Theor Appl Genet (1997) 95 : 1181–1189 © Springer-Verlag 1997

F. Hospital · L. Moreau · F. Lacoudre
A. Charcosset · A. Gallais

More on the efficiency of marker-assisted selection

Received: 11 July 1997 / Accepted: 4 August 1997

Abstract Computer simulations were used to study the efficiency of marker-assisted selection (MAS) based on an index combining the phenotypic value and the molecular score of individuals. The molecular score is computed from the effects attributed to markers by multiple regression of phenotype on marker genotype. The results show that in the first generation the ratio RE of the expected efficiency of MAS over the expected efficiency of purely phenotypic selection generally increases when considering: (1) larger population sizes, (2) lower heritability values of the trait, and (3) a higher type-I error risk of the regression. This is consistent with previously published results. However, at low heritabilities our results point out that response to MAS is more variable than response to phenotypic selection. Hence, when the difference of genetic gains is considered instead of their ratio, RE, the heritability values corresponding to maximal advantage of using MAS rather than phenotypic selection are still low, but higher than predicted based on RE. The study over several successive generations of the rate of fixation of QTLs shows that the higher efficiency of MAS on QTLs with large effects in early generations is balanced by a higher rate of fixation of unfavourable alleles at QTLs with small effects in later generations. This explains why MAS may become less efficient than phenotypic selection in the long term. MAS efficiency therefore depends on the genetic determinism of the trait. Finally, we investigate a modified MAS method involving an alternation of selection on markers with and without phenotypic evaluation. Our results indicate that such a selection method could at low cost, provide an important increase in the genetic gain per unit of time in practical breeding programs.

Key words Marker-assisted selection · Computer simulations

Communicated by G. Wenzel

Frédéric Hospital (✉) · L. Moreau · F. Lacoudre
A. Charcosset · A. Gallais
Station de Génétique Végétale, INRA/UPS/INA-PG,
Ferme du Moulon, 91190 Gif sur Yvette, France
Fax: (33)(1) 69 33 23 40
e-mail: fred@moulon.inra.fr

Introduction

The information provided by the genotype at molecular markers can be used in breeding programs to better estimate the genetic value of the individuals submitted to selection. Lande and Thompson (1990) proposed a method of marker-assisted selection (MAS) utilizing the linkage disequilibrium created by hybridization between inbred lines. Selection is performed on an index combining phenotypic and marker information, the latter being derived from multiple regression of the phenotype on the marker genotype. The efficiency of MAS compared with phenotypic selection has received considerable attention in the recent past.

The efficiency of MAS for the first generation was first investigated in the analytic approach of Lande and Thompson (1990). Recently, Moreau et al. (1997) extended this approach in the case of finite population size. Also, the efficiency of MAS over several successive generations has been studied using computer simulations (Zhang and Smith 1992, 1993; Gimelfarb and Lande 1994 a, b, 1995 ; Wittaker et al. 1995). The main conclusions are that MAS could be more efficient than purely phenotypic selection in quite large populations and for traits showing relatively low heritabilities. Simulations showed that additional genetic gain provided by MAS, compared with purely phenotypic selection, rapidly decreased when several successive cycles of selection were considered, and that MAS could become less efficient than phenotypic selection in the long term. This problem is more accute when the effects

associated with markers are not re-evaluated at each generation. Wittaker et al. (1995) proposed to take into account the position of markers when predicting the breeding value. However, these authors showed that this only slightly improved the efficiency of MAS. Also, Wittaker et al. (1995) and Moreau et al. (1997) focussed on the choice of the markers to be included in the selection index, a problem first mentioned by Gimelfarb and Lande (1994 a,b). Moreau et al. (1997) showed that the optimal number of markers depends on the size of the population and the genetic parameters employed, and that increasing the type-I error risk associated with the detection of the effects attributed to markers can increase the efficiency of MAS at low heritabilities.

Our aim in this paper is to gain more insight into the efficiency of marker-assisted selection using computer simulations. We will first study the efficiency of MAS in the first generation, to see how the analytical results of Moreau et al. (1997), obtained with a simplified genetic model (unlinked quantitative trait loci, one marker per QTL, effects detected by ANOVA), compare with the simulation results obtained with a more realistic genetic model and multiple regression. In particular, we will focus on the choice of the type-I error risk associated with the introduction of markers in the selection index. The variation of the response to MAS will also be considered. Then, we will study the efficiency of marker-assisted selection over several successive generations, and the dynamics of rates of fixation of QTLs, in order to better understand the limitations of this selection method and its effects on genetic variability. Finally, we will investigate the optimal use of marker information over several successive generations by alternate selection on markers with and without phenotypic evaluation, in order to reduce experimental costs and increase the genetic gain per unit of time.

Methods

The efficiency of marker-assisted selection was studied by computer simulations in the F_2 and following populations derived from a cross between two homozygous inbred lines. We consider an additive genetic model. The quantitative trait submitted to selection is determined by q quantitative trait loci (QTLs), with two alleles per QTL (one favourable, one unfavourable). In addition we consider that molecular markers evenly spread on the genetic map are available. We assume that the original parental lines carry different alleles at each locus (marker or QTL).

Simulations

The phenotypic value (P_i) of individual i is computed as the sum of its genotypic (G_i) and environmental (E_i) values:

$$P_i = G_i + E_i. \tag{1}$$

The environmental value is assumed to be a random normal variable with mean 0 and variance σ^2_E. The genetic value is computed as:

$$G_i = \sum_{q=1}^{nq} x_q \theta_{iq}, \tag{2}$$

where x_q is the effect of the QTL q, and θ_{iq} is the number of favourable alleles carried by individual i at locus q, and nq is the total number of QTLs. We consider here $nq = 25$ QTLs. Following Lande and Thompson (1990), we assume that the genetic variances at the QTLs in the original F_2 follow a geometric series, so that:

$$x_q = a^{(q-1)/2}, \tag{3}$$

with $a = 9/11$, corresponding to an effective number of ten QTLs (Lande 1981).

It is assumed throughout this paper that there is no interference in recombination. We consider a genetic map of ten chromosomes, each 100 cM long, on which molecular markers are evenly spread with a given density, and with one marker at each chromosome end. Unless specified, results were obtained for 110 markers (i.e. with a 10-cM interval between adjacent markers on the same chromosome).

Simulation results are replicated. The number of replicates may vary with the parameter set from a few hundred to a thousand, and is indicated below where appropriate. For each replicate, QTL positions are first drawn at random over the entire genome. Also, the favourable allele at each locus is attributed at random to each of the two parental lines with equal probability, so that the phase can take any value between total coupling and total repulsion. The requested number of individuals forming the F_2 population is then generated. The genotypic value G_i is computed for each individual based on its genotype at the QTLs. The variance of G_i among these individuals is computed subsequently and will be denoted as $\hat{\sigma}^2_G$. Given the value of $\hat{\sigma}^2_G$ in the F_2, the expected environmental variance σ^2_E is then chosen such that it leads to the requested heritability h^2 in the F_2; σ^2_E then remains constant in the following generations. At each generation, one-tenth (10%) of the individuals are selected (see below), and then mated at random to form the next generation. The various parameters (see below) are computed in each replicate prior to selection.

Marker-assisted selection

Marker-assisted selection is performed using the method proposed by Lande and Thompson (1990). Multiple regression of the phenotype on the marker genotype was performed using SAS (1988). At each generation, regression is performed once, considering all the markers. Markers entering the model are selected using the stepwise variable selection method, for which two significance levels (F statistics) must be specified: *sle* for entry into the model, and *sls* for staying in the model. The molecular score M_i of individual i is then computed as:

$$M_i = \sum_{m=1}^{nm^*} c_m \theta_{im}, \tag{4}$$

where c_m is the effect attributed to marker m in the regression, θ_{im} is the genotype of individual i at marker m, and nm^* is the number of markers with significant effects in the regression.

The selection index value I_i for individual i is written as:

$$I_i = b_P P_i + b_M M_i. \tag{5}$$

The coefficients b_P and b_M are estimated using:

$$\hat{b}_M = \frac{\hat{\sigma}^2_P - \hat{\sigma}^2_G}{\hat{\sigma}^2_P - \hat{\sigma}^2_M} \quad \text{and} \quad \hat{b}_P = \frac{\hat{\sigma}^2_G - \hat{\sigma}^2_M}{\hat{\sigma}^2_P - \hat{\sigma}^2_M}, \tag{6}$$

where $\hat{\sigma}_P^2$, $\hat{\sigma}_G^2$ and $\hat{\sigma}_M^2$ are the estimates of phenotypic variance, genetic variance, and the genetic variance explained by the markers, respectively. Given the phenotypic variance, the genetic variance explained by the markers is estimated from the adjusted R^2 of the regression, noting that:

$$R^2_{\text{adj}} = 1 - \frac{N-1}{N - nm^* - 1}(1 - R^2) = \frac{\hat{\sigma}_M^2}{\hat{\sigma}_P^2}. \quad (7)$$

It was observed that, in some cases, the estimate $\hat{\sigma}_M^2$ of the genetic variance explained by markers exceeded the estimated genetic variance $\hat{\sigma}_G^2$. In such circumstances, applying (6) strictly would attribute a negative coefficient to the phenotypic value in the selection index. This was already noted by Wittaker et al. (1995). To avoid this, the value of b_P was given a lower bound. Trials performed using different bounding values (data not shown) showed that the best results were obtained with a low but strictly positive value (e.g., 10^{-3}). The interesting conclusion is that, even when almost all the genetic variance is apparently explained by the markers, it is still important to include the phenotype in the selection index, since phenotypic value can help in discriminating between individuals with identical molecular scores.

At each generation, selection can be performed on the phenotypic value only (P), on the marker-phenotype index (I), or on molecular score only (Mo), and the corresponding genetic gains are computed as:

$$\Delta G(t) = \frac{\bar{G}(t) - \bar{G}(0)}{\sigma_G(0)}, \quad (8)$$

where $\bar{G}(t)$ is the genetic mean of the population at generation t ($t = 0$ standing for the initial F_2), and where $\sigma_G(0)$ is the genetic standard deviation in the F_2.

The relative efficiency of marker-assisted selection was defined by Lande and Thompson (1990) as the ratio of the selection response obtained with the index defined in (5) over the selection response obtained with classical phenotypic selection. In the simulations, relative efficiency was computed as:

$$\text{RE} = \frac{\overline{\Delta G_I}(t)}{\overline{\Delta G_P}(t)}, \quad (9)$$

where $\overline{\Delta G_I}(t)$ and $\overline{\Delta G_P}(t)$ are the averages over replicates of the genetic gains (8) at generation t when selection is performed with the suffixed methods. Since individuals are randomly sampled, genetic conditions may vary between replicates. In the first generation, it is possible to ensure that the comparison is made in the same conditions for the two methods by applying marker-assisted selection and phenotypic selection to the same sample of individuals for each replicate. This was done to obtain the genetic gains in Figs. 1 and 2. This is not possible when several successive generations of selection are considered; thus genetic gains in other figures were estimated in independent sets of replicates.

Results and discussion

Relative efficiency in the first generation

Simulations of marker-assisted selection and phenotypic selection were performed for different population sizes, heritabilities, and significance levels of the regression. The corresponding relative efficiencies (9) after one generation of selection are shown in Fig. 1.

The relative efficiency of marker-assisted selection appears to depend mostly on population size. In small populations, effects attributed to markers are poorly estimated, and the power of detection is low (for given *sle* and *sls* values), while expected response to phenotypic selection in the first generation does not depend on population size. At a given heritability, RE increases with population size. Also, the effect of population size is more important at low heritabilities, because in this situation marker effects are poorly estimated and the power of detection decreases (Charcosset and Gallais 1996). This is consistent with the results of Lande and Thompson (1990), Zhang and Smith (1993), and Gimelfarb and Lande (1994 a), and was investigated in detail by Moreau et al. (1997).

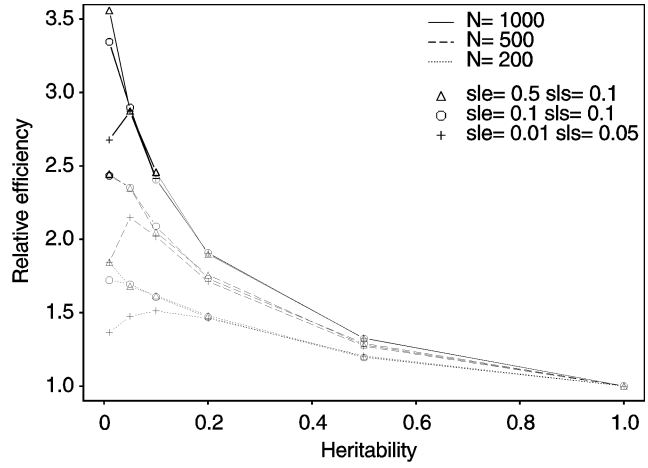

Fig. 1 Relative efficiency of marker-assisted selection in the first generation. RE from simulation results (ordinate) at different expected heritabilities ($h^2 = 0.01$, 0.05, 0.1, 0.2, 0.5, or 1, abscissa). At each heritability value, simulations were performed for three populations sizes N (line type, see legend) and three significance levels *sle* and *sls* (for symbols, see legend). Each data point shows the average over 300 replicates for $N = 1000$ and $N = 500$, and over 1000 replicates for $N = 200$

Heritability has contrasting effects on RE. When heritability tends to 1, genetic values are almost perfectly estimated by phenotypic values, so that marker-assisted selection can hardly do any better than phenotypic selection, and RE tends to 1. This effect was shown theoretically by Lande and Thompson (1990), and is verified in Fig. 1. When heritability tends to 0, the evolution of RE depends on the significance level. At a low significance level (1%), RE decreases when heritability tends to zero (Fig. 1). This was predicted by the analytic approach of Moreau et al. (1997) and is due to a decrease in the power of detection. At a high significance level (50%), RE increases when heritability tends to zero (Fig. 1). In this case, the increase in the power of detection overrides the increase in the detection of false positives. This was also predicted by Moreau et al. (1997). The only difference between the results in Fig. 1 and the analytic results of Moreau et al. (1997) is that a decrease in RE when heritability tends to zero was predicted by Moreau et al. (1997, Fig. 3) at

medium values of the significance level (10%), but is not observed in our Fig. 1. This may arise from the discrepancy in the assumptions regarding the genetic model (markers assumed unlinked in Moreau et al.) and more likely by the statistical method employed (the one-way ANOVA in Moreau et al. yields a lower power of detection compared with the multiple regression considered here, Jansen and Stam 1994). Note also that RE is very difficult to estimate in the simulations at low heritability values when both the response to marker-assisted selection and to phenotypic selection tends to zero (see below).

The effects of other parameters of the model on the relative efficiency of MAS were investigated, but were found of limited impact. The corresponding results are not shown, but only briefly mentioned. The distance between neighboring markers was varied from 5 cM to 50 cM, and the number of markers was varied accordingly (from 210 to 30, respectively). The efficiency of MAS was generally reduced with increasing distance between markers. In the first generation, the RE was also reduced for very small distances between markers, so that the 'optimal' distance was about 20 cM, which is consistent with the results of Gimelfarb and Lande (1994 a). According to Gimelfarb and Lande (1995), this is due to the fact that, when the distance between markers is small, the co-linearity in the regression increases, and then it is not always the nearest marker to a QTL which is included in the index. Note that this was no longer observed in the following generations, so that an optimal RE was then obtained for a distance between adjacent markers of about 5–10 cM. In any case, the results obtained with different distances between markers were not very different (except for very large population sizes), so that using low densities of markers could be a way of reducing the cost of MAS without affecting its efficiency.

Variation of response to selection in the first generation

In Fig. 1, the efficiencies of marker-assisted selection and phenotypic selection were studied using the parameter RE defined in (9) in order to compare our simulation results with previously published results. However, this parameter is not the most relevant when one is concerned with the putative efficiencies of both selection methods in one given experiment (the practical situation), because the variabilities of genetic progress around their means are not taken into account. We then compared the efficiencies of both methods for each replicate in the simulations.

Computing the ratio of genetic gains in one given replicate is meaningless, because numerator and denominator are very variable, can be close to zero, and/or can be of different signs. Rather, we chose to study for each replicate the *difference*, $\Delta G_I - \Delta G_P$, between genetic gains under the two methods, which gives

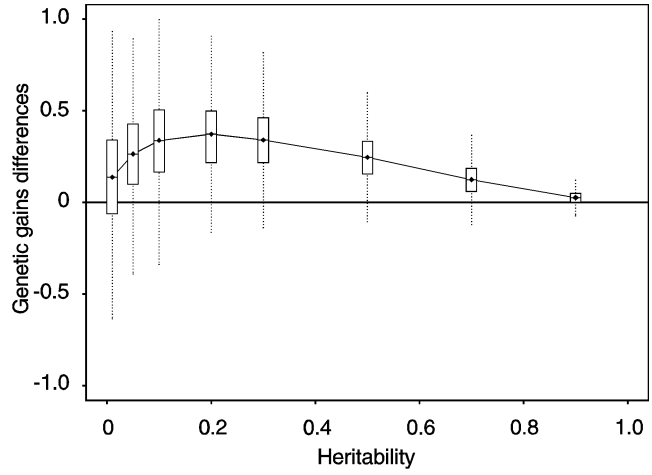

Fig. 2 Variability of additional genetic gain under marker-assisted selection. For the same simulations as Fig. 1 in the case $N = 200$, $sle = 0.5$ and $sls = 0.1$, *box-plots* of the distribution of the differences $\Delta G_I(1) - \Delta G_P(1)$ (ordinate, in standard units) over replicates are shown for different expected heritabilities ($h^2 = 0.01$, 0.05, 0.1, 0.2, 0.3, 0.5, 0.7 or 0.9, abscissa). *Diamonds* give the median, and *boxes* give the upper and lower quartiles. The span of whiskers (*dotted lines*) is 1.5-times the inter-quartile range

the algebraic advantage of using MAS rather than phenotypic selection. This is presented in Fig. 2 from the same simulations as Fig. 1 in the case where $N = 200$ and $sle = 0.5$. Though the ratio RE of genetic gains was found previously to be maximal at the lowest heritability value investigated ($h^2 = 0.01$, see Fig. 1), this does not correspond to maximal advantage (obtained for $h^2 = 0.2$ in the conditions of Fig. 2). Moreover, Fig. 2 shows that the advantage can be negative in some replicates, and that its variability is higher at low heritabilities, corresponding to a higher risk, in a given experiment, of marker-assisted selection being *less* efficient than phenotypic selection. For a breeder who is generally concerned with one single experiment, this may be considered to be too high a risk. Rather, the breeder may prefer to work in conditions where the chosen selection method is assured to be the most efficient in any case. This reinforces the interest of using MAS at medium heritability values (0.2–0.5). For larger population sizes (500, 1000) all values are moved upward (data not shown) so that advantages are less likely to be negative, but the general conclusions drawn from Fig. 2 still hold.

Long-term effects of marker-assisted selection

Responses to phenotypic and marker-assisted selection were compared over several successive generations. The corresponding results are given in Fig. 3, showing the genetic gains with the two methods over 50 generations.

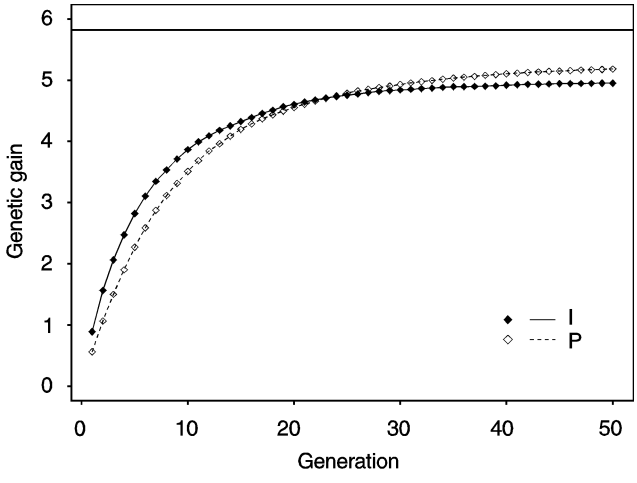

Fig. 3 Responses to phenotypic and marker-assisted selection over several successive generations. The genetic gains $\overline{\Delta G}(t)$ (ordinate, in standard units) at generation t (abscissa) are given for selection on the marker-phenotype index I (*filled diamonds and solid line*) or for purely phenotypic selection P (*open diamonds and dashed line*). The *horizontal line* at ordinate 5.82 shows the maximum possible genetic gain given QTL effects. Simulation results averaged over 200 replicates. $N = 200$, $sle = 0.5$, $sls = 0.1$, $h^2 = 0.1$

After 50 generations, the genetic gains provided by both marker-assisted and phenotypic selection are close to the maximum possible genetic gain of 5.82 [i.e. twice the expected genetic mean in the F_2 with the geometric-series distribution of QTL effects (3)], though this maximum expected value is never reached by either of the selection methods, even after a greater number of generations (data not shown). The ratio RE of genetic gains in Fig. 3 (data not shown) is approximately intermediate between the ones obtained by Gimelfarb and Lande for total coupling and total repulsion. Note that our simulations include random sampling of both phase and QTL positions in each replicate.

It can be seen from Fig. 3 that, in the long term, the response to marker-assisted selection becomes lower than the response to purely phenotypic selection. This was already observed by Gimelfarb and Lande (1994 a) for total repulsion, and was probably also observed for total coupling, though not enough generations are shown in their paper. In the particular case of Fig. 3, this occurs after generation 24. In order to understand why marker-assisted selection becomes less efficient than phenotypic selection in the long term, we studied the fixation of both alleles at each QTL in the simulations of Fig. 3.

The results are shown in Fig. 4 giving for the two methods and for each QTL the total fixation rate (i.e. fixation of either the favourable or the unfavourable allele) and the percentage of fixation for the unfavourable allele in the total. As expected, it can be seen from Fig. 4 (top) that total fixation is faster for QTLs with larger effects, and faster under marker-assisted selection than under phenotypic selection. This is consistent with the results of Zhang and Smith (1992). Also, the unfavourable allele is almost never fixed at QTLs with the largest effects, and is more often fixed at QTLs with smaller effects. It can be deduced from Fig. 4 (top and bottom) that fixation of the unfavourable allele is more frequent under marker-assisted selection than under phenotypic selection. Also, the discrepancy between fixations under the two methods is more important for QTLs with small effects. Note that the percentage of fixation for the unfavourable allele remains approximately constant until complete fixation at all QTLs (data not shown).

This explains why marker-assisted selection is less efficient than phenotypic selection in the long term. With the geometric series of QTL effects considered here, marker-assisted selection is more efficient than phenotypic selection on QTLs with the largest effects. This induces a higher selection intensity on QTLs with large effects and hence increases the probability of fixation of the unfavourable allele at QTLs with small effects, due to hitch-hiking (see for example Hospital and Chevalet 1996).

In the conditions of Figs. 3 and 4, the difference between response to selection under the two methods at the limit is not very important (Fig. 3). Since the favourable alleles at QTLs with large effects are almost always fixed under both marker-assisted selection and phenotypic selection (Fig. 4), the additional gain under phenotypic selection at the limit in Fig. 3 is mostly due to QTLs with small effects. Hence, the long-term superiority of phenotypic selection could be slightly more important if a larger number of QTLs was considered in the geometric series, since increasing nq in (3) amounts to adding more QTLs with small effects.

Efficiency of alternate selection on markers only

The efficiency of a MAS method called 'selection without marker re-evaluation' was investigated by Gimelfarb and Lande (1994 a). In this method, in the first cycle, the phenotype is evaluated, and all markers are genotyped and submitted to regression. Then, in subsequent cycles, the phenotype is evaluated but only the markers selected in the first cycle are genotyped and submitted to regression. The cost of genotyping is hence reduced but, strictly speaking, the effects attributed to markers *are* indeed evaluated at each cycle. The important consequence is that a cycle of selection 'without marker re-evaluation' in Gimelfarb and Lande (1994 a) does include the agronomic evaluation of all individuals. Thus, the duration of such a cycle is the same as the duration of a cycle of purely phenotypic selection, or of a cycle of MAS 'with marker re-evaluation'. In these conditions, the authors found that RE 'without re-evaluation' was always inferior to RE 'with

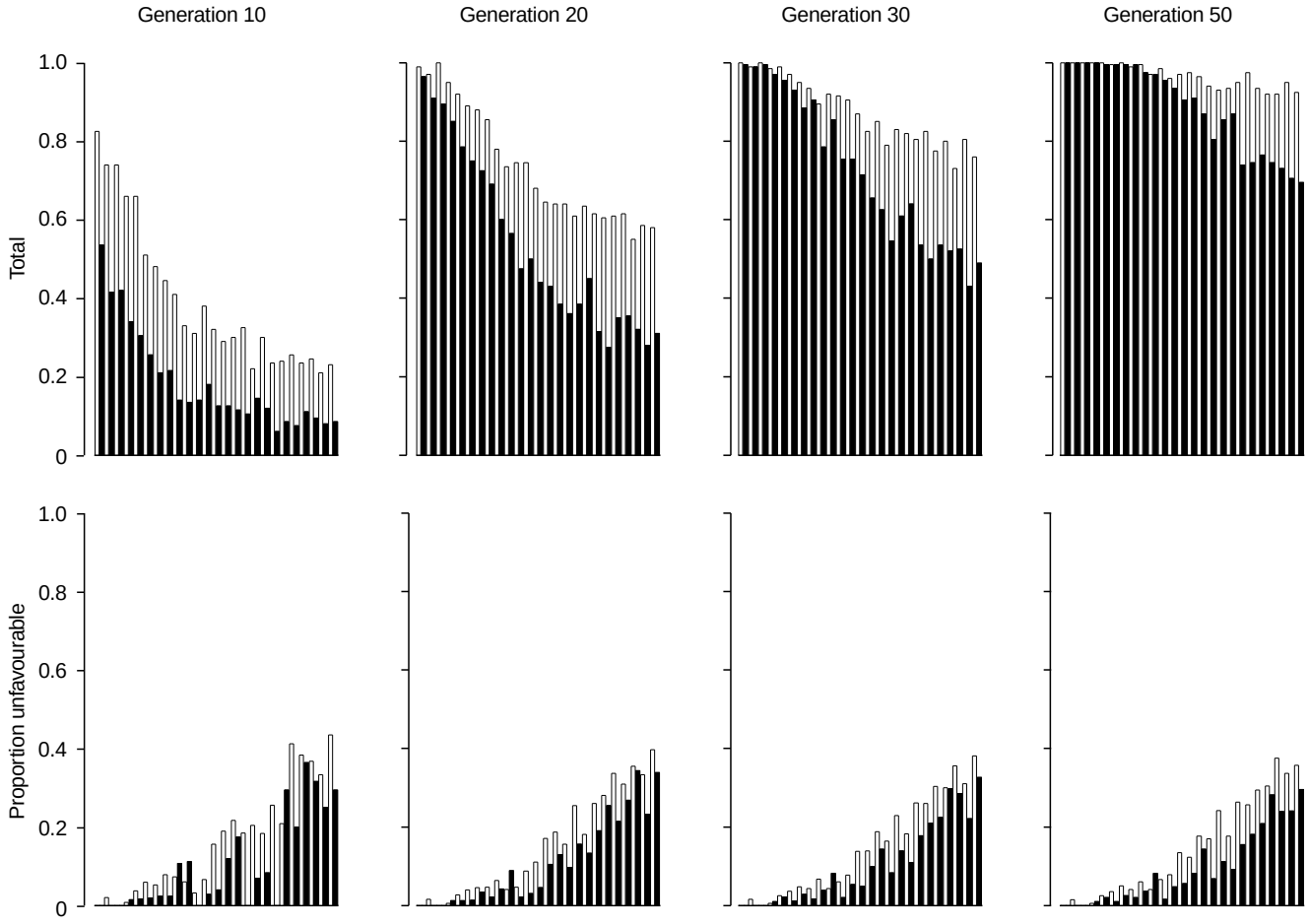

Fig. 4 Fixation rates for marker-assisted selection (*white*) and phenotypic selection (*black*) at different generations for the same simulations as Fig. 3. Abscissa: QTLs ranked with decreasing effects from left to right. Ordinate: proportion of replicates for which either of the two alleles was fixed (top) and corresponding percentage of replicates for which fixation is for the unfavourable allele (bottom)

re-evaluation' at each cycle. The same conclusion was also obtained by Zhang and Smith (1992) using the BLUP animal model.

Here we want to investigate a different method of selection on 'markers-only' (Mo). In this method, only the markers selected in a previous cycle with phenotypic evaluation are genotyped, but there is no phenotypic evaluation, and the effects attributed to those markers are not re-evaluated (the effects evaluated previously are used directly). The effects attributed to markers in a cycle of selection on markers-only may not be as well estimated as in the method proposed by Gimelfarb and Lande (1994 a). But, the important difference is that a cycle of selection on markers-only no longer necessitates the agronomic evaluation of the individuals. Compared with a cycle of selection on I or P, the cost of a cycle of selection on markers-only is then reduced and, more importantly, the duration of such a selection cycle can also be reduced in some cases. In fact, when the trait submitted to selection necessitates progeny testing (e.g. grain yield in maize, dairy production in cattle) a selection cycle involving agronomic measurement can last several years. Conversely, the duration of a cycle of selection on markers-only is restricted to the time necessary for the individuals to be genotyped and mated. In plants such a cycle can last 1 year, or less if off-season generations are available.

Once effects attributed to markers have been evaluated, it is possible to exploit marker information without phenotypic re-evaluation on a few following cycles, but not endlessly because linkage disequilibrium between markers and quantitative trait loci vanishes, and because markers submitted to selection get rapidly fixed. Hence, we propose to consider marker-assisted selection as an alternation of cycles of selection on index (I) with phenotypic evaluation (that allow effects attributed to markers to be re-evaluated) followed by one or a few cycles of selection on markers-only (Mo) without phenotypic evaluation. The efficiency of such a selection method is investigated in Figs. 5 and 6.

In Fig. 5, the genetic gains after one cycle of selection on the marker-phenotype index (I), possibly followed by one (I-Mo) or two (I-Mo-Mo) cycles of selection only on the markers detected in I cycle, are compared

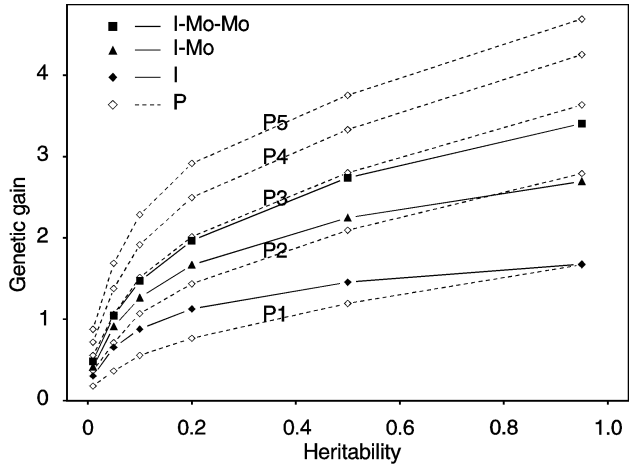

Fig. 5 Efficiency of alternate selection on markers with and without phenotypic evaluation in the first cycle. At different expected heritabilities ($h^2 = 0.01$, 0.05, 0.1, 0.2, 0.5 or 0.95, abscissa), the genetic gains (ordinate, in standard units) after one single generation of selection on index I with phenotypic evaluation (*filled diamonds*), or one generation of selection on I with phenotypic evaluation followed by one (*triangles*) or two (*squares*) generations of selection on marker score only (*Mo*) without phenotypic evaluation, can be compared with the genetic gain after one (*P1*) to five (*P5*) cycles of purely phenotypic selection (*open diamonds and dashed lines*, from bottom to top). $N = 200$, $sle = 0.2$, $sls = 0.1$

with the genetic gains provided by one (P1) to five (P5) cycles of purely phenotypic selection, for different heritabilities. The results in Fig. 5 show that, after the first cycle of selection on the marker-phenotype index, important additional genetic gains can be expected from one or even two cycles of selection on markers-only, even with the small population size considered ($N = 200$). At high heritabilities ($h^2 \geq 0.5$), one cycle of selection on the index followed by one cycle of selection on markers-only provide approximately the same genetic gain as two cycles of purely phenotypic selection, so that the additional gain provided by selection on markers-only is of about one cycle of phenotypic selection. This gain is even more important at lower heritabilities (about 1.5 cycle of phenotypic selection for $h^2 = 0.1$). In the conditions of Fig. 5, the additional gain provided by two cycles of selection on markers-only is approximately two cycles of phenotypic selection for any heritability.

The cost of a cycle of selection on markers-only is much lower than the cost of a cycle of MAS with phenotypic evaluation, because it does not include the measurement of the agronomic value of the individuals, and because molecular genotyping is only performed on the small subset of markers for which a significant effect has been detected during the previous evaluation step. Moreover, large population sizes are needed in marker-assisted selection mostly in order to increase the power of detection and the precision of the estimation of the effects attributed to markers, that is during the phenotypic evaluation step, and not during selection on markers-only without phenotypic evaluation. Hence, considering a large population size in the evaluation step, and smaller population sizes for selection on markers-only, might also reduce the cost of marker-assisted selection without reducing its efficiency very much. This deserves further investigation.

It is important to notice that when marker-assisted selection is considered as an alternation of cycles of selection with and without phenotypic evaluation, marker-assisted selection becomes more efficient than purely phenotypic selection over several successive cycles, even for traits with a high heritability ($h^2 \geq 0.5$). Again, this reinforces the interest of marker-assisted selection for traits with medium-to-high heritabilities, compared with the previous conclusions derived from the work of Lande and Thompson (1990).

The genetic gains per unit of time provided by several successive cycles (I-Mo-Mo) are shown in Fig. 6 for $N = 200$ and $h^2 = 0.5$, and can be compared with the gains provided by purely I or P selection. We consider, as an example, the case of an annual plant (e.g., maize) and a breeding scheme involving off-season generations, so that the duration of one P or I cycle is 2 years, and the duration of Mo cycles is 1 year. As expected, the additional gain provided by selection on markers-only (Mo) decreases as advanced selection cycles are considered. Yet, it is evident that performing selection on markers-only is efficient after the first two or three cycles of selection on I.

With the breeding scheme considered in Fig. 6, two cycles (I-Mo-Mo) last 6 years, and provide a genetic

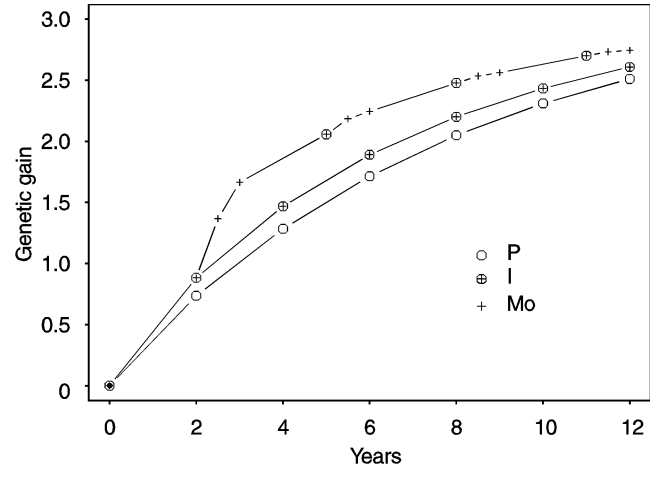

Fig. 6 Efficiency of alternate selection on markers with and without phenotypic evaluation over several successive cycles. Genetic gains (ordinate, in standard units) over 12 years (abscissa) of selection on phenotype only (*P*), or of repeated selection on marker-phenotype index with phenotypic evaluation (*I*), or of alternate selection on index I with phenotypic evaluation followed by selection on markers only without phenotypic evaluation (*Mo*). $N = 200$, $sle = 0.2$, $sls = 0.1$, $h^2 = 0.5$

gain of 2.24 standard units. Over the same duration, three P cycles are performed, providing a genetic gain of 1.71 standard units. Hence, even with the small population size considered in Fig. 6, after 6 years alternate selection on markers-only (I-Mo-Mo) provides up to a 31% additional genetic gain against phenotypic selection (P), compared with 11% for repeated selection on I against P. To put it in another way, in the conditions of Fig. 6 two (I-Mo-Mo) cycles in 6 years provide approximately the same genetic gain as 5 cycles of P in 10 years. Here, the gain is a gain in time, which can be of great importance when there is an advantage for breeding companies to release new improved genetic material on the market before competitors.

Finally, it is seen that, in the conditions of Fig. 6, performing Mo selection after the third I cycle provides only limited additional genetic gain. After 12 years, the genetic gain provided by alternate selection on markers-only (I-Mo-Mo) is only 9% greater than the gain provided by purely P selection. Note however that the genetic gain after 12 years of alternate selection on markers-only is obtained with only four cycles involving phenotypic evaluation, while six cycles of phenotypic evaluation are necessary in the case of purely P selection.

Whether alternate selection on markers-only is less expensive than purely phenotypic selection depends on the respective costs of molecular genotyping and agronomic evaluation. But, in any case, it is evident from Fig. 6 that alternate selection on markers-only (I-Mo-Mo) is more efficient *and* less expensive than repeated selection on I. In 6 years, alternate selection on markers-only provides 18% more genetic gain than repeated selection on I with only two cycles of evaluation instead of three. The total cost of the experiment is hence importantly reduced by about one-times the cost of the agronomic evaluation of all individuals plus their genotyping at all markers (minus twice the genotyping of all individuals at the few markers used for selection on markers-only, which is very low). In 12 years, the genetic gain is still 5% greater, with four cycles of evaluation instead of six.

Trials performed with $N = 500$ and the same conditions as in Fig. 6 (data not shown) indicate that even with a larger population size the additional genetic gain provided by Mo selection is limited after the second cycle (I-Mo-Mo). But, the genetic gain is greatly increased in the first two cycles, so that with $N = 500$, the genetic gains after 6 and 12 years are 42% and 14% greater, respectively, than the gains provided by purely P selection for the same times. As already noted in the case of purely I selection, this is because genetic gain under P selection for the first six cycles hardly depends on population size, while effects attributed to markers are better estimated in larger populations.

The study of the efficiency of alternate selection on markers-only was performed here under particular conditions (Fig. 6). An analytic study of the genetic gains per unit of time provided by alternate selection on markers-only in different breeding schemes was undertaken by Gallais et al. (1997). The results agree well with the simulations and show that using alternate selection on markers-only is expected to increase genetic gain per unit of time in the early selection cycles, compared with repeated marker-assisted selection on I.

The decrease of genetic variability under alternate selection on markers-only is expected to be faster than for repeated selection on I. As a consequence, the fixation of unfavourable alleles at QTLs with small effects due to hitch-hiking is also expected to be more important. Hence, long-term response to alternate selection on markers-only is expected to be even lower than what was previously observed for repeated selection on I (Fig. 3). Alternate selection on markers-only appears to be mostly interesting for a short-term objective; for example, for the fast production of new improved genotypes. At the extreme, an efficient marker-assisted breeding scheme could comprise only one cycle of phenotypic evaluation with a very large population size, allowing marker effects to be estimated as well as possible, followed by several cycles of selection on markers-only (possibly with a smaller population size) until complete fixation. The optimization of such a breeding scheme deserves more consideration.

Conclusions

Our simulation results obtained with a realistic genetic model validate the approximations made by Moreau et al. (1997). The RE in the first generation is higher when population size is larger, and when heritability is lower, though not too low. Also, increasing type-I error risk for the detection of the effects attributed to markers is beneficial in general, and should be recommended at low heritabilities.

After the first generation, recurrent selection on the marker-phenotype index is still more efficient than purely phenotypic selection for a few generations, but the advantage of using marker-assisted selection declines rapidly. Moreover, marker-assisted selection may become *less* efficient than phenotypic selection in the long term. This is consistent with the results of Gimelfarb and Lande (1994 a). Our results indicate that this is because the rate of fixation of unfavourable alleles at QTLs with small effects is higher under marker-assisted selection than under phenotypic selection. This drawback could be a consequence of the strong selection applied to QTLs with large effects under marker-assisted selection in early generations. One way of solving this problem is by reducing selection intensity (Hospital and Chevalet 1993), but this would reduce the efficiency of marker-assisted selection, which is not desired. In any case, this effect is of little practical importance, because it takes place after

a number of generations greater than the usual length of most breeding programs, and because, if QTL effects follow a geometric-series distribution, the loss on small-effect QTLs in the long term is small, compared with the gain on large-effect QTLs in the short term.

The main conclusion drawn from previously published works based on RE in the first generation, was that marker-assisted selection was only interesting for selection on quantitative traits with low heritabilities. Our results show that this conclusion must be reconsidered. First, if the additional genetic gain provided by marker-assisted selection, compared with phenotypic selection, is on an average highest at low heritability values (0.1 to 0.2), it is also more variable at these heritabilities, so that using markers is then more risky, especially when the population size is small. Conversely, using markers at medium heritability values (0.5 to 0.7) provides a smaller gain on average, but this gain is more assured. Second, and more importantly, if marker-assisted selection is compared with phenotypic selection over several successive generations in the framework of a breeding program involving an alternation of generations with and without phenotypic evaluation, then marker-assisted selection is also of interest if heritability is high. When heritability is high, the effects attributed to markers are better estimated in the phenotypic evaluation step, so that selection on markers-only without phenotypic evaluation is then efficient in the next generation, even for small population sizes. Moreover, the cost of marker-assisted selection in this context is greatly reduced. It seems to us that the work on marker-assisted selection should now focus on the optimization of such breeding schemes, which provide the most promising way of using markers and phenotype in selection strategies.

References

Charcosset A, Gallais A (1996) Estimation of the contribution of quantitative trait loci (QTL) to the variance of a quantitative trait by means of genetic markers. Theor Appl Genet 93: 1193–1201

Gallais A, Dillmann C, Hospital F (1997) An analytical approach of marker-assisted selection with selection on markers only. Proc 10th Meeting EUCARPIA Section Biometrics in Plant Breeding, Poznań, Poland, 14–16 May, 1997

Gimelfarb A, Lande R (1994 a) Simulation of marker-assisted selection in hybrid populations. Genet Res 63: 39–47

Gimelfarb A, Lande R (1994 b) Simulation of marker-assisted selection for non-additive traits. Genet Res 64: 127–136

Gimelfarb A, Lande R (1995) Marker-assisted selection and marker-QTL associations in hybrid populations. Theor Appl Genet 91: 522–528

Hospital F, Chevalet C (1993) Effects of population size and linkage on optimal selection intensity. Theor Appl Genet 86: 775–780

Hospital F, Chevalet C (1996) Interactions of selection, linkage and drift in the dynamics of polygenic characters. Genet Res 67: 77–87

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136: 1447–1455

Lande R (1981) The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99: 541–553

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

Moreau L, Charcosset A, Hospital F, Gallais A (1997) Marker-assisted selection efficiency in populations of finite size. Genetics (in press)

SAS (1988) SAS user's guide: statistics, version 6. SAS Institute, Cary, North Carolina

Wittaker JC, Curnow RN, Haley CS, Thompson R (1995) Using marker-maps in marker-assisted selection. Genet Res 66: 255–265

Zhang W, Smith C (1992) Computer simulation of marker-assisted selection utilizing linkage disequilibrium. Theor Appl Genet 83: 813–820

Zhang W, Smith C (1993) Simulation of marker-assisted selection utilizing linkage disequilibrium: the effects of several additional factors. Theor Appl Genet 86: 492–496