POPULATION GENETICS OF INCREASED HYBRID PERFORMANCE BETWEEN TWO MAIZE (*Zea mays* L.) POPULATIONS UNDER RECIPROCAL RECURRENT SELECTION

Joanne A. Labate,[†] Kendall R. Lamkey,*[†] Michael Lee[†] and Wendy L. Woodman[†]*
**USDA-ARS and [†]Department of Agronomy, Iowa State University, Ames, Iowa 50011*

Heterosis, the superiority in one or more characteristics of crossbred organisms relative to their inbred parents, is the basis of the modern cultivars utilized in maize. Heterosis is of interest in nondomesticated species due to its relevance to the question “how much polymorphism is maintained in natural populations due to selection?” (Berger, 1976). For maize and certain other domesticated species that employ inbred lines to produce commercial hybrids, knowledge of the mechanisms of gene action producing heterosis could contribute to advances in breeding techniques.

One method used to evaluate the existence of heterosis involves measuring multilocus heterozygosity levels in individuals sampled from a population with molecular genetic markers and correlating heterozygosity with a trait believed to reflect fitness, e.g., fecundity, viability, growth rate, or developmental stability. A vast number of these studies, many involving natural populations, have been published during the previous three decades. A general consensus is that a significant positive correlation between multilocus heterozygosity and fitness surrogates has been documented for a systematically wide range of organisms, although it is not a universal phenomenon (recently reviewed by Britten, 1996; Mitton, 1994; Zouros and Foltz, 1987).

The two genetic mechanisms most commonly invoked to explain heterosis are dominance and overdominance. The dominance hypothesis explains heterozygote superiority as a result of the masking of deleterious recessive alleles in an individual, whereas the hypothesis of overdominance postulates an advantage of heterozygosity *per se*, e.g., through differences in biochemical properties of homozygote versus heterozygote encoded single-locus products (Berger, 1976). Dominance cannot be distinguished in practice from “pseudooverdominance”, “associative overdominance”, or “dominance-correlation heterosis”. These are all synonyms of heterosis due to the joint action of genes associated in negative gametic phase disequilibrium. Some causes of gametic disequilibrium are directional selection, recombination suppression, inbreeding, or small effective population size (see Houle, 1989 for references).

Heterosis is relevant to the study of several subdisciplines within biology (e.g., plant and animal breeding, mating system evolution, developmental genetics); many good reviews differing slightly in their emphases have been published recently. Sedcole (1981) reviewed examples from plant breeding from approximately 1930 to 1980. Tsaftaris (1995) provided a review of recent molecular techniques used to study heterosis in plants, e.g., looking at RNA amount polymorphism (RAP), protein amount polymorphism (PAP), or DNA methylation levels. A review of heterosis as it relates to plant inbreeding depression can be found in Ritland (1996).

Berger (1976) reviewed theoretical mechanisms for the superiority of heterozygosity per se for protein polymorphisms; many of these have intuitive appeal. Presently, there are only a few well-documented instances of overdominance as a mechanistic explanation of heterosis in natural populations (see Mitton, 1994). The same can be said for domesticated species. One maize example is often cited: Schwartz (1973) found that active and stable heterodimers of alcohol dehydrogenase (Adh) are made up of two monomers, one of which is inactive (but stable) and the other of which is labile (but relatively active). The paucity of examples of single-locus heterosis may not be due to its infrequency, but may be because it is difficult to study, and overdominance is infrequently the sole supporting hypothesis.

Crow (1993, p. 15) recently reviewed genetic evidence that has led to the disfavor of the overdominance hypothesis in lieu of simple dominance as an explanation of heterosis in maize. Most importantly, researchers have found positive evidence for pseudooverdominance. This came from experiments in which hybrid maize populations were advanced several generations and recombination broke up linkages between favorable dominant and deleterious recessive alleles (e.g., Gardner and Lonnquist, 1959). Additional reasons for accepting the dominance hypothesis, according to Crow (1993), are a larger deleterious mutation load than originally generally believed (measured in a few species) and successful selection for relatively high yielding maize inbred lines (compared to early hybrids). The mutation load can explain the observed 15 to 20% grain yield increases observed in maize hybrids over their panmictic base populations, and high yielding inbred lines would not be possible if overdominance was the mechanism underlying high yield.

QUANTITATIVE GENETIC EVIDENCE FROM THE BSSS X BSCB1 RECIPROCAL RECURRENT SELECTION PROGRAM

In spite of the general acceptance of dominance as the explanation for heterosis in maize today, this was not true 50 years ago. Comstock et al. (1949) proposed a breeding method for maize that they termed ‘recurrent reciprocal selection’ (now known as reciprocal recurrent selection, RRS). Their motivation for developing the method was, as they stated, to discover a selection method that would be effective regardless of the level of dominant gene action. They proposed that RRS would be beneficial for instances in which overdominance, or situations analogous to overdominance (repulsion phase linkages), existed or when interactions of nonallelic genes (epistasis) were important; it would also exploit additive genetic effects. In theory, RRS is intended to improve the performance of an interpopulation cross of two genetically divergent populations. One ‘cycle’ of RRS involves development of genetic units within populations (e.g., S_1 lines, first-generation progenies from self-fertilized individuals), reciprocal crosses of genetic units between populations, phenotypic evaluation of these testcrosses, and selection of progenies based on testcross results. Selected progenies are then mated within each population. The next cycle of selection is initiated from these. RRS is designed to allow for genetic recombination within populations to maintain quantitative genetic variation, while minimizing inbreeding. The maintenance of two separate gene pools allows a different allele to be fixed within each population. For loci where this is achieved, interpopulation hybrids are assured to be heterozygous.

Two maize populations, Iowa Stiff Stalk Synthetic (BSSS) and Iowa Corn Borer Synthetic #1 (BSCB1), are currently in their 14th cycle of RRS in the Cooperative Federal-State maize breeding program at Iowa State University. Increased grain yield of the interpopulation cross has been the primary target of selection, with reduced grain moisture at harvest and increased resistance to root and stalk lodging as secondarily selected traits. Selection has been highly successful; mean grain yield of the interpopulation cross improved 77% by Cycle 11, relative to Cycle 0, with concurrent favorable responses in the other traits (Keeratinijakal and Lamkey, 1993a).

Midparent heterosis for BSSS(R) and BSCB1(R) was estimated as the difference between the mean of the interpopulation cross and the mean of the two parental populations. Inbreeding depression (the reduction in the mean value of a character produced by inbreeding) was measured for the interpopulation cross by selfing their F_1. Steady increases in heterosis and inbreeding depression for grain yield over 11 cycles were found (Keeratinijakal and Lamkey, 1993a). These were interpreted as resulting from an increase, over time, in heterozygosity of the interpopulation cross. Using Smith’s (1983) model Keeratinijakal and Lamkey (1993b) partitioned the genetic response to selection of BSSS(R) and BSCB1(R) into components due to additive and dominance effects and looked for evidence of overdominance. They found (partial to complete) dominance effects to be more important than additive effects in the interpopulation cross, with no evidence for overdominance. Diversity analysis (Moll and Hanson, 1984) of the two populations supported this interpretation. Directional dominance for grain yield and a difference in the frequencies of alleles affecting grain yield between the original populations were also inferred.

Similar results have been reported for other maize RRS programs. Eyherabide and Hallauer (1991a, 1991b) reported on reciprocal full-sib recurrent selection in the BS10 and BS11 populations. They found significant increases in midparent heterosis and inbreeding depression for grain yield in the interpopulation cross over eight cycles of selection. They also detected directional dominance and different frequencies of alleles with dominance effects for grain yield between the Cycle 0 populations. They suggested that selection had caused changes in frequencies of alleles with dominant effects in a different set of loci for each population or that different isoalleles with dominant effects had been selected in each population. Hanson and Moll (1986) also concluded that overdominant gene effects were not evident in the Jarvis and Indian Chief populations after 10 cycles of RRS; alleles having additive or dominant effects were selected.

MOLECULAR MARKER EVIDENCE FROM THE BSSS X BSCB1 RECIPROCAL RECURRENT SELECTION PROGRAM

We have genotyped samples from three populations within BSSS(R) and BSCB1(R), representing three different stages in their selective history (see Labate et al., 1997a for complete details). BSSS and BSCB1 synthetic populations trace back to 16 and 12 inbred lines, respectively. These collections of inbred lines are herein referred to as progenitor (P) populations. Cycle 0 populations were formed by several generations of random-mating bulked seed obtained from a series of crosses between progenitor inbred lines. These BSSS(R) and BSCB1(R) Cycle 0 populations were the starting material for RRS. Finally, we have genotypes from samples from both populations after twelve cycles of RRS (Cycle 12).

The molecular markers used were 82 nuclear genomic restriction fragment length polymorphism (RFLP) loci randomly distributed across all 20 chromosomal arms. The markers were assumed to be selectively neutral, i.e., the alleles at a locus would not differ measurably in their effects on the selected traits. The probes were chosen for their high levels of polymorphism and extensive coverage of the genome. One-hundred individuals from each Cycle 0 and Cycle 12 population were chosen at random for genotyping, as well as single individuals from each of 28 progenitor inbred lines (two of the BSSS progenitor inbred lines had been lost; however, the two parental lines of one of these were included). Each of the 82 RFLP probes was considered to be a single locus, and variants at each locus were assumed to be allelic.

Genetic Diversity

We found that mean gene diversity, expected heterozygosity under random-mating, was initially quite high within BSSS(R) and BSCB1(R). This can also be thought of as the probability of obtaining a heterozygote when two alleles are sampled at random from the population. This probability was around 60% in both progenitor populations. After 12 cycles of RRS, mean gene diversity had decreased to near 30% in each. Coinciding with this, the mean number of alleles per locus in BSSS(R) and BSCB1(R) dropped from about four to less than three. A further question was of interest. Looking at the *total* gene pool of BSSS(R) and BSCB1(R), what happened to genetic diversity over 12 cycles of RRS? If two alleles were sampled at random, one from each population, what would be the probability of obtaining a heterozygote? The increases in heterosis and inbreeding depression of the interpopulation cross seen in the quantitative genetic analyses suggested that the interpopulation cross was becoming more heterozygous. The pooled mean genetic diversity for the progenitor populations was estimated to be 63% and for the Cycle 12 populations, approximately 66%. The two estimates were not significantly different based on their standard errors.

Because of the assumption of selective neutrality of the RFLP markers, the lack of increase in interpopulation gene diversity was not completely unexpected. In fact, the estimated loss of mean genetic diversity *within* each population conformed to theoretical expectations (Nei, 1987, equation 13.12) of genetic drift of neutral alleles (i.e., random changes in allele frequency caused by gametic sampling each generation). We could see that, in the face of substantial loss of diversity within each population, the between population genetic diversity had remained high. Genetic diversity is a function of the numbers of alleles at a locus and allelic frequencies. This implied that, in general, different alleles had reached high frequencies in BSSS(R)C12 and BSCB1(R)C12.

Results from a principal components analysis (PCA) (Rohlf, 1994) of the 428 individuals sampled from BSSS(R) and BSCB1(R) populations are shown in Figure 1. Each point represents an individual separated in a 3-dimensional space based on the presence/absence of 391 alleles (genotypes for 82 loci) (unpublished data). The progenitor lines do not form two discrete clusters according to which population they formed. BSSS(R) and BSCB1(R) were initially nearly genetically identical. By Cycle 0, BSSS(R) and BSCB1(R) seem to be distinct from each other. In the absence of genetic drift and selection, the Cycle 0 populations should have remained clustered with the progenitors. We have inferred that maintenance for several decades of BSSS(R)C0 and BSCB1(R)C0 has altered their genetic constitutions. This was especially evident in BSSS(R), for which it seemed that many rare alleles present in P were not sampled in the modern representatives of Cycle 0 (Labate et al. 1997a, and unpublished data). By Cycle 12, BSSS(R) and BSCB1(R) were substantially diverged. The separation between the Cycle 0 and Cycle 12 populations include a component due to genetic drift, because a limited number of lines (10 - 20) were selected and recombined each cycle, and a component due to selection, because the recombined lines were not chosen at random.

So far, the results presented have focused on *mean* diversity, and genetic changes across *all* loci. By examining the data, it was clear that some of the loci had experienced extreme changes in allele frequencies over the course of selection. The pertinent question became, “Have any of the loci experienced allele frequency changes that were too large to be explained by genetic drift?”. Even though the markers fit a neutral model based on mean levels of gene diversity, this did not preclude that some of the allele frequency changes had been influenced by selection. This could have come about directly through selection or, more probably, through genetic hitchhiking. The hitchhiking effect is seen when selection at a locus changes the frequencies of neutral alleles at closely-linked loci and is conditioned on initial linkage disequilibrium between the loci.

Effective Population Size

Accurate knowledge of effective population size (N_e) is a key to discerning genetic changes brought about by drift from those that result from selection. Effective population size is defined as the number of individuals in an idealized (i.e., random mating) population that would undergo genetic drift at the same rate as the observed population. Under RRS, N_e is thought to be equal to the number of selected lines each cycle (Vencovsky, 1978). If all parents leave exactly the same number of offspring, N_e is expected to equal $2N - 1$ (Kimura, 1983, p. 41). When the number of selected lines has varied, N_e can be calculated as the harmonic mean of the number of selected lines over all cycles. Our empirical estimates based on the loss of mean genetic diversity between Cycle 0 and Cycle 12 supported an N_e

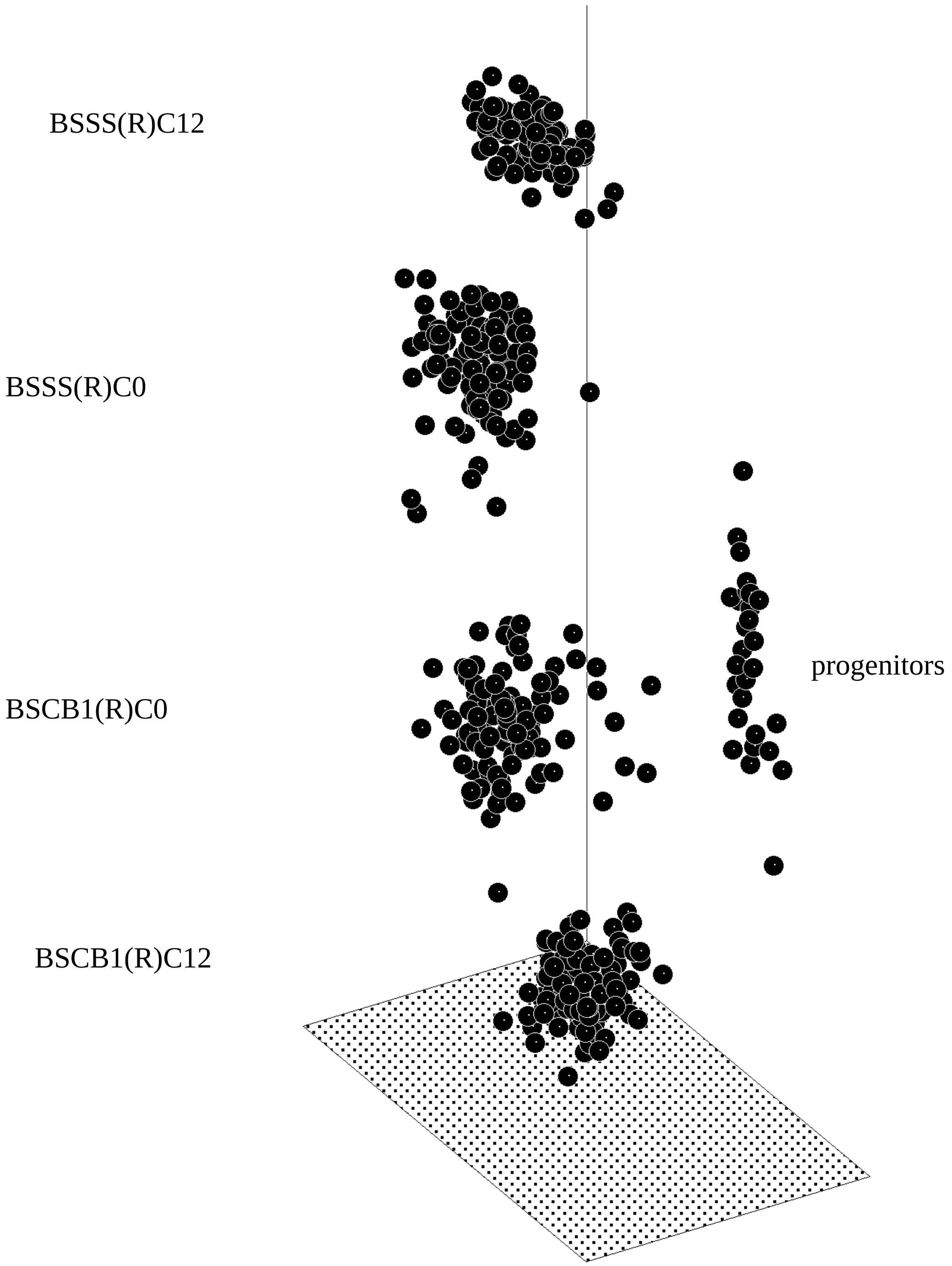

Figure 1. Principal components analysis of Iowa Stiff Stalk Synthetic (BSSS) and Iowa Corn Borer Synthetic #1 (BSCB1) based on genotypes of sampled individuals at 82 RFLP loci. The six sampled populations include progenitor inbred lines, populations before RRS (C0 populations), and populations after 12 cycles of RRS (C12 populations). Progenitor populations do not form two distinct groups.

equal to the harmonic mean of the number of selected lines, N_e = 12 (Labate et al., 1997a). A second method (Waples, 1989a), based on allele frequency changes across all loci, was used to estimate N_e for BSSS(R) and BSCB1(R) populations (Labate et al., 1997b). The two methods agreed; N_e is approximately the harmonic mean of the number of selected lines over all cycles. The 95% confidence intervals obtained for N_e using Waples' (1989a) method approached, but did not overlap with, ($2N$ -1).

Neutrality Tests

Given our estimates of N_e, we applied a test of selective neutrality (Waples, 1989b) to each of the 82 RFLP loci in BSSS(R) and BSCB1(R) populations. The null hypothesis was: the observed variation in allele frequency between two time points can be sufficiently explained as arising from the sampling of a population, of size N_e, that has undergone t generations of genetic drift.

We used estimated frequencies at time points Cycle 0 and Cycle 12 as initial and final allele frequencies, assumed N_e = 12 (or 23), and t = 12 generations (cycles). Because allele frequency changes at many of the loci between Cycle 0 and Cycle 12 were too large to be explained by genetic drift alone, we interpreted these changes as positive evidence for directional selection and/or genetic hitchhiking. The null hypothesis of drift was rejected for 11 and 17 loci in BSSS(R) and BSCB1(R), respectively, using Waples' test at a probability level of 5%. The loci were found on all chromosomes and were spread throughout the genome. These "nonneutral" loci fit a pattern of complementary genetic changes between the two populations. Only one was shared between BSSS(R) and BSCB1(R), and at that locus a different allele was reaching high frequency within each population.

The observed allele frequencies at the 27 loci are illustrated in Figure 2. Frequencies of nonneutral alleles are shown at Cycle 0 and Cycle 12 for both populations. Looking within a population at nonneutral alleles identified for that population, rejection of the null hypothesis was associated with an approximately 60% change in an allele's frequency.

We then estimated gene diversity of the interpopulation cross, comparing the 55 neutral loci to the 27 nonneutral loci (Labate et al., 1997b). The nonneutral loci increased in mean expected heterozygosity of the interpopulation cross between Cycle 0 (0.664 ± 0.0352) and Cycle 12 (0.776 ± 0.0537) whereas the 55 neutral loci did not (Cycle 0 = 0.603 ± 0.0243, Cycle 12 = 0.595 ± 0.0384). Comparing the two populations, the 11 nonneutral loci in BSSS(R) contributed to the increase in interpopulation heterozygosity more than the 17 nonneutral loci in BSCB1(R). A partial explanation for this can be found by studying Figure 2, parts c and d. Many of the 17 nonneutral BSCB1(R) alleles were at high frequencies in BSSS(R) at Cycle 0 and remained high in BSSS(R) at Cycle 12 (e.g., bnl835, bnl749, umc155). These loci underwent marked *decreases* in interpopulation expected heterozygosity.

One prediction under RRS is that if a favorable allele exists in both populations, selection will be more effective for that allele in the population within which it is more common (Cress, 1967). At about half of the nonneutral loci, the

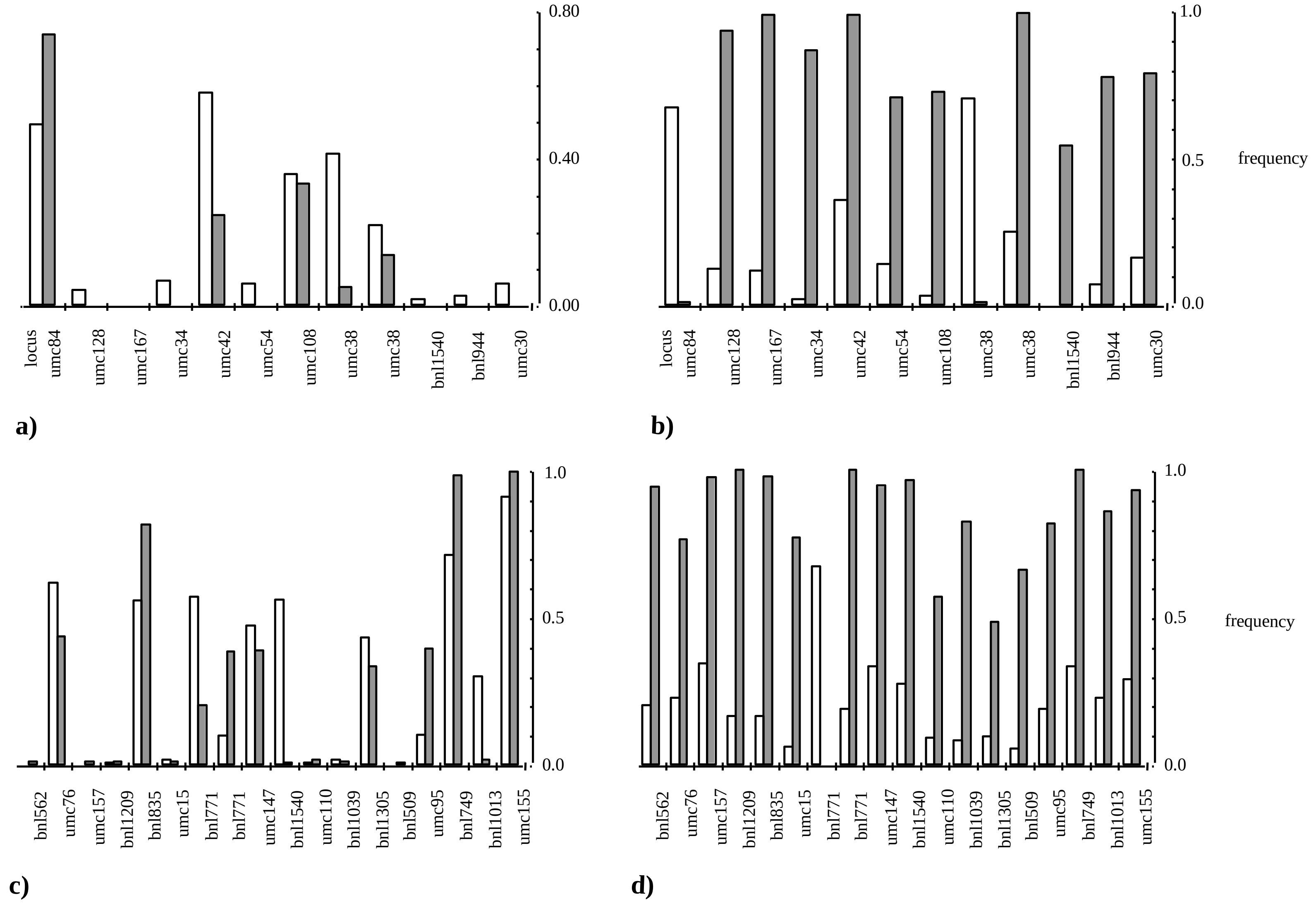

Figure 2. Allele frequencies at Cycle 0 (white bars) and Cycle 12 (filled bars) for 27 nonneutral loci identified in the BSSS(R) and BSCB1(R) populations. a) frequencies in BSCB1(R) for 11 nonneutral loci in BSSS(R), b) frequencies in BSSS(R) for 11 nonneutral loci in BSSS(R), c) frequencies in BSSS(R) for 17 nonneutral loci in BSCB1(R), d) frequencies in BSCB1(R) for 17 nonneutral loci in BSCB1(R).

"favored" allele was at an initial frequency of less than 10% in the reciprocal population and remained low. The other loci didn't conform to this predicted pattern (Fig. 2). Possible reasons for this are 1) at most loci, there were more than two alleles in BSSS(R) and BSCB1(R), so the dynamics of selection were not predicted by this simple model; 2) intralocus, complete dominance was not the genetic mechanism for increasing the selected allele; or 3) in the instance of genetic hitchhiking, interlocus correlation (two-locus disequilibrium) patterns were different within BSSS(R) and BSCB1(R).

CONCLUSIONS

Heterosis for grain yield in the interpopulation cross has increased in the BSSS(R) and BSCB1(R) RRS program, and the two populations have become quite genetically diverged from each other. The use of molecular markers has provided some insight into the roles of selection and genetic drift in BSSS(R) and BSCB1(R). Theoretical studies (Li, 1978) have shown that the absolute value of the selection coefficient for an allele must be greater than $1/N_e$ for selection to overcome genetic drift. This assumes a Wright-Fisher model of random genetic drift of neutral alleles (see Hartl and Clark, 1989, p. 351). The selection coefficient is the relative gametic contribution of a particular genotype compared with the most favored genotype in the population (Falconer and Mackay, 1996, p. 26). Our findings imply that a large fraction of loci in the maize genome, about 33% of those surveyed, were affected by selection. If $N_e = 12$ as estimated, then selection coefficients were at least 8%.

Although yield has not been the only agronomic trait selected, it has been emphasized. If yield is affected by many loci that are densely distributed throughout the genome and that carry large phenotypic effects, it is easy to understand why fixation of the most favored genotype in an inbred line derived from an improved population is difficult. Other population genetic studies where molecular markers were used also found that a large fraction of scored loci affected yield (Stuber et al., 1980; Stuber et al., 1992), although some studies (e.g., Brown and Allard, 1971; Kahler, 1983) have found that genetic drift could explain observed allele frequency changes. The earlier studies used allozyme loci; DNA-based markers are much more informative in maize.

Stuber et al. (1992), using 67 RFLP loci and nine isozyme loci, genotyped sets of lines descending from a cross originating between two maize inbred lines. When they regressed mean trait value on percent heterozygous marker loci, they found a high correlation between grain yield and proportion of heterozygous markers. A large fraction of the genome was found to affect yield (markers significantly associated with yield were found on all 10 chromosomes), even though this experimental design was limited to detecting regions polymorphic between the two original inbreds.

Reciprocally selected populations should continue to provide a suitable experimental system within which to study relationships between multilocus heterozygosity and phenotype. In this genetic system recombination is prohibited at the interpopulation level, allowing fixation of balanced intralocus or interlocus gene action in the interpopulation cross. Testing theories of gene action requires estimation of parameters such as mutation rates, selection pressure, recombination distances, and inbreeding coefficients (Zouros and Foltz, 1987). It should be possible to obtain more accurate measures of these parameters in maize selection programs than in natural populations.

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