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Allozyme frequencies, heterozygosity and genetic distances following S_1 recurrent selection in two synthetic maize populations

Abstract Three cycles of S_1 recurrent selection for yield were carried out in two synthetic maize populations, EPS6 from humid Spain and EPS7 from arid Spain. One-hundred S_1 lines were evaluated from each cycle of selection and the ten highest-yielding S_1 lines were recombined to produce the next cycle. Changes in variability and genetic distances in two synthetic maize populations, following three cycles of recurrent selection, recombining ten S_1 lines in each cycle, were determined. Isozyme analysis was performed on 125 seedlings per cycle of selection (four cycles in each of two populations). Regressions of each allozyme frequency on cycles of selection were performed, genetic distances between populations were determined, and simple correlations between genetic distances and heterosis were calculated. The average heterozygosity per locus was also calculated for each population. Regression analysis did not reveal any common trend between EPS6 and EPS7 for changes in allele frequencies presumably due to selection. The number of polymorphic loci, the mean alleles per locus, and the mean heterozygosity did not show any reduction in variability. Finally, selection did not affect genetic distances among cycles of selection. The agronomic evaluation of the selection program, after three cycles of selection, revealed that the genetic variance was not significantly reduced for most traits, and that the heterosis among cycles of selection of both populations had not changed significantly. The conclusions based on isozyme data supported the deductions made from agronomic data. Three cycles of selection neither caused relevant changes on variability nor on genetic distance among cycles of selection of both maize synthetic populations. These data did not indicate any basis for increasing the number of S_1 lines recombined for recurrent selection.

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Introduction

Maize breeders often employ recurrent selection methods based on selfed (S_1 or S_2) lines (Hallauer et al. 1988). The main limitation of this method is that variability is significantly reduced, and inbreeding depression increased, after a few cycles of selection, particularly when a low number of selfed lines (for example, ten S_1 lines) are used to produce the next cycle of selection (Hallauer and Sears 1973; Smith 1984; Helms et al. 1989; Benson and Hallauer 1994). The recombination of ten S_1 lines may cause a founder effect, e.g. a significant reduction on variability and random changes of allele frequencies.

Recurrent selection based on S_1 lines has been carried out on two synthetic maize populations, EPS6 from humid Spain and EPS7 from arid Spain, that represent the base of a new heterotic pattern (Ordás 1991). One-hundred S_1 lines were evaluated from each cycle of selection and the ten highest-yielding S_1 lines were recombined to produce the next cycle.

Agronomic evaluation, after three cycles of selection, revealed that the genetic variance was significantly reduced for yield but was still different from zero after three cycles of selection in both populations. The heterosis for yield among cycles of selection of both populations did not change significantly. No evidence of inbreeding depression was detected (Vales 1996).

Isozymes are useful tools to study the genetic structure of populations (Richardson et al. 1990; Hattemer 1991). However, the assumption of all genotypes being selectively equivalent, required in these kind of studies, is not always fulfilled. Stuber and Moll (1972), Stuber et al. (1980), Kahler (1983), and Pollak et al. (1984) found significant associations between allozyme

frequencies and agronomic traits in selection programs. These associations were not consistent over different germplasms (Pollak et al. 1984).

Alterations of allozyme frequencies due to selection would cause biases in the estimation of allele frequencies, as well as a reduction of neutral polymorphism and expected heterozygosity (Kaplan et al. 1989; Stephan et al. 1992). There is experimental evidence showing that allozyme biochemical function differs (Watt 1994) and this departure from neutrality may cause some bias in the estimation of changes in genetic parameters. Nevertheless, Skibinski et al. (1993) found that a major percentage of protein variation can be explained by variation in neutral mutation rate and a minor percentage by strong selection.

The recombination of few individuals, e.g. ten S$_1$ lines, during the recurrent selection program carried out in EPS6 and EPS7, may have resulted in reduced variability and changed genetic distances due to random drift. These effects may be assessed using isozymes. Reductions on variability would decrease future gains. Additionally, the synthetics EPS6 and EPS7 form the base of a new heterotic pattern that is being improved by reciprocal recurrent selection. Intrapopulation selection might have altered genetic distances between both synthetics. Changes in genetic distances would restrict heterosis and future gains throughout the subsequent interpopulation selection.

The objectives of the present work were to study the changes in variability and genetic distances in two synthetic maize populations, following three cycles of recurrent selection, recombining ten S$_1$ lines in each cycle.

Materials and methods

Plant material

Two synthetic maize populations, EPS6 from humid Spain and EPS7 from arid Spain, were improved for yield using three cycles of S$_1$ recurrent selection. These synthetics are the base of a new heterotic pattern (Ordás 1991). One-hundred S$_1$ lines were evaluated and the ten lines with the highest yield were recombined to start the next cycle of selection. The populations resulting from each cycle of selection were randomly mated a second time.

The eight populations used for the isozyme study were the original synthetics (EPS6 C0 and EPS7 C0) and the three subsequent cycles of selection (EPS6 C1 and EPS7 C1, EPS6 C2 and EPS7 C2, and EPS6 C3 and EPS7 C3). A third recombination was made preceding the agronomic evaluation (Vales 1996) and the isozyme study.

The first recombination was part of the selection program, it was made among the ten S$_1$ lines selected for yield; 15 plants per S$_1$ line were planted, five plants from each S$_1$ line were used as males, pollen of the 50 male plants was mixed and used to pollinate five different plants of each S$_1$ line. Thus 100 plants were recombined at random from each cycle to produce the next cycle. For the second and third recombinations, at least 50 plants from each cycle were used as males and each male was used once to pollinate one female plant; thus at least 100 plants were randomly recombined. Therefore, the selected populations are assumed to be at Hardy-Weinberg equilib-

rium at each locus. Also, intermating is expected to counteract the linkage disequilibrium presumably accumulated with the selection process.

Isozyme techniques

At least 150 seeds from each cycle of selection were germinated on wet filter paper in a dark chamber at 25°C. For isozyme analysis we used 125 seedlings per population to avoid problems due to small sample sizes (Lessios 1992). Extraction methods, electrophoretic techniques and scoring procedures were performed according to Stuber et al. (1988), with minor modifications (Revilla and Tracy 1995). Each plant was analyzed for 11 enzyme systems encoded by 20 loci (*Acp1, Adh1, Cat3, Dia1, Dia2, Enp1, Glu1, Got1, Got2, Got3, Idh1, Idh2, Mdh1, Mdh2, Mdh3, Mdh4, Mdh5, Pgm1, Pgm2* and *Phi1*). Banding patterns were used to calculate the frequencies of genotypes and allozymes. Alleles 6 and 7 of the *Glu1* locus were scored as one unique allele to avoid misclassification. Finally, null alleles were not considered and, therefore, some over-estimation of homozygous and under-estimation of heterozygosity may have occurred if null alleles were present.

Statistical analysis

Linear regressions of each allozyme frequency on cycles of selection were calculated to determine if there were changes in allozyme frequencies due to selection. Linear regression is known to over-estimate trends. However, if there are no common trend in both populations and only weak trends are found, we may assume that isozyme variation cannot be explained by selection. Genetic distances between populations were determined (Nei 1972) and simple correlations between genetic distances and heterosis were calculated. Average heterozygosity per locus was estimated for each population (Nei 1978). The unbiased estimator proposed by Nei (1978) was used for every calculation because the number of individuals changed for different loci due to missing data. We wanted to quantify the potential consequences of the founder effect on allozyme frequencies. A reduction in the number and frequency of some alleles would cause irrecoverable reductions of heterozygosity. However, other effects may be confounded with the founder effect. Heterozygous deficiency would reflect deviations from ideal conditions due to effects such as small sample size and non-random mating. Observed and expected heterozygotes were compared by χ^2 tests (Curie-Cohen 1982; Lessios 1992). Computations were performed using the computer packages NTSYS-PC (Rohlf 1989) and SAS (1989).

Results and discussion

Over the four cycles of the synthetic populations EPS6 and EPS7, 15 (75%) and 12 (60%) of the 20 loci had more than one allele, respectively (Table 1). The proportion of polymorphic loci, neglecting the alleles with a frequency lower than 0.05, was 55% for EPS6 C1, 50% for EPS6 C0, EPS7 C0, EPS6 C2 and EPS6 C3, 40% for EPS7 C1 and EPS7 C3, and 25% for EPS7 C2. Thirty nine alleles were found across the 20 loci (1.95 alleles per locus) scored for the 11 isozyme systems. Disregarding the alleles with a frequency lower than 0.05, the number of alleles per locus was 1.70 for EPS6 C1 and EPS6 C2, 1.65 for EPS6 C3, 1.60 for EPS6 C0 and EPS7 C0, 1.45 for EPS7 C1, 1.40 for EPS7 C3, and 1.25 for EPS7 C2. The variability found in these two

Table 1 Frequency of allozymes at 16 loci for each selection cycle in two maize populations. The b values associated with the frequency changes over selection cycles in each population are included

Enzyme system	Locus (chromosome)	Allozyme	EPS6					EPS7				
			C0	C1	C2	C3	b	C0	C1	C2	C3	b
Acid phosphatase	*Acp1* (9S)	2	0.31	0.14	0.17	0.31	0.00	0.58	0.76	0.70	0.60	0.00
		4	0.69	0.86	0.83	0.69	0.00	0.42	0.24	0.30	0.40	0.00
Alcohol dehydrogenase	*Adh1* (1L)	4	0.82	0.80	0.55	0.48	− 0.13	0.93	0.99	1.00	1.00	0.02
		6	0.18	0.20	0.45	0.52	0.13	0.08	0.01	0.00	0.00	− 0.02
Catalase	*Cat3* (?)	7	0.35	0.22	0.18	0.22	− 0.04	0.17	0.27	0.02	0.06	− 0.04
		9	0.57	0.63	0.56	0.68	0.02	0.83	0.73	0.98	0.94	− 0.06
		12	0.08	0.16	0.26	0.11	0.02	0.00	0.00	0.00	0.00	0.06
Endopeptidase	*Enp1* (6L)	6	0.66	0.43	0.54	0.45	− 0.05	0.16	0.02	0.02	0.12	− 0.03
		8	0.04	0.07	0.08	0.17	0.04	0.10	0.00	0.00	0.00	− 0.01
		12	0.30	0.47	0.38	0.38	0.01	0.74	0.98	0.98	0.86	− 0.03
β-Glucosidase	*Glu1* (10L)	2	0.54	0.29	0.20	0.35	− 0.07	0.44	0.69	0.61	0.86	0.12
		3	0.08	0.18	0.17	0.03	− 0.02	0.03	0.00	0.00	0.00	− 0.01
		6	0.38	0.53	0.64	0.61	0.08	0.54	0.29	0.39	0.14	− 0.11
Glutamate oxalacetate transaminase	*Got1* (3L)	4	1.00	0.98	1.00	1.00	0.00	1.00	1.00	1.00	0.99	− 0.01
		6	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	Got2 (5L)	2	0.00	0.06	0.00	0.03	0.00	0.08	0.05	0.10	0.09	0.01
		4	1.00	0.94	1.00	0.97	0.00	0.92	0.96	0.90	0.91	0.00
Isocitrate dehydrogenase	*Idh1* (8L)	4	0.91	0.87	0.84	0.97	0.02	1.00	1.00	1.00	1.00	0.00
		6	0.09	0.13	0.16	0.03	− 0.01	0.00	0.00	0.00	0.00	0.00
	Idh2 (6L)	4	0.79	0.84	0.57	0.85	− 0.01	0.96	0.85	0.98	0.97	0.01
		6	0.21	0.16	0.43	0.15	0.01	0.04	0.15	0.02	0.04	− 0.01
Malate dehydrogenase	*Mdh1* (8)	1	0.01	0.00	0.00	0.12	0.03	0.50	0.55	0.48	0.46	− 0.02
		6	0.99	1.00	1.00	0.88	− 0.03	0.50	0.45	0.52	0.54	0.02
	Mdh2 (6L)	3	0.41	0.45	0.30	0.33	− 0.04	0.15	0.25	0.39	0.17	0.02
		3.5	0.01	0.04	0.23	0.24	0.09	0.06	0.12	0.01	0.00	− 0.03
		6	0.58	0.51	0.48	0.43	− 0.05*	0.79	0.64	0.60	0.83	0.01
	Mdh4 (1L)	12	0.97	1.00	1.00	1.00	0.01	1.00	1.00	1.00	1.00	0.00
		14.5	0.03	0.00	0.00	0.00	− 0.01	0.00	0.00	0.00	0.00	0.00
	Mdh5 (5S)	12	0.70	0.62	0.65	0.73	0.01	1.00	1.00	1.00	1.00	0.00
		15	0.30	0.38	0.35	0.27	− 0.01	0.00	0.00	0.00	0.00	0.00
Phosphoglucomutase	*Pgm2* (5S)	3	0.01	0.14	0.10	0.13	0.03	0.10	0.26	0.05	0.27	0.00
		4	0.99	0.86	0.90	0.87	− 0.03	0.90	0.74	0.95	0.73	0.03
Phosphohexose isomerase	*Phi1* (1L)	4	0.93	0.97	1.00	1.00	0.03	0.87	0.93	0.98	1.00	− 0.04
		5	0.07	0.03	0.00	0.00	− 0.03	0.13	0.07	0.02	0.00	0.04

* Significant at $P = 0.05$

synthetics is lower than in the races Corn Belt Dent (Smith 1986), Northern Flint or Southern Dent (Doebley et al. 1988), but is higher than the variability found in many single populations (Reedy et al. 1995; Revilla and Tracy 1995).

The first hypothesis to be considered is that the changes observed in allozyme frequencies could be due to selection. This was tested by the linear regression of allozyme frequencies on cycles of selection. The only significant regression was for the allele *Mdh2-6* in EPS6, and for the alleles *Phi1-5* and *Cat3-7* in EPS7. No common trend was observed between EPS6 and EPS7 for the changes in allele frequency presumably due to selection. The frequency of the allele *Adh1-4* decreases in EPS6 and increases in EPS7; the frequency of the allele *Adh1-6* increases in EPS6 and decreases in

EPS7; and the frequency of the allele *Glu1-6* increases in EPS6 and decreases in EPS7. Considering that linear regression over-estimates the amount and significance of the trends and that most trends were weak and non-significant, we may conclude that a major proportion of allozyme variation is not related to selection.

Many authors have found changes in the allozyme frequencies that are associated with selection for yield or other agronomic traits. Stuber and Moll (1972) reported a high correlation between *Acp1* and yield. Pollak et al. (1984) also found associations between *Acp1* and yield, maturity and leaf variables, as well as *Got1*, *Prx1* (Peroxidase-1) and *Adh1*. Kahler (1983) observed changes in *Adh1*, *Mdh2* and *Acp1* related to yield selection. Stuber et al. (1980) found associations

between *Acp1*, *Glu1*, *Pgm1* and *Mdh1* and yield. These four studies were related with selection programs for yield and the only common locus among them was *Acp1*, which did not significantly change in our study.

Given that two generations of random mating with more than 100 plants per generation and population were performed, differences between the observed and the expected frequencies of heterozygotes would reflect effects such as natural selection, small sample size, scoring mistakes, mutation or migration (contamination). The χ^2 tests for differences between the observed and expected heterozygotes were significant for 13 of the 134 tests performed (data not shown). Adjusting the significance levels for multiple tests with the standard Bonferroni technique of dividing the pre-determined significance level, α, by the number of tests, the number of significant χ^2 tests is severely reduced depending on the criteria used to fix the minimum number of expected heterozygotes. Lessios (1992) discusses several rules proposed in the literature for testing electrophoretic data. In the most restrictive of these, the minimum number of expected heterozygotes should be 20. Following this last criterion 51 of the χ^2 tests may be taken into account and, given the tabulated value $\chi^2_{0.05/51(1)} \cong 10.828$, only the test for *Enp1*-6-12 was significant.

Variability is expected to decrease due to random drift because of the recombination of only ten of the S_1 lines. The data already summarized did not show a reduction in variability along successive cycles of selection. This observation was supported by the mean heterozygosity which was H = 0.19646 ($\sigma^2 = 0.04850$) for EPS6 C0, H = 0.21277 ($\sigma^2 = 0.05152$) for EPS6 C1, H = 0.22530 ($\sigma^2 = 0.06431$) for EPS6 C2, H = 0.22055 ($\sigma^2 = 0.05827$) for EPS6 C3, H = 0.16579 ($\sigma^2 = 0.03783$) for EPS7 C0, H = 0.15685 ($\sigma^2 = 0.03996$) for EPS7 C1, H = 0.11609 ($\sigma^2 = 0.03553$) for EPS7 C2, and H = 0.12320 ($\sigma^2 = 0.02958$) for EPS7 C3. No significant differences between pairs of mean heterozygosities were found. Thus, no reduction of heterozygosity was detected even though only ten S_1 lines were recombined in each cycle of selection. Accordingly, the agronomic data showed that the genetic variance of non-selected traits was not significantly reduced after three cycles of selection for yield (Vales 1996). The theoretically expected reduction of variability following selection and the recombination of only a few individuals (Hallauer and Sears 1973; Smith 1984; Helms et al. 1989; Benson and Hallauer 1994) was not found.

This conclusion, inferred from isozyme data, confirms the lack of inbreeding depression revealed by agronomic data (Vales 1996) and is in agreement with several reports where inbreeding depression was not detected after several cycles of selection using selfed lines (Helms et al. 1989; Lamkey 1992; Holthaus and Lamkey 1995).

Random drift may affect the frequency of rare alleles. For example, in the EPS6 population, the *Mdh1-1* allele, with a frequency $P = 0.004$ (SE = 0.004) in EPS6 C0, was not found in EPS6 C1 and EPS6 C2, but had a frequency $P = 0.120$ (SE = 0.021) in EPS6 C3. The allele *Phi1-5* had $P = 0.074$ (SE = 0.017) in EPS6 C0, $P = 0.028$ (SE = 0.012) in EPS6 C1 and was not found in the subsequent cycles. The allele *Got2-2* was present in EPS6 C1 ($P = 0.058$, SE = 0.013) and EPS6 C3 ($P = 0.03$, SE = 0.009). Finally, the allele *Got1-6* was present in EPS6 C1 ($P = 0.017$, SE = 0.007). Given that the probability, L_s, that a sample of size N fails to include an allele with a population frequency P is $L_s = (1 - P)^{2N}$ under ideal conditions (Bengtsson et al. 1995), and that $N = 10$ in the selection program, the probability of losing an allele in a cycle of selection by random drift would be $L_s = 0.923$ when $P = 0.004$, and $L_s = 0.567$ when $P = 0.028$. These probabilities would satisfactorily explain why the rare alleles *Mdh1-1* and *Phi1-5*, respectively, disappeared in subsequent cycles due to random drift. On the other hand, those alleles that did not appear in earlier cycles but did appear in later cycles (such as *Mdh1-1* in EPS6 C3, *Got2-2* and *Got1-6* in EPS6 C1) must be at very low frequencies in the earlier cycles because $N > 100$ in most of the samples used for isozyme scoring, and, assuming that their frequencies were similar in the earlier cycles, the probability of missing the alleles with $N = 100$ would be $L_s = 7.8 \times 10^{-12}$ when $P = 0.120$, $L_s = 6.5 \times 10^{-6}$ when $P = 0.058$, and $L_s = 0.032$ when $P = 0.017$, respectively. Therefore, random drift changed the frequency of rare alleles without having meaningful effects on allozyme variability.

Finally, we wanted to check if the non-significant changes in heterosis between cycles EPS6 and EPS7 were related to allozyme-based genetic distances. The correlation between genetic distances and heterosis among cycles EPS6 and EPS7 was low ($r = 0.1392$) and not significant. This low correlation is not surprising because the genetic distances do not significantly correlate with heterosis except when crosses among very close and very distant genotypes are included (Frei et al. 1986). Selection for yield did not affect genetic distances among cycles of selection. Changes in genetic distance or heterosis between cycles of selection due to random drift during the intrapopulation selection should not restrict the improvement of the EPS6 × EPS7 heterotic pattern.

In conclusion, no significant changes in variability and genetic distance were found following S_1 recurrent selection, even though as few as ten S_1 lines were recombined from each cycle. Considering that increasing the number of S_1 lines selected and recombined would reduce gains and increase costs, and that the ten S_1 lines employed has neither reduced variability nor genetic distance between the two maize synthetics, there is clearly no rationale for increasing the number of S_1 lines recombined.

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