P. Revilla · M.I. Vales · R.A. Malvar · A. Ordás

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

---

P. Revilla (✉)· M.I. Vales · R.A. Malvar · A. Ordás

Misión Biológica de Galicia, CSIC, Apartado 28, 36080 Pontevedra, Spain.

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 were 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 among genetic distances and heterosis were calculated. Average heterozygosity per locus was also calculated for each population. The 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 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 increasing the number of S_1 lines recombined for recurrent selection.

Key words *Zea mays* · Selection · Allozymes · Heterozygosity · Genetic distance

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. 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 are 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 yielder S_1 lines were recombined to produce the next cycle.

The agronomic evaluation, after three cycles of selection, revealed that the genetic variance was significantly reduced for yield but it 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 (Hattemer 1991; Richardson et al. 1990). 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, 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 reduced variability and changed genetic distances due to random drift. These effects may be assessed using isozymes. Reductions on variability would decrease future gains. Besides, the synthetics EPS6 and EPS7 are 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 on genetic distances would restrict heterosis and future gains throughout the posterior interpopulation selection.

The objectives of this 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, 5 plants from each S_1 line were used as males, pollen of the 50 male plants was mixed and used to pollinate 5 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 equilibrium at each locus. Also, intermating is expected to counteract the linkage disequilibrium presumably accumulated with the selection process.

Isozyme techniques

At least 150 seeds of each cycle of selection were germinated on wet filter paper in a dark chamber at 25ºC. 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 small 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 frequencies of genotypes and allozymes. The alleles 6 and 7 of the locus *Glu1* were scored as one unique allele to avoid misclassifications. Finally, the null alleles were not considered and, therefore, some overestimation of homozygous and underestimation 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 on allozyme frequencies due to selection. Linear regression is known to overestimate trends. However, if there are no common trends 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 among 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 on number and frequencies 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

Fifteen (75%) and 12 (60%) of the 20 loci had more than one allele over the four cycles of the synthetic populations EPS6 and EPS7, 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 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 tested is that the changes observed in allozyme frequencies could be due to selection. This hypothesis was tested by 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 frequencies 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 overestimates the amount and significance of the trends and that most trends were weak and no significant, we may conclude that a major proportion of allozyme variation is not related with selection.

Many authors have found changes on the allozyme frequencies that are associated with selection for yield or other agronomic traits. Stuber and Moll (1972) found 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) found changes on *Adh1*, *Mdh2* and *Acp1* related to yield selection. Stuber et al. (1980) found associations between *Acp1*, *Glu1*, *Pgm1* and *Mdh1* and yield. Those four studies were related with selection programs for yield and the only coincidence among them was on *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 observed and expected heterozygous were significant for 13 of the 134 χ^2 test performed (not shown). Adjusting the significant levels for multiple tests with the standard Bonferroni technique of dividing the predetermined significant 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. In the most restrictive suggestion, the minimum number of expected heterozygous should be 20. Following this last criterion 51 χ^2 tests may be taken into account and, given that the tabulated value $\chi^2_{.05/51(1)} \cong 10.828$, only the χ^2 test for *Enp*1-6-12 was significant.

Variability is expected to decrease due to random drift because of the recombination of ten S_1 lines. The data summarized before did not show a reduction in

variability along successive cycles of selection. This observation was supported by mean heterozygosity that was H = 0.19646 (σ^2 = 0.04850) for EPS6 C0, H = 0.21277 (σ^2 = 0.05152) for EPS6 C1, H = 0.22530 (σ^2 = 0.06431) for EPS6 C2, H = 0.22055 (σ^2 = 0.05827) for EPS6 C3, H = 0.16579 (σ^2 = 0.03783) for EPS7 C0, H = 0.15685 (σ^2 = 0.03996) for EPS7 C1, H = 0.11609 (σ^2 = 0.03553) for EPS7 C2, and H = 0.12320 (σ^2 = 0.02958) for EPS7 C3. No significant differences between pairs of mean heterozygosities were found. Thus, no reduction of heterezygosity 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 recombination of 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 it is in agreement with some reports in where inbreeding depression was not detected after several cycles of selection using selfed lines (Helms et al. 1989; Holthaus and Lamkey 1995; Lamkey 1992).

Random drift may affect frequencies of rare alleles. For example, in the population EPS6, the allele *Mdh1-1*, with a frequency p = 0.004 (SE = 0.004) in EPS6 C0, was not found in EPS6 C1 and EPS6 C2 but it 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 posterior 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 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 previous cycles but did appear in later cycles (like *Mdh1-1* in EPS6 C3, *Got2-2* and *Got1-6* in EPS6 C1) must be at very low frequencies in the previous cycles because the sample size was $N > 100$ in most of the samples used for isozyme scoring, and then, assuming that their frequencies were similar in the previous 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 nonsignificant changes in heterosis between cycles of EPS6 and EPS7 were related to allozyme-based genetic distances. The correlation between genetic distances and heterosis among cycles of 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 on genetic distance or heterosis between cycles of selection due to random drift during the intrapopulation selection should not restrict the improvement of the heterotic pattern EPS6 x EPS7.

As a conclusion, no significant changes on 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 use ten S_1 lines has neither reduced variability nor genetic distance between the two maize synthetics, there is no reason for increasing the number of S_1 lines recombined.

Acknowledgments Research supported by the Committee for Science and Technology of Spain (project cod. AGF95-0891-C02-01) and Excma. Diputación Provincial de Pontevedra, Spain. M.I.Vales acknowledges a fellowship from the Excma. Diputación Provincial de Pontevedra.

References

Bengtsson BO, Weibull P, Ghatnekar L (1995) The loss of alleles by sampling: A study of the common outbreeding grass *Festuca ovina* over three geographic scales. Hereditas 122:221-238

Benson DL, Hallauer AR (1994) Inbreeding depression rates in maize populations before and after recurrent selection. J of Hered 85:122-128

Curie-Cohen M (1982) Estimates of inbreeding in a natural population: A comparison of sampling properties. Genetics 100:339-358

Doebley J, Wendel JD, Smith JSC, Stuber CW, Goodman MM (1988) The origin of Cornbelt maize: the isozyme evidence. Econ Bot 42:120-131

Frei OM, Stuber CW, Goodman MM (1986) Uses of allozymes as genetic markers for predicting performance in maize single cross hybrids. Crop Sci 26:37-42

Hallauer AR, Russell WA, Lamkey KR (1988) Corn breeding. In: Sprague GF, Dudley JW (eds) Corn and corn improvement. Agron Monogr 18, 3rd ed ASA, CSSA, and SSSA Madison, WI, pp 463-564

Hallauer AR, Sears J (1973) Changes in quantitative traits associated with inbreeding in a synthetic variety of maize. Crop Sci 13:327-330

Hattemer HM (1991) Genetic analysis and population genetics. In: Fineschi, S, ME Malvoti F Cannata, HH Hattemer (eds) Biochemical markers in the population genetics of forest trees SPB Academic Publishing bv, The Hague, The Netherlands, pp 5-22

Helms TC, Hallauer AR, Smith OS (1989) Genetic drift and selection evaluated from recurrent selection programs in maize. Crop Sci 29:602-607

Holthaus JF, Lamkey KR (1995) Population means and genetic variances in selected and unselected Iowa Stiff Stalk Synthetic maize populations. Crop Sci 35:1581-1589

Kahler AL (1983) Effect of half-sib and S_1 recurrent selection for increased grain yield on allozyme polymorphisms in maize (*Zea mays* L). Crop Sci 23:572-576

Kaplan NL, Hudson RR, Langley CH (1989) The hitchhiking effect revisited Genetics. 123:887-899

Lamkey KR (1992) Fifty years of recurrent selection in the Iowa Stiff Stalk Synthetic maize population. Maydica 37: 19-28

Lessios HA (1992) Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Mar Biol 112:517-523

Nei M (1972) Genetic distance between populations. Am Nat 106:283-292

Nei M (1978) Estimates of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590

Ordás A (1991) Heterosis in crosses between American and Spanish populations of maize. Crop Sci 31:931-935

Pollak LM, Gardner CO, Parkhurst AM (1984) Relationships between enzyme marker loci and morphological traits in two mass selected maize populations. Crop Sci 24:174-179

Reedy ME, Knapp AD, Lamkey KR (1995) Isozyme allelic frequency changes following maize (*Zea mays* L) germplasm regeneration. Maydica 40:269-273

Revilla P, Tracy WF (1995) Isozyme variation and phylogenetic relationships among open-pollinated sweet corn cultivars. Crop Sci 35:219-227

Richardson BJ, Baverstock PR, Adams M (1990) Allozyme electrophoresis A handbook for animal systematics and population studies. Academic Press Inc. San Diego, CA, USA

Rohlf FJ (1989) NTSYS-PC numerical taxonomy and multivariate analysis system. Version 1.5 Exeter Publ, Setauket, NY, USA

SAS Institute Inc (1989) SAS/STAT User's Guide. Version 609 Cary, NC, USA

Skibinski DOF, Woodwark M, Ward RD (1993) A quantitative test of the neutral theory using pooled allozyme data. Genetics 135:233-248

Smith JSC (1984) Genetic variability within US hybrid maize: Multivariate analysis of allozyme data. Crop Sci 24:1041-1046

Smith JSC (1986) Genetic diversity within the Corn Belt Dent racial complex of maize (*Zea mays* L). Maydica 31: 349-367

Stephan W, Wiehe THE, Lenz MW (1992) The effect of strongly selected substitutions on neutral polymorphism: analytical results based on diffusion theory. Theor Popul Biol 41:237-254

Stuber CW, Moll RH (1972) Frequency changes of isozyme alleles in a selection experiment for grain yield in maize. Crop Sci 12:337-340

Stuber CW, Moll RH, Goodman MM, Schaffer H, Weir BS (1980) Allozyme frequency changes associated with selection for increased grain yield in maize. Genetics 95:225-236

Stuber CW, Wendel JF, Goodman MM, Smith JSC (1988) Techniques and scoring procedures for starch gel electrophoresis of enzymes from maize (*Zea mays* L). North Carolina Agric Res Ser Tech Bull 286 North Carolina Sta Univ Raleigh, NC, USA

Vales MI (1996) Tres ciclos de selección recurrente por productividad en dos poblaciones de maíz. Ph.D Thesis University of Vigo, Pontevedra, Spain

Watt WB (1994) Allozymes in evolutionary genetics. Self-impose burden or extraordinary tool?. Genetics 136:11-16

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
ϑ-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	*Got1* (3L)	4	1.00	0.98	1.00	1.00	0.00	1.00	1.00	1.00	0.99	-0.01
transaminase		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