Phenotypic analysis of intermated B73 x Mo17 (IBM) populations

by

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CHAPTER 1. GENERAL INTRODUCTION

Thesis organization

This thesis is divided in four chapters: a general literature review, two manuscripts and general conclusions. The literature review (Chapter 1) covers three topics: i) the effect of intermating on population parameters reported by previous studies, ii) a description of the development and importance of B73 x Mo17 intermated populations, and iii) a description of how different factors, such as pollen genotype and post-flowering source-sink ratio, may have an effect on the protein, oil and starch concentration in maize kernels, which provides background information for the second manuscript. The first manuscript (Chapter 2) reports phenotypic comparisons between the IBM and IBM-10 populations in terms of genetic variances, means and correlation coefficients estimated for a number of quantitative traits. The second manuscript (Chapter 3) reports the comparison between open and self-pollinated kernels for quality traits in the IBM and IBM-10 populations. The thesis also includes two appendices: one contains description of the formulas used (Appendix A), and other contains figures and tables not used in the chapters (Appendix B).

Literature review

i) Effect of intermating on quantitative parameters of populations

Population parameters such as genetic variances, means and covariance between traits can be influenced by linkage disequilibrium (Falconer and Mackay, 1996). Intermating within a population creates more opportunities for recombination, and therefore may contribute to reduce linkage disequilibrium and may produce changes in those population parameters.

Effect of intermating on genetic variances

Quantitative genetic theory indicates that estimates of additive genetic variance are biased positively when loci affecting a trait are linked in coupling-phase. In contrast, additive variance estimates are biased negatively when the loci affecting a trait are linked in

repulsion-phase. Estimates of dominance genetic variance are positively biased for both types of linkage phase (Comstock and Robinson, 1948).

In maize, earlier studies have been conducted using intermating as a tool to reduce the potential bias on the estimation of genetic variance components caused by linkage and for a better estimation of the average degree of dominance of genes (e.g., Comstock and Robinson, 1948, 1952; Gardner and Lonnquist, 1959; Moll et al., 1964; Moreno-Gonzalez et. al., 1975; Han and Hallauer, 1989; Cook, 1998). In the case of grain yield, a trait evaluated in most of these studies, most of the studies reported a decrease in the estimates of dominance variance with intermating, suggesting the presence of linked loci coding for this trait.

The effects of intermating on the genetic variance for several traits have been investigated in populations derived from the cross between B73 and Mo17 (Covarrubias-Prieto, 1988; Han and Hallauer, 1989; Cook, 1998). These studies differed in the number of cycles of intermating and in the type of progeny evaluated for the estimation of the genetic variance. Using backcross progeny and the Design III genetic model (Comstock and Robison, 1952), the variance components for yield and several plant and ears traits of the F2 generation (F2 syn0) were compared with the estimates obtained from a population created after 5 cycles of intermating (F2syn5) and 10 cycles of intermating (F2syn10) (Han and Hallauer, 1989; Cook, 1998). Both studies reported a decrease in the dominance variance and changes in additive variance in different directions depending on the trait. For example, the additive variance for ear diameter was approximately three times smaller in the F2syn5 than in the F2syn0, while the additive variance for ear height was larger in the F2Syn5 and F2syn10 than in the F2syn0. Even though these observations suggested the presence of coupling and repulsion linkage effect in the F2 generation, the actual phase of linkage was not assessed for any trait or at any locus. Direct comparison of the variance components between F2syn5 and F2syn10 populations could not be obtained because of the confounding environmental effects. Differences between the F2syn0 and F2syn5 in term of genetic variability among S1 progeny had a similar trend to that observed for the additive variance estimated with the Design III genetic model (Covarrubias-Prieto, 1987; Han and Hallauer, 1989).

Previous studies have shown the effect of intermating on the genetic variance for kernel quality traits in maize (Moreno-Gonzalez et al., 1975; Dudley, 1994; Dudley et al.,

2004). Two maize strains divergently selected for kernel oil concentration, Illinois High Oil (IHO) and Illinois Low Oil (ILO) were mated, and the F2 generation (Syn0) was intermated for 4 generations to produce the Syn4 (Moreno-Gonzalez et al., 1975). Genetic variance components for kernel oil concentration were estimated for the Syn0 and Syn4 using the Design III genetic model (Comstock and Robison 1952). The additive variance for oil concentration for the Syn4 was smaller than the estimate for the Syn0. The dominance variance for oil concentration was a small component of the total genetic variance. The same experimental approach was used to evaluate the Syn0 and Syn4 from the cross of two divergent maize strains selected for protein concentration, Illinois High Protein (IHP) and Illinois Low Protein (ILP) (Dudley, 1994). The additive variance for protein concentration was smaller in the Syn4 than in the Syn0. In addition, the genetic variance for protein and starch among S1 and testcross progeny was found to be smaller in the Syn4 than in the Syn0 (Dudley et al., 2004). The observed reduction in the genetic variance estimates after intermating in the IHO x ILO and IHP x ILP populations were attributed to recombination and subsequent change in allele configuration between loci linked in coupling phase in the parental strains; although in both experiments the linkage phase was not directly assessed.

The effects of intermating on genetic variances were also evaluated in other plant species. In cotton (*Gossypium hirsutum L.*), the F2 generation of a population was compared with a population created after six cycles of intermating the F2 generation (Miller and Rawlings, 1967). The F1 resulted from a cross of two inbred lines. The parental lines differed for the evaluated traits, except for fiber length. The estimates of genetic variance were lower after 6 cycles of intermating than in the F2 for six traits. For fiber length, the genetic variance was lower in the F2. In view of the results and the differences between the parental lines for all the evaluated traits except for fiber length, Miller and Rawlings suggested that coupling-phase linkages might be predominant for these characters in the parental lines. For fiber length, there was not significant segregation in the F2 generation, which made the authors suggest repulsion-phase linkage for this trait.

In three populations of wheat (*Triticum aestivum L.*), the genetic variance for a number of traits changed with three cycles of intermating. However the observed changes in genetic variance were not consistent across generation of intermating and populations (Altman and Bush, 1984).

Effect of intermating on correlation between traits

Linkage and pleiotropy are two causes of genetic correlation between traits (Falconer and Mackay, 1996). Correlation between traits caused by linkage is expected to decrease as the chances of recombination in a population are increased (Falconer and Mackay, 1996). In experiments with B73 x Mo17 intermating populations, 19 out of 91 (20.9 %) pairs of traits showed differences in their genetic correlation between F2syn0 and F2syn5 (Covarrubias-Prieto, 1987). In 13 out of the 19 cases the genetic correlation was larger in the F2syn5 than in the F2syn0, suggesting that the predominant type of linkage phase between loci was repulsion, although the actual linkage phase was not assessed for any of these pair of traits. Differences in correlation coefficients between F2syn0 and F2syn10 were observed for a number of traits, but they were not tested statistically (Cook, 1998). In IHP x ILP population, intermating was not accompanied by changes in correlations between the kernel quality traits (Dudley et al., 2004). The genotypic and phenotypic correlations between protein and starch was consistently high ($|r| > 0.8$) across the generations of intermating.

The correlation among traits tended to decrease with intermating of the F2 generation in three cotton populations (Miller and Rawlings, 1967; Meredith and Bridge, 1971). Furthermore, intermating permitted a reduction in the unfavorable correlation between two traits, yield and fiber strength.

Effect of intermating on population mean

If there is no interaction between linked loci controlling a given trait, then intermating is not expected to influence the population mean. However, when interactions among linked loci are present, then the influence of intermating on the mean values of the trait will depend on the magnitude of the linkage between the interacting genes (Mather and Jinks, 1982).

A number of investigations in different plant species reported changes in the population mean with intermating (Miller and Rawlings, 1967; Humphrey et al., 1969; Meredith and Bridge, 1971; Moreno-Gonzalez et al. 1975; Altman et al., 1984; Covarrubias-Prieto, 1987; Cook, 1998). The population mean for a number of traits in the F2syn0 of the cross between B73 and Mo17 differed from the mean estimated in the F2syn5 and F2syn10 (Covarrubias-Prieto, 1987; Cook, 1998). For example, the mean for plant height and ear height of S1 progeny was lower in the F2syn5 than in the F2syn0, whereas the mean for ear row number was larger in the F2syn5 than in the F2syn0. Backcross progeny of the F2syn10

showed larger mean for ear height and lower mean for grain moisture and day to silk than the F2syn0. In the cross IHO x ILO, the mean for kernel oil concentration was smaller in the Syn4 than in the Syn0 (Moreno-Gonzalez et al., 1975). Changes in allele frequency during the intermating cycles as a consequence of inadvertent or natural selection and recombination between interacting genes were mentioned as possible causes of the changes in the population mean. However none of these hypotheses were directly tested.

In tobacco (*Nicotiana tabacum L.*), the mean for yield and leaf width decreased slightly but significantly through five generations of intermating an F2 generation of a synthetic population of eight elite inbred varieties (Humphrey et al., 1969). Disruption of blocks of linked genes with favorable epistatic interaction was mentioned as the possible cause of the decrease in the mean, but like in the case of maize described above, linkage between genes was not directly monitored.

ii) IBM and IBM-10 populations: development and importance

The IBM and IBM-10 populations were derived from the single-cross hybrid of inbred B73 (female) and Mo17 (male). B73 was derived from the Iowa Stiff Stalk Synthetic population (BSSS) after five cycles of recurrent selection (Russell, 1972). B73 was a commercially valuable inbred covering 16% of total U.S. seed requirements in 1980 (Troyer, 2000). This inbred line has ears with 18 to 20 rows, kernels of yellow endosperm, tassel with upright branches, and erect leaves (Russell, 1972; USDA, ARS-GRIN, 2006a). Mo17 was developed from a population created by crossing inbred lines CI187-2 x C103 (Zuber, 1973). Mo17 has ears with 10 to 12 rows and kernels with endosperm of more vitreous consistency than in B73 kernels (Zuber, 1973; USDA, ARS-GRIN, 2006b). The single-cross hybrid B73 x Mo17 had an outstanding performance, and was grown extensively throughout the United State Corn Belt during the late 1970s and early 1980s. Recycled versions of these inbred lines are still grown (Troyer, 2000).

B73 and Mo17 were selected as parents of the IBM and IBM-10 populations because of their high degree of polymorphism at many loci and wide usage within many pedigrees. B73 is used in many genetic studies and will be the first maize inbred to have its genome sequenced. The IBM population was developed by self-pollinating the F1 hybrid from the cross B73 x Mo17. Within the F2 generation, 250 plants were random mated by making plant

to plant crosses. A single kernel from each F2 ear was bulked and used as parents for the next cycle of intermating. This random mating procedure was repeated during 4 generations, allowing more opportunities for recombination events to occur. Recombinant inbred lines (RILs) were then developed from this intermated population by eight cycles of self-pollination without intentional selection through single seed descent (Lee et al., 2002).

Increasing the number of cycles of intermating in a population would allow a better estimation of the genetic distance between adjacent loci as well as their relative positions along a chromosome since the number of recombination events would increase with each cycle of intermating. The effects of five cycles of intermating on recombination events have been reported for the cross B73 x Mo17 (Lee et al., 2002). The F2 generation and a generation created after 5 cycles of intermating the F2 (F2syn5) were evaluated at 190 RFLP loci. F2syn5 showed an increase in the recombination fraction at 91 % of the evaluated intervals between loci and therefore produced an expansion of the estimated genetic map length in cM. Winkler et al. (2003) determined that the expansion of the genetic map depends on the type of mapping population and also on the DNA marker coverage used in the study. A set of equations was developed in order to relate the recombination fraction observed after *t* generations of intermating with the F2-adjusted recombination rate in intermated recombinant inbred populations (Winkler et al., 2003).

The IBM population has become the reference population in different mapping studies and thus an important resource for maize genetic mapping (Maize Genetics and Genomics Database, www.maizegdb.org). Currently, it serves as the core resource for the integration of the genetic map with the B73 physical map (Maize Mapping Project, www.maizemap.org). In order to increase the genetic map resolution, a second population, IBM-10 was developed after six additional cycles of intermating. Subsequently, a set of doubled-haploid lines were developed by DuPont using a proprietary process for this purpose. The effects of the six additional cycles of intermating on recombination fraction have been reported for the IBM-10 population (Jaqueth, 2003). An increase of 1.53 fold in the recombination fraction between adjacent SSR loci was observed at chromosome one from the IBM-10 population. Similar results were found for the other chromosomes (unpublished). Because of the additional recombination, the IBM-10 population should have greater utility of genetic mapping and for linking genotypic and phenotypic variation.

Several authors have recognized the positive effects of increased recombination on the identification of genomic regions associated with phenotypic variation (Austin and Lee, 1996; Graham et al., 1997; Dudley et al. 2004). Furthermore, Darvasi and Soller (1995) conducted a simulation study where they determined that the confidence interval for QTL location narrows during the first 10 cycles of intermating the F2 generation of the mapping population. Single QTLs for plant and ear height detected in F2:3 families from the Mo17 x H99 mapping population were resolved into multiple linked QTLs with smaller phenotypic effect using RILs (F6:7) from the same population (Austin and Lee, 1996). Additional cycles of recombination at a specific chromosomal region allowed the dissection of a QTL for grain yield that explained approximately 20 % of the phenotypic variance into two smaller QTL that were linked in repulsion in the parental inbreds (Graham et al., 1997). The number of marker-QTL association for protein and starch was reduced after 4 cycles of intermating the F2 generation of the cross of two maize strains contrasting for protein and starch concentration in the kernel, Illinois High Protein (IHP) and Illinois Low protein (ILP) (Dudley et al., 2004). This result was expected because intermating should increase the chances of recombination between marker loci and QTL and between linked QTLs.

iii) Factors affecting the kernel protein, oil and starch concentration

Pollen effect on the concentration of oil, protein and starch in the maize kernel

Xenia describes any immediate effect a pollen has on the embryo or endosperm of seed plants (Kiesselbach, 1999).There are several reports regarding xenia effects in maize for several kernel traits (e.g. Miller and Brimhall, 1951; Perenzin et al., 1980; Poneleit and Egli, 1983; Bulant et al., 2000).

The knowledge of the effect of pollen on the concentration of kernel quality traits such as protein, oil and starch is important in the selection of adequate sources of kernels to accurately assess these traits. Open-pollinated kernels are easier to produce than self-pollinated kernels, but if it provides misleading information then the plant breeder needs to use self-pollinated kernels.

The effect of the pollen on the kernel oil concentration was investigated in maize by performing cross-pollination between genotypes showing substantial differences in the kernel oil fraction (Brunson et al., 1948; Miller and Brimhall, 1951; Curtis et al., 1956; Letchworth

and Lambert, 1998). Pollen from high-oil genotypes lead to increase the embryo size and also the oil concentration in the embryo of genotypes with relatively low concentration of oil in the kernel. However, the effect of the pollen upon the oil percentage of a high-oil variety was relatively small (Brunson et al., 1948; Miller and Brinhall, 1951; Curtis et al., 1956). Furthermore, the regression of total oil percentage on proportion of embryo and percentage of oil in the embryo was high and positive; indicating that high oil content is associated with these two variables. Based on these observations, it was concluded that studies concerning the inheritance of kernel oil concentration should be conducted using self-pollinated ears. Xenia effect on oil concentration is being exploited for the production of maize kernels with high oil concentration by growing a blend of male sterile hybrid of good agronomic performance with a high-oil male-fertile pollinator. This grain production system is known as TopCross (Registered trademark of DuPont Specialty Grains, Des Moines, IA).

Increased oil concentration in the kernel as a consequence of xenia was accompanied by an increase in the lysine content and the percentage of protein in the embryo; although the protein concentration in the whole kernel did not change (Miller and Brimhall, 1951; Thomison, 2003). Quality Protein Maize (QPM) genotypes fertilized with pollen of the normal-endosperm maize cultivar showed a decrease in the kernel tryptophan concentration; however, the protein concentration was not affected (Pixley and Bjarnason, 1994). In other investigation, the protein concentration in kernels of a high-protein hybrid (HP) (13.2 g kg^{-1}) fertilized with pollen of a low-protein hybrid (LP) (9.8 g kg^{-1}) did not differ with the protein concentration measured in SP kernels of the HP hybrid. Similarly, protein concentration for the reciprocal cross LP x HP was not different from the SP kernels of the LP hybrid. That indicates that the pollen genotype did not affect the protein concentration in the HP x LP reciprocal crosses, and the female parent was important in determining the protein concentration in the kernel (Letchworth and Lambert, 1998).

Xenia effect was detected on the activity of enzymes involved in starch biosynthetic pathway (Rahman et al., 1998; Bulant et al., 2000, Tracy, 2000). Homozygous mutant genotypes for the Su1 gene (Sugary 1) fertilized with pollen carrying the dominant allele for that gene increase the activity of the starch debranching isoamylase enzyme and the starch concentration in the kernel. The ADPGlucose pyrophosphorylase activity and the starch content were larger in cross-pollinated than in the self-pollinated kernels. In other

investigation, an inbred line of small kernel size had larger number of endosperm cells when pollinated with pollen of genotypes with large kernel size than when it was self-pollinated (Jones et al., 1996).

The effect of the pollen on protein, oil and starch concentration in the kernel was also evaluated by comparing open and self-pollinated ears in a set of 12 hybrids (Letchworth and Lambert, 1998). The kernels of self-pollinated ears had higher protein and lower starch concentration than the open-pollinated kernels. However, the differences in starch and protein concentration between open-pollinated and self-pollinated kernels were smaller than the differences observed between genotypes for these traits. For oil, on average, the open-pollinated kernels had higher oil concentration than the self-pollinated kernels. However, the differences in oil concentration between open-pollinated and self-pollinated kernels were not consistent across genotypes.

Effects of post-flowering source-sink ratio on protein, oil and starch concentration in maize grain

The post-flowering availability of assimilate (source) per growing kernel (sink) influences the kernel growth rate, final weight and composition (Jones and Simmons, 1983). The source-sink ratio depends on the genotype-environment combination. The source-sink ratio can be altered by crop management factors such as planting date, plant density, nutrient availability, water and others.

The flow and remobilization of C and N to the kernel during the grain filling period depend on the particular source-sink ratio of the plant (Jones and Simmons, 1983). Increase in the post-flowering source-sink ratio was accompanied by increase in the nitrogen flow to the grain per unit of carbohydrate flow to the grain (Uhart and Andrade, 1995). Studies using maize hybrids suggest that post-flowering source-sink ratio is positively associated with protein concentration (Jones and Simmons, 1983; Borras et al., 2002) and negatively associated with starch concentration in the kernel (Borras et al., 2002). In these investigations, variation in the post-flowering source-sink ratio was generated either by defoliation or reducing the number of kernels per ear with restricted pollination.

Protein was found to be the component most affected by variation in the source-sink ratio (Borras et al., 2002). In two maize hybrids, increased post-flowering source-sink ratio promoted reductions in starch concentration and increases in protein concentration, up to a

threshold of assimilate availability per kernel after which no response was detected. The threshold of source-sink ratio above which the starch concentration stopped declining was smaller than the threshold above which the protein concentration stopped increasing. No association was found between the assimilate availability per kernel and the oil concentration.

General objectives

The general objectives of this thesis were i) to determine whether the six additional cycles of intermating in the development of the IBM-10 population had effects on the genetic variances, means and phenotypic correlations for quantitative traits, and ii) to determine the degree to which values of protein, oil and starch concentration in open-pollinated kernels resemble those in the self-pollinated kernels of the inbred lines from the IBM and IBM-10 populations.

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PHENOTYPIC ANALYSIS OF INTERMATED B73 x Mo17 (IBM) POPULATIONS

Abstract

Intermating within a population creates more opportunities for recombination, thereby increasing the probabilities of observing recombination events between linked loci. Thus, more reliable genetic maps can be constructed. IBM and IBM-10 are two intermated recombinant inbred line populations that have been developed to facilitate high resolution genetic mapping. Both populations were derived from the cross between maize inbreds B73 and Mo17. The populations differ in the number of cycles of intermating of the F2 generation before the initiation of line derivation. IBM was created after 4 cycles of intermating. IBM-10 was created after 6 additional cycles of intermating of the F2 generation, and show increased recombination fraction between adjacent loci compared with IBM. Recombination and subsequent change in allele configuration between linked loci may produce change in population parameters such as variances and covariance between traits. This study was designed to determine whether the six additional cycles of intermating in the development of the IBM-10 population had effects on the genetic variances, means and phenotypic correlations for quantitative traits. Two hundred and forty four lines of each population were grown in two separate but adjacent experiments in Ames in 2006. Sixteen traits were measured in each population. The estimate of genetic variance for kernel oil concentration (OIL) and number of kernels per ear row (EKR) were significantly larger in the IBM-10 than in the IBM population (1.5 and 1.3 times larger for OIL and EKR, respectively). There was no difference in phenotypic correlations between populations. The mean for plant height and ear rows were significantly larger for IBM-10 than IBM. The differences in genetic variance for OIL and EKR between populations could be due to recombination and the subsequent change in allele configuration between linked loci affecting each of these traits. This could be favorable for the estimation of location and effect of QTL controlling these traits. The phenotypic characterization of the IBM-10 lines would be useful for research purposes such as QTL detection as a prelude to finer-scale genetic mapping and sequence discovery.

Introduction

Genetic recombination is an important process that consists in the exchange of genetic material between non-sister chromatids of homologous chromosomes during meiosis. If recombination occurs, new allelic combinations would be created in each generation and phenotypic variation would be observed among individuals of a species. This process has been exploited by geneticists as a tool to construct genetic maps. The relative distance among loci in these genetic maps is based on recombination units, calculated from the frequency of recombinant individuals at a given locus. Genetic maps serve as a guide to locate genes and genomic regions associated with a quantitative trait (QTLs) along the genome of a species, and facilitate map-based cloning of a given gene when high resolution maps are available.

Within a given population, intermating creates more opportunities for recombination to occur; thus increasing the probability of recombination events between linked loci, and subsequently, genetics map with high resolution can be constructed. The intermated B73 x Mo17 (IBM) population is a resource widely used for maize genetic mapping (Maize Genetics and Genomics Database, www.maizegdb.org). Currently, it serves as the core resource for the integration of the genetic map with the B73 physical map (Maize Mapping Project, www.maizemap.org). The population was developed by intermating the F2 generation of the single cross between B73 x Mo17 for four generations before recombinant inbred lines (RILs) were derived (Lee et al. 2002). To increase the resolution of the genetic map, a second population, IBM-10 was developed after six additional cycles of intermating.

The effects of the additional cycles of intermating on recombination have been reported for the IBM-10 population (Jaqueth, 2003). A 1.53 fold increase in the recombination fraction between two adjacent loci was observed when comparing chromosome one of IBM and IBM-10. Similar results were found for the other chromosomes (unpublished).

Intermating may produce changes in population parameters such as genetic variance and covariance between traits. From quantitative genetic theory, it has been demonstrated that estimates of additive genetic variance are biased positively when loci affecting a trait are linked in coupling-phase, and negatively when linked in repulsion-phase. Dominance genetic variance estimates are positively biased for both types of linkage phase (Comstock and Robinson, 1948). From this standpoint, it is expected that several cycles of intermating

produce changes in allele configuration between linked loci, inducing changes in the genetic variance and in the correlations between traits.

The effects of intermating on genetic variances, correlation between traits and means in different populations derived from the cross between B73 x Mo17 have been reported (Covarrubias-Prieto, 1987; Han and Hallauer, 1989; Cook, 1998). These studies differed in the number of cycles of intermating and in the type of progeny being evaluated. Using backcross progeny, the variance components for yield and several plant and ears traits of the F2 generation (F2 syn0) were compared with the estimates obtained from a population created after 5 cycles of intermating (F2 syn5) and 10 cycles of intermating (F2 syn10) (Han and Hallauer, 1989; Cook, 1998). Both studies reported a decrease in the dominance variance and changes in additive variance suggesting the presence of coupling and repulsion linkages in the F2 generation; although, the actual phase of linkage among loci was not assessed for any given trait. In addition, the genetic variance estimated for S1 progeny for several traits differed between F2syn0 and F2syn5 (Covarrubias-Prieto, 1987). For example the genetic variance for prolificacy was lower in the F2syn0 than in the F2syn5, whereas for ear diameter, ear rows and days to silking, the genetic variance was larger in the F2syn0 than in the F2syn5. Changes in the correlation coefficients between traits from the F2syn0 to the F2syn5 and F2syn10 were detected, but no consistent trend was observed between populations (Covarrubias-Prieto, 1987; Cook, 1998). The population mean for a number of traits in the F2syn0 differed from the mean estimated in the F2syn5 and F2syn10. For example, the mean for plant height and ear height of S1 progeny was lower in the F2syn5 than in the F2syn0, whereas mean ear row number was greater in the F2syn5 than in the F2syn0. Backcross progeny of the F2syn10 had larger mean ear height and lower mean grain moisture and day to silk than the F2syn0. Change in allele frequency during the intermating cycles as consequence of inadvertent or natural selection and recombination between interacting genes were mentioned as possible causes of the changes in the population mean, however none of these hypotheses were tested directly.

The effect of intermating on population parameters was also reported for other maize populations (e.g., Moreno-Gonzalez et al., 1975; Covarrubias-Prieto, 1989; Dudley, 1994; Dudley et al., 2004). Two maize strains divergently selected for kernel protein concentration (Illinois High Protein [IHP] and Illinois Low Protein [ILP]) were mated, and the F2

generation (Syn0) was intermated for 4 generations to produce the Syn4 (Dudley et al., 2004). The genetic variance for protein and kernel starch concentration was lower in the syn4 than in the syn0 (Dudley et al., 2004). Similarly, the additive genetic variance for kernel oil concentration was reduced after 4 cycles of intermating the F2 generation of a cross of two maize strains contrasting for kernel oil concentration (Illinois High Oil x Illinois Low Oil) (Moreno-Gonzalez et al., 1975). These observations were attributed to recombination and subsequent change in allele configuration between loci linked in coupling phase in the F2 generation; although the linkage phase of the loci involved was not directly assessed. In the IHO x ILO population, there was a tendency for increase in mean kernel oil concentration with the generations of intermating (Moreno-Gonzalez et al., 1975).

In other species, the effect of intermating on genetic variance means and correlations between traits have been reported (Miller and Rawlings, 1967; Humphrey et al., 1969; Meredith and Bridge, 1971; Altman and Busch, 1984). In cotton, intermating allowed a reduction in unfavorable correlations between agronomic traits (Miller and Rawlings, 1969; Meredith and Bridge, 1971). In addition, the population showed a reduction of the genetic variances after six cycles of intermating for a number of traits contrasting between both parental lines (Miller and Rawlings, 1969). In three wheat populations, changes in genetic variances and correlation coefficients were detected with intermating, but these changes were not consistent across populations (Altman et al., 1984). In tobacco, the mean for a number of traits changed through six generations of intermating (Humphrey et al., 1969). In this case, disruption of blocks of linked genes with favorable epistatic interaction was mentioned as the possible cause of the decrease in the mean.

Comparison of IBM and IBM-10 in terms of population attributes such us genetic variances, means and correlation between traits could be interesting, because differences between populations in terms of these parameters may indicate recombination between linked QTLs controlling specific traits, which could have favorable effects for the estimation of their effect and location. Furthermore, with the emerging complete genomic sequence of B73 and limited genomic sequence of Mo17, phenotypic characterization of the IBM-10 lines could be interesting for research purposes such as QTL detection as a prelude to finer-scale genetic mapping and sequence discovery.

The research reported herein was designed to: 1) generate phenotypic data for quantitative traits in the IBM and IBM-10 populations, 2) estimate genetic variances, means and phenotypic correlations between quantitative traits in both populations, and 3) to determine whether the six additional cycles of intermating in the development of the IBM-10 population had effects on the genetic variances, means and phenotypic correlations for quantitative traits.

Materials and methods

Germplasm

The germplasm for this study included 244 recombinant inbred lines (RILs) of the IBM population, 244 doubled-haploid lines (DHLs) of the IBM-10 population and the inbred lines B73 and Mo17. The IBM population was derived from the single-cross hybrid of B73 (female) and Mo17 (male). B73 was derived from the Iowa Stiff Stalk Synthetic population (Russell, 1972). Mo17 was developed from Lancaster Sure Crop germplasm (Zuber, 1973). From the initial cross of B73 and Mo17, the F1 was self-pollinated to produce the F2 generation. Within the F2 generation, 250 plants were random mated by making plant to plant crosses. Reciprocal crosses were not made. A single kernel was taken from each ear to form a bulk of seed to be grown for the following cycle of intermating. This random mating procedure was repeated for 4 generations. Recombinant inbred lines were then developed from this intermated population by eight cycles of self pollination through single seed descent (Lee et al., 2002). The IBM population was intermated for six additional generations. After ten generations of random mating, doubled-haploid lines were then developed by DuPont using a proprietary process for producing doubled haploid lines.

Experimental design

The IBM and IBM-10 populations of inbred lines were planted in separate but adjacent experiments, at the Agronomy Agricultural Engineering Research Center, Ames, Iowa in 2006. In each experiment, the lines were grown in a row column alpha lattice experimental design with two replications of seven columns and 37 rows. B73 and Mo17 were randomized eight and seven times, respectively, within the replications in each experiment. Plots consisted of a row 3.8 m long with 0.76 m between rows. Plots were over-

planted, and the plant density was reduced to 12 plants per row when plants had 4 to 5 leaves completely developed. The planting date was May 6th, 2006. The field was fertilized with 18 kg of Nitrogen, 44 kg of Phosphorous and 120 kg of Potassium per ha in September 2005, and with 175 kg of urea per ha 15 days before planting. Metolachlor and Atrazine herbicides were sprayed and incorporated into the soil 11 days before planting, at the rate of 1.86 and 1.12 kg of active ingredient per ha, respectively. Neither herbicides nor insecticides were applied on the experiments after planting.

Traits

The traits measured on the lines of both populations are listed in table 1. The traits were selected based on their importance for agronomy and basic biology, and all of them being a matter of study in other investigations.

Growing degree days (GDD) were calculated according to the hourly adjusted average system method (Cross and Zuber, 1972). The hourly temperature was adjusted by a base temperature of 8°C (Jones and Kiniry, 1986). Tassel branch number (TBN) was counted on the tassel of four competitive plants within each plot. The data for TBN was transformed as $\log_{10}$ (number of branches) in order to fulfill the assumption of normal distribution of the error component for t-test and estimation of correlation between TBN and the other variables. Plant height (PH) and number of nodes with brace roots (BR) were measured on four and five competitive plants within each plot, respectively; and expressed on a plot mean basis. Ears diameter (ED), ear length (EL), ear rows (ER), number of kernel per ear row (EKR) and cob diameter (CD) were measured on four unselected, open-pollinated ears from each plot, and expressed as the mean of the four ears. Kernel weight (KW) was measured on 150 kernels obtained from the four open-pollinated ears used to assess the ear traits. For the assessment of kernel quality traits (protein [PRO], oil [OIL] and starch [STA]), between 3 and 4 ears were self-pollinated in each plot and equal quantities of their kernels were bulked for the analysis. Self-pollinated ears with few kernels were discarded to minimize the effect of high source-sink ratio on the chemical composition in the kernel. Previous studies have established that when there are abnormally few kernels on an ear, the kernel protein and starch concentrations tend to be inflated and deflated, respectively (Jones and Simmons, 1983; Borras et al., 2002). Self and open-pollinated ears with disease symptoms were discarded. The whole-kernel samples were analyzed by near-infrared transmittance, with an

Infratec™ 1241 Grain Analyzer (Foss Inc., www.foss.dk) and followed the same procedures for the analysis of the quality traits used in previous investigations (i.e. Letchworth and Lambert, 1998; Borras et al., 2002; Clark et al., 2006). Results were expressed as a concentration (g kg^{-1}) on a dry matter basis.

The assessment of cob glumes color was done on the self-pollinated ears. Lines were classified into two categories: red cob and white cob. The lines were also characterized in term of the average number of tillers per plant (TN), which was estimated as the ratio between the total number of tillers per plot and the total number of plants per plot.

Statistical procedures

Population genetic variances with their confidence intervals and population means were estimated by the PROC MIXED procedure of SAS software (version 9.1, SAS Institute, Inc., Cary, NC, www.sas.com). The following statistical mixed model was used:

$$Y_{ijkl} = \mu + \beta j + \alpha(\beta)_{ij} + \delta(\beta)_{kj} + \theta_l + \varepsilon_{ijkl}$$

where, μ is the overall mean, βj is the fixed effect of the j^{th} replication, $\alpha(\beta)_{ij}$ is the fixed effect of the i^{th} row within the j^{th} replication, $\delta(\beta)_{kj}$ is the fixed effect of the k^{th} column within the j^{th} replication, θ_l is the random effect of the l^{th} line, and ε_{ijkl} is the experimental error.

Genetic variances were estimated by the Restricted Maximum Likelihood method. The CL option of PROC MIXED procedure of SAS was used for the estimation of the confidence interval for the estimate of genetic variance. Levene's test for equality of variances was performed to test for differences in genetic variances between populations. The absolute residual of the Best Linear Unbiased Predictor (BLUP) for each inbred line was used as dispersion variable for the test in order to avoid any influence of differences in field variability between experiments on the comparison between the two populations. BLUP for each inbred line was estimated by PROC MIXED procedure of SAS, with the option SOLUTION for the random effects. Before comparison of the genetic variances, Quantile-Quantile plots of BLUPs were constructed for each trait to verify the assumption of normality. Then, the homogeneity of variance test was performed by PROC GLM procedure of SAS, using the MEANS option HOVTEST=LEVENE (TYPE=ABS).

The parental lines were included in the data set for the comparison between the population means. The difference between the population mean and the mean of the parents

was estimated for each population. The two estimates of differences were then tested for statistical difference with a t-test.

Phenotypic correlations among traits were calculated using the mean of each line over replications. The estimation was performed by PROC CORR procedure of SAS. Differences in correlation coefficients between populations were then tested using the Fisher's Z transformation, following the procedures described by Steel and Torrie (1980), which yields an approximate p-value. Because simultaneous multiple tests for differences were performed, the Bonferroni correction was applied to the p-value of each test.

Results

Genetic variances

The estimates of genetic variance for the evaluated traits are shown in table 2. For all traits, the genetic variance estimates were significant ($P < 0.05$) in both populations. IBM-10 had higher estimates of genetic variance for GDD, BR, EL, ED, ER, EKR, CD, PRO, OIL and STA. According to Levene's test, the differences in genetic variances between populations were statistically significant for OIL and EKR ($P < 0.001$ and $P = 0.01$, respectively). The estimates of genetic variances for OIL and EKR were 1.5 and 1.3 times larger in IBM-10 than in IBM, respectively. In addition, the P-values of the test for difference of genetic variances for ER, GDD and EL were low ($P = 0.054$, $P = 0.078$ and $P = 0.082$, respectively) relative to the P-values obtained for the comparisons of genetic variances for the other 9 traits ($P > 0.25$). The trend for increase in genetic variance for IBM-10 is shown in figure 1. For four traits (PH, TBN, KW and KD) the estimates of the genetic variance were smaller in the IBM-10 population, although the differences between the estimates were statistically non-significant.

Means

The relative performance of the parental inbred lines was consistent across the two experiments. Significant differences were detected between B73 and Mo17 for all traits ($P < 0.001$) except for number of tillers per plant ($P = 0.196$). B73 showed larger means than Mo17, except for KW, EL and PRO.

The estimates of the population means for the traits are presented in table 3. The population mean for ER and PH were significantly higher for IBM-10 ($P < 0.001$ and $P = 0.034$, respectively). For the other 12 traits, the differences between population means were relatively small and statistically non-significant.

Correlation among traits

The estimates of phenotypic correlations for all the trait-pair combinations (91) are shown in Table 4. Without adjustment for simultaneous multiple comparisons, the test for difference in phenotypic correlation between populations had a P-value less than 0.05 for only 5.5 % of the total trait pairs (GDD-EKR, GDD-OIL, PH-ER, TBN-ER, EL-KD). Furthermore, applying Bonferroni's adjustment, there was no evidence of significant difference in phenotypic correlation between IBM and IBM-10 populations. Observing the absolute value of the correlations, the change in the phenotypic correlations did not follow a particular trend, since the number of correlations that tend to increase and decrease from IBM to IBM-10 is roughly equal (42 and 49, respectively). In both experiments, most of the estimated correlations were relatively low ($|r| < 0.3$ for 79 out of 91 correlations). Nevertheless, the following 5 pairs of traits showed a relatively high and consistent correlation across the populations: ER-ED, EKR-EL, CD-ED, KD-ED and STA-PRO ($|r| > 0.6$). The largest correlation was estimated between PRO and STA ($r = -0.80$ and $r = -0.82$ for IBM and IBM-10, respectively).

Tillers and cob color

Although the parental inbreds showed a very low average number of tillers per plant (TN = 0.08 and 0.05 for B73 and Mo17, respectively) in both populations, this trait was larger than 0.25 and 0.5 for around 19 % and 6 % of the recombinant inbred lines, respectively. The mean for the inbred showing the highest number of tillers per plant was 0.98 (standard error = 0.15) and 1.38 (standard error = 0.11) for the IBM and IBM-10 population, respectively.

Both B73 and Mo17, and the IBM RILs have red cob. Segregation for cob color was observed among the IBM-10 DHLs, as 4 of them (M0061, M0084, M0172, M0473) had white cob.

Discussion

Two sets of 244 lines, one each in the IBM and IBM-10 populations were characterized phenotypically for 16 traits. Means, genetic variances and phenotypic correlations were estimated for 14 quantitative traits in each population. Then, the populations were compared in terms of these estimates.

Evidences of significant differences in genetic variance between populations were found for two traits, OIL and EKR, for which IBM-10 had estimates 1.5 and 1.3 times larger than IBM, respectively. Furthermore, the estimated genetic variances were numerically larger in the IBM-10 for 10 out of 14 traits. For traits where most of the genetic variance is additive, quantitative genetic theory indicates that genetic variance may increase as a consequence of recombination between loci linked predominately in repulsion phase (Comstock and Robinson, 1948). In several maize populations, the genetic variance estimated for many traits including PH, EL, ED, ER, KW, OIL, PRO, STA, KD, CD was found to be mainly additive (Hallauer and Miranda 1988).

Previous studies involving B73 x Mo17 populations have assessed genetic variance either for backcross progeny using the Design III genetic model developed by Comstock and Robinson (1952) or for S1 progeny (Covarrubias-Prieto, 1987; Han and Hallauer, 1989; Cook, 1998). Kernel oil concentration and EKR were not analyzed by these previous studies. In the case of EL, PH and KD, there was no evidence of change in genetic variance with intermating, similar to the result reported herein. For ER, the additive and dominance variance and the variance among S1 progeny were smaller in the F2syn5 than in the F2 generation, suggesting that recombination have occurred between loci controlling ER predominately linked in coupling phase in the F2 generation. Conversely, in the present study, the genetic variance for ER was 1.2 times larger in the IBM-10 than in the IBM population.

Recombination and subsequent reduction of linkage disequilibrium between linked loci is expected to be greater during the first generation of intermating (Hanson, 1959; Falconer and Mackey, 1996). This suggests that large differences in genetic variance should not be expected between IBM and IBM-10, which agrees with the results reported herein. Furthermore, according to the formula relating the recombination fraction observed in a

mapping population (R) with the recombination fraction adjusted to an F2 basis developed by Winkler et at. (2003), R should be 1.69 times larger in the IBM than in the F2 generation, and 1.36 times larger in the IBM-10 than in the IBM population. This suggest that the first 4 cycles of intermating of the F2 generation for the development of the IBM population have greater impact on recombination fractions than the six additional cycles of intermating performed for the development of the IBM-10 populations.

Significant changes in the population mean were evident for only two traits, PH and ER. In previous investigations with B73 x Mo17 intermated populations, the mean for PH of S1 progeny was smaller in the F2Syn5 than in the F2syn0 (Covarrubias-Prieto, 1987). In contrast, the mean for PH of testcross progeny was larger in the F2syn10 than in the F2 generation, although the difference was not significant (Cook, 1998). That suggests the mean for PH should have increased from the F2syn5 to the F2syn10, as shown in the present study. The ER mean of S1 progeny was larger in the F2syn5 than in the F2syn0 (Covarrubias-Prieto, 1987; Han and Hallauer, 1989). Similarly, in the present study the ER mean was larger in the IBM-10 than in IBM population.

Changes in allele frequency during the six additional cycles of intermating in the development of the IBM-10 population could be one of the causes of the observed differences in population mean. In such case, the frequency of alleles with positive effect for ER and PH should be larger in the IBM-10 than in IBM population. Previous studies showed variation in allele frequency between populations derived from the cross of B73 x Mo17 after different number of cycles of intermating (Lee et al., 2002; Lu et al., 2002; Jaqueth, 2003; Fu et al., 2006). Genetic regions with allelic ratios different from the expected (1:1, B73:Mo17) were detected in the genetic map of chromosome one of the IBM and IBM-10 populations (Jaqueth, 2003). However, the size of such regions varied between populations, being smaller in the IBM-10, which shows less number of loci deviating from the expected 1:1 segregation. Furthermore, other investigations indicate that in the IBM population the allele frequencies of a number of loci differ from expected ratio (1:1) (Lu et al., 2002; Fu et al., 2006). Changes in allele frequency would not only affect the population means, but also may produce changes in genetic variance (Falconer and Mackay, 1996). In the present study, there was not evidence of difference in the genetic variance for PH between the populations ($P = 0.9$). However, for ER the genetic variance was 1.2 larger in IBM-10 than IBM ($P = 0.054$).

Changes in means could be due to recombination between interacting linked genes. Theory indicates that if there is no interaction between linked loci controlling a given trait, then intermating is not expected to influence the population mean. However, when interactions among linked loci are present, then the influence of intermating on the mean values of a trait will depend on the magnitude of the linkage between the interacting genes (Mather and Jinks, 1982). If the observed differences in the mean between populations were due to recombination between interacting linked genes, there should be some IBM-10 DHLs showing increased phenotypic effect at some loci affecting PH and ER. Changes in the allele effects as a consequence of epistatic interactions may produce changes in the genetic variances. Effects of epistasis at the QTL level on the population mean and variance have been reported (Carlborg et al., 2006). Moreover, previous studies in different species have reported changes in the effect of QTL across genetic backgrounds (e.g. Tanksley and Hewitt, 1988; Doebley, 1995; Lukens and Doebley 1999; Leips and Mackay, 2000; Carlborg et al., 2006). For PH, epistasis between two or more QTLs linked to the same marker locus was found in a population derived from the cross between B73 and Mo17 (Cockerham and Zeng, 1996).

There was no evidence of differences in phenotypic correlations between IBM and IBM-10 populations. Linkage between loci affecting two different traits can be a cause of correlation. In that case, coupling linkage would cause positive correlation, whereas repulsion linkage would cause negative correlation. The correlation due to linkage can be dissipated by intermating, because of the increase in the chances of recombination between linked loci. In a previous study where the S1 progeny of the F2syn0 and F2syn5 of the cross B73 x Mo17 were evaluated for several traits, the same number of test for differences in phenotypic correlations were performed (91), but no adjustment for simultaneous multiple comparison was applied (Covarrubias-Prieto, 1987). Without considering Bonferroni's correction, the number of tests for difference in correlations that resulted in significance (approximated P-value < 0.05) was less in the current study than in the previous study mentioned above (5 and 15, respectively). These results may indicate that the first 5 cycles of intermating had larger effect in creating equilibrium between coupling and repulsion linkage existing among loci controlling different traits than the additional cycles of intermating in the IBM-10 development.

Segregation for cob color among IBM-10 DHLs could have resulted as a consequence of recombination between tightly linked genes. In maize, the p1 gene encodes a transcription factor that conditions red flavonoid pigmentation in floral organs such as kernel pericarp, cob glumes, tassel glumes, and silk (Coe et al., 1988). Within the p1 gene sequence there is a cob glume–specific regulatory sequence in the distal enhancer region. It was demonstrated that recombination between p1 and a tightly linked paralogous gene (p2) results in a chimeric gene with altered glume-specific regulatory sequence, with dramatic reduction or complete loss of pigmentation in cob glumes (Zhang, 2005). From these findings, it is possible that recombination within specific regions of p1 could generate the unusual cob color segregation observed in IBM-10 DHL lines.

The differences in genetic variances and means detected between IBM and IBM-10 populations, and the segregation in the cob color among the DHLs of the IBM-10 population could reflect the increased recombination fraction observed between adjacent loci in IBM-10 relative to IBM. Several authors have recognized the positive effects of an increase in the frequency of recombination events on the identification of genomic regions associated with quantitative traits (Austin and Lee, 1996; Graham et al., 1997; Dudley et al. 2004). Specifically, increasing the number of recombinants individuals for a given chromosomal region allows the dissection of single QTLs into two or multiple linked QTLs (Austin and Lee, 1996; Graham et al. 1997).

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Table 1. Traits measured on the lines of IBM and IBM-10 populations in 2006.

Trait	Abbreviation	Description
Growing degree days	GDD	Growing degree days in °C day from planting until the date when at least 50 % of the tassels in the plot are shedding pollen.
Plant height	PH	Height of the plant in cm from the ground level to the flag leaf collar.
Tassel branch number	TBM	Number of primary branches in the tassel.
Number of nodes with brace roots	BR	Number of nodes above ground bearing brace roots.
Ear length	EL	Length of the ear in mm from the base to the tip.
Ear diameter	ED	Diameter of the ear in mm at the middle of its length.
Ear number of rows	ER	Number of rows per ear at the height of 50 mm from the ear base.
Ear, number of kernel per row	EKR	Number of kernels in a single ear row.
Cob diameter	CD	Diameter of the cob in mm at the middle of its length.
Cob glumes color	CC	Color of the glumes of the cob.
Kernel depth	KD	Kernel depth in mm estimated as half the difference between ear diameter and cob diameter.
Kernel weight	KW	Weight of 1000 kernels in g.
Protein	PRO	Kernel protein concentration in g kg^{-1}.
Oil	OIL	Kernel oil concentration in g kg^{-1}.
Starch	STA	Kernel starch concentration in g kg^{-1}.

Table 2. Estimates of genetic variance (σ^2_G) and their confidence intervals for 14 traits in IBM and IBM-10 populations grown in 2006 in Ames, Iowa.

	IBM			IBM-10		
Trait	σ^2_G	LL[†]	UL	σ^2_G	LL	UL
GDD to anthesis [(°C day)^2]	902	747	1112	1061	885	1295
Plant height (cm^2)	277	231	338	266	223	324
Number of tassel branches ^{2}	6.15	5.12	7.52	5.74	4.77	7.05
Number of nodes with brace roots ^{2}	0.295	0.238	0.377	0.308	0.252	0.385
Ear length (mm^2)	285	230	364	315	261	388
Ear diameter (mm^2)	8.56	7.05	10.61	9.11	7.58	11.17
Ear number of rows^2	2.30	1.89	2.85	2.83	2.33	3.51
Ear, number of kernel per row ^{2}	19.0	14.9	25.2	25.4 **	20.7	31.8
Cob diameter (mm^2)	4.72	3.87	5.89	5.07	4.19	6.28
Kernel weight (g^2)	1809	1494	2235	1752	1454	2151
Kernel depth (mm^2)	0.772	0.618	0.993	0.730	0.575	0.957
Protein (g^2 kg^{-2})	113	90	146	120	96	154
Oil (g^2 kg^{-2})	9.3	7.6	11.5	13.6 ***	11.2	16.8
Starch (g^2 kg^{-2})	104	85	131	122	99	154

[†] LL and UL = lower limit and upper limit for the 95 % confidence interval.
, * indicates that the genetic variance is significantly higher in the IBM-10 population at the 0.01 and 0.001 probability levels, respectively.

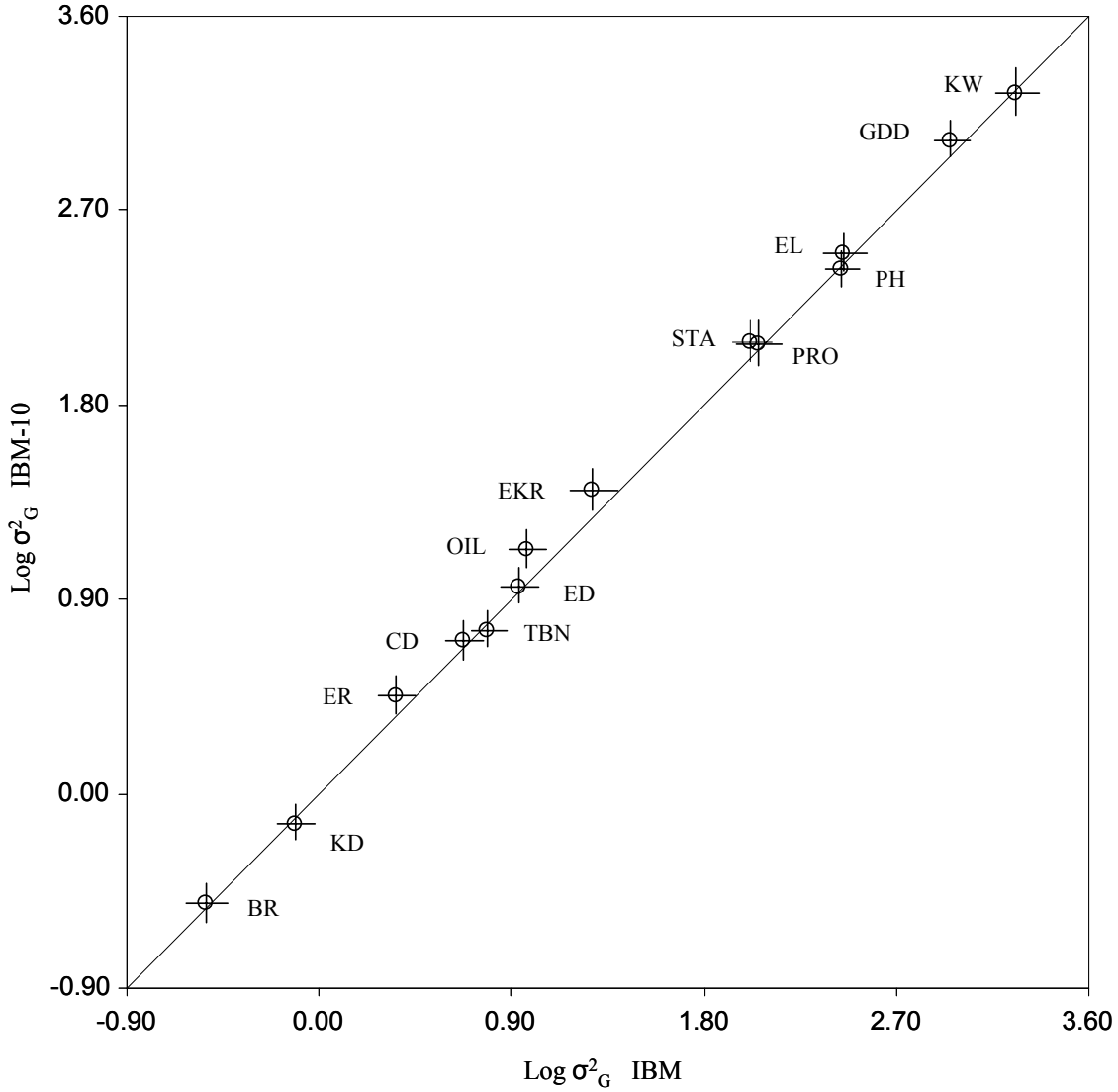

Figure 1. Graph of logarithm of genetic variance estimates (log σ^2_G) for quantitative traits in IBM population plotted against the log σ^2_G estimated in IBM-10 population. Horizontal bars represent the logarithm of confidence intervals for σ^2_G in the IBM population. Vertical bars represent the logarithm of confidence intervals for σ^2_G in the IBM-10 population. Interceptions between log σ^2_G located above the diagonal line indicate larger log σ^2_G for the IBM-10 population. KD = kernel depth, CD = cob diameter, ED = ear diameter, BR = number of nodes above ground with race rots, ER = number of ear rows, EL = ear length, TBN = number of tassel branches, OIL = kernel oil concentration, EKR = number of kernel per ear row, STA = kernel starch concentration, PRO = kernel protein concentration, PH = plant height, GDD = growing degree days to 50 % of anthesis, KW = kernel weight.

Table 3. Estimates of means for 14 traits in the IBM and IBM-10 populations and in parental inbreed lines B73 and Mo17[¶] grown in 2006 in Ames, Iowa.

	IBM			IBM-10			B73	Mo17[£]
Trait	Mean (SE)[†]	Min[§]	Max	Mean (SE)	Min	Max	Mean (SE)	Mean (SE)
GDD to anthesis (°C day)	907 (2.00)	831	1003	914 (2.14)	847	1010	934 (1.96)	917 (2.10)
Plant height (cm)	172 (1.09)	129	214	180 (1.07)**	135	231	190 (1.09	166 (1.14)
Number of tassel branches	7.10 (0.16)	3.01	17.86	7.49 (0.16)	3.61	19.38	6.90 (0.09	5.90 (0.10)
Number of nodes with brace roots	1.82 (0.04)	0.64	3.31	1.77 (0.04)	0.73	3.19	3.07 (0.05)	1.83 (0.05)
Ear length (mm)	160 (1.18)	115	203	161 (1.18)	112	207	151 (1.06)	185 (1.12)
Ear diameter (mm)	41.6 (0.20)	32.6	49.7	41.9 (0.20)	33.8	49.4	47.1 (0.26)	36.7 (0.27)
Ear number of rows	14.0 (0.10)	10.4	19.1	14.4 (0.11)*	10.7	19.3	18.0 (0.13)	10.6 (0.13)
Ear, number of kernel per row	31.2 (0.32)	20.5	40.9	32.3 (0.34)	16.4	44.5	31.2 (0.39)	28.8 (0.44)
Cob diameter (mm)	26.7 (0.15)	21.1	34.8	26.8 (0.15)	21.6	33.0	31.0 (0.15)	22.1 (0.16)
Kernel weight (g)	266 (2.84)	150	369	257 (2.76)	163	412	264 (3.42)	336 (3.47)
Kernel depth (mm)	7.50 (0.06)	5.35	9.77	7.58 (0.06)	5.74	10.01	8.00 (0.14)	7.29 (0.15)
Protein (g kg^{-1})	125 (0.76)	100	157	127 (0.78)	104	154	116 (1.88)	132 (1.99)
Oil (g kg^{-1})	38.5 (0.20)	32.7	48.6	39.1 (0.25)	31.8	50.2	40.5 (0.27)	37.0 (0.30)
Starch (g kg^{-1})	697 (0.70)	672	717	695 (0.76)	670	720	702 (1.26)	694 (1.38)

[¶] Mean of the parental lines estimated across experiments.
[£] The mean of Mo17 differs from B73 for every trait at the 0.001 probability level.
[†] Standard error of the mean.
[§] Min and Max = (population mean + minimum BLUP) and (population mean + maximum BLUP).
*, ** Indicate that the mean is significantly higher in the IBM-10 than in the IBM population at the 0.05 and 0.01 probability levels, respectively.

Table 4. Estimates of phenotypic correlations among 14 traits measured in the IBM and IBM-10 populations grown in 2006 in Ames, Iowa.

		PH	TBN	BR	EL	ED	ER	EKR	CD	KD	KW	PRO	OIL	STA
GDD	IBM	0.45**	0.15*	0.14*	-0.04	-0.05	-0.06	-0.42**	-0.06	-0.07	0.32**	-0.11	0.20**	0.00
	IBM-10	0.34**	0.05	0.29**	-0.21**	-0.10	-0.11	-0.59**	-0.06	-0.10	0.34**	-0.04	0.02	0.05
PH	IBM		0.17**	0.00	0.00	-0.01	-0.13*	-0.20**	-0.01	-0.01	0.28**	-0.17**	0.13*	0.07
	IBM-10		0.00	-0.05	0.13*	0.10	0.08	-0.09	0.10	0.03	0.27**	-0.12	0.02	0.10
TBN	IBM			0.13*	-0.01	0.06	0.09	-0.09	-0.04	0.12	0.03	-0.09	-0.04	0.05
	IBM-10			-0.02	0.00	0.06	-0.09	0.00	0.02	0.08	0.05	0.05	-0.05	-0.03
BR	IBM				-0.11	-0.01	-0.10	-0.19**	-0.07	0.08	0.13*	0.15*	0.08	-0.16*
	IBM-10				-0.14*	-0.14*	-0.14*	-0.29**	-0.13*	-0.06	0.16*	0.10	0.03	-0.05
EL	IBM					0.01	-0.08	0.63**	0.18**	-0.24**	0.02	-0.09	-0.05	0.13*
	IBM-10					0.05	-0.08	0.62**	0.11	-0.06	0.18**	-0.14*	-0.07	0.16*
ED	IBM						0.64**	0.14*	0.76**	0.65**	0.25**	-0.10	-0.06	0.09
	IBM-10						0.62**	0.15*	0.78**	0.64**	0.26**	-0.14*	0.07	0.06
ER	IBM							0.16*	0.56**	0.33**	-0.19**	-0.15*	0.10	0.04
	IBM-10							0.11	0.55**	0.33**	-0.26**	-0.18**	0.21**	0.02
EKR	IBM								0.18**	0.03	-0.41**	-0.07	0.01	0.06
	IBM-10								0.12	0.11	-0.34**	-0.15*	0.03	0.09
CD	IBM									0.03	0.12	-0.13*	-0.10	0.17**
	IBM-10									0.03	0.06	-0.06	-0.02	0.02
KD	IBM										0.25**	-0.03	0.03	-0.04
	IBM-10										0.30**	-0.15*	0.14*	0.07
KW	IBM											-0.05	-0.07	0.13*
	IBM-10											-0.06	-0.11	0.16*
PRO	IBM												-0.15*	-0.80**
	IBM-10												-0.09	-0.82**
OIL	IBM													-0.38**
	IBM-10													-0.42**

*, ** indicate that the phenotypic correlation is significantly different from 0 at the 0.05 and 0.01 probability level, respectively.

COMPARISON BETWEEN OPEN AND SELF-POLLINATED KERNELS FOR QUALITY TRAITS IN THE IBM AND IBM-10 POPULATIONS

Introduction

In maize, open and self-pollinated ears are two alternative sources of kernels for the assessment of quality traits such as kernel protein, oil and starch concentration. Open and self-pollinated ears may differ in the pollen genotype and in the post-flowering source-sink ratio. These factors may influence the kernel composition. Therefore, the concentration of protein, oil and starch in open-pollinated (OP) kernels may not resemble those in self-pollinated (SP) kernels. Using OP kernels for the assessment of quality traits save time and other resources required by the self-pollination process in the production of SP kernels. However, if OP kernels provide misleading information, then plant breeders need to use SP kernels. Therefore, determining the resemblance between OP and SP kernels in terms of protein, oil and starch concentration may have important practical implications in the improvement these traits.

The effect of the pollen on the development of the kernel components is known as xenia. Several studies have reported xenia effect on the kernel oil concentration (i.e. Miller and Brimhall, 1951; Curtis et al., 1956; Alexander and Lambert, 1968). Pollen from high-oil genotypes (i.e. more than 60 g kg^{-1}) was found to increase the embryo size and also the oil concentration in the embryo of kernels of low and normal-oil genotypes (i.e. less than 50 g kg^{-1}), whereas the effect of the pollen genotype upon these variables on a high-oil genotypes was relatively small (Miller and Brimhall, 1951; Curtis et al., 1956; Lambert, 2001). In high-oil genotypes OP kernels had lower oil concentration than the SP kernels, whereas in low-oil genotypes OP kernels had higher oil concentration than the SP kernels (Letchworth and Lambert, 1998).

Increased oil concentration in the kernel as a consequence of xenia was accompanied by an increase in the percentage of high quality protein and the percentage of protein in the embryo; although the protein concentration in the whole kernel did not change (Miller and Brimhall, 1951; Thomison, 2003). High and low-protein genotypes did not show difference

in protein concentration between self and cross-pollinated kernels (Letchworth and Lambert, 1998). In other investigations, SP kernels had more protein concentration than OP kernels (East and Jones, 1920; Letchworth and Lambert, 1998).

Xenia effect was detected on the activity of enzymes involved in starch biosynthetic pathway (Rahman et al., 1998; Bulant et al., 2000, Tracy, 2000). Homozygous mutant genotypes for the Su1 gene (Sugary 1) fertilized with pollen carrying the dominant allele for that gene increase the activity of the starch debranching isoamylase enzyme and the kernel starch concentration. Increase in oil concentration as a consequence of xenia effect was accompanied by an increase in the embryo: endosperm ratio and a reduction in the starch concentration (Lambert, 1998; Thomison, 2003).

Open-pollinated and SP ears may differ in the number of kernels. The number of kernels in a self-pollinated ear depends not only on plant growth at the stage of flowering (Tollenar et al., 1992; Andrade et al., 1999) but also on factors associated with the efficiency of the hand pollination, such as the amount and quality of the pollen applied to the silks and the percentage of silk emerged at the day of pollination. Fewer kernels in SP ears compared to OP ears was reported by East and Jones (1920). In that case, the number of plants required to produce enough kernels for the quality trait assessment will be larger if SP ears constitute the selected source of kernels. This may represent a disadvantage with limited availability of plants per genotype. Furthermore, the number of kernels per ear and the protein content are negatively correlated (Hayes and Garber, 1919). Differences in the number of kernels between OP and SP ears represent differences in the amount of assimilate supply (source) per growing kernel (sink) during the filling period. Post-flowering source-sink ratio is positively associated with protein kernel concentration (Jones and Simmons, 1983; Borras et al., 2002), and negatively associated with starch kernel concentration (Borras et al., 2002). No association was found between the assimilate availability per growing kernel and the kernel oil concentration (Borras et al., 2002).

In the present study, the differences between the composition of OP and SP kernels are being investigated with two populations of recombinant inbred lines that have been developed to facilitate high resolution genetic mapping, the IBM and IBM-10 populations. These populations were derived from the cross between inbred lines B73 and Mo17. These inbred lines were of relevant importance for the US maize breeding, and recycled versions

are still grown (Troyer, 2000). B73 and Mo17 differ in kernel protein, oil and starch concentration, and are polymorphic at many loci. B73 was used in many genetic studies and will be the first maize inbred to have its genome sequenced.

The objective of this study were (i) to determine the degree to which values of protein, oil and starch concentration in OP kernels resemble those in SP kernels; (ii) to estimate and compare the mean for protein, oil and starch concentration between OP and SP kernels; and (iii) to estimate and compare the genetic variance for protein, oil and starch concentration between OP and SP kernels.

Materials and methods

Germplasm

The study included 244 recombinants inbred lines (RILs) of the IBM population and 244 double haploid lines (DHLs) of the IBM-10 population. The IBM population was derived from the single-cross hybrid of inbred B73 (female) and Mo17 (male). B73 was derived from the Iowa Stiff Stalk Synthetic population (Russell, 1972). Mo17 was developed from Lancaster Sure Crop germplasm (Zuber, 1973). From the initial cross of B73 and Mo17, the F1 was self-pollinated to produce the F2 generation. Within the F2 generation, 250 plants were random mated by making plant to plant crosses. A single kernel was taken from each ear to form a bulk of seed to be grown for the following cycle of intermating. This random mating procedure was repeated for 4 generations (Lee et al., 2002). Recombinant inbred lines were then developed from this intermated population by eight generations of self pollination through single seed descent (Lee et al., 2002). The IBM population was intermated for six additional generations. After ten generations of random mating, doubled-haploid lines were then developed by DuPont using a proprietary process for producing doubled haploid lines.

Experimental design

Each population was planted in separate but adjacent experiments at the Agronomy Agricultural Engineering Research Center (AAERC), Ames, Iowa. In each experiment, the lines were grown in a row-column alpha lattice experimental design with two replications of 7 columns and 37 rows. Plots consisted of a row 3.8 m long with 0.76 m between rows. Plots

were over-planted, and the plant density was reduced to 12 plants per row when plants had 4 to 5 leaves completely developed. The planting date was May 6th, 2006. The field was fertilized with 18 kg of Nitrogen, 44 kg of Phosphorous and 120 kg of Potassium per ha on September 2005, and with 175 Kg of urea per ha 15 days before planting. Metolachlor and Atrazine herbicides were sprayed and incorporated into the soil 11 days before planting, at the rate of 1.86 and 1.12 kg of active ingredient per ha, respectively. Neither herbicides nor insecticides were applied on the experiments after planting.

Trait evaluation

For the evaluation of the kernel quality traits, two samples of kernel were obtained from each plot, self-pollinated (SP) and open-pollinated (OP). For the SP sample, 4 ear shoots per plot were covered with shoot bags to prevent pollination. To obtain uniform silk growth, the ear shoots were cut one or two days before manual pollination. The SP ears were covered with a pollinating bag used to collect pollen from the tassel, and remained covered until harvest. For the OP sample, 4 open-pollinated ears were chosen without selection from each plot. Self and open-pollinated ears were hand-harvested after physiological maturity, and then air-dried at 37.5°C until moisture content reached approximately 11 %. The ears were shelled by hand. Self-pollinated ears with few kernels were discarded to minimize the effect of high source-sink ratio on the chemical composition in the kernel. Previous studies have established that when there are abnormally few kernels on an ear, the protein and starch kernel concentration tend to be inflated and deflated, respectively (Jones and Simmons, 1983; Borras et al., 2002). Self-pollinated and OP ears with disease symptoms were discarded. The whole-kernel samples were analyzed by near-infrared transmittance, with an Infratec^{TM} 1241 Grain Analyzer (Foss Inc., www.foss.dk). The same procedures for the analysis of the quality traits were used in previous investigations (i.e. Letchworth and Lambert, 1998; Borras et al., 2002; Clark et al., 2006). Results were expressed as concentration (g kg^{-1}) on a dry matter basis.

The difference between OP and SP values of protein, oil and starch were also examined across values of growing degree days needed to reach 50 % of anthesis (GDD). Growing degree days were calculated according to the hourly adjusted average method (Cross and Zuber, 1972). The hourly temperature was adjusted by a base temperature of 8°C (Jones and Kiniry, 1986).

Statistical procedures

Population means and genetic variances with their confidence intervals were estimated by the PROC MIXED procedure of SAS software (version 9.1, SAS Institute, Inc., Cary, NC, www.sas.com). The following statistical mixed model was used:

$$Y_{ijkl} = \mu + \beta j + \alpha(\beta)_{ij} + \delta(\beta)_{kj} + \theta_l + \varepsilon_{ijkl}$$

where, μ is the overall mean, βj is the fixed effect of the j^{th} replication, $\alpha(\beta)_{ij}$ is the fixed effect of the i^{th} row within the j^{th} replication, $\delta(\beta)_{kj}$ is the fixed effect of the k^{th} column within the j^{th} replication, θ_l is the random effect of the l^{th} line, and ε_{ijkl} is the experimental error.

Because two samples of seed obtained from the plot are not independent, differences in mean between pollination treatments were tested performing a paired t-test with PAIRED statement of the PROC TTEST procedure of SAS, using the OP and SP mean of the lines over replications.

Genetic variances were estimated by the Restricted Maximum Likelihood method. The CL option of PROC MIXED procedure of SAS was used for the estimation of the confidence interval for the estimate of genetic variance. Levene's test for equality of variances was performed to test for differences in genetic variances between OP and SP. The test was performed with the PAIRED statement of the PROC TTEST procedure of SAS. The dispersion variable was the difference between the absolute residual of OP and SP values of each line.

Pearson correlation coefficients were calculated among values obtained from OP and SP kernels, for protein, oil and starch. The estimation was performed by PROC CORR procedure of SAS. The mean of each line over replication was used for the calculations.

Results

Correlation between values obtained from open and self-pollinated kernels

The main objective of this study was to determine the degree to which values of protein, oil and starch concentration in OP kernels resembled those in SP kernel. The sample correlation between values of the same quality trait measured in OP and SP kernels were significant ($P < 0.001$) and consistent across the two populations (Table 1). The estimated sample correlation for protein values were smaller than the correlations estimated for oil and

starch. The average sample correlation between OP and SP values across populations was 0.65, 0.80 and 0.75 for protein, oil and starch, respectively. The trend of correlation between OP and SP values for protein, oil and starch is shown in figures 1, 2 and 3, respectively.

The resemblance between OP and SP values in genotypes with higher protein, oil and starch concentration is of importance for selection purposes. If in each population the upper 20 % of the genotypes (49) were selected based on OP mean BLUP for protein concentration and compared with the upper 20% of genotypes selected based on SP mean BLUP values of the same trait, the selected fractions would have 45 % and 47 % of the genotypes in common for the IBM and IBM-10 population, respectively. If the selection is based on oil concentration, the selected fraction would have 61 % and 75 % of the genotypes in common for the IBM and IBM-10 population, respectively. In the case of selection for starch concentration, 57 % and 63 % of the genotypes would be common in the selected fractions for the IBM and IBM-10 population, respectively.

Population mean

The difference in population mean for protein, oil and starch concentration between OP and SP kernels were statistically significant ($P < 0.001$) and consistent across the two populations (Table 2). On average, the SP kernels had 1.07 and 1.09 times more protein concentration than the OP kernels for the IBM and IBM-10 population, respectively. The average difference between OP and SP values of protein across populations was 9 g kg^{-1} (0.9 %). Conversely, the mean oil concentrations were 1.04 and 1.05 times greater in the OP than in the SP kernels for the IBM and IBM-10, respectively. The average difference between OP and SP values of oil across populations was 2 g kg^{-1} (0.2 %). For starch, the mean concentration in the OP kernels was 1.01 times greater than in the SP kernels for both populations. The average difference between OP and SP values of starch across populations was 7 g kg^{-1} (0.7 %).

For each trait, the difference between OP and SP values were also examined for the groups of genotypes that represent the 20 % of the population with higher values in the SP kernels (upper 20%) and for the group of genotypes that make the 20 % with lower values in the SP kernels (lower 20 %) (Table 2). For protein concentration, SP kernels had larger values than the OP kernels in both extremes of the populations (upper 20 % and lower 20 %). However, the differences between SP and OP values were significantly higher in the upper

20 % than in the lower 20 % ($P < 0.001$). For oil concentration, the upper 20 % did no show differences between OP and SP values; whereas in the lower 20 % the OP kernels had larger oil concentration than the SP kernels ($P < 0.001$). For starch concentration, OP values were larger than the SP values in both extremes of the populations (upper 20 % and lower 20 %); although the differences between OP and SP values were significantly higher in the lower 20 % than in the upper 20 %.

The difference between OP and SP kernel composition was examined across values of growing degree days needed to reach 50% of anthesis (GDD). For the three quality traits, there was a trend for a decrease in the difference between SP and OP values with increase in the values of GDD (Figure 4, 5 and 6). In both populations, the estimated sample correlations between GDD and the difference between OP and SP values ($r_{GDD,OP-SP}$) were larger (absolute value) for protein than for oil and starch (the average $\left| r_{GDD,OP-SP} \right|$ across populations was 0.46, 0.26 and 0.33 for protein, oil and starch, respectively; $P < 0.001$).

Genetic variances

Significant genetic variances were estimated for the three quality traits ($P < 0.05$) (Table 3). The genetic variances for oil and starch concentration in SP kernels were significantly greater than in the OP kernels ($P < 0.05$). For protein concentration, the variances for SP and OP kernels were not significantly different ($P > 0.05$).

Discussion

A set of 244 lines in the IBM and IBM-10 population were evaluated for their kernel protein, oil and starch concentration, using two different sources of kernels: self-pollinated and open-pollinated ears. The hypothesis was that concentration of protein, oil and starch was not the same in open-pollinated than in self-pollinated kernels. The hypothesis was based on evidence of xenia, the effect of the pollen on the development of the kernel, and the post-flowering source-sink ratio effect on kernel components reported by previous studies. The results indicate that the OP and SP kernel differ in composition. Moreover, selection based on OP and SP values may result in different set of selected genotypes.

On average, the SP kernels had higher protein and lower starch concentrations when compared to the OP kernels in the IBM and IBM-10 populations of inbred lines. This

observation agrees with results reported by East and Jones (1920) for protein and by Letchworth and Lambert (1998) for protein and starch. Differences in the number of growing kernels per plant, and therefore in the post-flowering source-sink ratio, between the SP and OP plants for each particular genotype may be one of the reasons for the observed differences in the concentration of protein and starch in the kernel. If the self-pollinated ears had less number of kernels, those kernels would be growing under a higher post-flowering source-sink ratio than the OP kernels. The increase in the post-flowering source-sink ratio was found to be positively correlated with protein concentration and negatively correlated with starch concentration (Borras et al., 2002).

The population means for oil concentration estimated with OP kernels were larger than in the SP kernels. This observation agrees with the results reported by Letchworth and Lambert (1998). In addition, the genetic variance for oil concentration of SP kernels was 1.4 and 1.3 times larger than in the OP kernels in the IBM and IBM-10 population, respectively.

Moreover, there was no difference in the oil concentration between OP and SP kernel of genotypes with relatively high oil concentration (upper 20 %), whereas the group of genotypes with relatively low oil concentration (lower 20 %) showed significantly more oil concentration in the OP kernels than in the SP kernels (Table 2). The observed differences in mean and genetic variances for oil concentration between OP and SP kernels could be due to xenia effect. Previous studies reported that pollen from high-oil genotypes increased the oil concentration of OP kernel of genotypes with relatively low oil concentration, whereas the effect of pollen upon the oil concentration of high-oil genotypes was relatively small (Brunson et al., 1948; Miller and Brimhall, 1951; Curtis et al., 1956). Therefore, it can be hypothesize that in the evaluated populations, pollen from high oil genotypes might have increased the oil concentration in the OP kernels of genotypes with relatively lower oil concentration. In that case, the differences in oil concentration between genotypes contrasting in oil concentration would be less in the OP kernels than in the SP kernels, which would result in smaller genetic variance in the OP kernels than in the SP kernels.

The xenia effect on oil fraction can be related to the increase of embryo:endosperm ratio. In such case, OP kernels are expected to have larger embryo:endosperm ratio than the SP kernels. Therefore the starch concentration should be lower in the OP kernels than in the SP kernels. The results reported herein for the mean starch concentration does not support the

mentioned hypothesis, because on average the OP kernels had larger starch concentration than the SP kernels. As was already mentioned, higher starch concentration in OP kernels could be associated with lower number of kernels in SP ears than in OP ears, and the subsequent smaller post-flowering source-sink ratio in the OP plants than in the SP plants. Interestingly, the difference in starch concentration between OP and SP kernels was significantly ($P < 0.001$) smaller in the group of low-oil genotypes (4 g kg^{-1} and 5 g kg^{-1} for the IBM and IBM-10, respectively) than in the group of high-oil genotypes (9 g kg^{-1} and 10 g kg^{-1} for the IBM and IBM-10, respectively). This observation suggests that the difference in oil concentration between OP and SP kernels was reduced in the low-oil genotypes due to xenia effect.

There was a tendency for reduced differences between OP and SP values with increase in the thermal time required to reach anthesis. This trend was clearer for kernel protein concentration. In maize, the number of leaves per plant increases with the thermal time required to reach anthesis. That suggests differences in source capacity between early and late flowering genotypes. If late flowering genotypes are less source limited (e.g. larger post-flowering source-sink ratio) than the early flowering genotypes, the effect of increased source-sink ratio in SP plants on the kernel components could be smaller in the late flowering genotypes than in the early flowering genotypes. In addition, it is expected that less number of genotypes will contribute to the pollination of OP ears of late flowering genotypes than in early flowering genotypes. In that case, the chances of xenia effect on kernel components should be less in the late flowering genotypes than in the early flowering genotypes. Further characterization of early and late flowering inbreds of the IBM and IBM-10 populations in terms of xenia and post-flowering source-sink ratio effects on kernel components may provide information to explain the observed trend.

Further studies designed to examine differences in composition between OP and SP kernels should take into account the effect of different mechanisms on the development of the ears. The success of self-pollination process can be affected by the environmental condition during flowering time. For example, rainy weather may negatively affect the quality of the pollen that is collected for self-pollination, leading to less number of fertilized ovules per ear and subsequently an increase in the post-flowering source-sink ratio. The microenvironment surrounding the ear during its development may be different in SP ears

than in OP ears. Self-pollinated ears that remain covered by the pollination bags during their growth do not have incidence of solar radiation on the husks and this may affect the temperature surrounding the ear. Competition for resources (e.g. water, radiation, nutrients) is another mechanism that may affect the ear development. Within a plot of inbred lines, the plants that flower first could be the dominant ones, which usually have greater ability for kernel production. Therefore, it is important to randomly select the open and self-pollinated plants from the plots.

The present study was based on observations made in one single environment. The solar radiation and temperature values during flowering and grain filling periods were close to the average values for the site (see figures 6 and 7 in Appendix B). Rainfalls were below the typical values during the pre-flowering period and above the typical values during flowering and grain filling periods (see figure 8 in Appendix B). Environmental factors may affect the oil concentration, although in numerous studies the genotype effect on oil values was significantly larger than the effect of the environment (Genter et al., 1956; Jellum et al., 1966; Jellum et al., 1973). Kernel protein and starch concentration are largely affected by environmental factors such as level of Nitrogen fertilization, water availability during flowering and grain filling period and plant density (Genter et al., 1956; Pollmer et al., 1978; Borras et al., 2003). As such, further studies exploring other environmental conditions would be of interest.

Conclusions

In summary, the observations of the present study indicate that OP and SP kernels differed in composition. Open and self-pollinated plants may differ primarily in the pollen genotype. The results reported herein suggest xenia effect on the oil fraction. This conclusion is based on the larger mean and smaller genetic variance for oil concentration in the OP kernels than in the SP kernels; and in the larger difference between OP and SP kernel for the genotypes with low oil than in the genotypes with relatively high oil concentration. Based on the observations made in these populations and in this environment, accurate oil concentration assessment should be obtained from SP kernels. Higher protein concentration in SP kernels than in the OP kernels suggests that self-pollinated plants had less number of

kernels and subsequently higher source-sink ratio than the open-pollinated plants, although the number of kernels per plant was not assessed in the present study. Further investigations regarding the differences in composition between OP and SP kernels should assess the number of kernels per plant in order to examine the actual difference in sink capacity between OP and SP plants. For each genotype, the open-pollinated plants should express the maximum sink capacity permitted by the environment; therefore OP ear should be used for the assessment of protein concentration. With respect to starch concentration, lower mean in the SP kernels than in the OP kernels suggest highest post-flowering source-sink ratio in the self-pollinated plants than in the open-pollinated plants, as was indicated for protein. Interesting, the difference in starch concentration between OP and SP kernels in the genotypes with low oil concentration was smaller than in the genotypes with high oil concentration, which suggests that xenia also affected the starch fraction. Further studies are needed to examine the relative importance of the effects of xenia and post-flowering source-sink ratio on the kernel starch concentration.

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Table 1. Estimates of sample correlation between open-pollinated and self-pollinated values of kernel protein, oil and starch concentration in the IBM and IBM-10 populations, grown in 2006 in Ames, Iowa.

Population	Protein	Oil	Starch
IBM	0.64***	0.78***	0.76***
IBM-10	0.66***	0.81***	0.74***

*** indicates that the correlation is significantly different from 0 at the 0.001 probability level.

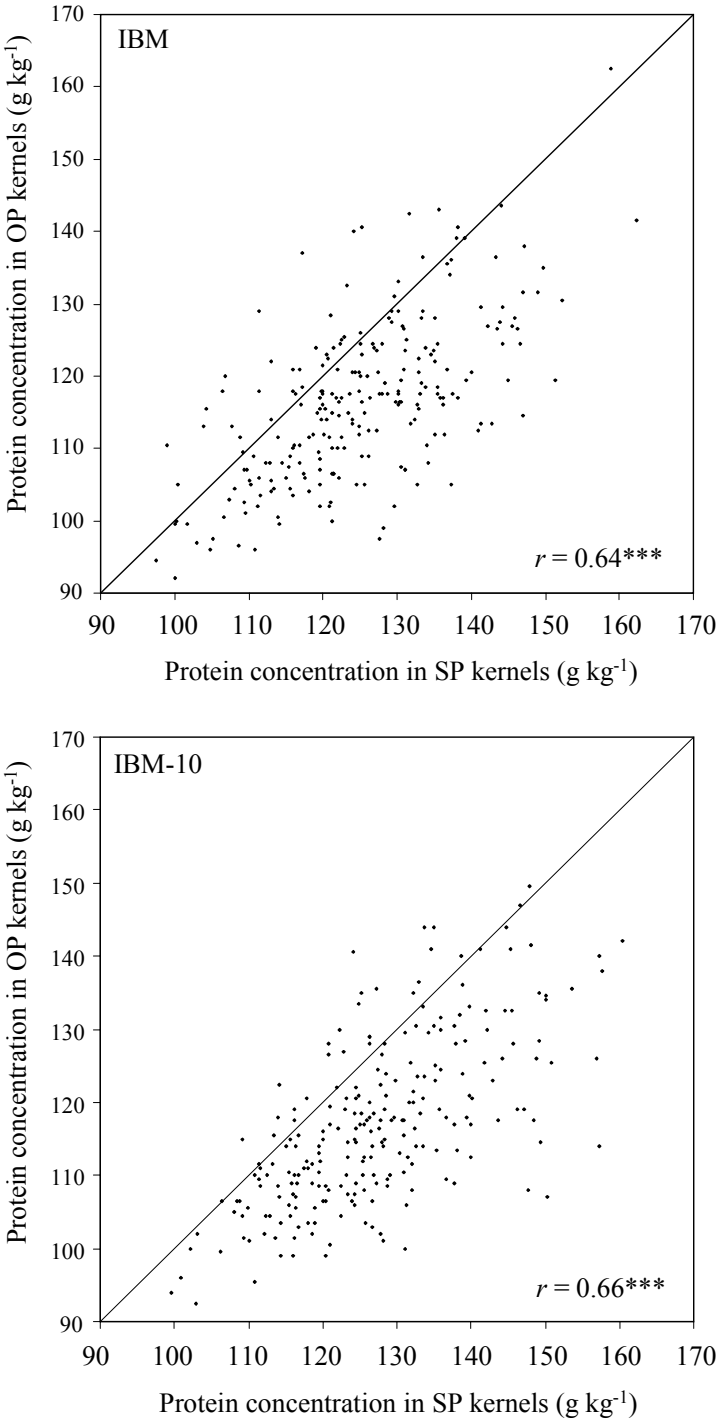

Figure 1. Graphs of mean protein concentration in self-pollinated (SP) kernels of inbred lines of the IBM and IBM-10 populations plotted against the mean protein concentration in open-pollinated (OP) kernels of the same genotypes. The line represents the diagonal. *** indicates that the correlation (*r*) is significantly different from 0 at the 0.001 probability level.

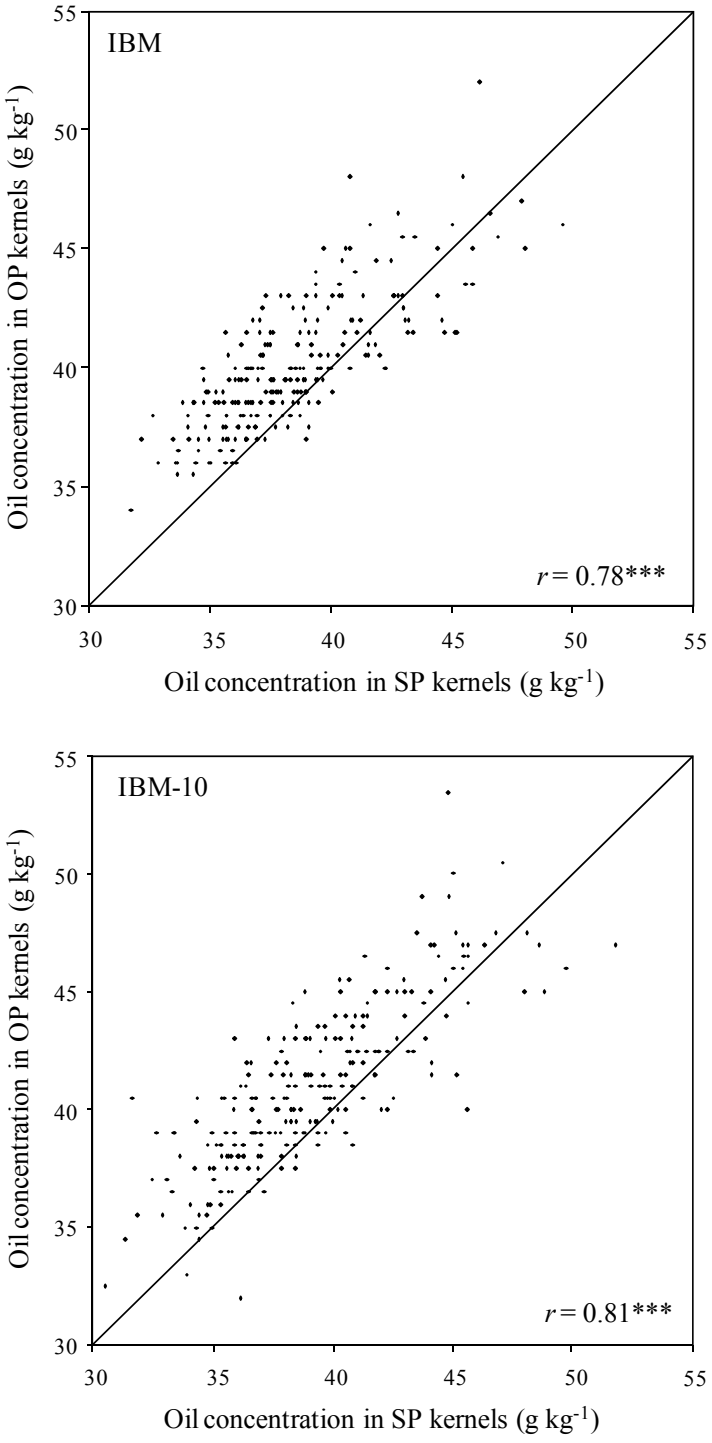

Figure 2. Graphs of mean oil concentration in self-pollinated (SP) kernels of inbred lines of the IBM and IBM-10 populations plotted against the mean oil concentration in open-pollinated (OP) kernels of the same genotypes. The line represents the diagonal. *** indicates that the correlation (*r*) is significantly different from 0 at the 0.001 probability level.

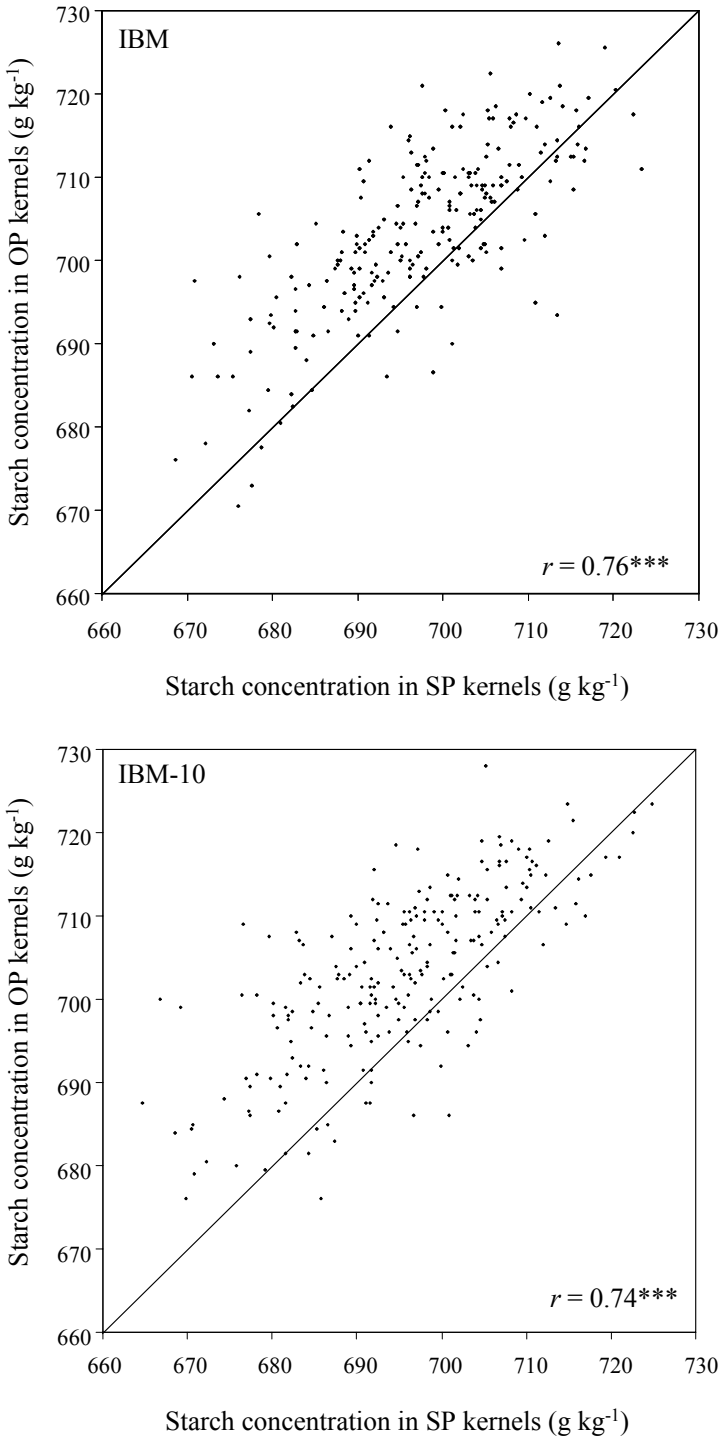

Figure 3. Graphs of mean starch concentration in self-pollinated (SP) kernels of inbred lines of the IBM and IBM-10 populations plotted against the mean starch concentration in open-pollinated (OP) kernels of the same genotypes. The line represents the diagonal. *** indicates that the correlation (*r*) is significantly different from 0 at the 0.001 probability level.

Table 2. Estimates of population mean for protein, oil and starch concentration measured in self-pollinated (SP) and open-pollinated (OP) kernels of inbred lines of the IBM and IBM-10 populations, grown in 2006 in Ames, Iowa.

Pop	SP	OP	OP-SP [¶]		
			all genotypes	Lower 20% [§]	Upper 20%
			Protein (g kg^{-1})		
IBM	125 (0.76)[†]	117 (0.71)	-8.5 (0.63)***	-2.7 (1.12)*	-15.6 (1.43)***
IBM-10	127 (0.78)	117 (0.73)	-10.3 (0.63)***	-5.0 (0.80)***	-17.5 (1.62)***
			Oil (g kg^{-1})		
IBM	38.5 (0.20)	40.1 (0.18)	1.7 (0.13)***	2.8 (0.20)***	-0.16 (0.31)
IBM-10	39.1 (0.25)	41.0 (0.21)	1.9 (0.15)***	3.0 (0.29)***	0.54 (0.40)
			Starch (g kg^{-1})		
IBM	697 (0.70)	704 (0.65)	6.2 (0.48)***	10.2 (1.02)***	0.7 (1.00)
IBM-10	695 (0.76)	703 (0.66)	8.0 (0.53)***	14.6 (1.20)***	3.1 (0.84)***

[¶] OP-SP = difference between the mean of OP and SP values.
[†] Values in parenthesis are the standard error of the mean.
[§] Lower 20 % and Upper 20 % = 20 % of the population with the lower SP values and 20 % of the population with higher SP values, respectively.
*, *** indicate that the difference between the mean of OP and SP values is significantly different from 0 at the 0.05 and 0.001 probability level, respectively.

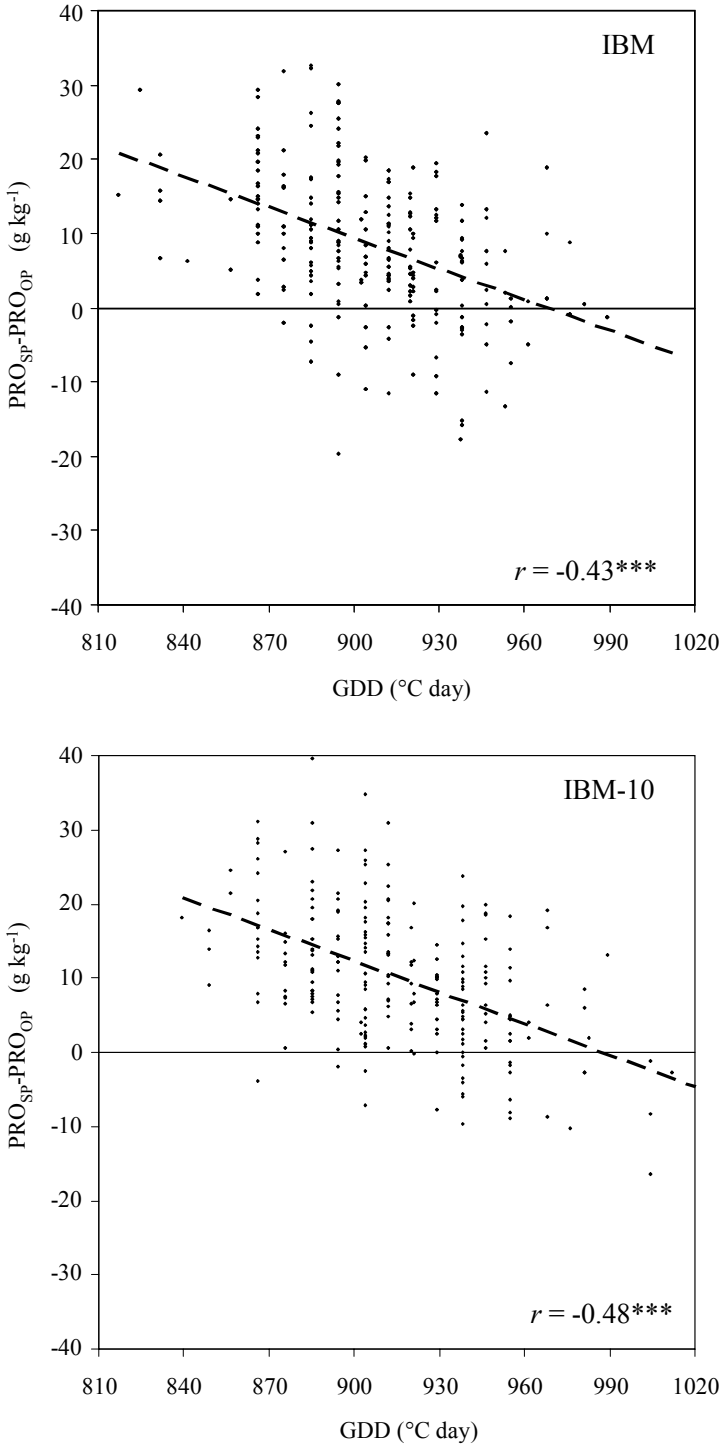

Figure 4. Difference in protein concentration between self and open-pollinated kernels (PRO_{SP}-PRO_{OP}) in relation to growing degree days to anthesis (GDD) for the IBM and IBM-10 populations. The dashed lines represent the trend line. *** indicates that the correlation (*r*) is significantly different from 0 at the 0.001 probability level.

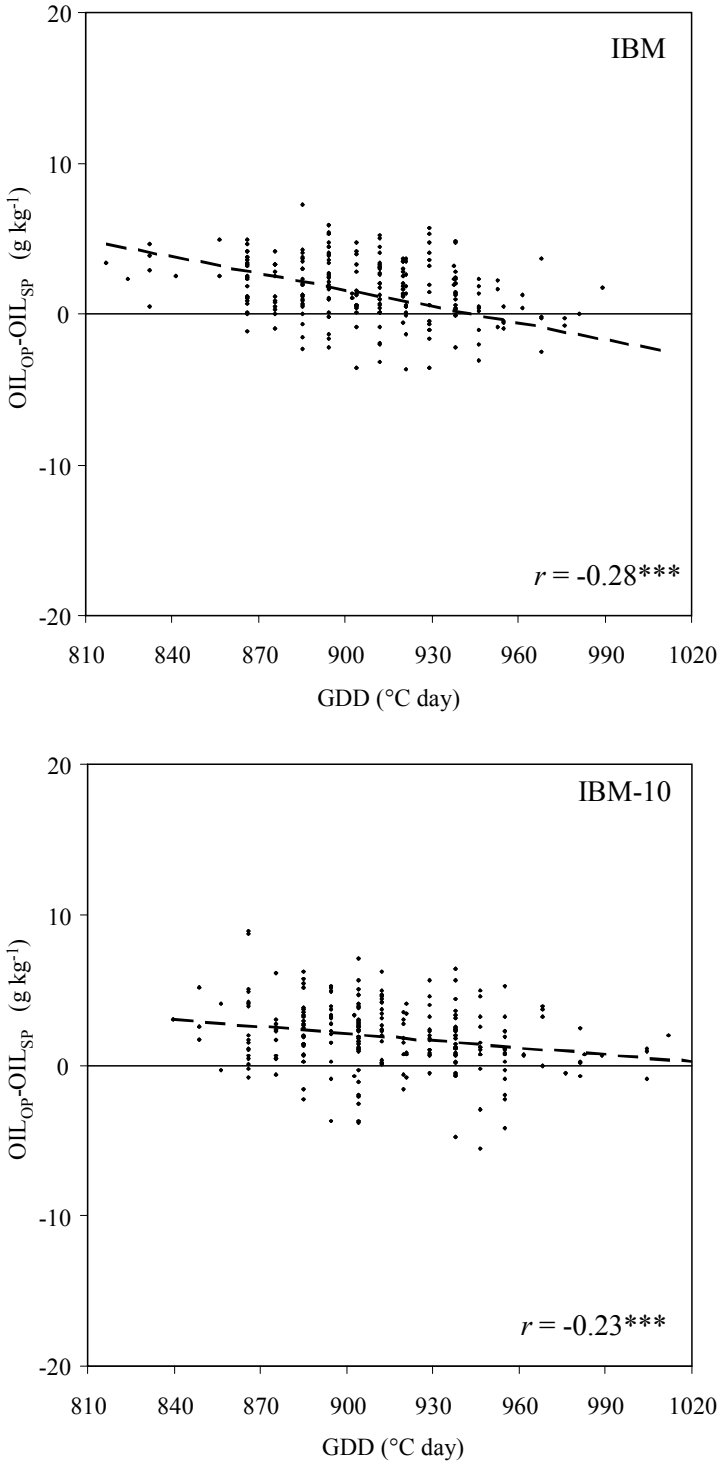

Figure 5. Difference in oil concentration between open and self-pollinated kernels (OIL_{OP}-OIL_{SP}) in relation to growing degree days to anthesis (GDD) for the IBM and IBM-10 populations. The dashed lines represent the trend line. *** indicates that the correlation (*r*) is significantly different from 0 at the 0.001 probability level.

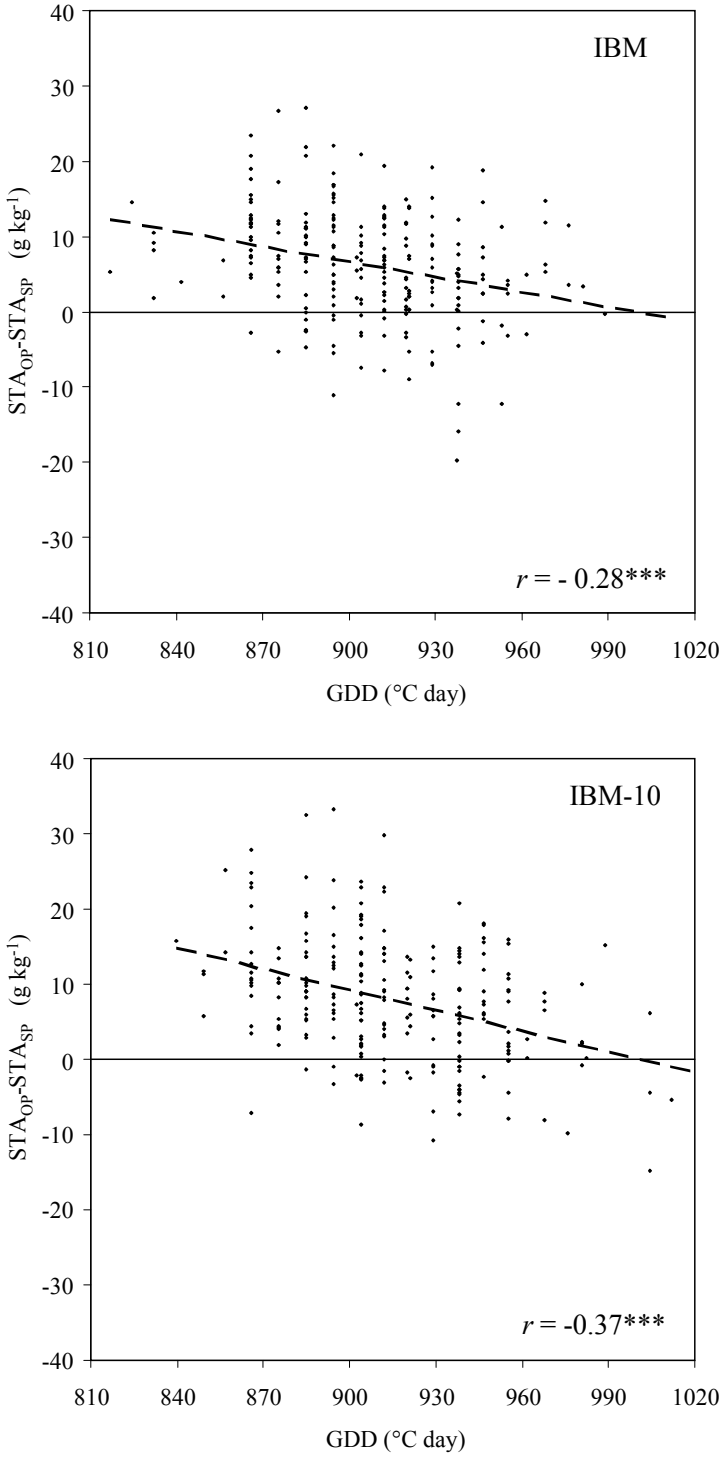

Figure 6. Difference in starch concentration between open and self-pollinated kernels (STA_{OP}-STA_{SP}) in relation to growing degree days to anthesis (GDD) for the IBM and IBM-10 populations. The dashed lines represent the trend line. *** indicates that the correlation (*r*) is significantly different from 0 at the 0.001 probability level.

Table 3. Estimates of genetic variance (σ^2_G) for protein, oil and starch concentration measured on open-pollinated (OP) and self-pollinated (SP) kernels in the IBM and IBM-10 populations, grown in 2006 in Ames, Iowa.

	SP			OP		
Population	σ^2_G	LL[§]	UL	σ^2_G	LL	UL
			Protein ($g^2\ kg^{-2}$)			
IBM	113	90	146	110	90	136
IBM-10	120	96	154	121	100	149
			Oil ($g^2\ kg^{-2}$)			
IBM	9.3 **	7.6	11.5	6.6	5.4	8.2
IBM-10	13.6 *	11.2	16.8	10.5	8.7	12.9
			Starch ($g^2\ kg^{-2}$)			
IBM	104 *	85	131	93	77	114
IBM-10	122 **	99	154	99	83	122

[§] LL and UL = lower limit and upper limit for the 95 % confidence interval.
*, ** indicates that the genetic variance is significantly higher in the self-pollinated kernel at the 0.05 and 0.01 probability levels, respectively.

CHAPTER 4. GENERAL CONCLUSIONS

Two sets of 244 lines, one each in the IBM and IBM-10 populations were characterized phenotypically for 16 traits. Means, genetic variances and phenotypic correlations were estimated for 14 quantitative traits in each population. IBM-10 had larger genetic variance for kernel oil concentration and number of kernels per ear row than IBM. The population means for plant height and number of ear rows were greater in the IBM-10 than in the IBM population. There was no evidence of differences in phenotypic correlations between the populations.

The differences in genetic variances and means detected between IBM and IBM-10 populations, and the segregation in the cob color among the double haploid lines of the IBM-10 population could reflect the increased recombination fraction observed between adjacent loci in IBM-10 relative to IBM. Several authors have recognized the positive effects of an increase in the frequency of recombination events on the identification of genomic regions associated with quantitative traits (Austin and Lee, 1996; Graham et al., 1997; Dudley et al., 2004). Specifically, increasing the number of recombinant individuals for a given chromosomal region allows the dissection of single QTLs into two or multiple linked QTLs (Austin and Lee, 1996; Graham et al., 1997). Moreover, the phenotypic characterization of the IBM-10 double haploid lines can be used for research purposes such as detection of QTL for the evaluated traits. Quantitative Traits Loci detected on this high resolution mapping population will facilitate the identification and selection of candidate sequences in the emerging physical map, contributing toward linking phenotype and genotype.

Open-pollinated (OP) and self-pollinated (SP) kernels differed in composition. Selection for kernel protein, oil and starch concentration based on open and self-pollinated values may result in different sets of selected genotypes.

The results obtained in this study suggest xenia effect on the oil fraction of the recombinant inbred lines of the IBM and IBM-10 populations. This conclusion is based on the larger mean and smaller genetic variance for oil concentration in the OP kernels than in the SP kernels; and in the larger difference between OP and SP kernel for the genotypes with low oil than in the genotypes with relatively high oil concentration. Based on the

observations made in these populations and in this environment, accurate oil concentration assessment should be obtained from SP kernels.

Higher protein concentration in SP kernels than in the OP kernels suggests that self-pollinated plants had less number of kernels and subsequently higher source-sink ratio than the open-pollinated plants, although the number of kernels per plant was not assessed in the present study. Further investigations regarding the differences in composition between OP and SP kernels should assess the number of kernels per plant in order to examine the actual difference in sink capacity between OP and SP plants. For each genotype, the open-pollinated plants should express the maximum sink capacity permitted by the environment; therefore OP ear should be used for the assessment of protein concentration. With respect to starch concentration, lower mean in the SP kernels than in the OP kernels suggests highest post-flowering source-sink ratio in the self-pollinated plants than in the open-pollinated plants, as was indicated for protein. However, the difference in starch concentration between OP and SP kernels in the genotypes with low oil concentration was smaller than in the genotypes with high oil concentration, suggesting that xenia also affected the starch fraction. Further studies are needed to examine the relative importance of the effects of xenia and post-flowering source-sink ratio on the kernel starch concentration.

APPENDIX A. DESCRIPTION OF FORMULAS USED

Statistical mixed model used for the estimation of genotypic variances in the IBM and IBM-10 population

$$Y_{ijkl} = \mu + \beta j + \alpha(\beta)_{ij} + \delta(\beta)_{kj} + \theta_l + \varepsilon_{ijkl}$$

where

μ = overall mean,

βj = fixed effect of the j^{th} replication

$\alpha(\beta)_{ij}$ = fixed effect of the i^{th} row within the j^{th} replication

$\delta(\beta)_{kj}$ = fixed effect of the k^{th} column within the j^{th} replication

θ_l = random effect of the l^{th} line

ε_{ijkl} = the experimental error

The genetic variances were estimated by the Restricted Maximum Likelihood method.

Hourly adjusted average system method for the estimation of growing degree days to anthesis (Cross and Zuber, 1972)

$$GDD = \sum_{i}^{n}{}_{1} \left(\left(\sum_{i}^{24}{}_{1} \; X_{ij}^{Hr'} - 8 \right) / 24 \right)$$

where

n = number of days the thermal units are accumulated.

X_{ij}^{Hr} = temperature for the j^{th} hour of the i^{th} day in °C.

$X_{ij}^{Hr'} = X_{ij}^{Hr}$ if $X_{ij}^{Hr} > 8$

$X_{ij}^{Hr'} = 8$ if $X_{ij}^{Hr} < 8$

Test for differences in genetic variances between populations: Levene's test

The Levene test was defined as:

H_0: $\sigma^2_{IBM} = \sigma^2_{IBM\text{-}10}$

H_A: $\sigma^2_{IBM} \neq \sigma^2_{IBM\text{-}10}$

The Test statistic (W) is estimated as follow

$$W = \frac{(N-k)}{(N-1)} \frac{\Sigma_{i=1}^{k} N_i (\bar{Z}_{i.} - \bar{Z}_{..})^2}{\Sigma_{i=1}^{k} \Sigma_{j=1}^{N_i} (Z_{ij} - \bar{Z}_{i.})^2}$$

where N is the sample size (sum of the number of lines of both populations), N_i is the sample size of the ith population, k is the number of populations being compared; Z_{ij} is the dispersion variable estimated as follow

Z_{ij} = | BLUP of the jth line of the ith population– mean BLUP of the ith population |

$Z_{i.}$ is the mean of the dispersion variable for the ith population, and $Z_{..}$ is the overall mean for the Z_{ij}.

The Levene test rejects the hypothesis that the variance of the populations are equal if

$$W > F_{(\alpha,\ k-1,\ N-k)}$$

T-test for differences between population means

$$t = \frac{diff_{IBM\text{-}10} - diff_{IBM}}{\sqrt{SE\ diff_{IBM\text{-}10}^{\ 2} + SE\ diff_{IBM}^{\ 2}}}$$

where

$diff_{IBM\text{-}10}$ = IBM-10 DHL mean – parental inbreds mean in IBM-10 experiment

$diff_{IBM}$ = IBM RIL mean – parental inbreds mean in IBM experiment

$SE\ diff_{IBM\text{-}10}$ = standard error for the estimate of $diff_{IBM\text{-}10}$

$SE\ diff_{IBM}$ = standard error for the estimate of $diff_{IBM}$

Degrees of freedom = $n_1 + n_2 - 2$,

where, n_1 and n_2 are the number of inbred lines evaluated in the IBM-10 and IBM population, respectively.

Test for differences between correlation coefficients

The significance of the difference between the correlation coefficients estimated in the IBM and IBM-10 population were tested according to the formula presented by Steel and Torrie (1980). Fisher's Z transformation is applied to the correlation coefficients (r) as follow

$$Z_r = 0.5 \ln [(1+r)/(1-r)]$$

where r is the correlation coefficient.

The test statistic (Z') is estimated as follow

$$Z' = \frac{Z_1 - Z_2}{\sqrt{[1/(n_1\text{-}3) + 1/(n_2\text{-}3)]}}$$

where
Z_1 and Z_2 represent the transformed correlation coefficients of the IBM-10 and IBM population, respectively; and n_1 and n_2 are the number of inbred lines evaluated in the IBM-10 and IBM population, respectively.

APPENDIX B. ADDITIONAL TABLES AND FIGURES

Table 1. Estimates of sample correlation between BLUP estimates of open-pollinated and self-pollinated values of kernel protein, oil and starch concentration in the IBM and IBM-10 populations.

Population	Protein	Oil	Starch
IBM	0.60***	0.76***	0.72***
IBM-10	0.66***	0.78***	0.72***

*** indicates that the correlation is significantly different from 0 at the 0.001 probability level.

Table 2. Phenotypic correlations[¶] (above diagonal) and correlation of BLUP estimates (below diagonal) between 14 traits measured in the IBM and IBM-10 populations grown in 2006 in Ames, Iowa.

		GDD	PH	TBN	BR	EL	ED	ER	EKR	CD	KD	KW	PRO	OIL	STA
GDD	IBM		0.45**	0.15*	0.14*	-0.04	-0.05	-0.06	-0.42**	-0.06	-0.07	0.32**	-0.11	0.20**	0.00
	IBM-10		0.34**	0.05	0.29**	-0.21**	-0.10	-0.11	-0.59**	-0.06	-0.10	0.34**	-0.04	0.02	0.05
PH	IBM	0.46**		0.17**	0.00	0.00	-0.01	-0.13*	-0.20**	-0.01	-0.01	0.28**	-0.17**	0.13*	0.07
	IBM-10	0.34**		0.00	-0.05	0.13*	0.10	0.08	-0.09	0.10	0.03	0.27**	-0.12	0.02	0.10
TBN	IBM	0.14*	0.15*		0.13*	-0.01	0.06	0.09	-0.09	-0.04	0.12	0.03	-0.09	-0.04	0.05
	IBM-10	0.05	-0.01		-0.02	0.00	0.06	-0.09	0.00	0.02	0.08	0.05	0.05	-0.05	-0.03
BR	IBM	0.15*	-0.01	0.12		-0.11	-0.01	-0.10	-0.19**	-0.07	0.08	0.13*	0.15*	0.08	-0.16*
	IBM-10	0.31**	-0.02	-0.01		-0.14*	-0.14*	-0.14*	-0.29**	-0.13*	-0.06	0.16*	0.10	0.03	-0.05
EL	IBM	-0.06	-0.04	-0.05	-0.09		0.01	-0.08	0.63**	0.18**	-0.24**	0.02	-0.09	-0.05	0.13*
	IBM-10	-0.21**	0.13*	0.01	-0.11		0.05	-0.08	0.62**	0.11	-0.06	0.18**	-0.14*	-0.07	0.16*
ED	IBM	-0.06	-0.02	0.09	0.00	0.01		0.64**	0.14*	0.76**	0.65**	0.25**	-0.10	-0.06	0.09
	IBM-10	-0.12	0.10	0.07	-0.16*	0.05		0.62**	0.15*	0.78**	0.64**	0.26**	-0.14*	0.07	0.06
ER	IBM	-0.08	-0.14**	0.10	-0.12	-0.07	0.63**		0.16*	0.56**	0.33**	-0.19**	-0.15*	0.10	0.04
	IBM-10	-0.12	0.07	-0.07	-0.15*	-0.08	0.62**		0.11	0.55**	0.33**	-0.26**	-0.18**	0.21**	0.02
EKR	IBM	-0.43**	-0.24	-0.09	-0.18**	0.65**	0.12	0.17**		0.18**	0.03	-0.41**	-0.07	0.01	0.06
	IBM-10	-0.59**	-0.09	0.00	-0.27**	0.62**	0.17**	0.10		0.12	0.11	-0.34**	-0.15*	0.03	0.09
CD	IBM	-0.05	-0.02	-0.03	-0.07	0.18**	0.75**	0.57**	0.14		0.03	0.12	-0.13*	-0.10	0.17**
	IBM-10	-0.06	0.11	0.01	-0.13*	0.11	0.77**	0.56**	0.13*		0.03	0.06	-0.06	-0.02	0.02
KD	IBM	-0.07	0.00	0.15*	0.06	-0.20**	0.65**	0.32**	0.03	0.01		0.25**	-0.03	0.03	-0.04
	IBM-10	-0.13*	0.03	0.11	-0.08	-0.04	0.64**	0.31**	0.12	0.02		0.30**	-0.15*	0.14*	0.07
KW	IBM	0.33**	0.31**	0.03	0.14*	0.05	0.28**	-0.20**	-0.39**	0.13*	0.28**		-0.05	-0.07	0.13*
	IBM-10	0.35**	0.29**	0.05	0.16*	0.18**	0.25**	-0.25**	-0.32**	0.06	0.30**		-0.06	-0.11	0.16*
PRO	IBM	-0.09	-0.14*	-0.11	0.11	-0.04	-0.08	-0.11	-0.01	-0.09	-0.03	-0.07		-0.15*	-0.80**
	IBM-10	-0.05	-0.12	0.03	0.10	-0.15*	-0.13*	-0.18**	-0.15*	-0.06	-0.15*	-0.05		-0.09	-0.82**
OIL	IBM	0.19**	0.13*	-0.02	0.07	-0.06	-0.07	0.09	-0.02	-0.08	-0.01	-0.05	-0.16		-0.38**
	IBM-10	0.04	0.02	-0.04	0.02	-0.08	0.05	0.18**	0.02	-0.01	0.10	-0.11	-0.10		-0.42**
STA	IBM	-0.02	0.03	0.06	-0.11	0.10	0.07	0.00	0.02	0.12	-0.02	0.13*	-0.80**	-0.37**	
	IBM-10	0.05	0.11	-0.02	-0.05	0.18**	0.06	0.03	0.10	0.03	0.07	0.15*	-0.82**	-0.41**	

[¶] Correlation between mean values of each inbred line over replications.

*, ** indicate that the phenotypic correlation is significantly different from 0 at the 0.05 and 0.01 probability levels, respectively.

Table 3. Phenotypic correlations[¶] (above diagonal) and genotypic correlations (below diagonal) between 14 traits measured in the IBM and IBM-10 populations grown in 2006 in Ames, Iowa.

		GDD	PH	TBN	BR	EL	ED	ER	EKR	CD	KD	KW	PRO	OIL	STA
GDD	IBM		0.45**	0.15*	0.14*	-0.04	-0.05	-0.06	-0.42**	-0.06	-0.07	0.32**	-0.11	0.20**	0.00
	IBM-10		0.34**	0.05	0.29**	-0.21**	-0.10	-0.11	-0.59**	-0.06	-0.10	0.34**	-0.04	0.02	0.05
PH	IBM	0.48		0.17**	0.00	0.00	-0.01	-0.13*	-0.20**	-0.01	-0.01	0.28**	-0.17**	0.13*	0.07
	IBM-10	0.36		0.00	-0.05	0.13*	0.10	0.08	-0.09	0.10	0.03	0.27**	-0.12	0.02	0.10
TBN	IBM	0.18	0.18		0.13*	-0.01	0.06	0.09	-0.09	-0.04	0.12	0.03	-0.09	-0.04	0.05
	IBM-10	0.06	0.00		-0.02	0.00	0.06	-0.09	0.00	0.02	0.08	0.05	0.05	-0.05	-0.03
BR	IBM	0.16	0.02	0.13		-0.11	-0.01	-0.10	-0.19**	-0.07	0.08	0.13*	0.15*	0.08	-0.16*
	IBM-10	0.32	-0.04	-0.01		-0.14*	-0.14*	-0.14*	-0.29**	-0.13*	-0.06	0.16*	0.10	0.03	-0.05
EL	IBM	-0.05	-0.02	-0.01	-0.13		0.01	-0.08	0.63**	0.18**	-0.24**	0.02	-0.09	-0.05	0.13*
	IBM-10	-0.22	0.14	0.00	-0.17		0.05	-0.08	0.62**	0.11	-0.06	0.18**	-0.14*	-0.07	0.16*
ED	IBM	-0.04	-0.02	0.08	-0.03	0.01		0.64**	0.14*	0.76**	0.65**	0.25**	-0.10	-0.06	0.09
	IBM-10	-0.10	0.10	0.08	-0.16	0.03		0.62**	0.15*	0.78**	0.64**	0.26**	-0.14*	0.07	0.06
ER	IBM	-0.07	-0.13	0.09	-0.12	-0.09	0.68		0.16*	0.56**	0.33**	-0.19**	-0.15*	0.10	0.04
	IBM-10	-0.12	0.07	-0.10	-0.15	-0.08	0.64		0.11	0.55**	0.33**	-0.26**	-0.18**	0.21**	0.02
EKR	IBM	-0.49	-0.22	-0.13	-0.20	0.63	0.20	0.18		0.18**	0.03	-0.41**	-0.07	0.01	0.06
	IBM-10	-0.64	-0.10	0.01	-0.33	0.63	0.14	0.12		0.12	0.11	-0.34**	-0.15*	0.03	0.09
CD	IBM	-0.05	-0.03	-0.03	-0.10	0.24	0.81	0.61	0.25		0.03	0.12	-0.13*	-0.10	0.17**
	IBM-10	-0.05	0.10	0.02	-0.15	0.11	0.83	0.59	0.13		0.03	0.06	-0.06	-0.02	0.02
KD	IBM	-0.03	0.02	0.15	0.11	-0.25	0.68	0.38	0.02	0.10		0.25**	-0.03	0.03	-0.04
	IBM-10	-0.11	0.03	0.11	-0.09	-0.09	0.67	0.36	0.07	0.14		0.30**	-0.15*	0.14*	0.07
KW	IBM	0.34	0.30	0.04	0.15	0.05	0.26	-0.20	-0.42	0.11	0.30		-0.05	-0.07	0.13*
	IBM-10	0.37	0.27	0.06	0.19	0.16	0.24	-0.29	-0.36	0.05	0.35		-0.06	-0.11	0.16*
PRO	IBM	-0.12	-0.20	-0.11	0.17	-0.11	-0.14	-0.18	-0.10	-0.16	-0.05	-0.09		-0.15*	-0.80**
	IBM-10	-0.05	-0.15	0.06	0.12	-0.17	-0.16	-0.20	-0.18	-0.08	-0.19	-0.07		-0.09	-0.82**
OIL	IBM	0.21	0.14	-0.05	0.09	-0.06	-0.06	0.13	0.01	-0.10	0.03	-0.06	-0.12		-0.38**
	IBM-10	0.02	0.03	-0.06	0.03	-0.08	0.08	0.24	0.03	-0.02	0.17	-0.11	-0.06		-0.42**
STA	IBM	0.00	0.08	0.07	-0.18	0.15	0.10	0.04	0.07	0.19	-0.04	0.16	-0.79	-0.43	
	IBM-10	0.06	0.12	-0.04	-0.06	0.19	0.07	0.01	0.10	0.02	0.09	0.18	-0.82	-0.47	

[¶] Correlation between mean values of each inbred line over replications.

*, ** indicate that the phenotypic correlation is significantly different from 0 at the 0.05 and 0.01 probability levels, respectively.

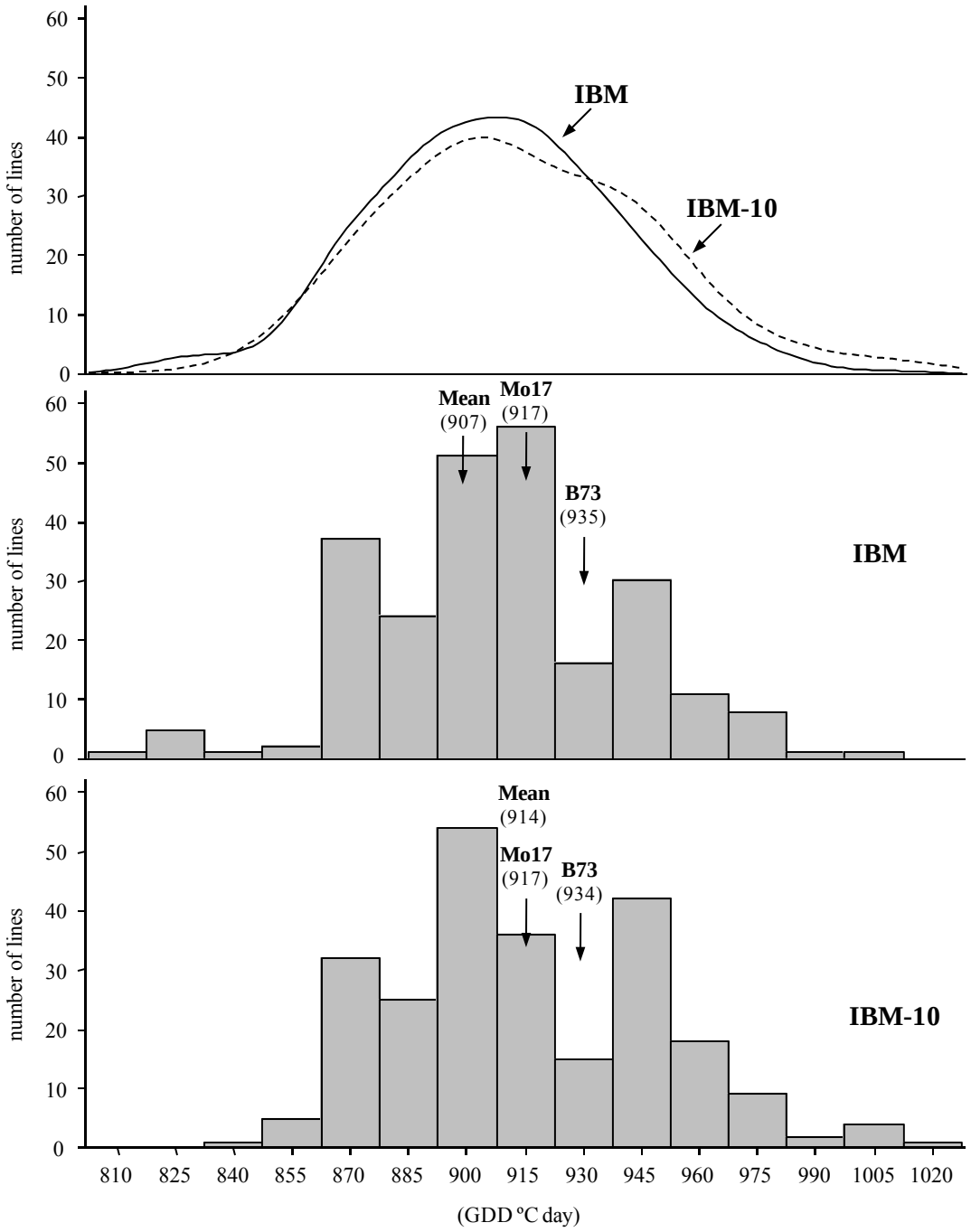

Figure 1. Histograms of growing degree days to 50 % of anthesis (GDD) in the IBM and IBM-10 populations, grown in 2006 in Ames, Iowa. Values in parenthesis under Mo17 and B73 are the mean of the respective parental inbred line over replications. The value in parenthesis under Mean is the population mean.

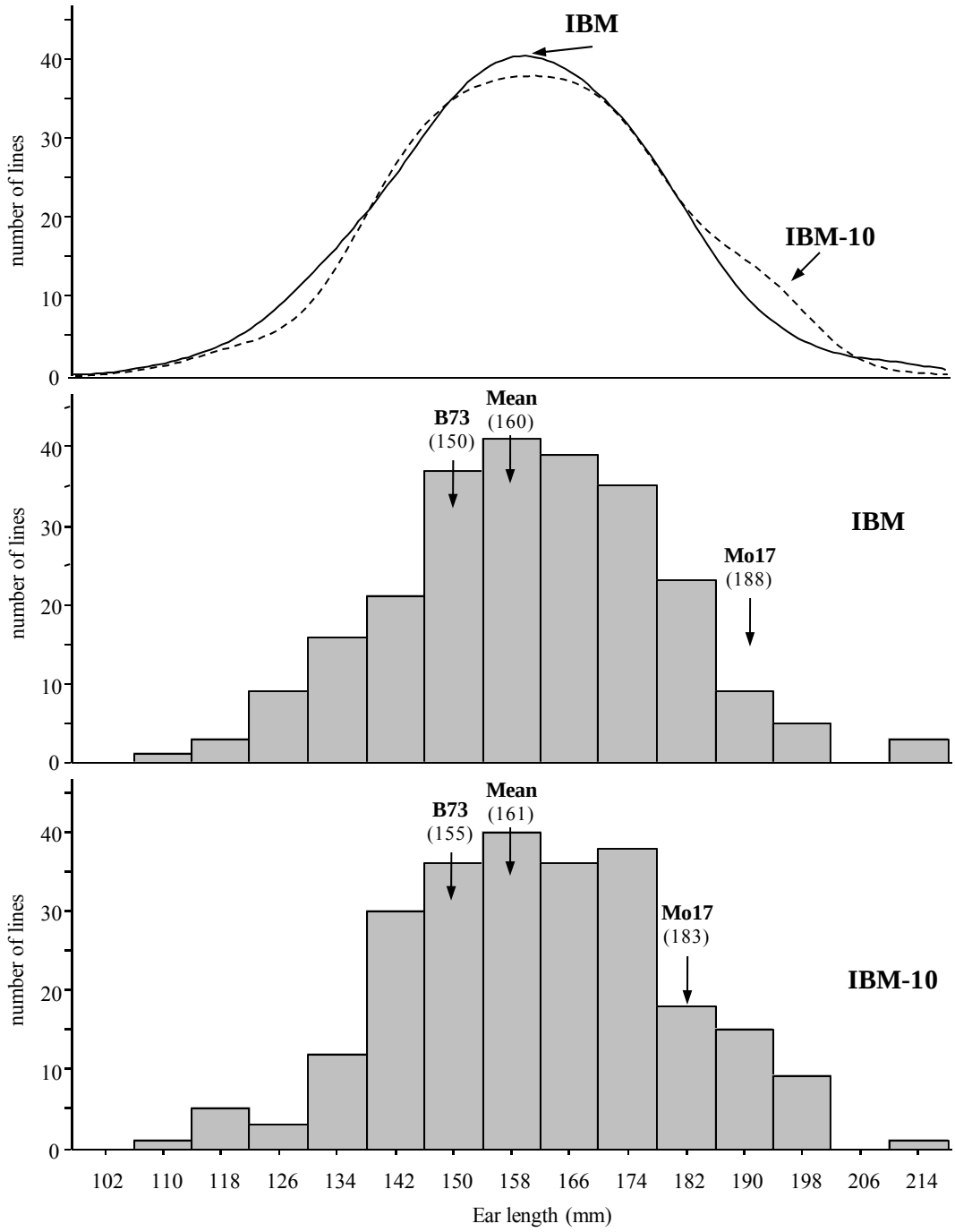

Figure 2. Histograms of ear length in the IBM and IBM-10 populations, grown in 2006 in Ames, Iowa. Values in parenthesis under Mo17 and B73 are the mean of the respective parental inbred line over replications. The value in parenthesis under Mean is the population mean.

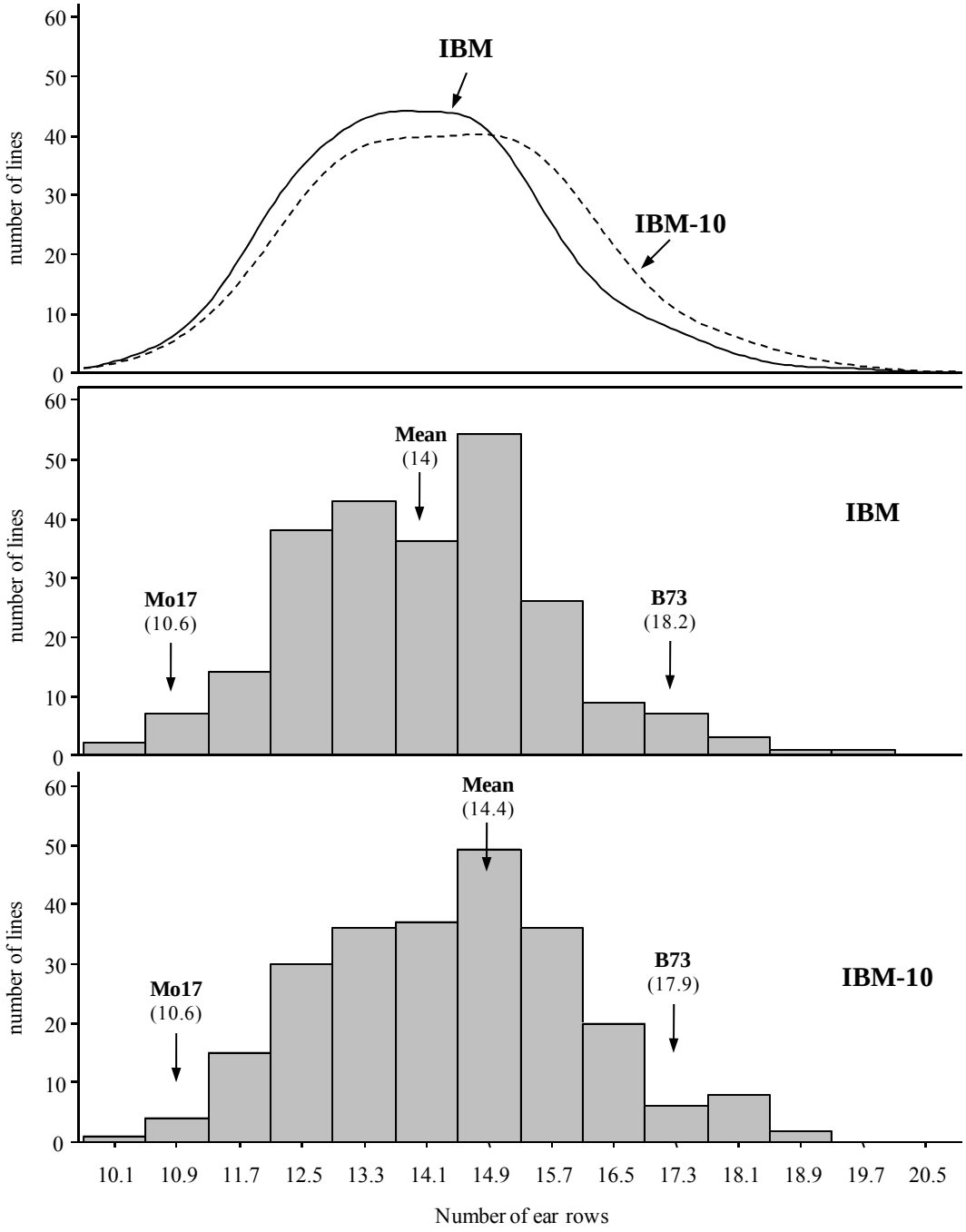

Figure 3. Histograms of number of ear rows in the IBM and IBM-10 populations, grown in 2006 in Ames, Iowa. Values in parenthesis under Mo17 and B73 are the mean of the respective parental inbred line over replications. The value in parenthesis under Mean is the population mean.

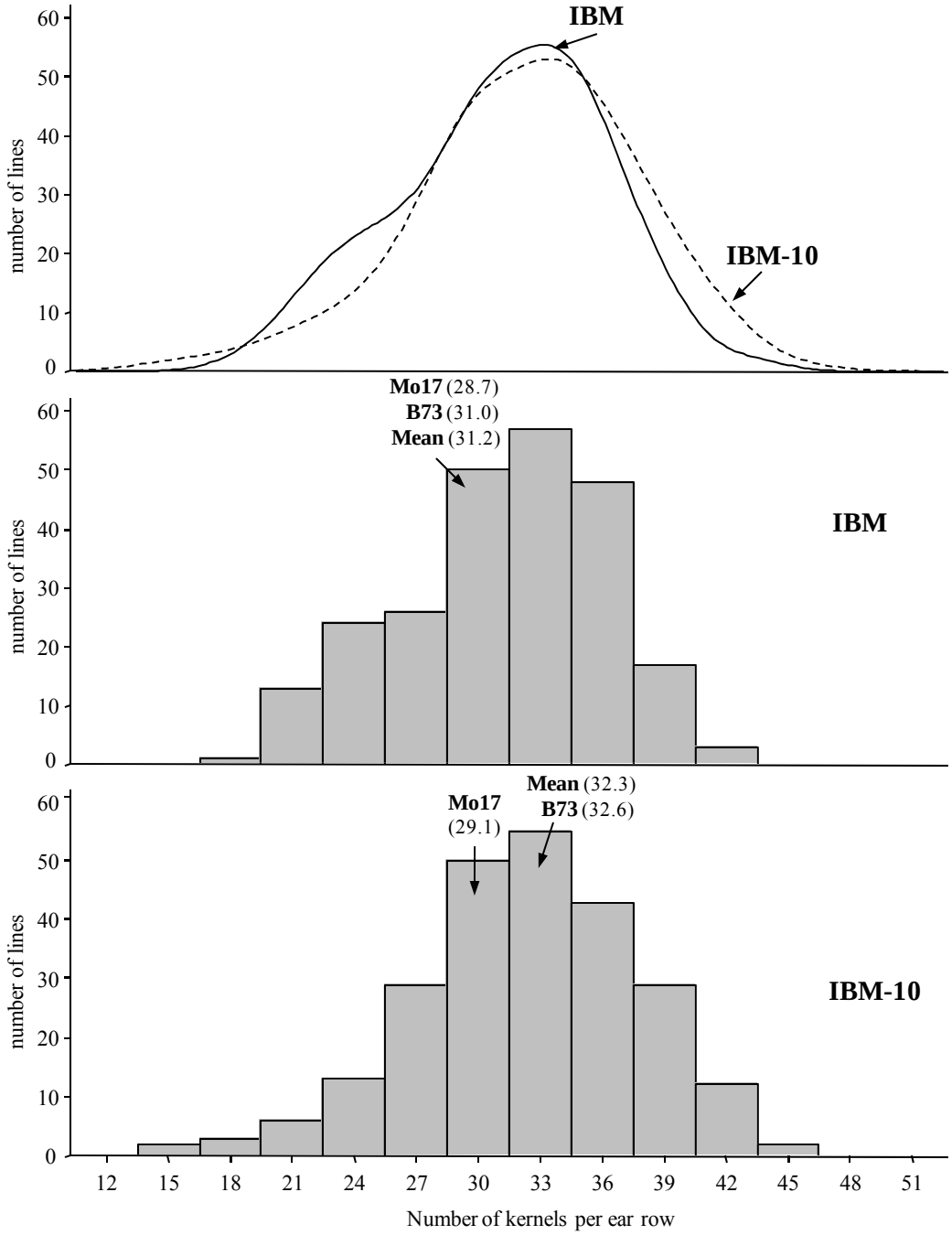

Figure 4. Histograms of number of kernels per ear row in the IBM and IBM-10 populations, grown in 2006 in Ames, Iowa. Values in parenthesis next to Mo17 and B73 are the mean of the respective parental inbred line over replications. The value in parenthesis next to Mean is the population mean.

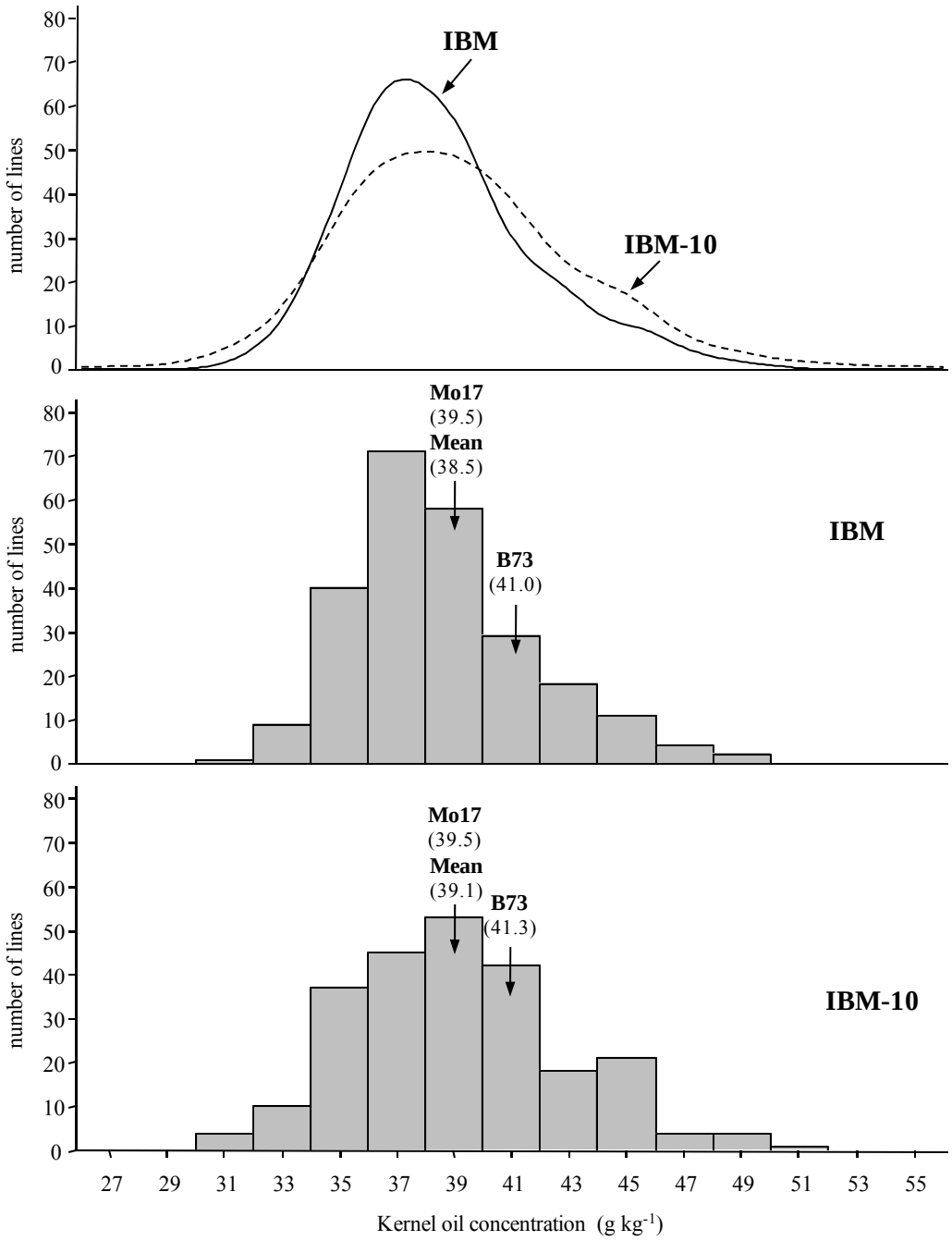

Figure 5. Histograms of kernel oil concentration in the IBM and IBM-10 populations, grown in 2006 in Ames, Iowa. Values in parenthesis under Mo17 and B73 are the mean of the respective parental inbred line over replications. The value in parenthesis under Mean is the population mean.

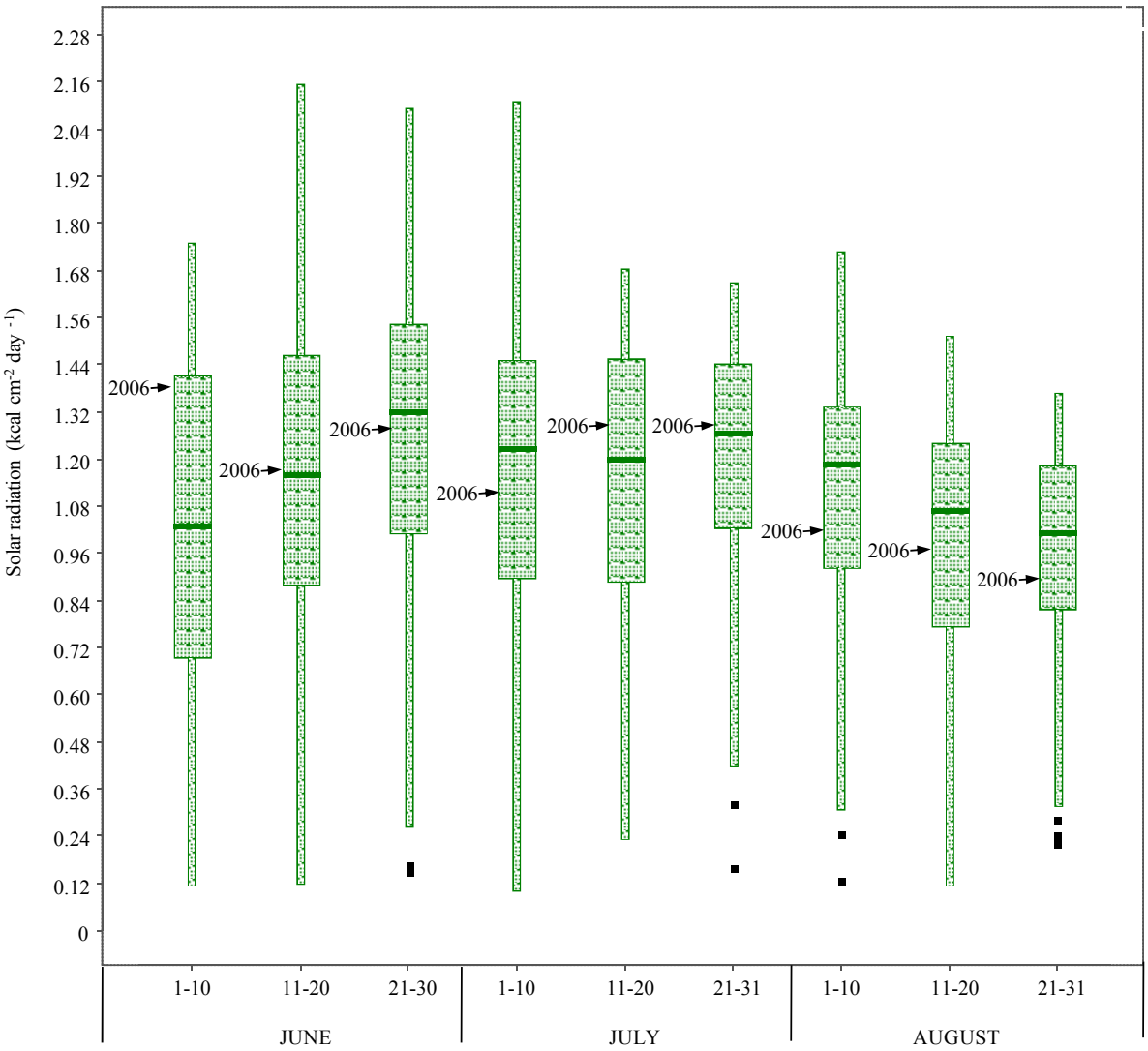

Figure 6. Box plots showing the distribution of daily solar radiation (kcal cm^{-2} day^{-1}) during June, July and August for the period 1986 – 2006. The arrow indicates daily mean solar radiation for 2006. The dots represent unusual values. Data was available on an hour mean basis. Data source: Iowa Environmental Mesonet (IEM) http://mesonet.agron.iastate.edu/index.phtml

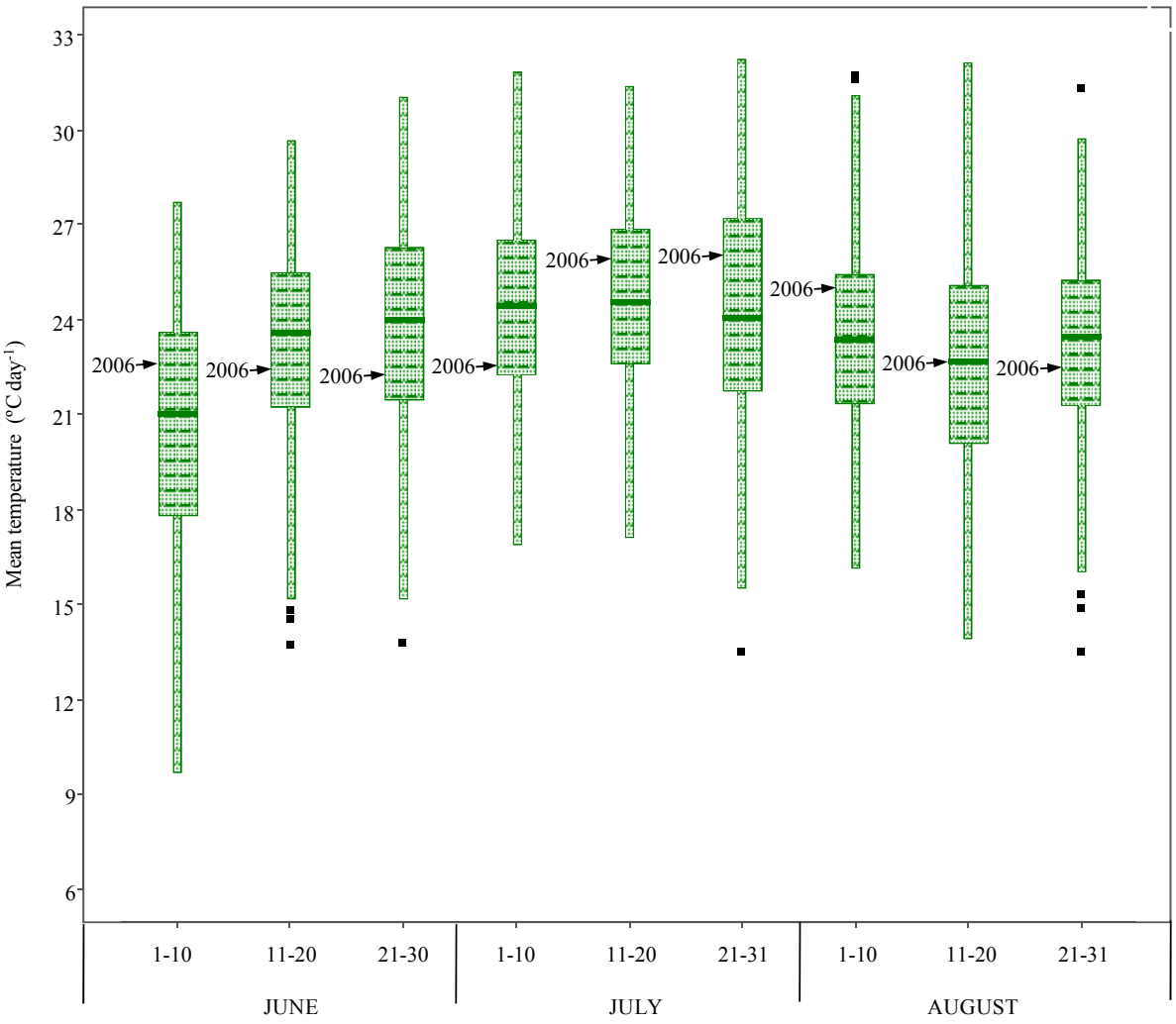

Figure 7. Box plots showing the distribution of daily mean temperature (ºC day^{-1}) during June, July and August for the period 1986 – 2006. The arrow indicates daily mean temperature for 2006. The dots represent unusual values. Data was available on an hour mean basis. Data source: Iowa Environmental Mesonet (IEM) http://mesonet.agron.iastate.edu/index.phtml

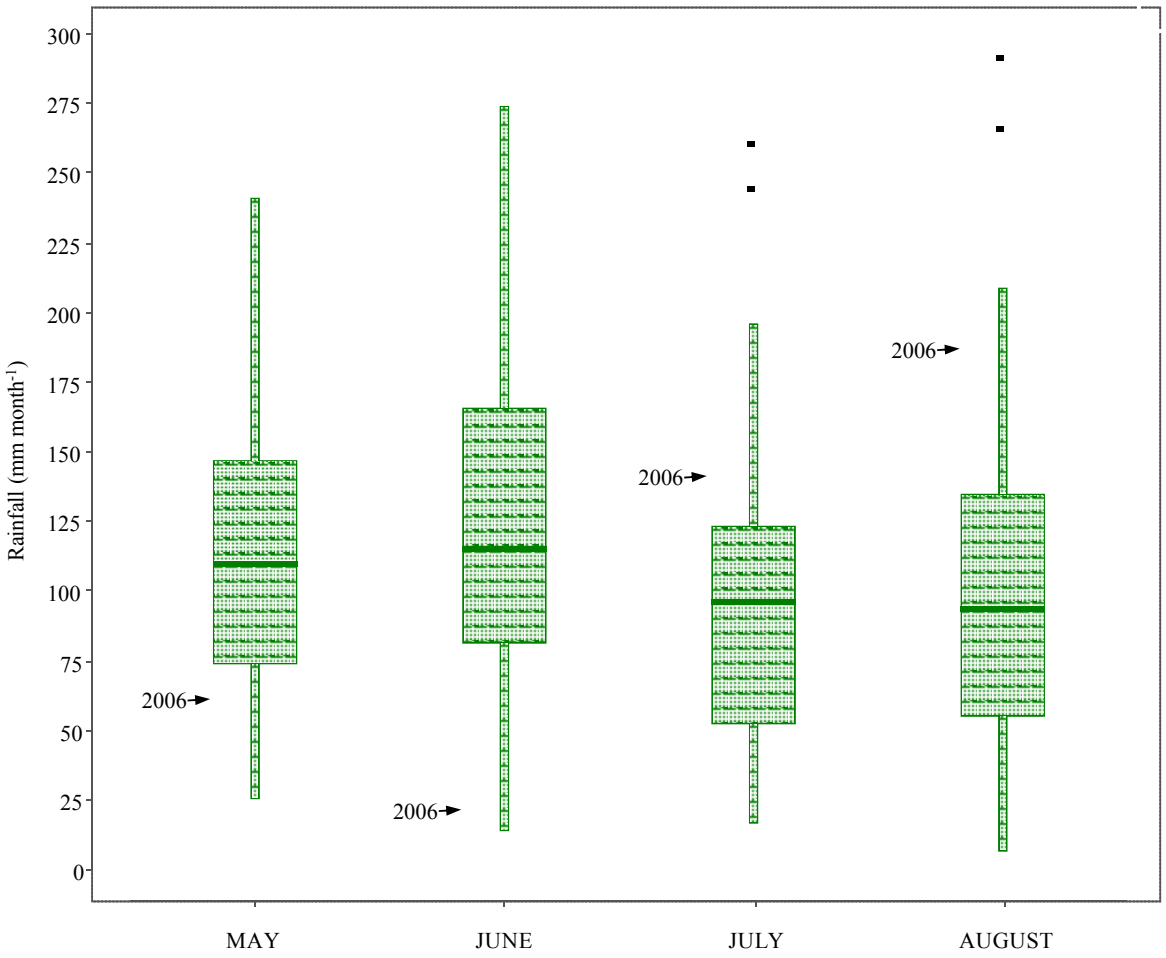

Figure 8. Box plots showing the distribution of rainfall (mm month^{-1}) of May, June, July and August for the period 1951 – 2006. The arrow indicates the monthly rainfall for 2006. The dots represent unusual values. Data was available on a monthly mean basis. Data source: Iowa Environmental Mesonet (IEM) http://mesonet.agron.iastate.edu/index.phtml