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Effect of recombination in the parent populations on the means and combining ability variances in hybrid populations of maize (*Zea mays* L.)

Abstract Recombination of selected genotypes plays a key role in plant breeding for generating new base populations. We investigated the influence of recombination in two parent populations on the means and combining ability variances of their hybrid population by (1) quantitative genetic theory and (2) experiments with maize. The two parent populations were founded by four early flint and four early dent inbred lines, respectively. Each population was studied in three generations: Syn-0, the four inbred lines themselves; Syn*-1, the six intrapool single crosses (SC); and Syn*-2, the three intrapool double crosses (DC). Four interpool hybrid populations were created: (1) all 16 SC and (2) all 36 DC were produced from generations Syn-0 and Syn*-1, respectively, (3) 168 biparental progenies (BIP) of type flint × dent (female × male), and (4) 168 BIP of type dent × flint were produced according to NC-design I with randomly sampled plants of generation Syn*-2. The half-sib and full-sib families obtained in this manner were evaluated for grain yield, dry matter concentration and plant height. According to theoretical results, differences in the population means of these hybrid populations indicate the presence of various types of epistasis. Changes in combining ability variances from SC to DC reflect different levels of parental inbreeding (F = 1 vs F = 0), whereas changes from DC to BIP only reflect the effects of recombination and are attributable to covariances between additive and dominance effects caused by linkage disequilibrium in the Syn-0 generations. The experimental results showed a significant decline in yield from DC to BIP due to a loss of gene combinations with favourable epistatic effects. Estimates of σ^2_{GCA} attributable to flint or dent lines decreased or remained unchanged from SC to DC, but generally increased in the BIP populations. The

consequences of these trends for developing improved interpool hybrids are discussed.

Introduction

All breeding categories commence with a "phase of procuring initial variation", which provides the base populations for subsequent breeding steps (Schnell 1982). In an advanced program for hybrid varieties, the parental base populations are commonly created by intermating selected genotypes. The early generations of such base populations (e.g., F_2 and backcross populations or synthetics from elite inbred lines) are expected to be in linkage disequilibrium (LD). The same applies to the selected genotypes of a population undergoing recurrent selection. In both cases, intermating is practised to reduce LD and thereby increase genetic variation by means of recombination.

For subsequent selection measures, it is important to quantify the effect of recombination in the parent populations on the distribution properties of their hybrid population. In a theoretical study, Comstock and Robinson (1952) investigated the effects of LD on the genetic variance components of NC-design III progenies. They concluded that the approach to linkage equilibrium could result in a decrease or increase of the general combining-ability variance (σ^2_{GCA}), depending upon the preponderance of coupling or repulsion-phase linkages in the parents, respectively. In contrast, the variance due to specific combining ability (σ^2_{SCA}) is expected to decrease in either case. Theoretical results about the influence of parental LD on the covariance between relatives are available for random-mating populations (Gallais 1974; Weir et al. 1980) and hybrid populations (Melchinger 1988).

Numerous experimental studies have been conducted in maize demonstrating that recombination in the parental populations usually leads to a reduction in the means

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of hybrid populations due to a loss of gene combinations with favourable epistatic effects (for a review see Melchinger 1984). Limited experimental information is available in the literature about the effect of recombination in the parental populations on the heritable variance of their hybrid populations. Lamkey et al. (1995) compared the testcross means and genetic variances of F_2 and F_2-Syn-8 populations generated from the cross of U.S. maize inbreds B73 and B84 and found a substantial effect of recombination on both.

The objectives of our study were to (1) investigate the influence of recombination in two parent populations on the means and combining ability variances of their hybrid populations by deriving quantitative-genetic expectations and by estimating pertinent parameters in an experiment with maize, (2) discuss the experimental results relative to the theoretical expectations, and (3) assess the chances of selecting parents for superior interpool hybrids.

Theory

Assumptions

Let parents $P_1^I, P_2^I, \ldots P_{n^I}^I$ and $P_1^{II}, P_2^{II}, \ldots P_{n^{II}}^{II}$ be homozygous inbreds constituting the Syn-0 generation of parent populations π^I and π^{II}, respectively. Subsequent generations are produced either by continued random mating or symmetric outbreeding and denoted as Syn-t or Syn*-t, respectively. Symmetric outbreeding consists of all possible matings between unrelated genotypes of generation Syn*-(t − 1) (for details see Melchinger 1984). The hybrid populations $\pi^I \times \pi^{II}$ are produced by factorial matings between all genotypes or a random sample of genotypes from a given generation of π^I and π^{II}, depending upon whether the generation used (Syn-t or Syn*-t) consists of a finite or infinite number of different genotypes, respectively. For illustration, we refer to the mating design employed in our experimental study described below. We assume no selection of gametes or genotypes in producing the Syn or Syn* generations of the hybrid populations.

Means

The expected mean $\mu_{\pi^I \times \pi^{II}}$ of a hybrid population between any generations Syn-t or Syn*-t from π^I and π^{II} can be expressed in terms of a model, which allows for digenic epistasis but no linkage (Melchinger 1988):

$$\mu_{\pi^I \times \pi^{II}} = {}_e\mu_{\pi^I \times \pi^{II}} + \xi^I aa^I + \xi^{II} aa^{II} + \xi^I \xi^{II} dd. \tag{1}$$

Here, ${}_e\mu_{\pi^I \times \pi^{II}}$ = mean of hybrid population $\pi^I \times \pi^{II}$, when the parental gametic arrays are in linkage equilibrium. For this situation, Schnell (1965) coined the term gene-orthogonal population and gave the partitioning into dually defined gene effects, which are used here; aa^I = sum of additive × additive epistatic effects over all loci pairs,

Table 1 Values of coefficients ξ, ϕ and ψ in Eqs. (1) and (3) to (5) for the various generations

Generation of parent population	Hybrid population	ξ	ϕ	ψ
Syn-0	SC	1	1	1
Syn*-1	DC	1/3	1/3	1/3
Syn*-2	BIP	0	1/3	0

weighted by the gamete frequencies of gametes produced by generation Syn-0 of π^I; aa^{II} = defined analogously with respect to π^{II}; dd = sum of dominance × dominance epistatic effects over all loci pairs, weighted by the product of gamete frequencies in the gametic arrays produced by generation Syn-0 of $\pi^I \times \pi^{II}$; ξ^I, ξ^{II} = functions of unigametic descent measures defined by Cockerham and Weir (1973) and the number of founder inbreds as described by Melchinger (1988). They are generation dependent. Numerical values are given in Table 1.

An extension of Eq. (1) to include linkage and epistasis between triplets of loci was given by Melchinger (1988).

Variances

The genotypic variance (σ_g^2) among interpool hybrids produced from crosses between generations Syn-t or Syn*-t of π^I and π^{II} can be partitioned into the variances of general combining abilities ($\sigma_{GCA^I}^2, \sigma_{GCA^{II}}^2$) of parents from π^I and π^{II}, respectively, and the variance of specific combining ability (σ_{SCA}^2):

$$\sigma_g^2 = \sigma_{GCA^I}^2 + \sigma_{GCA^{II}}^2 + \sigma_{SCA}^2. \tag{2}$$

In the absence of epistasis and linkage, the following quantitative-genetic expectations can be obtained from the general results of Melchinger (1988):

$$\sigma_{GCA^I}^2 = \phi^I \sigma_{A^I}^2 + \psi^I C_{ab^I}, \tag{3}$$

$$\sigma_{GCA^{II}}^2 = \phi^{II} \sigma_{A^{II}}^2 + \psi^{II} C_{ab^{II}}, \tag{4}$$

$$\sigma_{SCA}^2 = \phi^I \phi^{II} \sigma_D^2 + \psi^I \psi^{II} C_{cd}. \tag{5}$$

Here, $\sigma_{A^I}^2$ = sum of the variance of additive effects of genes from π^I added over all loci; C_{ab^I} = sum of the covariance of additive effects of genes from π^I added over all loci pairs; $\sigma_{A^{II}}^2, C_{ab^{II}}$ = defined analogously with respect to π^{II}; σ_D^2 = sum of the variance of dominance effects of gene pairs from $\pi^I \times \pi^{II}$ added over all loci; and C_{cd} = sum of the covariance of dominance effects of gene pairs from $\pi^I \times \pi^{II}$ added over all loci pairs; ϕ^I, ϕ^{II}, ψ^I, ψ^{II} = functions of digametic descent measures defined by Cockerham and Weir (1973) for pairs of genes (homologous in the case of ϕ^I and ϕ^{II} and non-homolo-

gous in the case of ψ^I and ψ^{II}) from population π^I and π^{II}, respectively, and the number of founder inbreds as described by Melchinger (1988). Both depend on (1) the number of founder inbreds (n^I and n^{II} in π^I and π^{II}, respectively) constituting generation Syn-0 and (2) the generation Syn-t or Syn*-t considered. Numerical values are given in Table 1.

Extensions of formulae (3) to (5) are available to include linkage and additive × additive epistasis (Melchinger 1984, 1988).

Materials and methods

Plant materials

The two parent populations in the present study were founded from four early flint ($n^I = 4$) and four early dent ($n^{II} = 4$) homozygous inbred lines, respectively. Population π^I was established from flint inbreds DS8, DS10, DS15 and DS514, the latter containing about 50% dent ancestors. Population π^{II} was established from dent inbreds CO117, CO125, V3 and DS504. Lines CO117, CO125 and V3 are North American in origin, whereas all lines designated by DS had been developed in Germany by F.W. Schnell. While the set of flint inbred lines is completely unrelated to the set of dent lines, the flint lines DS8 and DS10 have an open-pollinated population as a common ancestor.

In each of the two parent populations, three generations exhibiting different degrees of LD were created: (1) Syn-0, the four inbred lines themselves; (2) Syn*-1, the six possible intrapool single crosses (SC); and (3) Syn*-2, a mixture of equal parts of the three possible intrapool double crosses (DC).

In each of the three generations, the two parent populations π^I and π^{II} were crossed with each other to produce hybrid populations according to the following mating designs. (1) For crosses between generations Syn-0, a factorial mating scheme was used. The four flint inbreds were crossed in all possible combinations with the four dent inbreds yielding a total of 16 flint × dent SC. (2) The same mating design was employed for crosses between the Syn*-1 generations, i.e., the six flint SC were mated to the six dent SC, producing all possible 36 flint × dent DC. Crosses between the Syn*-2 generations were made according to a NC-design I (Comstock and Robinson 1952). (3) Fifty six randomly chosen plants from the dent population, serving as males, were each mated to a random but different sample of three plants of the flint population, yielding a set of 168 biparental progenies (BIP$_{f \times d}$). Analogously, 56 flint males were each mated to a sample of three different dent females to produce another set of 168 biparental progenies (BIP$_{d \times f}$). All hybrid seed was produced in the same year to assure that the differences among the three groups of hybrids would not be confounded with the age and origin of the seed. In producing the BIP, special attention was paid to avoid selection or assortative mating.

Field experiments

The four interpool hybrid populations were evaluated in two adjacent experiments conducted at Stuttgart-Hohenheim, Germany, in 2 years. Each experiment included one set of five checks, 16 SC, 36 DC and 168 BIP. The experimental design for both experiments was a 15 × 15 lattice with two replications and single-row plots of 20 plants spaced 62.5 cm apart. The trials were overplanted and thinned in the four- to seven-leaf stage to a final stand of about 76,900 plants ha^{-1}. The experiments were machine-harvested by a picker and the ears subsequently dried to 0% moisture. Data were collected on plant height (measured in cm from the ground level to the lowest tassel branch), dry matter concentration of the ear (calculated in g kg^{-1} from ear dry weight and ear fresh weight), and grain yield (in Mg ha^{-1}, adjusted to 15.5% moisture).

Statistical analyses

Lattice analyses of variance were performed on the data from each experiment within each year. Adjusted entry means and effective error mean squares were used to compute combined analyses of variance over years. The sums of squares for hybrids [219 degrees of freedom (df)] in each experiment were subdivided for the SC, DC and BIP populations (with 15 df, 35 df and 167 df, respectively) and orthogonal contrasts among the three hybrid populations. Within the SC and DC, there was a further subdivision of the df into GCA effects due to flint parents (3 df and 5 df, respectively) and due to dent parents (3 df and 5 df, respectively) as well as SCA effects (9 df and 25 df, respectively). Within the BIP, there was a further subdivision of df according to the usual parametrization for NC-design I experiments into effects of males (57 df) and females within males (116 df). A corresponding subdivision was conducted on the entry by year sums of squares.

A multiple regression model similar to the conventional generation-means analysis (Haymann 1958) was applied to the means of the SC, DC, BIP$_{f \times d}$, and BIP$_{d \times f}$ populations to estimate parameters $e\mu_{\pi^I \times \pi^{II}}$, $aa = aa^I + aa^{II}$, and dd. The genetic expectations of the means of the four hybrid populations were obtained from Eq. (1) by inserting the numerical values of ξ given in Table 1. Models without epistatic effects (Model 1), with aa effects (Model 2), as well as with both aa and dd effects (Model 3) were fitted in stepwise regression.

The genetic parameters for all three models were estimated by weighted least squares:

$$\hat{\beta} = (X'WX)^{-1}X'Wy$$

where, $\hat{\beta}$ = column vector of estimated genetic parameters; X = a matrix with elements that are a function of the parent generation of the respective population of interpool hybrids; W = a matrix with the inverse of the variances of the hybrid populations on the diagonal and zero on the off-diagonal; and y = column vector of the hybrid population means.

Standard errors of parameter estimates were computed as the square root of the associated diagonal elements of the $(X'WX)^{-1}$ matrix. The adequacy of each model was evaluated with a chi-square test (Mather and Jinks 1982):

$$\chi^2 = \sum [(O - E)^2 \times V]$$

where, O = observed hybrid population mean; E = expected hybrid population mean; V = inverse of the variance of the hybrid population mean.

Estimates of GCA and SCA effects of SC and DC were obtained from the means across both experiments and years. GCA and SCA effects associated with the SC and DC were treated as fixed effects, because the 16 SC and 36 DC were identical to their reference populations Syn-0^I×Syn-0^{II} and Syn*-1^I × Syn*-1^{II}, respectively. In contrast, the GCA and SCA variances pertaining to the BIP were regarded as random effects because their parents represent random samples from the infinite populations Syn*-2^I and Syn*-2^{II}. The corresponding variances $(\sigma^2_{GCA^I}, \sigma^2_{GCA^{II}}, \sigma^2_{SCA})$ were for all four hybrid populations defined according to the rules given by Scheffé (1959). The entry × year interactions were partitioned in an analogous manner. In this case, the GCA × year and SCA × year interactions were considered as random effects in the statistical model. Estimates of the variance components $\sigma^2_{GCA^I}, \sigma^2_{GCA^{II}}, \sigma^2_{SCA}, \sigma^2_{GCA^I \times Y}, \sigma^2_{GCA^{II} \times Y}, \sigma^2_{SCA \times Y}$ and σ^2_e (error variance), and their standard errors (SE), were calculated according to procedures described by Searle (1971). In the case of the BIP, estimates of GCA and SCA variances were obtained by substituting σ^2_m and $\sigma^2_{f:m}$, the variance due to males and females within males, respectively, by the following identities: $\sigma^2_m = \sigma^2_{GCA^{II}}$, $\sigma^2_{f:m} = \sigma^2_{GCA^I} + \sigma^2_{SCA}$ for the BIP$_{f \times d}$, and $\sigma^2_m = \sigma^2_{GCA^I}$, $\sigma^2_{f:m} = \sigma^2_{GCA^{II}} + \sigma^2_{SCA}$ for the BIP$_{d \times f}$, and analogous

relationships for the corresponding interaction variances $\sigma^2_{m \times Y}$ and $\sigma^2_{f:m \times Y}$. For testing the significance of GCA and SCA variances as well as the corresponding interaction variances, F-tests were calculated according to the approximation of Satterthwaite (1946).

Results

Frequency distributions

For all three traits, the DC showed a narrower distribution and a smaller range than the SC (Fig. 1). Compared with the DC, the BIP displayed an expanded variation and a shallower distribution approaching a Gaussian (normal) distribution for all traits. The long lower and upper tails of the SC histograms for grain yield were attributable to crosses of flint lines DS115 and DS514, respectively. The yield distributions of the BIP were shifted downwards relative to the SC and DC. Consequently, inspite of an increased genetic variation in the BIP, the mean of the upper 25% fraction was higher for the DC and even the top ranking hybrids in each set of the 168 BIP yielded less than the best two of the 36 DC.

The distributions of the SC and DC for dry matter concentration were closely centered around the population mean. Recombination in the parent populations generated a considerable increase in the variation of the BIP with a large number of crosses (5–11%) transgressing the ranges of the SC and the DC in both directions. The SC displayed a wide range for plant height with a remote upper and lower extreme. The variation in the DC was considerably reduced. Recombination extended the variation from the DC to the BIP, leading to a similar range as for the SC, yet with a more continuous phenotypic distribution.

Means

The mean performance of interpool SC was slightly lower than that of interpool DC for all three traits, but the difference was not significant ($P < 0.05$) for any of the traits (Table 2). A significant ($P < 0.05$) reduction in the means from DC to BIP was observed for grain yield. The DC outperformed the $BIP_{f \times d}$ by 5.4% and the $BIP_{d \times f}$ by 11.0%. A similar trend was found for plant height. No significant ($P < 0.05$) differences among the means of the four hybrid populations were found for dry matter concentration.

The non-epistatic model (Model 1) was sufficient to describe the means of the four hybrid populations for dry matter concentration ($R^2 = 83.8\%$) but explained only a small proportion ($R^2 < 20\%$) of the variation among hybrid populations means for grain yield (χ^2-test significant at $P < 0.05$) and plant height (Table 2). Inclusion of additive $\times$ additive (aa) epistatic effects (Model 2) improved the fit only slightly for grain yield, whereas further inclusion of dominance $\times$ dominance (dd) epistatic effects substantially increased the R^2 values above 86% for both traits. Estimates of aa and dd effects (Model 3) were positive and negative, respectively, and exceeded

Fig. 1 Frequency distributions of the four maize hybrid populations for grain yield, dry matter concentration, and plant height. The *dashed line* refers to the mean of the respective population; *arrowheads* indicate the means of the upper and lower quartiles
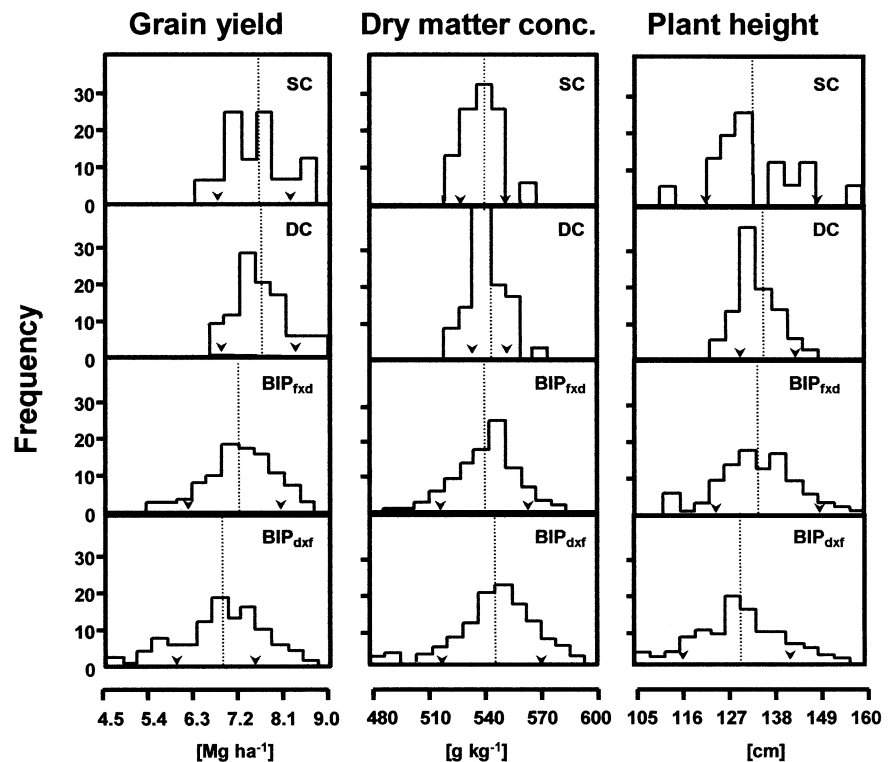
Hybrid population	No. of entries	Grain yield Mg ha^{-1}	Dry matter concentration g kg^{-1}	Plant height cm
Means				
SC	32[a]	7.51 ± 0.26[b]	539.0 ± 19.9	134.2 ± 2.0
DC	72[a]	7.64 ± 0.14	542.9 ± 9.9	137.1 ± 1.4
$BIP_{f\times d}$	168	7.27 ± 0.23	543.1 ± 17.8	134.5 ± 2.8
$BIP_{d\times f}$	168	6.81 ± 0.27	546.0 ± 20.6	130.2 ± 3.2
Genetic effects[c]				
Model 1				
$_e\mu_{I\times II}$		7.44 ± 0.14	542.5 ± 0.9	135.3 ± 1.1
χ^2 (3[d])		8.28*	0.07	4.42
R^{2e}		16.9	83.8	19.6
Model 2				
$_e\mu_{I\times II}$		7.30 ± 0.20	544.5 ± 0.5	135.3 ± 1.9
aa		0.41 ± 0.42	−5.3 ± 1.0	0.2 ± 3.5
χ^2 (2[d])		6.25*	0.01	4.41
R^{2e}		37.3	98.5	19.7
Model 3				
$_e\mu_{I\times II}$		7.07 ± 0.14	544.2 ± 0.7	132.6 ± 1.3
aa		2.37 ± 0.78	−3.2 ± 3.6	19.7 ± 6.5
dd		−1.94 ± 0.73	−2.0 ± 3.3	−18.1 ± 5.8
χ^2 (1[d])		1.39	0.01	0.76
R^{2e}		86.1	98.7	86.2

Table 3 General combining ability (GCA) effects and their standard errors (SE) of four flint and four dent maize inbred lines estimated from SC hybrids for three agronomic traits measured at one location in two years

Parent line	Grain yield Mg ha^{-1}	Dry matter concentration g kg^{-1}	Plant height cm
Flint			
DS514	−0.54*	0.1	−8.5**
DS8	−0.24	−1.3	2.1
DS1	0.12	2.9	−1.3
DS115	0.67**	−1.8	7.8**
Dent			
V3	−0.32	−3.2	3.2
DS504	−0.26	5.5	−0.8
CO125	0.22	11.9**	7.2*
CO117	0.35	−3.3	−9.6**
SE	0.11	1.8	1.3

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

twice their standard error for both grain yield and plant height.

GCA and SCA effects and variance components

Estimates of GCA effects obtained for grain yield from the analysis of SC were significant ($P < 0.05$) only for flint lines DS115 and DS514 (Table 3). GCA effects of the dent lines and SCA effects (data not shown) were rel-atively unimportant. For plant height, significant ($P < 0.05$) GCA effects were found for flint inbreds DS115 and DS514, and dent inbreds CO117 and CO125. Little variation among GCA effects was observed for dry matter concentration except for the highly significant ($P < 0.01$) negative value for dent inbred CO125.

Estimates of $\sigma^2_{GCA_I}, \sigma^2_{GCA_{II}}$ and σ^2_{SCA} for grain yield indicated no change from SC to DC (Table 4). The BIP showed a substantial increase for all three variance components. In toto, the genotypic variance $\hat{\sigma}^2_g$ was about twice as large for the BIP than the DC. No significant changes in the GCA and SCA variances from SC to DC were observed for dry matter concentration, but there was a significant increase in GCA variances from DC to the BIP. For plant height, GCA and SCA variances showed a marked decline from SC to DC. Again, $\hat{\sigma}^2_g$ showed a four-fold increase from DC to BIP due to a substantial increase in $\hat{\sigma}^2_{GCA_I}$ and $\hat{\sigma}^2_{SCA}$.

Discussion

The influence of recombination on important population parameters, such as the mean and genetic variance, can be investigated most effectively by a comparison of populations having identical genotypic frequencies at each individual locus but differing with respect to the joint frequencies of two and more loci. In technical terms, one has to create populations differing solely in the LD of their parental gametic arrays. Other factors changing the population structure, such as inbreeding or selection, should be absent.

Table 4 Estimates of variance components in three types of maize hybrid populations for three agronomic traits measured in trials at one location in 2 years

Variance component[a]	Hybrid population[a]	Grain yield Mg ha^{-1}	Dry matter concentration g kg^{-1}	Plant height cm
$\sigma^2_{GCA^I}$ (flint)	SC	18.4 ± 14.8[b]	−22 ± 9	36.0 ± 15.9
	DC	18.1** ± 3.4	14* ± 8	2.4 ± 4.2
	BIP	25.5** ± 6.7	173** ± 39	33.4** ± 9.7
$\sigma^2_{GCA^{II}}$ (dent)	SC	5.1 ± 8.4	36 ± 44	37.3 ± 21.8
	DC	5.8** ± 1.6	35 ± 21	13.9** ± 2.2
	BIP	6.7 ± 4.7	100** ± 26	12.2** ± 7.8
σ^2_{SCA}	SC	−0.5 ± 3.7	24 ± 18	16.7* ± 6.4
	DC	1.1 ± 2.3	−4 ± 8	1.3 ± 2.3
	BIP	9.1* ± 7.0	−22 ± 35	25.7** ± 11.2
$\sigma^2_{GCA^I \times Y}$	SC	0.3 ± 2.9	40** ± 19	−2.1 ± 1.1
	DC	−0.3 ± 1.4	5 ± 6	7.1** ± 4.0
	BIP	0.4 ± 2.9	27** ± 10	1.2 ± 3.5
$\sigma^2_{GCA^{II} \times Y}$	SC	6.7* ± 5.2	23** ± 16	0.6 ± 3.2
	DC	1.4 ± 0.8	16** ± 9	−2.3 ± 0.8
	BIP	3.1 ± 2.7	−11 ± 10	−2.9 ± 3.9
$\sigma^2_{SCA \times Y}$	SC	2.5 ± 5.2	31* ± 20	−0.7 ± 5.4
	DC	1.6 ± 3.2	17* ± 12	−5.9 ± 3.0
	BIP	4.3 ± 4.3	35** ± 15	13.7** ± 5.9
σ^2_e [c]		48.7	132	59.5

*,**Significant at the 0.05 and 0.01 probability levels, respectively
[a] For abbreviations see Materials and methods

[b] Square root of an unbiased estimate for the sampling variance of the corresponding variance component estimate
[c] Estimate of effective error, pooled over years and both experiments

Factors influencing the means of hybrid populations

In the present study, special care was taken to study the effects of recombination under *ceteris paribus* conditions. The entries from all four hybrid populations were interpool crosses and, consequently, non-inbred with a similar level of heterozygosity. Moreover, since the 16 SC and 36 DC are identical with the Syn-0^I × Syn-0^{II} and the Syn*-1^I × Syn*-1^{II} populations, respectively, and special precautions were taken to avoid selection or assortative mating in producing the 168 BIP$_{f \times d}$ and 168 BIP$_{d \times f}$ progenies, deviations in genotype frequencies caused by these systematic forces or by sampling should be negligible. However, we cannot rule out that maternal, reciprocal and competition effects may have contributed to differences between the four hybrid populations.

Maternal effects may have hampered the growth of SC because their seed is produced on inbred lines as opposed to heterozygous seed parents for the other three groups. Reduced vigor of the seed parent may lead to a poorer seed weight and quality, and consequently to reduced early vigor and plant growth, particularly under unfavourable climatic conditions as those prevailing at our test site. In European maize germplasm of similar type, Schnell and Singh (1978) found a 3.1% yield increase in three-way crosses, if the parental single cross served as parent instead of the parental inbred line. Maternal effects of this magnitude were also observed in reciprocal forms of backcrosses (Melchinger et al. 1985). Altogether, differences in maternal inbreeding may have

resulted in a lower SC mean and, thus, introduced a bias in the estimated *aa* and *dd* effects.

Reciprocal effects may have influenced the comparison between the means of the hybrid populations, because three of them (SC, DC, BIP$_{f \times d}$) were produced on flint seed parents, whereas the 168 BIP$_{d \times f}$ were produced on dent seed parents. According to breeder's experience, flint × dent hybrids have a yield advantage over their reciprocal forms in the marginal maize-growing areas of Central Europe. For grain yield, the mean reciprocal difference in favour of flint × dent single crosses amounted to 2.8% (Mann and Pollmer 1981) and 2.0% (Melchinger et al. 1985) in studies with similar germplasm. Consequently, reciprocal differences of this type can explain the lower yield of BIP$_{d \times f}$ progenies in comparison with the BIP$_{f \times d}$ progenies, thereby introducing a bias in the estimates of epistatic effects.

Competition effects may also influence our comparisons, because SC are genetically uniform, whereas the entries in the DC and BIP consist of mixtures of closely related genotypes. Genetically heterogeneous stands of maize could have an advantage over comparable uniform stands, because the genotypes best adapted to the prevailing environment might grow more vigorously and tend to suppress less-adapted competitors. However, most experiments on intra-plot competition in maize failed to show a significant net gain of hybrid mixtures over the mean of their components in pure stands (for a review see Geiger and Becker 1984).

In spite of the foregoing factors, there is strong evidence that recombination in the parental populations led

to a substantial reduction in the performance level of the hybrid populations in our study. For unlinked loci pairs, the initial LD present in the gametic array produced by the Syn-0 is expected to decline to 1/3 and 0 in the Syn*-1 and Syn*-2 generations, respectively (see parameter ξ in Table 1). Hence, if gene combinations with favourable epistatic effects were present in the parental lines, those combinations would occur less frequently in the DC and reach linkage equilibrium for unlinked loci in the BIP. Thus, with recombination in the parent populations, a decline in the performance level of the corresponding hybrid populations should be a rather common phenomenon, if the initial parents are highly selected inbred lines. However, when the founder genotypes are close to linkage equilibrium as is expected for unselected lines originating from the same germplasm pool, recombination in the parents is expected to entail no changes in the means of the corresponding hybrid populations.

These predictions based on our theoretical results are in excellent agreement with numerous experiments reported in the literature (for a review see Melchinger 1984). The populations compared in the studies reviewed by this author comprised diallel sets of SC and complete or balanced sets of three-way crosses (TC) and DC produced from the same set of parent lines. For grain yield, the means (weighted by the no. of hybrids averaged across experiments) amounted to 97.4% for TC and 96.1% for DC, relative to SC (100%) produced from selected parents. However, with unselected parent lines no significant yield differences existed between the three groups.

In the present study, recombination had the most drastic effect on unlinked loci because LD between linked loci is reduced at a lower rate (Melchinger 1984). In order to study the effects of recombination on the reduction of LD between linked loci, Lamkey et al. (1995) compared the testcross means of generations F_2 and F_2-Syn-8 (obtained by eight generations of random mating) from the cross of the elite U.S. inbreds B73 × B84. They also observed a significant reduction in grain yield and ear height that was explained by the decline in the frequency of favourable epistatic effects between linked loci.

The analysis of hybrid population means investigated in this study has essentially the same limitations as the classical generation-means analysis (Haymann 1958) in that the estimated parameters (*aa*, *dd*) represent sums of effects over all loci pairs influencing a given trait. Because positive and negative terms cancel each other in the sum, the estimates measure only the net effect but do not reflect the importance of epistasis at the level of individual pairs of loci. The latter is in principle possible with QTL mapping studies; however, most experiments published on maize hitherto have failed to reveal significant epistasis between the detected QTL for grain yield (Stuber et al. 1992; Schön et al. 1994). Recently, Jannick and Jansen (2001) devised an experimental design that allows a genome-wide test for epistasis at the QTL level. It is based on a comparison of different populations similar to the approach presented here. Thus, it might be possible to combine our approach of testing for epistasis at the level of the entire genome with modern approaches of QTL mapping and detection of epistasis at the level of individual QTLs.

General and specific combining ability variances

In principle, the estimates of $\sigma^2_{GCA^I}, \sigma^2_{GCA^{II}}$ and σ^2_{SCA} could be affected by the same factors, which were discussed when comparing the population means. However, the results reported by Mann et al. (1981) for similar plant materials indicate that the variance of maternal and reciprocal effects is only marginal. According to our knowledge, no experimental information is available regarding the effects of competition on the estimates of combining ability variances. Taken as a whole, it appears safe to assume that the possible bias in GCA and SCA variances attributable to these factors is small compared to the standard error of the estimates.

Based on quantitative-genetic theory [Eqs. (3) to (5), Table 1], the ratio of SC to DC is 3:1 for GCA variances and 9:1 for SCA variances because of changes in the inbreeding level of the parents. An alternative but more direct way to derive this result is by using Jenkins' (1934) Method B for prediction of DC from the performance of the non-parental SC. The variance components estimated from our experiments were in harmony with these expectations in all those cases, in which the estimates exceeded their standard errors.

Changes in combining-ability variances from DC to BIP are attributable to a reduction in LD because here the effects of recombination are not confounded with the effects of parental inbreeding [Eqs. (3) to (5), Table 1]. In the absence of epistasis, the changes in the GCA variances depend exclusively on covariances of additive effects in the respective parent populations arising from LD. Likewise, changes in σ^2_{SCA} depend only on covariances between dominance effects. As opposed to changes in the population means, epistasis can be only of secondary importance in altering the combining ability variances from DC to BIP, because the coefficients of variances and covariances involving epistatic effects change only little (Melchinger 1984). Thus, the GCA and SCA variances can increase or decrease from DC to BIP depending on the sign of the covariances of additive effects or dominance effects, respectively.

Under certain circumstances, the distribution of the GCA values in the initial parent population may indicate the trend in the pertinent GCA variances. For example, if the founder inbreds of a parent population are of diverse origin but similar in their GCA, there is preponderance of negative covariances of additive effects and, consequently, the GCA variance is expected to increase with recombination. On the other hand, if the initial lines are divided into two groups with striking differences in GCA, it can be reckoned that a great number of loci pairs have positive covariances of additive effects and, conse-

quently, recombination most-likely reduces the GCA variance.

Our experimental data clearly demonstrate that recombination may cause sizeable changes in the combining ability variances. With two exceptions, the magnitude of σ^2_{GCA} and σ^2_{SCA} increased from DC to BIP, and for dry matter concentration and plant height, estimates for the BIP were multiples of those for the DC. The different behaviour of the GCA variances of the flint and dent parents illustrates that the kind of changes entailed by recombination strongly depends on the source materials. Since the extent of the initial LD is of crucial importance, it is postulated that changes in combining ability variances are of greater magnitude for genotypes of highly diverse origin than for genotypes from narrow-based breeding populations.

Significance of recombination in maize breeding

The extent to which recombination alters the genotypic distribution of a population is of great interest to breeders, because the success of subsequent selection steps depends mainly on the behaviour of the population mean and the components of the genetic variance. The most common procedure practised in commercial hybrid maize breeding is to develop new inbred lines from crosses between elite inbreds with proven outstanding combining ability. From the breeders point of view, a crucial question is whether allowances for further recombination in the parental populations will enhance the chances to select new lines resulting in improved hybrids. In the present study, recombination in the parent populations resulted in two opposing trends. On the one hand, the mean performance of the hybrid population declined through the disruption of gene combinations with favourable epistatic effects, thereby reducing the chances for the selection of superior hybrid genotypes. On the other hand, a larger utilisable genetic variance was released through recombination, which opens the possibility of achieving an increased selection gain. For grain yield, the first trend was dominating over the second trend and, consequently, the probability of recovering better lines from the intermated populations was rather low. For example, the mean of the upper 25% fraction in BIP was considerably inferior in yield to the top DC and SC.

Recombination is also of crucial importance in recurrent selection programs. While selection generates a negative LD even if loci are unlinked (Nei 1963; Bulmer 1976), recombination is practised to restore genetic variation for continued improvement in succeeding cycles. Here, an important question is how many generations of intermating are to be interposed so that linkage equilibrium is at least partly restored before initiating the next selection cycle. Obviously, the answer depends on the recurrent selection method and the selection intensity employed.

Despite the fundamental role of recombination in creating new genetic variability in selection programs, our present experimental knowledge of its significance is still rather limited. More research is urgently needed to quantify the effect of recombination on genetic variance components. In view of the large standard errors associated with second degree statistics, further experiments with a larger sample size are required to clarify whether the findings of our study are specific to our plant materials or are to be considered representative for highly selected source materials.

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