenies ($x_{\bar{F}_3}$) derived from each cross. To exclude possible group effects due to different test environments and/or testers, mean products and mean squares were first calculated separately for the six crosses in each set and then pooled across the six sets for calculating correlations. A similar procedure was applied to calculate correlations among $\hat{\sigma}_g^2$ for each cross with estimates of GD, mid-parent heterosis, and the absolute value of the difference between the TC means of parents ($P_1 - P_2$).

Analysis of combined predictors

In addition to linear regression, we also evaluated multiple linear-regression models for the prediction of $\hat{\sigma}_g^2$ by using: (1) both $|P_1 - P_2|$ and GD estimates based on RFLPs as regressors, and (2) a quadratic function of $|P_1 - P_2|$. In both models, we accounted for possible group effects due to different test environments and/or testers using standard procedures (Snedecor and Cochran 1980).

Coefficient of coancestry (f)

The estimates of coefficient of coancestry (f) were taken from Messmer et al. (1992), wherein f was calculated as described by Falconer and Mackay (1996). For all pairs of lines without known parentage, f was set to zero. Two lines were designated as unrelated if their coancestry was smaller than 0.1.

Results

Testcross means

Because the sets had different testers and were evaluated in different environments, no direct comparison for the level of TC performance between sets was made. No significant ($P < 0.05$) difference was found between the mean TC performance of the four parent lines ($\bar{x}_P$), the six F_1 crosses ($\bar{x}_{F_1}$), and the 120 F_3 progenies ($\bar{x}_{F_3}$) within each set, for dry matter content and grain yield (Table 2). Only for plant height was $\bar{x}_{F_3}$ significantly ($P < 0.05$) smaller than $\bar{x}_P$ and $\bar{x}_{F_1}$ in set 5, and greater than these means in set 6.

Significant ($P < 0.05$) differences existed for TC means across environments among the four parent lines in all six sets and three traits, except in set 2 for plant height and grain yield (data not shown). A wide range in the TC means of the four parents was observed for plant height in sets 1 and 6, for dry matter content in sets 1, 4 and 5, and for grain yield in sets 4 and 5 (see maximum values for $|P_1 - P_2|$ in Table 3). In contrast, a narrow range existed among the TC means of the parents for plant height and grain yield in set 2 and for dry matter content in set 3.

Testcross variance $\hat{\sigma}_g^2$ among F_3 progenies

The means of over the six crosses in each set revealed no common pattern across the six sets (Table 3). A high number of crosses with significant ($P < 0.01$) $\hat{\sigma}_g^2$ estimates and large means for $\hat{\sigma}_g^2$ were observed for plant height in all five sets in which this trait was determined, for dry matter content in sets 1, 3, 4 and 6, and for grain yield in sets 2 and 3. In the other sets, $\hat{\sigma}_g^2$ values for individual crosses were often not significantly ($P < 0.05$) greater than zero and smaller than their corresponding standard errors. Bartlett's test revealed heterogeneity for $\hat{\sigma}_g^2$ among the six crosses within each set in most cases, with the exception of dry matter content in set 2 and grain yield in sets 1 and 6. This was also reflected in the wide range of $\hat{\sigma}_g^2$ among the six crosses in most sets.

Mid-parent heterosis in F_1 crosses

Estimates of MPH for plant height and grain yield were significantly ($P < 0.05$) positive for all crosses, except for cross NS2 × NS3 between closely related lines, with a coancestry coefficient $f = 0.50$. The average level of MPH was similar in all sets except for smaller values in set 2 for both plant height and grain yield and a greater value in set 4 for grain yield. The MPH of individual crosses for dry matter content varied in sign and was in many cases not significantly ($P < 0.05$) different from zero.

Variation for RFLP and AFLP markers

All 101 DNA clones revealed RFLPs among the 22 inbreds with at least one restriction enzyme. Only 9 (4.6%) of the 194 clone-enzyme combinations were monomorphic across all inbreds. Altogether, 957 distinct RFLP bands were detected across the 22 inbreds for the 194 clone-enzyme combinations assayed.

In the AFLP assays, the 13 primer combinations yielded a total of 691 markers (selectively amplified DNA fragments ranging in size from 60 to 600 bp). Most of these bands (70%) were polymorphic across all 21 inbreds assayed. The number of polymorphic bands per primer combination ranged from 17 to 91 with an average of 38.1, and was thus about eight times that observed per clone-enzyme combination with RFLPs. The number of monomorphic bands per primer combinations varied from 7 to 19 with an average of 12.9. Each inbred had a unique profile with regard to both RFLPs and AFLPs.

Genetic distances between parent lines

Estimates of the GD between parent lines determined from 194 RFLP markers varied from 0.70 for cross NS5 × NS6 in set 6 to 0.30 for D405 × D406 in set 5 and 0.17 for NS2 × NS3 in set 2 (Table 3). In comparison with the other three sets of flint lines, the six line-combinations in set 2 had low GD values, suggesting some relatedness between all four lines constituting this set. For the two sets of dent inbreds, the lower mean GD in set 5 (0.56) than in set 6 (0.63) was attributable to the low GD (0.30) of D405 × D406, a pair of related lines with $f = 0.45$ (Boppenmaier et al. 1993).

Estimates of the GD between combinations of parent lines determined from 691 AFLP markers ranged from 0.57 for cross D405 × KW13 in set 5 to 0.17 for D405 × D406 in set 5 and 0.14 for NS2 × NS3 in

Table 2 Means of testcross progenies in maize, produced from parent lines, their F_1 cross, and F_3 progenies crossed to single-cross testers, evaluated in two environments for plant height, dry matter content, and grain yield

Set	Cross $P_1 \times P_2$	Plant height (cm)			Dry matter content (%)			Grain yield (q ha^{-1})		
		$\bar{P}$[a]	F_1	$\bar{F}_3$[b]	$\bar{P}$	F_1	$\bar{F}_3$	$\bar{P}$	F_1	$\bar{F}_3$
1	KW4 × F1772	206.2	196.2	201.8	64.3	64.0	64.3	99.4	93.0	99.6
	KW4 × F251	191.6	201.9	192.8	66.8	67.0	66.0	91.6	100.2	94.2
	KW4 × KW20	204.4	200.0	197.8	66.2	65.9	65.8	98.8	98.0	97.6
	F1772 × F251	192.4	193.8	191.2	66.0	64.9	65.8	92.2	102.3	94.5
	F1772 × KW20	205.6	200.0	203.7	65.4	66.8	65.2	99.3	100.3	99.7
	F251 × KW20	190.5	194.4	188.8	67.9	65.5	66.9	91.5	91.1	94.6
	Mean[c]	198.4a	197.7a	196.0a	66.1a	65.7a	65.7a	95.5a	97.5a	96.5a
2	NS1 × NS2	246.6	242.8	247.7	59.9	59.7	59.5	100.4	96.3	97.5
	NS1 × NS3	246.1	238.9	245.2	59.5	59.6	59.1	102.3	92.5	99.5
	NS1 × NS4	249.1	254.4	241.4	59.2	58.9	58.7	103.6	102.1	99.2
	NS2 × NS3	246.9	245.9	243.7	60.9	60.6	60.9	103.8	98.1	98.5
	NS2 × NS4	249.9	248.4	245.2	60.5	60.1	60.3	105.1	103.7	100.3
	NS3 × NS4	249.4	244.0	247.0	60.1	59.7	60.0	106.9	98.2	103.8
	Mean	248.0a	245.9a	245.0a	60.0a	59.8a	59.7a	103.7a	98.5a	99.8a
3	DK105 × KW8	232.2	235.0	235.1	63.0	63.0	62.6	95.4	93.9	95.1
	DK105 × KW7	225.5	223.8	224.9	63.5	63.2	63.4	93.2	91.1	86.0
	DK105 × Co255	230.8	234.7	233.5	63.6	64.0	62.3	92.4	88.5	91.0
	KW8 × KW7	223.7	220.3	233.7	62.8	62.8	63.0	89.3	85.1	83.7
	KW8 × Co255	229.1	228.7	231.2	62.9	62.6	62.1	88.5	89.1	88.1
	KW7 × Co255	222.3	221.5	227.4	63.3	62.8	62.3	86.3	87.8	88.2
	Mean	227.3a	227.3a	230.9a	63.2a	63.1a	62.6a	90.8a	89.2a	88.7a
4	D503 × DK105	–[d]	–	–	62.5	62.4	62.5	84.0	87.7	82.2
	D503 × KW4	–	–	–	62.8	61.5	61.0	83.4	84.0	87.8
	D503 × F1444	–	–	–	62.2	60.7	60.4	78.5	79.2	81.6
	DK105 × KW4	–	–	–	60.4	60.1	60.6	94.1	92.2	93.7
	DK105 × F1444	–	–	–	59.8	59.4	59.6	89.2	87.6	84.5
	KW4 × F1444	–	–	–	60.1	59.6	59.8	88.6	80.0	85.6
	Mean	–	–	–	61.3a	60.6a	60.6a	86.3a	85.1a	85.9a
5	D44 × D405	196.0	191.7	192.4	61.7	61.7	63.1	68.7	68.4	70.1
	D44 × D406	195.9	198.5	193.6	61.2	61.1	60.3	74.6	72.2	71.4
	D44 × KW13	195.7	194.1	194.1	60.6	59.9	59.9	77.9	70.3	71.0
	D405 × D405	204.6	203.0	205.7	59.9	59.9	59.9	75.0	70.4	72.6
	D405 × KW13	204.4	200.7	203.4	59.3	59.4	58.9	78.4	79.0	76.2
	D406 × KW13	204.3	202.9	197.1	58.7	58.9	59.2	84.3	75.2	78.3
	Mean	200.1a	198.5a	197.7b	60.2a	60.1a	60.2a	76.5a	72.6a	73.3a
6	NS8 × NS5	230.8	227.2	233.7	57.5	58.7	57.2	73.4	76.7	73.7
	NS8 × NS6	224.8	227.9	227.0	57.7	57.1	57.8	74.5	78.0	72.1
	NS8 × NS9	225.2	223.1	226.0	58.4	58.4	57.9	75.9	74.6	72.9
	NS5 × NS6	236.3	234.1	242.4	56.4	57.0	55.4	78.7	74.0	75.4
	NS5 × NS9	236.6	239.9	239.3	57.1	57.0	56.6	80.1	79.6	74.9
	NS6 × NS9	230.6	235.6	236.1	57.3	57.5	56.6	81.2	86.6	81.9
	Mean	230.7a	231.3a	234.1b	57.4a	57.6a	56.9a	77.3a	78.2a	75.1a

[a] $\bar{P} = (P_1 + P_2)/2$
[b] $\bar{F}_3$ = Mean of testcrosses of 20 random F_3 progenies from the respective cross
[c] Means followed by the same letter for a given trait were not different at the 0.05 probability level using at a *t*-test
[d] Not determined

set 2 (Table 3). Except for set 2 with a lower mean-GD (0.23), all other five sets had a similar mean-GD ranging from 0.43 to 0.48.

Phenotypic correlations

Correlations among the TC means of the parents ($\bar{P}$), F_1 crosses, and corresponding F_3 progenies ($\bar{F}_3$), were significantly ($P < 0.01$) positive for all traits and greatest ($r > 0.76$) between $\bar{P}$ and $\bar{F}_3$ for dry matter content and grain yield (Table 4). For all three traits, $\hat{\sigma}_g^2$ was positively associated with the parental GD, determined either by RFLPs or AFLPs, but the correlation was significant ($P < 0.05$) in only one case. Correlations of MPH with $\hat{\sigma}_g^2$ were low ($|r| < 0.21$) and varied in sign for the three traits. Likewise, $|P_1 - P_2|$ had a moderate positive ($0.29 \leq r \leq 0.37$) correlation

Table 3 Estimates of genetic distance (GD) between parents (P_1, P_2) based on 194 RFLP markers or 691 AFLP markers, absolute difference ($|P_1 - P_2|$) of testcross performance of parents, mid-parent heterosis (MPH) in their F_1 cross, and testcross variance (σ_g^2) among F_3 progenies from $P_1 \times P_2$ evaluated in two environments for plant height (PHT), dry matter content (DMC) of grain, and grain yield (GY)

Set	Cross $P_1 \times P_2$	GD		$\lvert P_1 - P_2 \rvert$			MPH[a]			σ_g^2		
		RFLPs	AFLPs	PHT (cm)	DMC (%)	GY (q ha^{-1})	PHT (cm)	DMC[c] (%)	GY (q ha^{-1})	PHT (cm)	DMC (%)	GY (q ha^{-1})
1	KW4 × F1772	0.62	0.43	2.5	1.6	1.1	89.20	5.55	46.87	9.87	0.91**	9.20
	KW4 × F251	0.49	0.47	27.7	3.4	14.5	–[b]	–	–	14.24	1.47**	23.68**
	KW4 × KW20	0.59	0.49	1.2	2.1	0.2	–	–	–	26.96**	1.26**	14.96
	F1772 × F251	0.56	0.41	30.2	5.0	15.6	88.30	− 0.75	47.94	47.12**	0.52*	11.79
	F1772 × KW20	0.58	0.49	3.7	3.8	1.3	73.35	2.80	42.77	17.05*	1.94**	15.55*
	F251 × KW20	0.56	0.44	26.6	1.2	14.3	76.65	− 6.85	36.12	36.53**	0.21	12.53
	Mean	0.57	0.45	15.3	2.8	7.8	81.88	0.19	43.43	25.29	1.05	14.62
2	NS1 × NS2	0.48	0.28	1.6	2.7	2.9	40.85	2.65	6.10	53.28**	0.37	13.53
	NS1 × NS3	0.47	0.28	0.5	1.9	6.7	52.05	7.05	36.83	47.05**	0.22	8.02
	NS1 × NS4	0.47	–[b]	6.6	1.3	9.3	36.35	8.70	44.42	34.01**	0.84**	27.56**
	NS2 × NS3	0.17	0.14	1.0	0.8	3.7	29.60	− 1.30	− 2.06[NS]	7.30*	0.07	14.01*
	NS2 × NS4	0.46	–	5.0	1.4	6.3	44.55	2.75	20.48	9.92	0.39*	27.02*
	NS3 × NS4	0.45	–	6.1	0.6	2.6	53.35	2.75	20.05	44.55**	0.32*	34.29*
	Mean	0.42	0.23	3.5	1.4	5.2	42.79	3.77	20.97	32.69	0.37	20.80
3	DK105 × KW8	0.41	0.37	3.5	1.3	7.8	59.15	6.90	40.89	15.09*	0.50**	1.55
	DK105 × KW7	0.63	0.43	17.0	0.4	12.2	46.25	7.35	46.17	84.29**	0.84**	34.71**
	DK105 × Co255	0.64	0.50	6.3	0.2	13.7	100.80	5.75	61.47	19.11**	0.93**	29.99**
	KW8 × KW7	0.55	0.40	13.5	0.8	4.4	74.60	4.80	31.52	33.47**	1.06**	15.55*
	KW8 × Co255	0.61	0.46	2.8	1.0	5.9	80.40	1.30	34.48	24.36**	0.39*	26.64*
	KW7 × Co255	0.60	0.41	10.7	0.2	1.5	87.50	0.65	42.18	14.48	0.73**	10.84
	Mean	0.57	0.43	9.0	0.6	7.6	74.78	4.46	42.79	31.80	0.74	18.43
4	D503 × DK105	0.47	0.40	–[b]	4.8	21.4	77.50	2.60	47.90	–[b]	1.06**	31.25**
	DK503 × KW4	0.63	0.46	–	4.1	20.2	–[b]	–	–	–	0.43*	18.16
	D503 × F1444	0.60	0.47	–	5.4	10.4	92.10	6.80	64.97	–	0.45*	6.58
	DK105 × KW4	0.63	0.46	–	0.7	1.2	–[b]	–	–	–	0.20	20.46*
	DK105 × F1444	0.63	0.48	–	0.5	11.0	67.05	11.25	72.99	–	0.20	0.01
	KW4 × F1444	0.61	0.43	–	1.3	9.8	82.90	8.10	61.02	–	0.50*	13.13
	Mean	0.59	0.45	–	2.8	12.3	79.89	7.19	61.72	–	0.47	14.93
5	D44 × D405	0.59	0.56	17.3	2.6	0.8	65.00	4.25	54.44	19.04**	0.66**	0.01
	D44 × D406	0.59	0.50	17.2	3.8	12.7	61.25	4.30	51.46	66.14**	0.19	21.70*
	D44 × KW13	0.66	0.52	16.7	4.9	19.3	78.30	1.30	61.92	30.20**	0.31	20.46*
	D405 × D406	0.30	0.17	0.1	1.2	11.8	37.50	6.85	36.26	13.72	0.21	17.95
	D405 × KW13	0.61	0.57	0.6	2.3	18.5	69.90	8.65	68.62	24.14**	0.11	8.77
	D406 × KW13	0.60	0.51	0.5	1.1	6.6	65.80	6.15	64.65	34.58**	0.17	4.75
	Mean	0.56	0.47	8.7	2.6	11.6	62.96	5.25	56.23	31.31	0.28	12.28
6	NS8 × NS5	0.55	0.45	22.9	2.6	8.4	75.40	0.70	56.33	33.08**	1.02**	7.61
	NS8 × NS6	0.68	0.46	10.9	2.2	10.5	82.95	− 1.30	42.16	64.59**	0.33*	12.67
	NS8 × NS9	0.58	0.49	11.6	0.7	13.4	82.10	6.35	59.14	13.04	0.23	16.19
	NS5 × NS6	0.70	0.49	12.0	0.4	2.1	82.50	− 4.30	53.98	28.02**	0.54**	21.49*
	NS5 × NS9	0.60	0.49	11.3	1.8	5.1	58.40	1.25	46.63	59.34**	0.33*	15.70
	NS6 × NS9	0.64	0.50	0.7	1.5	3.0	76.65	0.45	50.47	52.81**	0.70**	12.49
	Mean	0.63	0.48	11.6	1.5	7.1	76.33	0.53	51.79	41.81	0.53	14.36

*,** Significant at the 0.05 and 0.01 probability levels, respectively
[a] Except for the one marked as NS, all the estimates of MPH for PHT and GY are significant at the 0.05 probability level
[b] Not determined, see Materials and methods
[c] Determined only in Hohenheim (1990)

with $\hat{\sigma}_g^2$ for all traits, especially grain yield. Similar results were obtained for the polynomial regression between these two traits (data not shown). The coefficients of determination (R^2) for all three traits in the multiple regression models with $|P_1 - P_2|$ and GD estimates determined from the RFLP data were less than 0.20. The GD estimates determined from RFLPs were tightly correlated with GD estimates determined from AFLPs ($r = 0.85$**), and this also applied when the three crosses among related lines ($f > 0.0$) were excluded from the calculations ($r = 0.62$**).

Table 4 Correlation coefficients (r) between testcross means of parents, their F_1 crosses (F_1) and corresponding F_3 progenies ($\bar{F}_3$), and between testcross variance among F_3 progenies ($\hat{\sigma}^2_g$), genetic distance (GD) of their parents determined with 194 RFLP and 691 AFLP markers, mid-parent heterosis (MPH) of their F_1 cross, and difference between the TC means of the two parents ($|P_1 - P_2|$) for plant height, dry matter content, and grain yield in European maize

Variable		Correlation r (X, Y)		
X	Y	Plant height	Dry matter content	Grain yield
$\bar{P}$	F_1	0.41**	0.76**	0.50**
$\bar{P}$	$\bar{F}_3$	0.44**	0.81**	0.76**
F_1	$\bar{F}_3$	0.50**	0.70**	0.53**
$\hat{\sigma}^2_g$	GD-RFLPs	0.39*	0.01	0.08
$\hat{\sigma}^2_g$	GD-AFLPs	0.20	0.13	0.06
$\hat{\sigma}^2_g$	MPH	− 0.21	0.19	− 0.05
$\hat{\sigma}^2_g$	$\|P_1 - P_2\|$	0.29	0.29	0.37*

*,** Significant at the 0.05 and 0.01 probability levels, respectively

Discussion

The main aim of our present study was to identify predictors for two important components of Schnell's (1983) concept of 'usefulness', i.e. the mean μ and the genetic variance, σ^2_g, among segregating progenies, in the framework of hybrid breeding. This is because the breeding prospects of base populations are primarily determined by these two components (Melchinger 1987).

Prediction of TC means

Our results clearly demonstrate that inter-pool TC means of segregating generations derived from F_1 crosses can be predicted fairly accurately from the average TC performance ($\bar{P}$) of their parents (Table 4). For dry matter content and grain yield, the respective coefficient of determination (R^2) was greater than 58%. The TC performance of the parental F_1 cross had a lower power of prediction, most likely due to the greater standard error of this predictor compared with $\bar{P}$ (data not shown). Altogether, these findings corroborate both the theoretical expectations (Melchinger 1987) and the previous studies in the literature with individual pairs of parents (Lamkey et al. 1995; Melchinger et al. 1988) in that the TC means of F_2 or backcross populations, or later selfing generations derived from them, are predictable from the TC means of the parents.

This predictor for the mean TC performance of base populations has the following advantages for the maize breeder. First, the required information can be obtained within one year or two seasons: (1) TC seed can be produced in a greenhouse or off-season nursery, and (2) TC progenies must be evaluated in the target environment(s). Second, by testing the TC of n parent lines, it is possible to predict the mean TC performance of $n(n-1)/2$ possible F_2 populations and their selfing generations without even producing their parental F_1 crosses. Consequently, a linear increase in the testing expenditures is rewarded by a quadratic increase in the amount of information for the breeder. Third, from the TC performance of the parents one can additionally predict the mean TC performance of the $n(n-1)$ possible backcrosses (BC_1) to both parents and, if of interest, also of higher backcross generations based on the formulae given by Melchinger et al. (1988).

Significance of epistatic effects

The comparison of the TC means of the parents, the F_1 crosses, and the F_3 progenies for individual crosses, and averages over the six crosses for a given set, also provides a test for epistasis. This test has the advantage that it is not confounded with maternal or reciprocal effects, because all three generations served as pollen parents and were crossed onto a single-cross tester as the seed parent. In technical terms, the TC of the three generations (P, F_1, F_3) represent gamete-orthogonal populations (for definition see Melchinger 1988), which may differ in the linkage disequilibria among loci in the paternal gametic arrays but have identical expected genotype frequencies at each locus.

The absence of significant differences between the TC means of the three generations in most sets for all three traits suggests that: (1) epistasis was of minor importance in our materials, and/or (2) positive and negative epistatic effects cancelled each other (Table 2). Melchinger et al. (1988) also found no indication for epistasis for grain and forage traits from the comparison of the TC means of the parents, the F_1, F_2, and BC_1 generations of elite dent lines. In contrast, in a comparison of the TCs of these generations plus the TCs of the F_2-Syn8 generation from the cross B73 × B84, Lamkey et al. (1995) found significant epistatic effects for grain yield and grain moisture mainly due to recombination losses in the latter generation. The presence of positive epistatic gene complexes was also indicated in comparisons between balanced sets of single (2 W), three-way (3 W), and double crosses (4 W) between selected lines, in that the average yield performance decreased from 2 W to 3 W and 4 W by 2.6% and 3.9%, respectively (for a review see Melchinger 1984). By comparison, averaged across the six sets in our study the TC means for grain yield of the F_1 and F_3 generations decreased relative to the TC means of the parents only by 1.4% and 1.5%, respectively. Because the parent lines in our experiments were mostly the result of numerous cycles of re-cycling breeding, in contrast to the lines employed in most earlier studies, we hypothesize that epistasis was of greater importance in older materials than in modern elite lines.

Prediction of the TC variance in segregating populations

A positive association existed between $\hat{\sigma}_g^2$ and three predictors (GD-RFLPs, GD-AFLPs, $|P_1 - P_2|$) investigated in the present study (Table 4). Only MPH showed positive and negative correlations with $\hat{\sigma}_g^2$. However, all correlations were too small to be of predictive value for the breeder, irrespective of the trait considered. Even the quadratic relationship between $\hat{\sigma}_g^2$ and $|P_1 - P_2|$ and the multiple linear regression of $\hat{\sigma}_g^2$ on $|P_1 - P_2|$ and GD estimates hardly improved the prediction of $\hat{\sigma}_g^2$. In a study with oats (Moser and Lee 1994), combined estimates of RFLP-based genetic distance and genealogical distance were also not associated with $\hat{\sigma}_g^2$. Explanations for this disappointing result could be: (1) shortcomings in our experimental data, and/or (2) reasons of a more fundamental nature. Both are subsequently discussed in detail.

Our goal was to investigate the relationship between σ_g^2 and various predictors, Y, but our correlations were actually calculated between estimates of both variables. Consequently, errors in $\hat{\sigma}_g^2$ and $\hat{Y}$ will reduce the correlation $r(\hat{\sigma}_g^2, \hat{Y})$ relative to $r(\sigma_g^2, Y)$. This applies especially to $\hat{\sigma}_g^2$, for which the corresponding standard deviations were in most crosses of greater magnitude than $\hat{\sigma}_g^2$ for grain yield, of similar magnitude for dry matter content, and of smaller magnitude for plant height (data not shown). The large standard errors for $\hat{\sigma}_g^2$ are due to the small sample size (20) of the TC progenies sampled from each cross, which was very likely inadequate for obtaining reliable estimates of σ_g^2, and their evaluation in only two environments. Decreasing the standard errors of σ_g^2 substantially would require increasing the number of TC progenies per cross to over 100 and testing them in several (> 4) environments. However, this would be almost impossible and extremely demanding of the resources required for a greater number of crosses, as used in the present study and needed for a reliable estimation of the correlations.

Sizeable errors were also associated with estimates of MPH and $|P_1 - P_2|$ (data not shown), which could be reduced, at low expenditure, by increasing the number of test environments and replications per trial. In contrast, our estimates of GD based on RFLPs and AFLPs should be fairly accurate given the large number of markers assayed with each marker system and the good coverage of the entire genome.

The idea of using MPH as a predictor of σ_g^2 was originally proposed for the breeding of autogamous crop species, where σ_g^2 refers to the segregation variance for line *per se* performance. Under this setting a high correlation of σ_g^2 with MPH can be expected, when the degree of dominance (d/a) is constant and greater than zero (i.e. partial or complete dominance or over-dominance) at all segregating QTLs contributing to σ_g^2 (Melchinger, unpublished results). However, this consideration does not apply, if σ_g^2 refers to the segregation variance for TC performance. In this case, heterozygous QTLs contributing to the MPH in $P_1 \times P_2$ do not necessarily also contribute to σ_g^2, because differences in the alleles from P_1 and P_2 can be masked by dominant alleles in the tester. Conversely, QTLs with additive gene action may contribute to σ_g^2 for TC performance but not to MPH in $P_1 \times P_2$. In either case, this would result in a reduction of the correlation r (σ_g^2, MPH).

The rationale for predicting σ_g^2 by measures of the GD between P_1 and P_2 is that: (1) only heterozygous QTLs can contribute to σ_g^2, and (2) the GD determined from marker loci provides an indicator for the proportion of heterozygous QTLs in $P_1 \times P_2$. The latter can be expected for crosses between lines related by pedigree, because in this case the GD as well as the MPH and the proportion of heterozygous QTLs are a linear function of the coancestry coefficient f (Melchinger 1993). In our study, the three crosses between related lines (NS2 × NS3 with $f = 0.5$, D405 × D406 with $f = 0.45$, and D503 × DK105 with $f = 0.18$) showed a reduction in MPH corresponding to their f values, but no clear trend was found for $\hat{\sigma}_g^2$. For crosses between unrelated lines ($f = 0.0$), the expected relationship between GD estimates and $\hat{\sigma}_g^2$ parallels that between the GD estimates and the MPH investigated in detail by Charcosset and Essioux (1994). Accordingly, a close association between both variables can be expected only if each DNA marker employed for calculating the GD estimates is either identical to, or else extremely tightly linked with, a QTL for the trait and, *vice versa*, each polymorphic QTL is marked by one DNA marker. This would require: (1) a comprehensive knowledge about the location of QTLs for TC performance for each trait and germplasm pool, and (2) the calculation of trait-specific GD estimates. The latter requirement is supported by the low correlations ($|r| < 0.25$) among $\hat{\sigma}_g^2$ for the three traits in our study. Because information about the location of QTLs for grain traits in European maize is still lacking, the sets of RFLP and AFLP markers used for calculating our GD estimates were chosen under the premise of a uniform coverage of the entire genome. Increasing the number and density of markers will not necessarily improve the association between GD and $\hat{\sigma}_g^2$, as can be seen from the comparison of the respective correlations for the GD estimates based on RFLP and AFLP markers (Table 4).

The only predictor of σ_g^2 in our study which takes into account the influence of the tester is $|P_1 - P_2|$. A high correlation between the two variables can be expected if, for every cross $P_1 \times P_2$, all QTLs contributing to σ_g^2 are in coupling phase and have a similar genetic effect. This implies that for all QTLs contributing to $|P_1 - P_2|$, the allele increasing the TC performance originates always from the same parent. In contrast, QTLs in repulsion phase will contribute

equally to σ_g^2, but their contributions to $|P_1 - P_2|$ will cancel each other, at least partly, because of the summation of positive and negative terms. In the extreme case, $|P_1 - P_2|$ will be zero but σ_g^2 can be maximum if all QTLs for the TC performance of a given trait in a $P_1 \times P_2$ cross are in a heterozygous state and P_1 carries the favorable alleles at a half of these QTLs. A preponderance of coupling-phase QTLs can be expected in crosses between extreme parents with large values for $|P_1 - P_2|$. However, the parents chosen by breeders for establishing new base populations are generally elite lines with a similar TC performance for important traits. This applies also to the six sets of lines in our study as reflected by the relatively small range in estimates of $|P_1 - P_2|$ (Table 3). In summary, we can conclude that the predictive power of $|P_1 - P_2|$ is limited by the fact that large values of $|P_1 - P_2|$ are associated with large values of σ_g^2 whereas small values of $|P_1 - P_2|$ are not necessarily indicative of small values of σ_g^2.

Conclusions

We have demonstrated that the TC means of segregating populations derived from F_1 crosses can be predicted efficiently from the TC means of the parent lines. However, prediction of σ_g^2, the genetic variance among TC progenies from such populations, still remains unsolved. Neither MPH nor GD estimates based on RFLPs or AFLPs can be used for predicting σ_g^2 because both ignore the masking effect of the tester on σ_g^2 and have other shortcomings. Likewise, the difference $|P_1 - P_2|$ between the TC performance of the parents, which takes into consideration the effect of testers, gives a weak indication about the relative size of σ_g^2 only under certain circumstances.

Another lesson from this study is that an experimental investigation on the quality of predictors for σ_g^2 will almost certainly exceeds a manageable size. This is because the reliable estimation of σ_g^2 requires testing a large number of TC progenies from each F_1 cross, and a reliable estimation of the correlation $r(\hat{\sigma}_g^2, Y)$ requires the examination of a large number of crosses.

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