Theor Appl Genet (1988) 76:398–404

Isozyme marker loci associated with cold tolerance and maturity in maize *

R. A. Guse [1], J. G. Coors [2], P. N. Drolsom [2] and W. F. Tracy [2]

[1] Garst Seed Company, Research Department, Slater, IA 50244, USA
[2] Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA

Received October 10, 1987; Accepted March 9, 1988
Communicated by A. Hallauer

Summary. Two maize (*Zea mays* L.) populations, AS1(S) and ECR-A, were evaluated for allozyme frequency changes associated with selection for improved seedling emergence, early season vigor and early maturity. Eleven marker loci were examined and four loci were used for indirect selection in an attempt to modify cold tolerance and maturity. Allozyme-selected divergent subpopulations were produced by compositing selected S_1 progeny from cycle one (C1) of AS1(S) and from C2 of ECR-A. These subpopulations and S_1 generations from all cycles resulting from phenotypic selection, ECR-A C1 through C7 and AS1(S) CO through C6, were tested in cold tolerance and agronomic performance trials over five environments in 1986. Seedling emergence and seedling dry weight did not improve with phenotypic selection in ECR-A, while plant height, ear height, grain yield, grain moisture, days to mid-silk and days to mid-pollen were reduced significantly. Contrasts between divergent allozyme-selected subpopulations from ECR-A were significant for grain moisture and mid-pollen date. For AS1(S), seeding emergence increased, while plant and ear height decreased with phenotypic selection. Contrasts between allozyme-selected subpopulations were significant for plant and ear height. Changes associated with marker-based selection for AS1(S) were not in the same direction as with phenotypic selection. Selection for favorable allozyme genotypes may be effective in changing certain traits in populations that have been modified by direct selection, however results may not be predictable.

Key words: *Zea mays* L. – Electrophoresis – Recurrent selection – Population improvement

Introduction

Cold tolerance in maize (*Zea mays* L.) is an aggregate of traits incorporating the ability to germinate, emerge and establish a vigorous stand in cool, early-season environments. Associated with adequate early season performance, germplasm growing in a short, cool season must flower early and the grain must dry down quickly for harvest and storage. Unfortunately, direct selection for cold tolerance traits is difficult because uncontrollable environmental factors adversely affect reliability of field trials. The efficiency of maize improvement programs might be enhanced if molecular markers, such as allozymes, could be used to manipulate quantitative trait loci.

Data supporting the notion that allozymes could be used as markers of quantitative trait loci have gradually accumulated during the past 15 years. Stuber and Moll (1972) monitored allozyme frequency changes in a selection program for grain yield. Alleles at three peroxidase loci behaved neutrally to yield selection, whereas the frequency of the *Acp1-4* allele showed a correlation with grain yield of $r=0.9$ over selection cycles. Stuber et al. (1980) monitored frequency changes of alleles at 20 isozymes loci in 4 long-term maize selection studies. Eight isozyme loci showed significant directional allele frequency changes, and the same alleles at each locus were consistently associated with yield. Kahler (1983) examined the effect of half-sib and S_1 recurrent selection for increased grain yield on allozyme polymorphisms in

* Contribution from the Department of Agronomy, Wisconsin Agric. Exp. Stn., Madison, WI. Part of a thesis submitted by the senior author in partial fulfillment of the requirements for a Ph. D. received June, 1987. Research supported by the College of Agric. and Life Sci., University of Wisconsin-Madison, Dekalb-Pfizer Genetics, Garst Seed Company, and Pioneer Hi-Bred.

maize and concluded that directional selection for changing allozyme frequencies at five loci was feasible. Pollak et al. (1984) reported that four of nine isozyme marker loci assayed were strongly associated with particular quantitative traits of plants in the maize cultivar 'Hays Golden'. Edwards et al. (1987) investigated 2 F_2 maize populations and found that, for 25 quantitatively inherited traits, the cumulative effect of marker-linked regions of the genome explained between 8%–40% of the phenotypic variation. Using the same populations, Stuber and Edwards (1987) reported that marker-locus facilitated selection was nearly as effective as phenotypic selection for grain yield, ear height and ear number.

Allozyme associations with cold tolerance and associated traits also have been documented. Vallejos and Tanksley (1983), in an interspecific cross of *Lycopersicon esculentum* and a high-altitude, cold-tolerant *L. hirsutum*, detected a minimum of three loci associated with growth at low temperatures. Kahler et al. (1980) examined 31 populations of *Avena barbata* from diverse habitats in Israel using 7 isozyme systems. Principal component and multiple regression analyses revealed that temperature and altitude of the collection source were significantly correlated with particular allozyme genotypes. In an examination of 34 races of maize from Mexico, Doebley et al. (1985) found that the frequencies of 22 alleles were significantly correlated with altitude. For Bolivian maize, Goodman and Stuber (1983) reported 12 alleles that were significantly correlated with altitude. Associations of allozymes with traits related to cold tolerance and maturity would be expected in such germplasm.

The effectiveness of allozyme selection on manipulating quantitative traits has been studied by several investigators. Stuber et al. (1982) manipulated allozyme frequencies in the 'Jarvis Golden Prolific' maize cultivar to approximate those found after ten cycles of full-sib selection for increased grain yield in the same population. One cycle of allozyme-based selection was found to be equivalent to 1.5 cycles of full-sib selection. Frei et al. (1986) generated maize subpopulations from the maize synthetic 'F26' based on allozyme frequencies associated with high and low yielding S_2 progeny as evaluated in testcrosses. Selection was effective when allozyme-selected progeny were evaluated in testcrosses but not when they were evaluated as populations per se.

The objectives of this study were to: (1) determine allozyme frequency changes associated with recurrent selection for increased cold tolerance and early maturity in two maize populations; (2) identify marker loci, based on observed allozyme frequency changes, that could be used for indirect selection; (3) generate subpopulations with frequencies of marker alleles epected to provide specific phenotypic responses; and (4) evaluate the effect of allozyme selection on cold tolerance, maturity and related agronomic traits.

Materials and methods

Experimental materials

Two synthetic populations, ECR-A and AS1(S), were examined. ECR-A is a composite population produced by J. H. Lonnquist (University of Wisconsin-Madison) by intercrossing 16 open-pollinated varieties adapted to the northern U.S. Corn Belt. This composite was used as a base for a mass selection program to increase the ability to germinate, emerge and quickly grow to maturity in cool, wet soils in northern Wisconsin. In each cycle, approximately 7500 seeds were planted in isolation at Marshfield, Wisconsin in early spring. After emergence, slow-growing seedlings were removed. At harvest, approximately 250 ears were visually selected from the earliest, healthy plants with the driest grain. A balanced composite of seed was formed for the next cycle by compositing equal numbers of seed from each ear. Seven cycles have been completed. AS1(S) was formed by J. L. Geadelmann at the University of Minnesota by crossing the synthetic populations BS2 and ASA and allowing five generations of random mating. Recurrent selection using visual field evaluation of S_1 lines for early vigor was conducted in northern Minnesota. A 10% selection intensity was applied to approximately 190 S_1 progeny each cycle. Selection was based upon date of 50% emergence and 2 visual field seedling vigor evaluations recorded at approximately the 4 and 8 leaf stages of seedling growth. Selected S_1 lines were recombined one time per cycle.

Allozyme investigations

In order to identify specific alleles at marker loci that were associated with selection progress, allozymes for 11 loci (*Acp1, Adh1, Est8, Glu1, Got1, Got2, Mdh1, Mdh2, Pgd2, Pgm2* and *Phi1*) were assayed on 52–120 seedlings from C1 and C7 of ECR-A, and CO and C6 of AS1(S). Linkage relationships indicate that all marker loci segregate independently with the exception of *Adh1* and *Phi1*, which are located approximately 12 units apart on the long arm of chromosome one. These 11 loci were chosen based on resolution and repeatability of the enzyme system and degree of polymorphism. Starch gel electrophoresis techniques and methodology were derived from Cardy et al. (1983), Marty et al. (1984) and Stuber and Goodman (1983). The FORTRAN program 'Genestats' (Black and Krafsur 1985) was used for allelic frequency estimations and detection of significant differences between cycles of selection. Significant differences between the initial and final cycles of selection were determined by Chi-square tests for heterogenity of allozyme frequencies between the two selection cycles (Workman and Niswander 1970).

Allozymes whose frequencies differed significantly from the initial to final cycle of selection were chosen as marker candidates for that population. Intermediate cycles of selection [C3 and C5 for ECR-A, and C2 and C4 for AS1(S)] were then assayed for the appropriate marker loci using 74–104 seedlings. Statistical procedures developed by Wilson (1980), using transformed ($2\sin^{-1}\sqrt{p}$) allozyme frequencies, were used to test whether fluctuations in frequencies from cycle to cycle could be attributed to genetic drift alone, or whether directional selection had occurred. Linear and quadratic trends were tested using Chi-square tests incorporating sampling variation due to restricted population size during selection [assumed to be a constant of 250 for ECR-A and 20 for AS1(S)] and restricted number of plants sampled for genotypic analysis. If significant deviations from random drift were detected, and significant directional changes, as represented by linear trends, were observed, then evidence was judged sufficient to suggest that the

particular allozyme locus may be linked to one or more loci affecting traits shown to have changed with recurrent selection.

Indirect selection experiment

Five-day old coleoptile sections were excised and preserved from 500 S_0 seedlings [250 from ECR-A C2 and 250 from AS1(S) C1]. Seedlings were then transplanted to peat pots and transplanted to the field breeding nursery 2 weeks later. These plants were self-pollinated, and 164 plants from ECR-A C2 and 176 plants from AS1(S) C1 were harvested with care taken to impose as little selection pressure as possible. The coleoptile sections were assayed for the appropriate allozyme markers. The summation of the allozyme breeding value at each marker locus (2 = homozygous for desirable allele, 1 = heterozygous, 0 = desirable allele absent) resulted in the determination of the aggregate genotype. Bulked subpopulations were formed by compositing equal numbers of kernels from each selected S_1 ear based on divergence of allozyme frequencies as follows:

ECR-A C2(+) – 16 plants (out of the 164 that were self-pollinated) with the best aggregate genotype for the favorable alleles;

ECR-A C2(−) – 16 plants (out of the 164) with as few favorable alleles as possible;

AS1(S) C1(+) – 17 plants (out of the 176 that were self-pollinated) with the best aggregate genotype for the favorable alleles; and

AS1(S) C1(−) – 17 plants (out of the 176) with as few favorable alleles as possible.

Each cycle of selection from both populations (ECR-A C1 through C7, AS1(S) CO through C6) was self-pollinated during the same season as the material from which allozyme-selections were made, in order to provide appropriate material for field studies at comparable levels of inbreeding. Each cycle was represented by a balanced composite of equal number of kernels from each S_1 family. There were between 30–41 S_1 families representing each cycle. The four subpopulations, ECR-A C2(+), ECR-A C2(−), AS1(S) C1(+) and AS1(S) C1(−), plus each cycle of selection, were examined for cold tolerance and agronomic parameters in field evaluations.

Field evaluations

Cold tolerance and agronomic performance experiments were conducted in five environments in Wisconsin. The trials were grown at three locations, Madison, Arlington and Marshfield, with early (April 18, 19 and 30 for the three respective locations) and normal (May 8 and 9 at Madison and Arlington, respectively) planting dates. The experimental design at Madison and Arlington was a split-split plot design. The main plots were planting dates, sub-plots were populations, and sub-sub-plots were cycles of selection. At Marshfield, there was only one planting date (early) and, therefore, the experiment had a split-plot design. Six replications were planted at each planting date. Experimental units were 2-row plots, 5.5 m long with 76.2 cm between rows. Seventy-two kernels were planted in each two-row plot. Kernels were treated with captan {*N*-[(trichloromethyl)thio]-4-cyclohexene-1, 2-dicarboximide} prior to planting. Seedling emergence was recorded 30 days after planting. At 45 days after planting, plots were thinned to final stand, 53,818 plants ha^{-1}. Of the plants removed, ten were harvested at ground level and oven-dried to determine seedling dry weight. Data for percentage emergence and seedling dry weight were recorded for six replications at each early planting. Plant height, ear height, grain yield adjusted to 15.5% moisture and grain moisture at harvest were recorded for four replications at both early and normal plantings. Mid-pollen and mid-silk date were recorded for both planting dates at Madison. Plots were hand-harvested, shelled in the field with a mechanical husker/sheller, and a representative sample was used for moisture analysis.

Table 1. Loci and allozyme frequencies in AS1(S) C0 and C6, and in ECR-A C1 and C7

Locus	Allele	AS1(S)		ECR-A	
		C0	C6	C1	C7
Adh1	4	–	–	0.636	0.533*
	6	–	–	0.364	0.467*
Pgd2	2.8	0.267	0.550**	–	–
	5	0.733	0.450	–	–
Acp1	2	0.283	0.128**	0.229	0.186
	3	0.228	0.106**	0.207	0.219
	4	0.467	0.767**	0.564	0.595
	6	0.022	0.000	0.000	0.000
Glu1	1	0.546	0.444	0.040	0.022
	2	0.000	0.025	0.149	0.111
	3	0.000	0.000	0.018	0.017
	6	0.230	0.198	0.212	0.162
	7	0.217	0.333**	0.495	0.637**
	10	0.007	0.000	0.086	0.051
Est8	4	0.375	0.391	0.084	0.091
	4.5	0.050	0.052	0.079	0.097
	5	0.430	0.458	0.775	0.727
	6	0.145	0.099	0.062	0.085
Pgm2	1	0.000	0.000	0.046	0.008*
	3	0.064	0.057	0.225	0.237
	4	0.921	0.943	0.683	0.650
	8	0.014	0.000	0.046	0.104*
Phi1	2	0.000	0.000	0.004	0.000
	4	0.944	1.000**	0.913	0.870
	5	0.056	0.000**	0.083	0.130
Got1	4	0.775	0.950**	0.937	0.901
	6	0.225	0.050*	0.063	0.099
Got2	2	0.044	0.000*	0.283	0.202
	4	0.956	1.000*	0.717	0.798
Mdh1	6	0.271	0.280	0.635	0.694
	10.5	0.012	0.013	0.032	0.040
Mdh2	3	0.181	0.293*	0.151	0.048**
	3.5	0.229	0.167	0.008	0.032
	6	0.307	0.247	0.175	0.185

*,** Significant difference between C0 and C6 AS1(S) or between C1 and C7 for ECR-A at the 0.05 and 0.01 probability levels, respectively

Results and discussion

Allozyme investigations

If phenotypic selection was successful at modifying traits associated with cold tolerance and/or early maturity in

Table 2. Allele frequencies and Chi-square tests for deviations from random drift and for linear and quadratic trends in allelic frequencies for allozyme markers used in indirect selection for ECR-A and AS1(S)

Cycles	Adh1−6	Pgm2−8	Mdh2−3	Glu1−7	Acp1−4	Phi1−4
ECR-A:						
1	0.364	0.046	0.151	0.495	–	–
3	0.520	0.018	0.183	0.620	–	–
5	0.507	0.058	0.163	0.650	–	–
7	0.467	0.104	0.048	0.637	–	–
Deviations[a]	*	**	**	*		
Linear	nd	nd	*	*		
Quadratic	**	**	***	**		
C2 (−)[b]	0.367	0.000	0.438	0.156	–	–
C2 (+)	0.875	0.062	0.000	0.906	–	–
AS1(S):						
0	–	–	0.181	0.217	0.467	0.944
2	–	–	0.333	0.314	0.478	0.939
4	–	–	0.276	0.608	0.444	1.000
6	–	–	0.293	0.333	0.767	1.000
Deviations			ns	***	**	ns
Linear			ns	ns	ns	ns
Quadratic			ns	**	**	ns
C1 (−)	–	–	0.029	0.028	0.654	0.735
C1 (+)	–	–	0.471	0.711	0.906	0.912

[a] Test for deviations greater than expected from genetic drift
*,**,*** Significant Chi-square values at $P<0.10$, $P<0.05$ and $P<0.01$, respectively
[b] C2 (−), C2 (+)=composite of S_1 seed from 16 ECR-A plants with the worst and best aggregate isozyme genotype, respectively
C1 (−), C1 (+)=composite of S_1 seed from 17 AS1(S) plants with the worst and best aggregate isozyme genotype, respectively

these populations, enzyme loci that were originally in linkage disequilibrium with loci affected by selection should also show changes in allelic frequency. Conversely, if certain allozyme loci are linked to loci conditioning early season vigor and maturity, then successful manipulation of allozyme frequencies at these loci should alter the expression of the population phenotype.

Four and seven loci assayed in ECR-A and AS1(S), respectively, showed significant allele frequency differences between the initial and final cycles of selection (Table 1). Allozymes at four loci for AS1(S), *Acp1-4, Glu1-7, Mdh2-3* and *Phi1-4*, and at four loci for ECR-A, *Adh1-6, Glu1-7, Mdh2-3* and *Pgm2-8* were chosen marker candidates for indirect selection. Frequencies of these alleles at intermediate cycles are presented in Table 2.

Deviations greater than would be expected based on random genetic drift were observed at all four loci in ECR-A with $P<0.10$ (Table 2). Deviations for *Pgm2-8* and *Mdh2-3* were significant at $P<0.05$. In AS1(S), frequencies for two allozymes, *Acp1-4* and *Glu1-7*, had significant deviations ($P<0.05$, $P<0.01$, respectively). Linear trends, which are convincing indicators of directional selection, were only observed for *Mdh2-3* and *Glu1-7* in ECR-A. Quadratic responses for allele frequencies were detected for all allozymes where significant deviations occurred. Significant deviations and trends were much harder to detect in AS1(S), in spite of the fact that allele frequency changes appeared to be equivalent to those observed in ECR-A, because the restricted population size used for recombination in AS1(S) increased the likelihood of genetic drift. Similar findings have been reported by Kahler (1983). Nonetheless, all four loci, including *Mdh2* and *Phi1*, were used for divergent selection in AS1(S) because of the desirability of covering as much of the genome as possible. Large deviations and directional trends were observed at *Mdh2* in ECR-A, and *Phi1* is linked to *Adh1*, which was chosen for selection in ECR-A. Two of the marker alleles, *Glu1-7* and *Mdh2-3*, were used for both populations. However, the frequency of *Mdh2-3* in ECR-A progressed in the opposite direction from AS1(S).

A major objective of this study was to generate subpopulations with divergent frequencies of marker alleles expected to be linked with loci affecting phenotypic traits associated with cold tolerance and maturity. Of the individuals self-pollinated and harvested from ECR-A C2 and AS1(S) C1, approximately 10% were chosen to form the ECR-A C2(+) subpopulation. Another 10% were chosen to form the ECR-A C2(−) subpopulation. The same selection scheme was used for AS1(S) C1. In selecting plants for the divergent subpopulations, the frequencies of the favorable alleles were moved as close to fixa-

Table 3. Means for eight agronomic traits for seven cycles of recurrent selection for cold tolerance in ECR-A and AS1(S), and their respective allozyme-selected cycles

Cycle	EMERG[a] %	SDW (g)	PHT (cm)	EHT (cm)	YLD (Mg ha^{-1})	MOIS (%)	DMS (days)	DMP (days)
ECR-A:								
C1	78.4	1.81	160	68	3.10	29.4	70.3	68.9
C2	84.7	1.85	164	74	2.98	29.4	72.5	70.5
C3	77.7	1.95	146	63	2.31	27.5	67.8	66.6
C4	78.9	1.91	149	62	2.72	28.2	68.8	67.7
C5	83.0	1.79	157	67	3.09	28.3	68.8	67.8
C6	82.5	1.93	144	58	2.75	28.4	68.3	67.9
C7	79.6	1.83	150	62	2.41	27.6	69.0	67.8
C2 (−)[b]	83.7	1.79	159	66	3.36	29.7	72.4	70.8
C2 (+)	84.1	1.65	159	68	3.32	28.1	71.3	69.6
AS1(S):								
C0	82.2	1.64	190	86	3.65	33.0	77.8	76.0
C1	83.3	1.85	194	88	4.06	32.7	77.2	75.3
C2	87.4	1.69	191	82	3.73	32.0	77.3	75.1
C3	86.5	1.80	190	85	3.79	31.9	77.6	75.3
C4	89.9	1.77	184	82	4.16	33.0	76.7	75.3
C5	88.5	1.68	183	86	3.35	34.0	77.8	75.8
C6	88.9	1.86	181	81	3.71	32.0	76.8	75.6
C1 (−)	76.5	1.50	177	81	3.70	32.4	76.3	74.6
C1 (+)	74.8	1.70	196	88	4.07	32.8	77.5	75.5
Reps	18	18	20	20	20	12	12	12
Mean	82.5	1.77	171	75	3.34	30.6	73.7	72.1
LSD (0.05)	3.2	0.21	6	6	0.45	1.4	1.5	1.0
CV (%)	6.4	15.5	5.2	11.6	17.4	7.1	1.6	1.1

[a] EMERG = seedling emergence, SDW = seedling dry weight, PHT = plant height, EHT = ear height, YLD = grain yield, MOIS = grain moisture, DMS = days to mid-silk, DMP = days to mid-pollen

[b] C2 (−), C2 (+) = composite of S_1 seed from 16 ECR-A plants with the worst and best aggregate isozyme genotype, respectively. C1 (−), C1 (+) = composite of S_1 seed from 17 AS1(S) plants with the worst and best aggregate isozyme genotype, respectively

tion (0.0 or 1.0) as possible, depending on the allelic trend between the initial and final cycle of selection. Marker allele frequencies were changed in the desired direction in each of the four subpopulations (Table 2).

The five environments in which agronomic traits were measured covered a range of growing conditions. Grain yield means ranged from 2.79 Mg ha^{-1} to 4.00 Mg ha^{-1}. Early planting significantly decreased plant height and ear height at Arlington and plant height at Madison. Analyses of variance of all environments (not shown) demonstrated that all traits were significantly affected by the environment.

The 1986 spring growing conditions were favorable, even though measured heat units suggested good cold-stress environments. In cold-tolerance evaluations in 17 environments covering a 2 year period, Hoard and Crosbie (1986) reported an average of 295 and 534 heat units at 30 and 45 days after planting, respectively. In comparison, in this study heat units averaged 178 and 279 at 30 and 45 days after planting, respectively, for the 3 early planting dates. However, the lack of excessive moisture may have promoted seedling emergence and vigor. Analyses of variance over all cold tolerance environments (not shown) indicated both emergence and seedling dry weight were strongly affected by the environment, and that the location mean squares were highly significant for both traits.

Six of the eight traits examined for ECR-A changed significantly as a probable result of mass selection as indicated by significant linear regression trends (Tables 3 and 4). Plant and ear heights, grain moisture content, days to mid-pollen and mid-silk, and grain yield decreased significantly. Seedling emergence and seedling dry weight showed no significant linear or quadratic change over the seven cycles. Either the selection scheme employed in this population was only effective for maturity-related traits and not for early season performance, or the three environments used to evaluate early season performance in 1986 were not stressful enough to distinguish improvements in germination and seedling vigor. Recent cold-test evaluations of ECR-A under more controlled conditions in growth chambers indicated that the

Table 4. Regression coefficients for eight agronomic traits measured in seven cycles of recurrent selection for cold tolerance ECR-A and AS1(S)

Agronomic traits	ECR-A	AS1(S)
Seedling emergence		
linear	0.16	1.18**
quadratic	−0.09	−0.27
Seedling dry weight (g)		
linear	0.00	0.00
quadratic	−0.01	0.00
Plant height (cm)		
linear	−2.17**	−1.88**
quadratic	0.53*	−0.39
Ear height (cm)		
linear	−1.70**	−0.74*
quadratic	0.16	0.03
Grain yield (Mg ha^{-1})		
linear	−0.06**	−0.03
quadratic	0.01	−0.02
Grain moistuire (%)		
linear	−0.23**	0.01
quadratic	0.06	0.03
Days to mid-silk (days)		
linear	−0.40**	−0.09
quadratic	0.14*	0.01
Days to mid-pollen (days)		
linear	−0.26**	0.00
quadratic	0.11**	0.07

*,** Significant at the 0.05 and 0.01 probability levels, respectively. Orthogonal polynomial coefficients used for linear and quadratic regression were (−3 −2 −1 0 +1 +2 +3) and (+5 0 −3 −4 −3 0 +5), respectively

former explanation is more likely, and that selection for improved seedling emergence was not successful for this population (J. G. Coors, unpublished).

For traits showing no demonstrable change through selection, differences between divergent subpopulations [C2(+) versus C2(−)] would not be expected, and no such differences were observed for seedling emergence and seedling dry weight in ECR-A. Significant differences between C2(−) and C2(+) were detected only for grain moisture and days to mid-pollen shed. The direction of these differences were as expected if the extent of recombination between marker loci and loci affecting grain moisture and pollen date was not great. No significant differences were detected for any trait between ECR-A C2 and either divergent subpopulation originating from C2, suggesting that effects of allozyme-based selection were not great.

Of the eight traits examined for AS1(S), only seedling emergence, plant height and ear height changed significantly as a result of phenotypic selection (Tables 3 and 4). Regression coefficients indicate that seedling emergence improved at a rate of 1.18 percentage units per cycle, and that plant and ear heights were reduced by 1.88 cm and 0.74 cm per cycle, respectively. Previous information (Hexum 1984; Martiniello 1985) showed that seedling dry weight and grain yield also increased in AS1(S), but such trends were not apparent in this study.

In comparisons of divergent subpopulations [C1(+) versus C1(−)] for AS1(S), highly significant differences were detected for two of the three traits observed to change with phenotypic selection, plant height and ear height, while no significant differences were detected for any other trait. Plant height in the AS1(S) C1(+) subpopulation was over 19 cm taller than the AS1(S) C1(−) subpopulation, and the comparable difference for ear height was 7 cm. These differences are opposite from what occurred with phenotypic selection. If marker loci were linked to loci affecting plant and ear height, chance recombination within the small composites (17 S_1 plants) used to form the divergent subpopulations may be responsible for these observations. Four of the nine genes known to reduce plant height (*br, br2, D8* and *rd*) are located on the long arm of chromosome 1, and *D8* and *rd* are within 7 and 12 map units, respectively, of *Phi1*. Of the remaining genes, *rd2* is located on the long arm of chromosome 6 (specific location unknown) as is *Mdh2*, and *d3* is located near the centromere on the short arm of chromosome 9, close to the tentative placment of *Acp1*. If one or more of these genes were segregating in AS1(S), linkage phase would affect the direction of selection response.

It was apparent that both AS1(S) divergent subpopulations had markedly reduced seedling emergence from that of C2 and any other of the AS1(S) cycles. Seedling vigor also was somewhat reduced. Several explanations are possible. Perhaps alleles chosen for indirect selection were not strictly neutral with respect to seedling performance, and that allozyme-based selection led to some sort of physiological imbalance. It also may be that methods used to select for specific allozymes led to greater inbreeding, increasing the likelihood of uncovering linked recessive deleterious alleles and poor vigor in the divergent subpopulations of AS1(S).

Genetic drift may be partially responsible for significant comparisons between divergent subpopulations. However, no significant differences were detected between divergent allozyme-selected subpopulations in either ECR-A or AS1(S) for traits for which there were no significant changes associated with phenotypic selection, indicating that the effect of restricted sample size was not large. Genetic drift was a major factor in the ability to detect significant marker-locus associations in the two populations, ECR-A and AS1(S). Recombination in ECR-A was accomplished with 250 ears and the effect of random drift on gene frequency was minimal in the few cycles examined. This is in contrast to the inabil-

ity to convincingly document allozyme associations in AS1(S), where recombination was accomplished with 20 or fewer progeny. Populations resulting from recurrent selection, which are most amenable to detecting allozyme associations, are those with large effective population sizes and with documented improvements for specific traits. It appears likely that quantitative trait loci are distributed throughout the genome and that marker-loci can be identified using such germplasm.

Acknowledgements. We would like to thank Pioneer Hi-Bred International, Dekalb-Pfizer Genetics and the Garst Seed Company for providing financial support for this research. We also extend a special thanks to D. B. Johnson and J. R. Davis for their assistance with field evaluations, and to R. P. Guries for his advice and encouragement.

References

Black WC, Krafsur ES (1985) A FORTRAN program for analysis of genotypic frequencies and description of the breeding structure of populations. Theor Appl Genet 70:484–490

Cardy B, Stuber CW, Wendel JF, Goodman MM (1983) Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Institute of Statistics Monograph Series no. 1317

Doebley JF, Goodman MM, Stuber CW (1985) Isozyme variation in the races of maize from Mexico. Am J Bot 72:629–639

Edwards MD, Stuber CW, Wendel JF (1987) Molecular-marker-facilitated investigations of quantitative trait loci in maize. 1. Numbers, genomic distribution and types of gene action. Genetics 116:113–125

Frei OM, Stuber CW, Goodman MM (1986) Yield manipulation from selection on allozyme genotypes in a composite of elite corn lines. Crop Sci 26:917–921

Goodman MM, Stuber CW (1983) Races of maize. VI. Isozyme variation among races of maize in Bolivia. Maydica 28:169–187

Hexum DK (1984) Evaluation of visual S_1 recurrent selection for early vigor in maize. Diss Abstr B 45:3123

Hoard KG, Crosbie TM (1986) Effects of recurrent selection for cold tolerance on genotype-environment interactions for cold tolerance and agronomic traits in two maize populations. Crop Sci 26:238–242

Kahler AL (1983) Effect of half-sib and S1 recurrent selection for increased grain yield on allozyme polymorphisms in maize (*Zea mays* L.). Crop Sci 23:572–576

Kahler AL, Allard RW, Krzakowa M, Wehrhahn CF, Nevo E (1980) Associations between isozyme phenotypes and environment in the slender wild oat (*Avena barbata*) in Israel. Theor Appl Genet 56:31–47

Martiniello P (1985) Recurrent visual selection in S1 progenies for early vigor in maize (*Z. mays* L.). Maydica 30:301–308

Marty TL, O'Malley DM, Guries RP (1984) A manual for starch gel electrophoresis. University of Wisconsin-Madison, Department of Forestry, Staff Paper Series no. 20, 26 pp

Pollak LM, Gardner CO, Parkhurst AM (1984) Relationships between enzyme marker loci and morphological traits in two mass selected maize populations. Crop Sci 24:1174–1179

Stuber CW, Edwards MD (1987) Genotypic selection for improvement of quantitative traits in corn using molecular marker loci. Proc 41st Ann Corn and Sorghum Ind Res Conf, Chicago

Stuber CW, Goodman MM (1983) Allozyme genotypes for popular and historically important inbred lines of corn, *Zea mays* L. USDA-ARS, ARR-S-16. US Department of Agriculture, New Orleans, LA

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, Goodman MM, Moll RH (1982) Improvement of yield and ear number resulting from selection at allozyme loci in a maize population. Crop Sci 22:737–740

Vallejos CE, Tanksley SD (1983) Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato. Theor Appl Genet 66:241–247

Wilson S (1980) Analyzing gene-frequency data when the effective population size is finite. Genetics 95:489–502

Workman PL, Niswander JD (1970) Population studies on southwestern Indian tribes. 2. Local differentiation in the Papago. Am J Hum Genet 22:24–49