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Number and Fitness of Selected Individuals in Marker-Assisted and Phenotypic Recurrent Selection

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ABSTRACT

Selected individuals in recurrent selection usually have equal fitness, i.e., they contribute the same number of progenies to the next cycle of selection. Our objective was to determine if varying the fitness of selected individuals increases the response to recurrent selection. We developed and evaluated an optimum method (Unequal Fitness) and a simplified method (Better Half) for determining the appropriate fitness of selected individuals. By computer simulation we found that if the number of selected individuals (N_{Sel}) is constant, the short-term response (cycles 1–5) to phenotypic recurrent selection was generally higher with the Better Half and Unequal Fitness methods than with the Equal Fitness method. In practice, however, breeders would find it easier to change N_{Sel} than to manipulate the fitness of selected individuals. Reducing N_{Sel} often negated any short-term advantage of the Better Half and Unequal Fitness methods. Likewise, the Better Half and Unequal Fitness methods were not advantageous in marker-assisted recurrent selection, which is a short-term procedure. Across different N_{Sel} values, the Better Half and Unequal Fitness methods were superior to the Equal Fitness method for medium- and long-term phenotypic recurrent selection (cycles 6–30). We recommend the Better Half method over the Unequal Fitness method because of its simplicity and because it remained superior to the Equal Fitness method over more cycles of selection. As a rule-of-thumb, we suggest that N_{Sel} should be roughly equal to the number of cycles for which selection will be conducted. This rule of thumb leads to N_{Sel} values lower than those typically used in selection programs.

Recurrent selection involves evaluating a population and selecting the best individuals, recombining these selected individuals to form the next generation or cycle of selection, and repeating the entire procedure. Recurrent selection programs differ in the number of progenies that are evaluated (N) and the number of progenies that are subsequently selected and recombined (N_{Sel}).

The number of progenies selected and recombined in recurrent selection typically ranges from $N_{Sel} = 10$ to 30 (Kenworthy and Brim, 1979; Hallauer, 1992; Weyhrich et al., 1998). Reducing N_{Sel} while keeping N constant would increase both the selection differential and the response achieved in the next cycle of selection. A low N_{Sel}, however, could lead to a reduced genetic variance and, consequently, a lower response in future cycles of selection (Rawlings, 1979). Theoretical results as well as empirical studies with model species have indicated that N_{Sel} values of 30 to 45 are required to maintain medium-term and long-term response to selection (Robertson, 1960; Frankham et al., 1968; Jones et al., 1968). But empirical studies in maize (*Zea mays* L.) (Weyhrich et al., 1998; Guzman and Lamkey, 2000) and in soybean [*Glycine max* (L.) Merr.] (Brim and Burton, 1979) have indicated little advantage in selecting and recombining more than $N_{Sel} = 10$ progenies, at least in the short term (e.g., five cycles).

Regardless of N_{Sel}, breeders usually assign equal fitness to the selected individuals in recurrent selection. Fitness refers to the number of progenies contributed by an individual to the next generation. Suppose $N_{Sel} = 10$ individuals are selected from $N = 400$ individuals. If a population size of $N = 400$ is to be maintained across cycles, the $N_{Sel} = 10$ individuals are typically recombined in such a way that each selected individual contributes $2N/N_{Sel} = 80$ gametes to the next cycle. It seems arbitrary, however, to have the 10th-best individual contribute 80 gametes but to have the 11th-best individual not contribute any gametes to the next generation. Varying the fitness of the selected individuals could potentially increase the response to selection. In this scheme, the N individuals in a given cycle will be ranked according to the probability that they would produce superior progenies. The N_{Sel} individuals will then be recombined in a way that the best individual will have a higher fitness than the second-best individual, the second-best individual will have a higher fitness than the third-best individual, and so on. Depending on the criterion for defining superiority, some individuals will effectively have a fitness of zero.

Our objective in this study was to determine if varying the fitness of selected individuals increases the short-term, medium-term, and long-term response to recurrent selection. Specifically, we developed and evaluated an optimum method (Unequal Fitness) and a simplified method (Better Half) for determining the appropriate fitness of selected individuals. Optimizing the fitness of selected individuals needs to be more advantageous than simply reducing N_{Sel} to increase the selection response. We therefore studied the joint effects of having different numbers of selected individuals and different fitness of selected individuals in recurrent selection. We studied both recurrent selection based on phenotypic data and marker-assisted recurrent selection (MARS).

MATERIALS AND METHODS

Genetic Models and Selection Methods

We considered four models pertaining to the number of QTL controlling the trait and to the population size: (i) 10 QTL, $N = 100$; (ii) 40 QTL, $N = 100$; (iii) 100 QTL, $N = 100$;

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and (iv) 40 QTL, $N = 400$. The number of individuals selected and recombined was $N_{Sel} = 2$, 3, 4, 5, 10, 20, or 40. Phenotypic recurrent selection comprised 30 cycles. We considered the response in cycles 1 to 5 as short-term response, cycles 6 to 15 as medium-term response, and 16 to 30 as long-term response. The MARS procedure (Edwards and Johnson, 1994; Hospital et al., 1997; Johnson, 2001; Koebner, 2003; Johnson, 2004) comprised one cycle of selection based on an index of phenotypic data and marker scores (i.e., marker-assisted selection, Lande and Thompson, 1990), followed by three cycles of selection based on marker scores only (i.e., marker-based selection). For the 10 QTL, $N = 100$ model, we considered only the short-term response to phenotypic recurrent selection and MARS, given that medium-term or long-term selection for a trait controlled by only 10 QTL is unrealistic because the genetic variance is expected to be exhausted after a few cycles of selection.

We conducted 1000 repeats of each simulation experiment and averaged the results across the repeats. Selection responses were expressed in terms of units of the genetic standard deviation in cycle 0. The statistical significance ($P = 0.05$) of differences in selection response was determined with z-tests, using the variances of the selection response across the 1000 repeats of an experiment.

Genotypic and Phenotypic Values

In this study, genotypic and phenotypic values were defined in terms of testcross performance, which is appropriate for maize. But the results regarding the number and fitness of selected parents should generally apply to per se performance as well. Details of the procedures we used to simulate genotypic and phenotypic values have been described in previous articles (Bernardo, 2004; Bernardo and Charcosset, 2006). Each repeat of an experiment differed in the genetic map, the genotypes of the individuals sampled, and their phenotypic values.

The base population for both phenotypic recurrent selection and MARS was a simulated F_2 generation formed by selfing the F_1 between two parental inbreds. The F_2 population was segregating at 100 codominant marker loci and $l = 10$, 40, or 100 QTL. The sizes of the chromosomes and of the entire genome (1749 cM) corresponded to those in a published maize linkage map (Senior et al., 1996). The genome was divided into 100 bins of 1749/100 $\cong$ 17 cM. A marker was assumed randomly located (according to a uniform distribution) within $\pm$ 5 cM of the midpoint of each bin. The l QTL were randomly located among the 10 chromosomes according to a uniform distribution over the total genome. In each simulated cycle of selection, F_2 (in cycle 0) or S_0 individuals (after cycle 0) were selfed and testcrossed to an unrelated inbred tester. Testcross genotypic values were simulated according to metabolic control theory as outlined by Bost et al. (1999) and Bernardo and Charcosset (2006). The effects of the l QTL followed a geometric distribution, where few QTL had large effects and many QTL had small effects. Specifically, the first QTL had the largest effect, the second QTL had the second-largest effect, and the lth QTL had the smallest effect. The first parent had the favorable allele at even-numbered QTL and the less-favorable allele at odd-numbered QTL. Coupling and repulsion linkages were therefore generated at random, given that QTL positions were randomly assigned without regards to the magnitude of QTL effects.

Random nongenetic effects were added to the genotypic values to obtain phenotypic values. The random nongenetic effects had a normal distribution with a mean of zero and were scaled so that broad-sense heritability among testcrosses, on an entry-mean basis, was $H = 0.20$, 0.50, or 0.80 in the initial F_2 population. The amount of nongenetic variance (V_E), for each level of H, was constant across cycles of selection. Although the true values of the genetic variance (V_G) and V_E were known, these parameters were estimated in each cycle of phenotypic recurrent selection. Estimates of V_G and V_E in each cycle were later used in calculating the fitness of selected individuals.

Estimation of Marker Effects and Marker-Assisted Recurrent Selection

In MARS, markers associated with the trait were identified and their effects were estimated only in the initial F_2 population (i.e., cycle 0). First, multiple regression of phenotypic value on the number of marker alleles (0, 1, or 2) from the first parental inbred was performed on a chromosome-by-chromosome basis. Significant markers on each chromosome were identified by backward elimination. Second, multiple regression coefficients were obtained by jointly analyzing all the markers found significant in the per-chromosome analysis. Standard procedures were used to handle any singularities encountered in multiple regression analysis (Press et al. 1992, p. 56). Relaxed significance levels ($\alpha = 0.20$, 0.30, and 0.40), which have been found to maximize the response to MARS (Hospital et al., 1997), were used.

In practice, cycle 0 of MARS in maize involves marker-assisted selection in regular selection seasons and locations (Koebner, 2003; Johnson, 2004). Selection in cycle 0 was therefore based on both phenotypic and marker data. In contrast, cycles 1 to 3 of MARS in maize are conducted in an off-season (e.g., winter) nursery, where phenotypic evaluations are not meaningful but three generations can be grown in 1 yr (Koebner, 2003; Johnson, 2004). Selection in cycles 1 to 3 was therefore based on markers alone. We simulated the following procedures in MARS. In cycle 0, a marker score (Lande and Thompson, 1990) for each family was calculated from the multiple regression coefficients for the markers with significant effects. This marker score was then combined with the individual's phenotypic value in a least-squares selection index as outlined by Lande and Thompson (1990), but with the restriction that the weight for the phenotypic value was always positive (Hospital et al., 1997). In cycles 1 to 3, only the marker scores were calculated.

Equal Fitness and Better Half Methods

In the Equal Fitness method, the N individuals in each cycle were ranked according to their phenotypic mean (phenotypic recurrent selection), selection index value (cycle 0 in MARS), or marker score (cycles 1 to 3 in MARS). The top N_{Sel} individuals were selected, without considering whether the means of the poorest selected individual and of the best nonselected individual were significantly different. The number of pairwise crosses among the N_{Sel} selected individuals was $N_{Cross} = N_{Sel}(N_{Sel} - 1)/2$ (Fig. 1); selfs and reciprocal crosses were excluded. Each of the N_{Cross} pairwise crosses had an equal number or nearly equal number of progenies in the next cycle of selection. Suppose $N_{Sel} = 4$ individuals were selected out of $N = 100$. In this situation, each of the $N_{Cross} = 6$ crosses contributed $[|N/N_{Cross}|] = [|100/6|] = [|16.67|] = 16$ progenies to the next cycle, where the $[|\ |]$ symbol denotes the integer function. Given that 6 crosses $\times$ 16 progenies per cross would lead to only 96 progenies, a total of $100 - 96 = 4$ crosses were selected at random. Each of these four random crosses then contributed one additional progeny to the next cycle. On the other hand, N_{Cross} sometimes exceeded N, e.g., $N = 100$, $N_{Sel} = 20$, and $N_{Cross} = 20(19)/2 = 190$. In this situation, 100 out of the

	2	3	4	5	6	7	8	9	10
1	x	x	x	x	x	x	x	x	x
2		x	x	x	x	x	x	x	x
3			x	x	x	x	x	x	x
4				x	x	x	x	x	x
5					x	x	x	x	x
6						x	x	x	x
7							x	x	x
8								x	x
9									x

Equal Fitness

	2	3	4	5	6	7	8	9	10
1	x	x	x	x	x	x	x	x	x
2		x	x	x	x	x	x	x	
3			x	x	x	x	x		
4				x	x	x			
5					x				
6									
7									
8									
9									

Better Half

Fig. 1. Equal Fitness and Better Half methods. The numbers refer to the ranks of the parents, and an X indicates the corresponding pair of parents contributing progenies to the next cycle of selection. Selfs and reciprocal crosses are excluded.

190 crosses were selected at random, and each of these 100 crosses contributed one progeny to the next cycle.

In the Better Half method, the N individuals were ranked and selected as in the Equal Fitness method. For each of the pairwise crosses among the N_{Sel} individuals, the sum of the ranks of the two parents was calculated, and a pair of parents contributed progenies to the next cycle only if the sum of their ranks did not exceed $N_{Sel} + 1$. Suppose $N_{Sel} = 10$ individuals were selected. In this situation, only those pairs of parents whose sum of ranks did not exceed 11 contributed progenies to the next cycle (Fig. 1). The cross between individual 5 and 6, whose sum of ranks was 11, contributed progenies to the next generation. But the cross between 5 and 7, whose sum of ranks was 12, did not contribute progenies to the next generation. For $N_{Sel} = 10$, the number of crosses in the Better Half method was $N_{Cross} = 25$ (versus 45 in the Equal Fitness method). The procedures for determining the number of progenies for each of the N_{Cross} pairs were similar to those in the Equal Fitness method. As the name of the method implies, progenies were contributed only by roughly the superior half of all possible crosses among the N_{Sel} individuals.

Unequal Fitness

In the Unequal Fitness method, the number of progenies contributed by cross i was proportional to the probability that the cross would produce superior progeny. This probability was denoted by p_i. In this study a progeny was considered superior if it had a genotypic mean in the top 1% of the population. This percentage corresponded to that of the best individual in the most common population size ($N = 100$) we studied. In preliminary studies, we also considered upper-tail probabilities of 5 and 0.1%. Upper-tail probabilities of 5, 1, or 0.1% did not affect the usefulness of the Unequal Fitness method relative to the Equal Fitness and Better Half methods (results not shown). Compared with less-stringent probabilities, an upper-tail probability of 0.1% led to a slightly higher short-term response but a slightly lower long-term response.

Suppose cycle k has a mean of $\mu_{Cycle\ k}$ (Fig. 2) and a genetic variance of $V_{G(Cycle\ k)}$. The dotted vertical line in Fig. 2 depicts the threshold above which the top 1% of individuals in cycle k are found. Two individuals in cycle k are crossed to form Cross 1, and the progenies in Cross 1 have a mean (μ_1) greater than $\mu_{Cycle\ k}$ but a genetic variance less than $V_{G(Cycle\ k)}$. A second pair of individuals in cycle k are crossed to form Cross 2, and the progenies in Cross 2 have a mean (μ_2) greater than μ_1 but a genetic variance less than that in Cross 1. The probability of progenies exceeding the 1% threshold is depicted by p_1 in Cross 1 and p_2 in Cross 2 (Fig. 2). Given that p_2 is greater than p_1, the number of progenies contributed by Cross 2 would be greater than the number of progenies contributed by Cross 1.

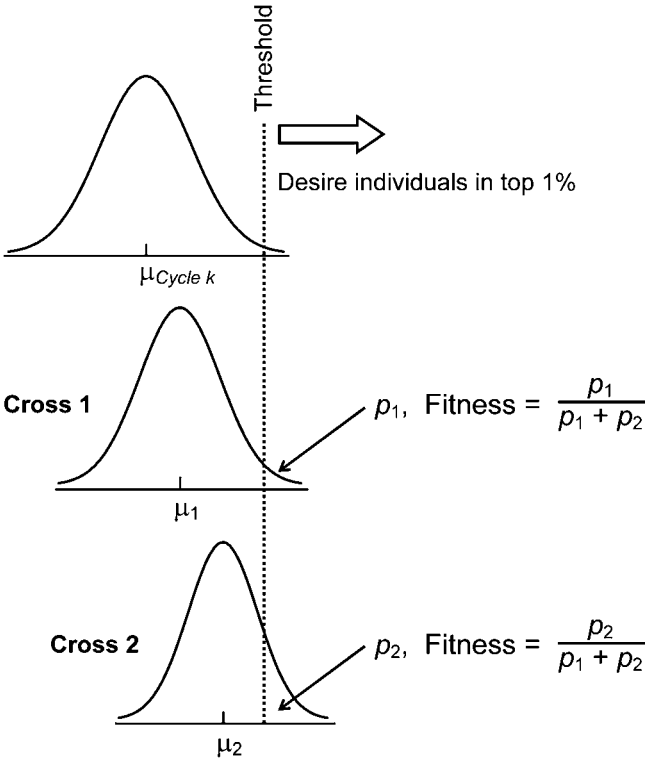

Fig. 2. Optimizing the fitness in crosses among selected parents.

The value of p_i was modeled assuming a normal distribution. Suppose cross i was created by crossing individuals j and j' in cycle k. Calculating p_i required information on the predicted mean (μ_i) and predicted genetic variance [$V_{G(i)}$] in cross i.

Unequal Fitness in Phenotypic Recurrent Selection

In phenotypic recurrent selection, the threshold in cycle k was estimated as $T = \mu_{Cycle\ k} + z_T [V_{G(Cycle\ k)}]^{1/2}$, where $\mu_{Cycle\ k}$ was the estimated mean phenotypic value in cycle k; $V_{G(Cycle\ k)}$ was the estimate of V_G in cycle k; and z_T was the one-sided z-value for the threshold (2.326 for 1%). Suppose v_j was the phenotypic value of individual j whereas $v_{j'}$ was the phenotypic value of individual j'. The mean of cross i was predicted as $\mu_i = \mu_{Cycle\ k} + H\,[1/2\,(v_j + v_{j'}) - \mu_{Cycle\ k}]$, where H was the estimate of broadbase heritability in cycle k. The genetic variance in cross i was predicted as $V_{G(i)} = [1 - 1/(2N_{Parents})]V_{G(Cycle\ k)} = 0.75V_{G(Cycle\ k)}$ (Lande, 1980), where $N_{Parents}$ was equal to 2 because each pairwise cross was formed by mating two individuals. In phenotypic recurrent selection, the crosses between the N_{Sel} individuals therefore differed in their predicted means but had the same predicted genetic variance.

The probability of superior progenies in cross i was then a function of $z_i = (T - \mu_i)/[V_{G(i)}]^{1/2}$. Assuming a standard normal distribution, p_i was equal to the one-sided probability that corresponded to z_i. The number of progenies to be contributed by cross i was determined through the following steps. First, the relative fitness of each cross (i.e., among the N_{Sel} individuals) was calculated as $p_{i'} = p_i/\Sigma p_i$. Second, the minimum, nonzero $p_{i'}$ among all crosses was determined. If the minimum $p_{i'}$ was less than $1/N$ (i.e., the minimum contribution to the next cycle was one progeny), then this minimum $p_{i'}$ was set equal to zero. Steps 1 and 2 were then repeated until the minimum, nonzero $p_{i'}$ was greater than $1/N$. Third, the number of progenies from each cross was calculated as $N_i = [|Np_{i'}|]$. Finally, the total number of progenies was calculated as ΣN_i. If, due to

rounding error, ΣN_i was less than N, then adjustments similar to those for the Equal Fitness method were made.

Unequal Fitness in Marker-Assisted Recurrent Selection

The availability of marker-effect information in MARS permitted the modeling of the variance in a given cross in addition to its mean. The procedures for the Unequal Fitness method in phenotypic recurrent selection and in MARS therefore differed only in the calculation of the predicted mean and genetic variance in a cross. Procedures for calculating T, z_i, p_i, $p_{i'}$, and N_i in MARS were similar to those used for phenotypic recurrent selection.

In MARS, $\mu_{Cycle\ k}$ and $V_{G(Cycle\ k)}$ were calculated from selection index values in cycle 0 and from marker scores in cycles 1, 2, and 3. Suppose cross i was created by crossing individuals j and j' in cycle k. In cycle 0, the predicted mean of cross i (μ_i) was calculated as the mean selection index value of individuals j and j'. Likewise, μ_i in cycles 1, 2, and 3 was calculated as the mean marker score of individuals j and j'.

Three methods were used to model the genetic variance within cross i: Expected Variance, Immediate Variance, and Equal Variance. Suppose the favorable allele at a significant marker was denoted by + and the less favorable allele was denoted by −. The estimated marker effect at the mth significant marker was b_m. In the Expected Variance method, the variance at the locus was modeled as $2pqb^2_m$ (Falconer, 1981, p. 116), where p was the frequency of the + allele and q was the frequency of the − allele. The variance due to the mth marker was therefore $0.5b^2_m$ if the parental genotypes of cross i were ++ and − − or were +− and +− (i.e., allele frequencies of 0.5); $0.375b^2_m$ if the parents were ++ and +− or were +− and − − (i.e., allele frequencies of 0.75 and 0.25); and zero if both parents were homozygous for the same allele (i.e., allele frequencies of 1 or 0). The expected variance in cross i was then calculated as the sum of variances across significant markers, assuming that the selected markers were independent.

The Expected Variance method modeled the genetic variance expected on random mating among the progenies of the cross, but it did not model the genetic variance expressed immediately in the cross. For example, the progenies from mating a ++ individual and a − − individual would all have the +− genotype. At this locus, the progenies will all have the same genotypic value in the cross [i.e., immediate $V_{G(i)} = 0$] but will have an expected variance greater than zero on random mating. In the Immediate Variance method, the variance due to the mth marker was calculated as $0.5b^2_m$ if both parents were +−; $0.25b^2_m$ if the parents were ++ and +− or were +− and − −; and zero for all other pairs of parental genotypes. The immediate variance in cross i was calculated as the sum of variances across significant markers.

In the Equal Variance method, $V_{G(i)}$ was assumed constant across all crosses. Specifically, $V_{G(i)}$ was modeled using the variance among selection index values in cycle 0 and among marker scores in cycles 1 to 3.

RESULTS AND DISCUSSION

Short-Term Response

For the same N_{Sel}, the response to short-term phenotypic selection (cycles 1–5) was generally higher with the Unequal Fitness and Better Half methods than with the Equal Fitness method, particularly when N_{Sel} was 10 or 20. Consider the genetic model of 40 QTL, $N = 100$ (Table 1). When N_{Sel} was 20 and heritability was $H = 0.20$, the responses in cycle 5 (in units of the genetic standard deviation in cycle 0) were 2.60 with Equal Fitness, 2.94 with Unequal Fitness, and 2.86 with the Better Half method. These responses with the Unequal Fitness and Better Half methods were significantly higher that with the Equal Fitness method [least significant differences ($P = 0.05$) in Table 1 ranged from 0.10 to 0.15]. The superiority of the Unequal Fitness and Better Half methods over the Equal Fitness method for short-term response was expected, as the Better Half and Unequal Fitness methods place more weight on the best selected individuals, thereby increasing the selection differential. Despite this general result, the differences between the methods were often small and inconsistent, particularly for small values of N_{Sel}.

In practice, breeders would find it easier to change N_{Sel} than to manipulate the fitness of selected individuals. In terms of short-term response, reducing N_{Sel} often negated any advantage of varying the fitness of selected individuals though the Unequal Fitness or Better Half methods. Consider the genetic model of 40 QTL, $N = 100$, and $H = 0.80$. At cycle 1, the response with a constant $N_{Sel} = 10$ was significantly higher with the Better Half method (1.69) than with Equal Fitness method (1.49). But when N_{Sel} was not held constant the maximum responses with the Better Half and Equal Fitness methods were no longer significantly different from each other (1.88 for the Better Half method with $N_{Sel} = 3$, versus 1.91 for the Equal Fitness method with $N_{Sel} = 2$). In the Equal Fitness method, the increased response due to a lower N_{Sel} was most evident in cycle 1, to the point that the Better Half and Unequal Fitness methods were never superior to the Equal Fitness method in the first cycle of selection (Table 1).

Among the 60 comparisons for short-term response (i.e., four genetic models, three levels of H, and cycles 1–5), the maximum response with the Unequal Fitness method was numerically higher than the maximum response with Equal Fitness method in 28 instances (47%; Fig. 3). The maximum response with the Better Half method was numerically higher than the maximum response with the Equal Fitness method in 42 instances (70%). Yet even with the large number of repeats (1000) of the simulation experiments, most of the differences were statistically insignificant ($P = 0.05$). The maximum response with the Unequal Fitness method was significantly higher than the maximum response with the Equal Fitness method in only two instances (3%). The maximum response with the Better Half method was significantly higher than the maximum response with the Equal Fitness method in only eight instances (13%). Overall, these results indicated that simply reducing N_{Sel} is superior to varying the fitness of selected individuals in short-term phenotypic selection. The appropriate N_{Sel} in phenotypic selection will be discussed later in the Rule-of-Thumb for N_{Sel} section.

Marker-assisted Recurrent Selection

The Equal Fitness, Unequal Fitness, and Better Half methods did not lead to significant differences in the

Table 1. Response (in units of the genetic standard deviation in cycle 0) to phenotypic recurrent selection with different numbers (N_{Sel}) and fitness of selected individuals in phenotypic recurrent selection.

	Equal Fitness						Unequal Fitness						Better Half				
Cycle	N_{Sel} = 2	N_{Sel} = 3	N_{Sel} = 4	N_{Sel} = 5	N_{Sel} = 10	N_{Sel} = 20	N_{Sel} = 3	N_{Sel} = 4	N_{Sel} = 5	N_{Sel} = 10	N_{Sel} = 20	N_{Sel} = 40	N_{Sel} = 3	N_{Sel} = 4	N_{Sel} = 5	N_{Sel} = 10	N_{Sel} = 20
40 QTL, N = 100, H = 0.20†																	
1	1.00‡	0.97	0.93	0.86	0.72	0.63	0.98	0.90	0.88	0.77	0.70	0.73	0.99	0.95	0.92	0.84	0.72
2	1.34	1.42	1.50	1.44	1.28	1.21	1.46	1.47	1.44	1.38	1.31	1.30	1.37	1.45	1.50	1.44	1.27
3	1.79	2.00	2.07	2.10	1.93	1.62	1.94	2.02	2.01	1.98	1.90	1.87	1.92	2.02	2.11	2.05	1.85
4	2.06	2.37	2.53	2.56	2.44	2.11	2.42	2.53	2.52	2.51	2.42	2.38	2.33	2.50	2.59	2.59	2.37
5	2.39	2.83	2.95	3.04	2.90	2.60	2.76	2.93	2.97	3.01	2.94	2.86	2.70	2.93	3.06	3.09	2.86
10				4.65	4.90	4.59				5.09[a]	5.04	4.89				5.14[a]	5.06
15				5.65	6.27	6.15				6.44	6.47[a]	6.30				6.42	6.55[a]
20				6.21	7.06	7.24				7.23	7.32	7.20				7.06	7.46[a]
25				6.38	7.66	7.97				7.70	7.87	7.77				7.49	8.10[a]
30				6.46	7.89	8.42				7.90	8.16	8.10				7.77	8.51
40 QTL, N = 100, H = 0.50																	
1	1.63	1.47	1.47	1.28	1.16	0.94	1.46	1.43	1.41	1.24	1.24	1.22	1.51	1.45	1.46	1.23	1.03
2	2.35	2.32	2.29	2.27	2.08	1.64	2.38	2.31	2.39	2.15	2.15	2.13	2.30	2.28	2.42	2.23	1.94
3	3.12	3.15	3.22	3.20	2.93	2.44	3.20	3.21	3.23	3.02	2.98	2.98	3.20	3.20	3.23	3.12	2.75
4	3.73	3.90	3.98	3.96	3.62	3.08	3.90	3.97	3.96	3.79	3.76	3.74	3.87	3.89	4.03	3.85	3.45
5	4.21	4.46	4.60	4.61	4.39	3.71	4.50	4.60	4.67	4.52	4.46	4.43	4.53	4.62	4.68	4.57	4.17
10				6.69	6.85	6.35				7.03[a]	6.98	6.94				7.01[a]	6.80
15				7.57	8.08	7.89				8.19	8.20[a]	8.17				8.15	8.24[a]
20				7.81	8.56	8.64				8.62	8.68	8.67				8.60	8.87[a]
25				7.96	8.80	9.01				8.77	8.84	8.84				8.67	9.04
30				8.02	8.84	9.08				8.81	8.91	8.95				8.78	9.20[a]
40 QTL, N = 100, H = 0.80																	
1	1.91	1.86	1.77	1.74	1.49	1.12	1.87	1.76	1.71	1.57	1.56	1.54	1.88	1.81	1.83	1.69	1.44
2	3.03	3.01	2.98	2.92	2.57	2.11	3.05	2.99	2.92	2.73	2.67	2.64	3.05	3.00	3.03	2.84	2.47
3	4.11	4.13	4.10	3.98	3.56	3.02	4.13	4.10	4.05	3.78	3.70	3.69	4.15	4.08	4.17	3.86	3.39
4	4.97	5.06	5.03	4.96	4.51	3.81	5.07	5.01	5.01	4.73	4.61	4.66	5.16	5.12	5.09	4.89	4.36
5	5.64	5.75	5.76	5.74	5.25	4.58	5.84	5.83	5.82	5.56	5.47	5.44	5.81	5.82	5.98[a]	5.69	5.14
10				7.96	7.96	7.42				8.18[a]	8.12	8.15				8.28[a]	8.04
15				8.53	8.84	8.78				8.92	8.94[a]	8.93				8.85	8.99[a]
20				8.50	8.97	9.15				9.08	9.09	9.07				9.05	9.30[a]
25				8.57	9.02	9.25				9.11	9.11	9.11				9.12	9.38[a]
30				8.55	8.99	9.26				9.11	9.14	9.15				9.04	9.31

† Number of QTL, number of individuals in each cycle of selection (N), and broad-sense heritability (H). Results are shown only for the 40 QTL, N = 100 model.

‡ In each cycle, an underline indicates the maximum response obtained across the three methods.

[a] Maximum response for Unequal Fitness or Better Half was significantly greater (P = 0.05) than the maximum response for Equal Fitness. The least significant difference ranged from approximately 0.10 to 0.15.

maximum responses (i.e., across different N_{Sel} values) to MARS (Table 2). Given that MARS involved one cycle of marker-assisted selection (based on phenotypic values and marker scores) followed by three cycles of marker-based selection (based on marker scores only), the response to MARS represented a short-term response. The lack of advantage of the Better Half and Unequal Fitness methods over the Equal Fitness method in MARS was therefore consistent with the similar lack of advantage in short-term phenotypic selection. As in short-term phenotypic selection, varying N_{Sel} was superior to varying the fitness of selected individuals in MARS.

Under the Unequal Fitness method, the three ways of modeling the genetic variance in a cross between two individuals (Expected Variance, Immediate Variance, and Equal Variance) did not lead to any significant difference or clear trend in the response to MARS. The (i) lack of superiority of the Unequal Fitness method over the Equal Fitness method and the (ii) lack of significant differences among the Expected Variance, Immediate

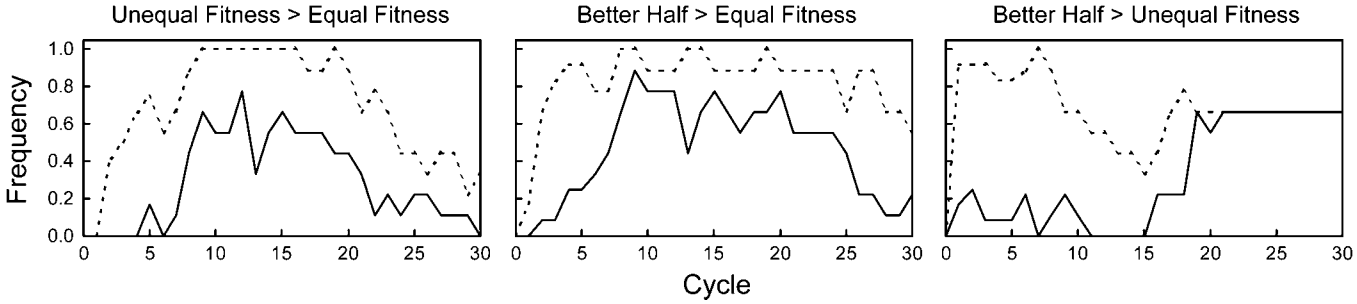

Fig. 3. Frequencies of maximum responses (across different N_{Sel}) being higher due to varying the fitness of selected individuals. The dotted lines indicate that the maximum response is numerically higher, whereas the solid lines indicate the maximum response is significantly higher at P = 0.05. Comparisons for short-term selection (cycles 1–5) are across four genetic models (10 QTL, N = 100; 40 QTL, N = 100; 100 QTL, N = 100; and 40 QTL, N = 400) and three levels of heritability (H = 0.20, 0.50, and 0.80). Comparisons for medium-term (cycles 6–15) and long-term (cycles 16–30) selection are across three genetic models (10 QTL, N = 100 excluded) and the three levels of H.

Table 2. Maximum response (in units of the genetic standard deviation in cycle 0) to marker-assisted recurrent selection for Equal Fitness, Unequal Fitness assuming three methods of modeling the variance in a cross (Immediate Variance, Expected Variance, and Equal Variance), and Better Half methods of determining the contribution of selected individuals to the next generation.

		Unequal Fitness			
Model	Equal Fitness	Expected Variance	Immediate Variance	Equal Variance	Better Half
10 QTL, N = 100, H = 0.20	1.68 (2)†	1.77‡ (3)	1.67 (5)	1.65 (3)	1.73 (3)
10 QTL, N = 100, H = 0.50	2.69 (2)	2.61 (5)	2.71 (3)	2.64 (4)	2.66 (3)
10 QTL, N = 100, H = 0.80	3.25 (2)	3.21 (3)	3.27 (4)	3.23 (4)	3.34 (3)
40 QTL, N = 100, H = 0.20	1.43 (4)	1.41 (4)	1.37 (10)	1.37 (4)	1.41 (5)
40 QTL, N = 100, H = 0.50	2.39 (2)	2.37 (5)	2.44 (5)	2.41 (5)	2.41 (5)
40 QTL, N = 100, H = 0.80	3.10 (3)	3.13 (3)	3.07 (5)	3.16 (3)	3.13 (5)
100 QTL, N = 100, H = 0.20	1.22 (10)	1.19 (10)	1.17 (10)	1.18 (10)	1.13 (10)
100 QTL, N = 100, H = 0.50	2.03 (10)	2.03 (3)	2.06 (5)	2.04 (4)	2.04 (5)
100 QTL, N = 100, H = 0.80	2.72 (4)	2.70 (5)	2.79 (4)	2.76 (4)	2.73 (4)
40 QTL, N = 400, H = 0.20	3.03 (3)	3.05 (4)	3.06 (3)	2.99 (10)	3.06 (4)
40 QTL, N = 400, H = 0.50	4.15 (4)	4.16 (4)	4.13 (4)	4.14 (4)	4.21 (4)
40 QTL, N = 400, H = 0.80	4.84 (4)	4.77 (4)	4.82 (4)	4.78 (4)	4.88 (4)

† Results are given for whichever significance level (0.20, 0.30, or 0.40) that led to the largest response. The number of selected individuals (N_{Sel}) that led to the largest response is in parentheses.

‡ An underline indicates the largest response obtained across methods. None of the maximum responses for Unequal Fitness or Better Half was significantly greater (P = 0.05) than the maximum response for Equal Fitness, with least significant differences ranging from approximately 0.12 to 0.14.

Variance, and Equal Variance methods could first be due to poor predictions of the genetic variance in a cross. In accordance with previous studies, we used relaxed significance levels (α = 0.20, 0.30, or 0.40) for detecting QTL and considered a population size (N = 100) typically used in MARS (Hospital et al., 1997; Johnson, 2001). The use of relaxed significance levels typically led to the detection of 20 to 40 markers with significant effects for the quantitative trait (results not shown). In a previous study we found that for the 100 QTL, N = 100, H = 0.20 genetic model, the use of α = 0.30 led to an average of 37 markers declared significant (Bernardo and Charcosset, 2006). But the variance due to these significant markers was twice the amount of V_G, indicating an overestimation of QTL effects (Beavis, 1994). Given these previous results, we initially speculated that estimating the effects of 20 to 40 markers from a population size of N = 100 would not lead to sufficiently precise predictions of genetic variances. The results for the 10 QTL, N = 100 model, the 40 QTL, N = 100 model, and the 100 QTL, N = 100 model confirmed this speculation. However, a larger population size of N = 400 (i.e., 40 QTL, N = 400 model) still did not lead to any advantage of the Unequal Fitness method over the Equal Fitness method in MARS. This result suggests that even larger population sizes should be used, although in practice population sizes larger than 400 would be prohibitive. This result may also suggest that the contribution of differences in genetic variances to the fitness of crosses is intrinsically limited when compared to that of the mean, but we were unable to confirm this speculation, which deserves further investigation.

Medium- and Long-Term Response

For medium-term (cycles 6–15) response to phenotypic selection, the Better Half and Unequal Fitness methods were usually superior to the Equal Fitness method (Fig. 3). Consider the genetic model of 40 QTL, N = 100, and H = 0.20 (Table 1). In cycles 1 to 5 (short-term response), the three methods lead to similar maximum responses. But in cycle 10 (medium-term response), the maximum responses with the Better Half (5.14) and Unequal Fitness methods (5.09) were both significantly higher than the maximum response with the Equal Fitness method (4.90).

Among 90 comparisons for medium-term response (i.e., three genetic models, three levels of H, and cycles 6–15), the maximum responses with the Unequal Fitness or Better Half methods were numerically higher than the maximum response with the Equal Fitness method in 82 instances (91%; Fig. 3). Furthermore, the maximum response with the Unequal Fitness method was significantly higher than the maximum response with the Equal Fitness method in 42 instances (47%), whereas the maximum response with the Better Half method was significantly higher than the maximum response with the Equal Fitness method in 59 instances (66%). The advantage of the Better Half method was greatest in the middle cycles of medium-term selection (cycles 9–12). Across all cycles, the maximum responses were usually numerically higher with the Better Half method than with the Unequal Fitness method, although most of the differences were insignificant. Overall, these results indicated that the Better Half method is useful for increasing the response to medium-term selection.

For long-term response (cycles 16–30), the superiority of the Better Half and Unequal Fitness methods over the Equal Fitness method tended to diminish toward the later cycles of selection (Fig. 3). Again consider the genetic model of 40 QTL, N = 100, and H = 0.20 (Table 1). Compared to the Equal Fitness method, the Better Half method continued to have significantly higher maximum responses in cycles 20 and 25. By cycle 30, however, the maximum response with the Better Half method was no longer significantly higher than the maximum response with the Equal Fitness method. For this genetic model, the maximum responses with the Unequal Fitness method in cycles 20, 25, and 30 were not significantly higher than the maximum responses with the Equal Fitness method. Among 135 comparisons for long-term response (i.e., three genetic models, three levels of H, and cycles 16–30), the maximum response with the Better Half method was significantly higher than the maximum

response with the Equal Fitness method in 62 instances (46%; Fig. 3). The maximum response with the Unequal Fitness method was significantly higher than the maximum response with Equal Fitness method in only 37 instances (27%).

Based on theory, we expected the Unequal Fitness method to be superior to the Better Half method. It was unclear why the Unequal Fitness method was not superior to the Better Half method. The Better Half method depends on rankings of selected individuals, whereas the Unequal Fitness method depends on estimates of their mean performance. In practice, ranks are generally considered more robust and less prone to error than estimates of mean performance, and this robustness may have contributed to the superiority of the Better Half method over the Unequal Fitness method. From a practical standpoint, the Better Half method is also simpler and easier to apply in a breeding program than the Unequal Fitness method.

There was no clear difference among methods in the variance of the selection response (i.e., across the 1000 repeats of the simulation experiments). In general, the effect of the Equal Fitness, Unequal Fitness, and Better Half methods on the variance of the response was minor compared with the effects of the cycle of selection (larger variance of response at later cycles), heritability (larger variance of response at lower H), and N_{Sel} (larger variance of response at lower N_{Sel}). Consider the 40 QTL, $N = 100$ genetic model. For $N_{Sel} = 20$ and $H = 0.80$, the variance of the response in cycle 1 was 1.60 with Equal Fitness, 1.60 with Unequal Fitness, and 1.59 with the Better Half method. For the Better Half method, this variance of the response in cycle 1 increased to 1.76 when H was reduced from 0.80 to 0.20, and it increased further to 2.02 when H was reduced from 0.80 to 0.20 and N_{Sel} was reduced from 20 to 3. With $H = 0.80$ and $N_{Sel} = 20$, the variance of the response increased from 1.59 in cycle 1 to 2.54 in cycle 30.

The effective population size is maximized when the selected parents contribute equal numbers of gametes to the next generation (Falconer, 1981, p. 67). For a given N_{Sel}, the effective population size is therefore larger with the Equal Fitness method than with the Unequal Fitness and Better Half methods. The results therefore indicated that, for a given N_{Sel}, the reduction in effective population size due to variable fitness in the Unequal Fitness and Better Half methods has only a very limited effect on the variance of the response.

Rule-of-Thumb for N_{Sel}

If changing N_{Sel} is preferable to varying the fitness of selected individuals, particularly in short-term selection, what value of N_{Sel} should be used? To maximize the genetic gain within cycle 0, the optimum number of selected individuals should be $N_{Sel} = 1$ (i.e., select the best individual in cycle 0 and develop an inbred or a cultivar directly from it). To maximize the genetic gain achieved in cycle 1, the best two individuals in cycle 0 should be selected and crossed to form cycle 1; selecting the third-best cycle 0 individual would decrease the selection differential and, consequently, decrease response to selection achieved in cycle 1. So if the number of selected individuals should be $N_{Sel} = 1$ to identify the best individual in cycle 0 and $N_{Sel} = 2$ to maximize the gain achieved in cycle 1, would the appropriate number of selected individuals be $N_{Sel} = 3$ for selection until cycle 2, $N_{Sel} = 4$ for selection until cycle 3, $N_{Sel} = 5$ for selection until cycle 4, and N_{Sel} for selection until cycle $N_{Sel} - 1$?

This rule-of-thumb for determining the optimum N_{Sel} is depicted by the dotted diagonal lines in Fig. 4, for $N_{Sel} = 2$, 3, 4, 5, 10, 15, 20, 25, 30, 35, and 40 and for cycles of selection equal to $N_{Sel} - 1$ until cycle 29. The size of the plotted circles in Fig. 4 is proportional to the number of times, across the different genetic models studied, a particular N_{Sel} led to the maximum response in a given cycle of selection, with N_{Sel} being constant across cycles of selection. For the Equal Fitness method, the rule-of-thumb gave good predictions of the optimum N_{Sel}, particularly for short-term selection. In medium- to long-term selection, the optimum N_{Sel} varied mostly between $N_{Sel} = 10$ and 30. Nevertheless, the rule-of-thumb provided good approximations of the mean of the optimum N_{Sel} values for medium- and long-term selection. This rule-of-thumb obviously recognizes that for short-term recurrent selection where maintaining

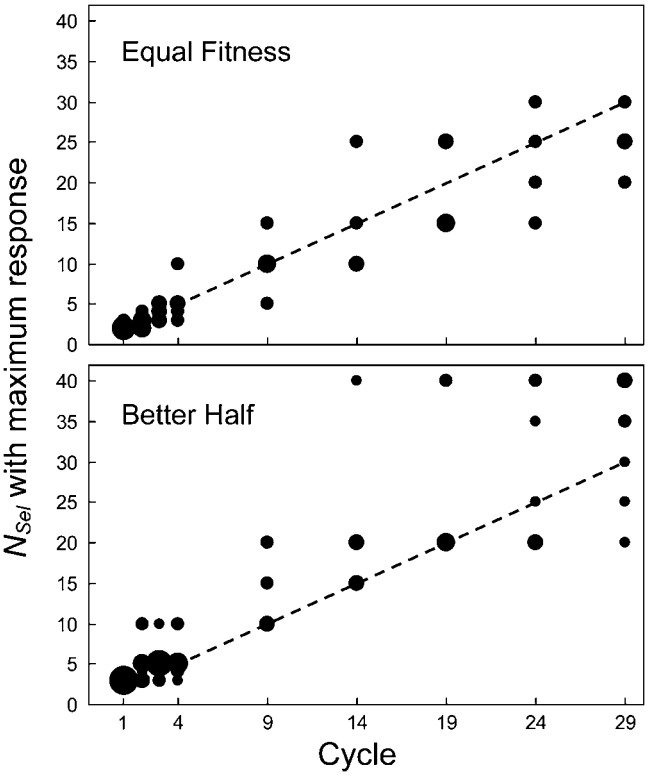

Fig. 4. Values of N_{Sel} that led to the maximum response to selection at different cycles of recurrent selection. N_{Sel} values of 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, and 40 were considered. The size of the plotted circles corresponds to the number of times, across the different genetic models studied, a particular N_{Sel} value led to the maximum response. The dotted lines depict the proposed rule-of-thumb that for cycle $N_{Sel} - 1$, the optimum number of individuals to select and recombine in each cycle is equal to N_{Sel}.

genetic variance is not of primary concern, selecting only a few individuals in each cycle would maximize the selection differential and the short-term response. On the other hand, in long-term selection a large number of individuals should be kept in each cycle to maintain genetic variance and sustain the response to selection. As we mentioned in the previous section, selecting a large number of individuals would also lead to less variability in the response.

For the Better Half method, the optimum N_{Sel} values were mostly larger than those predicted by the rule-of-thumb. This result was expected because, compared with the Equal Fitness method, the Better Half method achieves the same selection differential with a larger number of selected individuals. In this situation, the rule-of-thumb would be useful for determining the minimum number of individuals that should be selected and recombined using the Better Half method.

We were unable to derive an analytic proof for this simple rule-of-thumb. How useful it will be in practice remains to be seen, as breeders usually do not specify in advance how many cycles of recurrent selection will be conducted in a breeding population. Yet to the extent that the duration of a recurrent selection program is planned, this rule-of-thumb should provide a useful guide for determining N_{Sel}. As we previously mentioned, the number of individuals selected in recurrent selection programs typically ranges from $N_{Sel} = 10$ to 30 (Kenworthy and Brim, 1979; Hallauer, 1992; Weyhrich et al., 1998). These values of N_{Sel} are larger than those we are recommending for short-term selection. Perhaps part of the reason for these larger N_{Sel} values is because selection programs are initiated without specifying the number of cycles to be conducted, larger N_{Sel} values are used so that sufficient genetic variance will be retained should selection be continued in the long-term. We argue, however, that the low N_{Sel} values we recommend for short-term selection are consistent with those used in inbred development. Maize inbreds are usually developed from the cross between only two inbreds (Hallauer, 1992). We reason that if breeders can successfully develop elite inbreds from the cross of only two parental inbreds, then N_{Sel} values of 2 to 5 are reasonable for short-term recurrent selection.

In MARS, the N_{Sel} values that led to the maximum response ranged from 2 to 10 but were mostly between 2 and 4 (Table 2). These numbers of selected individuals are lower than those used in MARS in maize (Edwards and Johnson, 1994). Given that MARS comprises four cycles of selection, the rule-of-thumb indicates that $N_{Sel} = 5$ should be used in MARS. The differences in response in MARS with $N_{Sel} = 2, 3, 4$, or 5 were usually small (results not shown). Consider the genetic model of 40 QTL, $N = 100$. When heritability was $H = 0.20$, the response to MARS with the Equal Fitness method was 1.31 with $N_{Sel} = 2$, 1.36 with $N_{Sel} = 3$, 1.43 with $N_{Sel} = 4$, and 1.42 with $N_{Sel} = 5$. When the heritability increased to $H = 0.80$, the response was 3.10 with $N_{Sel} = 2$, 3.10 with $N_{Sel} = 3$, 3.09 with $N_{Sel} = 4$, and 3.04 with $N_{Sel} = 5$.

In conclusion, simply reducing N_{Sel} is superior to varying the fitness of selected individuals in short-term phenotypic selection. Varying the fitness of selected individuals is most useful in medium-term recurrent selection. We recommend the Better Half method over the Unequal Fitness method because of its simplicity and because it remained superior to the Equal Fitness method over more cycles of selection. Varying the fitness of selected individuals is not useful in marker-assisted recurrent selection, which is short-term. As a rule-of-thumb, we suggest that N_{Sel} should be roughly equal to the number of cycles for which selection will be conducted.

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REFERENCES

Beavis, W.D. 1994. The power and deceit of QTL experiments: Lessons from comparative QTL studies. p. 250–256. *In* Proc. 49th Annu. Corn Sorghum Ind. Res. Conf. 7–8 Dec. 1994, Chicago, IL. Am. Seed Trade Assoc., Washington, DC.

Bernardo, R. 2004. What proportion of declared QTL in plants are false? Theor. Appl. Genet. 109:419–424.

Bernardo, R., and A. Charcosset. 2006. Usefulness of gene information in marker-assisted recurrent selection: A simulation appraisal. Crop Sci. 46:614–621.

Bost, B., C. Dillmann, and D. de Vienne. 1999. Fluxes and metabolic pools as model traits for quantitative genetics. I. The L-shaped distribution of gene effects. Genetics 153:2001–2012.

Brim, C.A., and J.W. Burton. 1979. Recurrent selection in soybeans. II. Selection for increased percent protein in seeds. Crop Sci. 19:494–498.

Edwards, M., and L. Johnson. 1994. RFLPs for rapid recurrent selection. p. 33–40 *In* Analysis of molecular marker data. Joint Plant Breeding Symposium Series, Am. Soc. Hort. Sci., CSSA, Madison, WI.

Falconer, D.S. 1981. Introduction to quantitative genetics. 2nd ed. Longman, London.

Frankham, R., L.P. Jones, and J.S.F. Barker. 1968. The effects of population size and selection intensity in selection for a quantitative character in *Drosophila*. I. Short-term response to selection to selection. Genet. Res. 12:249–266.

Guzman, P.S., and K.R. Lamkey. 2000. Effective population size and genetic variability in the BS11 maize population. Crop Sci. 40: 338–346.

Hallauer, A.R. 1992. Recurrent selection in maize. Plant Breed. Rev. 9:115–179.

Hospital, F., L. Moreau, F. Lacoudre, A. Charcosset, and A. Gallais. 1997. More on the efficiency of marker-assisted selection. Theor. Appl. Genet. 95:1181–1189.

Johnson, L. 2001. Marker assisted sweet corn breeding: A model for specialty crops. p. 25–30. *In* Proc. 56th Annu. Corn Sorghum Ind. Res. Conf. 5–7 Dec. 2001, Chicago, IL. Am. Seed Trade Assoc., Washington, DC.

Johnson, R. 2004. Marker-assisted selection. Plant Breed. Rev. 24(1): 293–309.

Jones, L.P., R. Frankham, and J.S.F. Barker. 1968. The effects of population size and selection intensity in selection for a quantitative character in *Drosophila*. II. Long-term response to selection. Genet. Res. 12:249–266.

Kenworthy, W.J., and C.A. Brim. 1979. Recurrent selection in soybeans. I. Seed yield. Crop Sci. 19:315–318.

Koebner, R. 2003. MAS in cereals: Green for maize, amber for rice, still red for wheat and barley. *In* Marker assisted selection: A fast track to increase genetic gain in plant and animal breeding? 17–18 Oct. 2003, Turin, Italy. FAO, Rome. www.fao.org/biotech/docs/Koebner.pdf (verified 25 May 2006).

Lande, R. 1980. Genetic variation and phenotypic evolution during allopatric speciation. Am. Nat. 116:463–479.

Lande, R., and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756.

Press, W.H., S.A. Teukolsky, W.T. Vetterling, and B.P. Flannery. 1992. Numerical recipes in Fortran: The art of scientific computing. 2nd ed. Cambridge Univ. Press, New York.

Rawlings, J.O. 1979. Long- and short-term recurrent selection in finite populations—Choice of population size. p. 201–217. *In* F.T. Corbin (ed.) World Soybean Res. Conf. II: Proc. Westview Press, Boulder, CO.

Robertson, A. 1960. A theory of limits in artificial selection. Proc. R. Soc. Lond. B. Biol. Sci. 153:234–249.

Senior, M.L., E.C.L. Chin, M. Lee, J.S.C. Smith, and C.W. Stuber. 1996. Simple sequence repeat markers developed from maize sequences found in the GENBANK database: Map construction. Crop Sci. 36:1676–1683.

Weyhrich, R.A., K.R. Lamkey, and A.R. Hallauer. 1998. Effective population size and response to S_1–progeny selection in the BS11 maize population. Crop Sci. 38:1149–1158.