Efficient marker-based recurrent selection for multiple quantitative trait loci

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Summary

We studied the efficiency of recurrent selection based solely on marker genotypes (marker-based selection), in order to increase favourable allele frequency at 50 previously detected quantitative trait loci (QTLs). Two selection procedures were investigated, using computer simulations: (1) Truncation Selection (MTS), in which individuals are ranked based on marker score, and best individuals are selected for recombination; and (2) QTL Complementation Selection (QCS), in which individuals are selected such that their QTL composition complements those individuals already selected. Provided QTL locations are accurate, marker-based selection with a population size of 200 was very effective in rapidly increasing frequencies of favourable QTL alleles. QCS methods were more effective than MTS for improving the mean frequency and fixation of favourable QTL alleles. Marker-based selection was not very sensitive to a reduction in population size, and appears valuable to optimize the use of molecular markers in recurrent selection programmes.

1. Introduction

Lande & Thompson (1990) described a marker-assisted selection (MAS) scheme, in which selection progress is improved by combining phenotypic data with marker–trait associations (detected by multiple regression of phenotype on marker genotypes). The efficiency of this method has been studied by various authors using either analytic or simulation results (Zhang & Smith, 1992, 1993; Gimelfarb & Lande, 1994, 1995). The major conclusion of these studies is that population size is the most important parameter affecting the efficiency of marker-assisted selection, as it controls the power of detection of marker–trait associations (Moreau *et al.*, 1998). However, with a large experiment size, if marker-assisted selection is more effective than phenotypic selection in the first generation of selection, it may not be efficient enough to balance the additional cost induced by the molecular genotyping of individuals for a large number of markers (Moreau *et al.*, in press). Hospital *et al.*

(1997) proposed optimizing the use of molecular markers in recurrent selection programmes over several successive generations by alternating one selection cycle including phenotypic evaluation and detection of effects attributed to markers, with a few cycles of selection on 'markers only' (i.e. selection based solely on the genotype of markers with significant effect detected in the previous evaluation cycle). The authors showed that, even with a small experiment size (200), this alternate strategy of marker-assisted selection is efficient at reducing costs and increasing genetic gain per unit time compared with both recurrent selection on marker-phenotype index and phenotypic selection. However, with a small experiment size it is not efficient to perform more than two or three cycles of selection on 'markers only' after one evaluation cycle. With a small experiment size, marker effects are poorly estimated and need to be re-evaluated regularly. A better estimation of marker effects can be achieved by using a larger experiment size. Here, large populations are needed only during the evaluation cycles, and probably not during the cycles of selection on 'markers only'. Hence, an extreme strategy of marker-assisted selection based on

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the conclusions of Hospital *et al.* (1997) could consist of two distinct phases: (i) a single cycle of evaluation of marker effects with a very large population size, in order to detect a large number of quantitative trait loci (QTLs) with greater confidence, then (ii) an increase in the frequencies of favourable alleles at the QTLs detected in phase (i) through several generations of selection based only on the genotypes of the markers flanking those QTLs (marker-based recurrent selection), with as small a population size as possible.

The present paper is devoted to the study and optimization of such marker-based recurrent selection (MBRS), using stochastic computer simulations. Though phases (i) and (ii) above should eventually be studied simultaneously because of their possible interactions, it is first necessary to optimize phase (ii) (selection on detected QTLs), which has received much less attention in the past than phase (i) (QTL detection). In this first study, we formally disconnect QTL detection from QTL selection, reserving a combined study for future work. It is then important to note that we assume that phase (i) above has been successfully performed previously, and we focus only on phase (ii). In other words, the problems related to the efficiency of QTL detection (e.g. detection of false positive or 'ghost' QTLs) are not considered. Here, we do not wish to predict an overall efficiency of MAS in real conditions, but rather design the best marker-based selection method. In practice the overall efficiency of MAS will be a combination of the efficiency of QTL detection and the efficiency of marker-based selection, and hence probably below the sole efficiency of MBRS investigated here.

From this methodological standpoint, we consider a hypothetical starting point for marker-based selection, where in phase (i) a large number of QTLs (50) have been detected with good precision, and where each QTL is flanked by two markers. This large number of detected QTLs is consistent with experimental results obtained in maize (Openshaw & Frascaroli, 1997), provided that QTLs are pooled over several traits (e.g. in a multi-trait selection index).

2. Methods

(i) *Genetic model*

We consider 10 chromosomes, each carrying five QTLs. We assume no interference in recombination. For the sake of simplicity, we assume that QTLs represent single loci, located in the middle of their corresponding marker brackets. The distance between a QTL and its flanking markers is the same for all QTLs and is given by parameter d_{MQ} (thus the total marker bracket length is $2d_{MQ}$). The distance between adjacent marker brackets (i.e. the distance from the 'right' marker of a bracket to the 'left' marker of the next bracket) is fixed at d_{MM}. In addition to the markers flanking the QTLs and submitted to selection (simply called 'markers' herein), other markers not submitted to selection ('neutral markers') are considered in order to study the evolution of genetic variability outside QTL marker brackets. Neutral markers are evenly spread on the genome, except within QTL marker brackets, with fixed distance 2 cM between them. Positions of QTLs and markers on a chromosome are outlined in Fig. 1.

The initial population (generation 0), consists of 1000 F_2 (or F_4) individuals derived from a cross between two homozygous inbred lines. We assume that the F_1 is completely heterozygous for QTLs, markers of QTLs and neutral markers. Each marker bracket is attributed the same allele as its corresponding QTL in the parent lines. This is done for the sake of programming simplicity and has no impact on the results. However, the parent that brings the favourable allele is drawn at random and is allowed to differ from one QTL and corresponding marker bracket to the next, and from one simulation to another.

(ii) *Selection objective*

The selection objective is here to increase the frequency of the favourable allele at all QTLs in the population, up to complete fixation if possible. In this context, equal weights are assigned to QTLs in the selection procedures, regardless of the estimated effects of the QTLs. This strategy is consistent with the selection objective above, based on a deterministic prediction of the evolution of QTL allele frequencies (i.e. not taking account of drift), starting from equal frequencies in the F_2 (J. C. M. Dekkers, personal communication). However, attributing equal weights to QTLs might not be optimal, even for the selection objective, but the theory in this domain remains largely unexplored (see also Section 4). QTL weights might take account of the linkage between the QTLs: some trials were performed with QTL weights depending on the distance between QTLs carrying
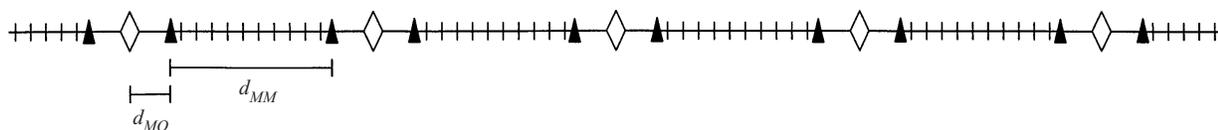

Fig. 1. Positions of QTLs and markers on a chromosome. ◇, QTL; ▲, selected flanking marker; |, neutral marker.

favourable alleles in different ways, but none provided a better selection response than equal weighting of QTLs (results not shown). Nevertheless, this deserves more theoretical work. Also, QTLs might be weighted according to favourable allele frequency (J. C. M. Dekkers, personal communication). This was not investigated in this paper. Rather, we suggest a selection method based on 'QTL complementation' (QCS, see below), which is another way of (implicitly) taking account of favourable QTL allele frequencies in the selection process.

Hence, QTLs are considered as equally important in all the selection procedures investigated here. If estimated QTL effects differ, one could wish to favour QTLs of large effects in the selection index. This would increase short-term genetic gain on large-effect QTLs, at the expense of a reduced overall genetic gain in the longer term. Such a strategy is a matter for economic considerations which are beyond the scope of the present paper and require specific methods (Dekkers & van Arendonk, 1998). Moreover, attributing unequal weights to QTLs might be a risky strategy if the observed distribution of detected QTL effects does not reflect the distribution of their true effects, due to various causes (Beavis, 1994; Bost *et al.*, in preparation).

(iii) *Selection scheme*

Starting from the initial population, we perform several cycles of marker-based recurrent selection (MBRS) as follows. At each generation (g), each individual among a total of $N(g)$ individuals is attributed a 'molecular score' (MS) based on its genotype at marker brackets. Then $N'(g)$ individuals are selected based on their molecular scores, and possibly other considerations (described below). These $N'(g)$ individuals are then mated at random and $N(g+1)$ offspring are generated, which form the population at the next generation ($g+1$). Various outputs are computed among the offspring *before* selection (e.g. alleles frequencies at QTLs and markers, fixation rates), and the process is iterated. Outputs are then averaged over 100 simulations with the same parameter set, each simulation starting from a different initial population (with parental genotypes drawn at random as described above).

The various aspects of the optimization of marker-based recurrent selection investigated include: computation of the MS, the selection method and selection intensity. In addition, the effects of different input parameters of the model were studied. Most simulations were performed with a 'base' parameter set (initial population F_2, $N(g > 0) = 200$, $d_{MQ} = 5$ cM, $d_{MM} = 20$ cM). The effects of the variation of parameters about their 'base' values were investigated, usually for one parameter at a time.

Table 1. *Bracket scores*

Marker genotypes		Exact[c]				
		$d_{MQ} = 5$ cM		$d_{MQ} = 10$ cM		
$\theta_1{}^a$	$\theta_2{}^b$	F_2	F_4	F_2	F_4	Approximate
0	0	0·005	0·014	0·020	0·050	0
0	1	0·502	0·509	0·510	0·531	0·5
1	0	0·502	0·509	0·510	0·531	0·5
1	1	1·000	1·000	1·000	1·000	1
0	2	1·000	1·000	1·000	1·000	1
2	0	1·000	1·000	1·000	1·000	1
1	2	1·498	1·491	1·490	1·469	1·5
2	1	1·498	1·491	1·490	1·469	1·5
2	2	1·995	1·986	1·980	1·950	2

[a,b] Number of favourable alleles at left and right markers, respectively.
[c] Expected number of favourable QTL alleles given marker genotypes.

(iv) *Molecular score (MS)*

Each individual is attributed a value (molecular score, MS) based on its genotype at the markers. To compute the molecular score, each marker bracket is first attributed a value based on the genotypes at the two markers and then these values are combined over all marker brackets.

Let $M_{1,q}$ and $M_{2,q}$ be respectively the 'left' and 'right' markers flanking QTL q and forming marker bracket q. The value attributed to this marker bracket is θ_q. Let $\theta_{1,q}$ and $\theta_{2,q}$ be the numbers of favourable alleles carried by $M_{1,q}$ and $M_{2,q}$, respectively. Since we want to increase the number of favourable alleles at the QTLs via selection on the marker brackets, we can set θ_q values to the expected number of favourable alleles at the QTL, given the genotypes at flanking markers (i.e. the probability of being heterozygous at the QTL, plus twice the probability of being homozygous for the favourable allele, given marker genotypes). These 'exact' values were computed using the algorithm provided by Hospital *et al.* (1996), and are given in Table 1 for F_2 and F_4 individuals. It is interesting to note that probabilities for marker genotypes $(\theta_{1,q}, \theta_{2,q}) = (0, 2)$, $(2, 0)$ and $(1, 1)$ are strictly equal to each other, and take a constant value of 1 regardless of recombination rate or generation number (with no selection). This is due to the symmetries in the frequencies of genotypes derived from a cross between two inbred lines (Hospital *et al.*, 1996). Conditional probabilities for other marker genotypes do depend on recombination rates, but, for the marker–QTL distances considered, it is seen from Table 1 that 'exact' conditional probabilities hardly differ numerically from the 'approximate' ones:

$$\theta_q = (\theta_{1,q} + \theta_{2,q})/2. \tag{1}$$

Indeed, simulations performed with either 'exact' or 'approximate' values gave very similar results (data not shown), and 'approximate' values of (1) were used for the sake of simplicity.

Different formulae for the combination of marker bracket scores into the molecular score were investigated but did not prove to be more efficient than the simple sum of bracket scores

$$MS = \sum_q \theta_q \qquad (2)$$

which was then used throughout.

(v) Truncation selection (MTS)

The first method of selection investigated is individual molecular score truncation selection (MTS), in which all $N(g)$ individuals are first ranked according to their MS values and then the best $N'(g)$ individuals are selected for reproduction.

Different values of $N'(g > 0)$ were investigated. Since population size in the initial generation (1000) was assumed here to differ from population size in subsequent generations, we wondered how many individuals should be selected in the initial generation. To be consistent, this number could have been chosen such that the ratio $N'(g)/N(g)$ is constant for any g. However, trials performed with different values (results not shown) indicated that $N'(0)/N(0) = 20/1000$ generally gave the best results after 10 generations of selection, regardless of the numbers of individuals selected in subsequent generations (though the impact of $N'(0)$ was never very important). This is consistent with our results on variable selection intensity (see Section 3). Hence, the fixed value $N'(0) = 20$ was used throughout.

A MTS strategy is described by one parameter: $N' = N'(g > 0)$, and will be referred to as MTS(N') hereafter.

(vi) QTL complementation selection (QCS)

The QTL complementation selection (QCS) method is intended to avoid negative fixations at QTLs, by ensuring that each favourable QTL allele is carried by at least n_T of the individuals selected. The algorithm used in the simulations is as follows.

As in MTS, individuals are first ranked based on their overall molecular scores. Individuals with equal MS are ranked based on their numbers of QTLs for which the favourable allele is 'present'. A favourable allele at QTL q is declared 'present' in an individual if the bracket score is above a given threshold ($\theta_q \geqslant \theta_T$). For example, if $\theta_T = 1$ we decide that the favourable QTL allele is present in an individual if this individual is expected to carry at least one copy of the favourable QTL allele, given the genotypes at flanking markers. Then: (i) the first N'_0 individuals are selected; (ii) we identify the QTLs for which the favourable alleles are 'present' in fewer than n_T selected individuals; (iii) among the remaining individuals, taken in order of decreasing MS, we look for the individual having favourable alleles 'present' at the greatest number of those QTLs identified in (ii). This individual is added to the subset of selected individuals. Steps (ii) and (iii) are iterated until either of the following conditions is met: favourable alleles at all QTLs are present in at least n_T individuals of the selected subset, or the number of individuals in the selected subset reaches a given maximal value (10), or it is not possible to find an individual in step (iii). N' is here the number of individuals selected at the end of the QCS procedure. If the complementation criterion is fulfilled during step (i), then the process is interrupted, so fewer than N'_0 individuals may be selected in some cases.

It is important to note that in step (i) individuals are taken based on their absolute MS values, while in step (iii) individuals are taken based on their ability to complement the subset of already selected individuals, which is a variable criterion. In step (iii), MS is a secondary criterion: if several individuals are found to complement the subset for the same number of QTLs, then only the individual with highest overall MS is selected. The QCS procedure above is as effective as a linear programming approach aimed at finding the best subset of N' individuals that would have both (i) desired frequency of all favourable QTL alleles, and (ii) largest MS value. Though linear programming was difficult to include in the simulation routine, in some trials it was checked that the individuals selected by the linear programming approach were often exactly the same as the individuals selected by the QCS simulation procedure.

A QCS strategy is described by three parameters: θ_T, the threshold for marker bracket score, above which a favourable QTL allele is declared 'present'; n_T, each QTL is requested to be 'present' in at least n_T selected individuals; N'_0, the size of the first kernel of individuals selected solely on their individual MS values, prior to complementation. Such a strategy will be referred to as QCS(θ_T, n_T, N'_0) hereafter.

(vii) Outputs

To investigate MBRS efficiency, we studied the variation over generation number g of the mean frequency $f(Q^+)$ of the favourable QTL alleles in the population. When the rate of improvement per generation decreases with time, small numerical differences in $f(Q^+)$ between two selection methods may hide important differences in selection efficiencies (when, for example, several additional generations of selection are needed to compensate for a small

difference in $f(Q^+)$). In order to provide a more complete evaluation of MBRS efficiency, we also studied the variation over generations of the percentages of QTLs fixed for the favourable (FixQ$^+$), or unfavourable (FixQ$^-$) allele, the fixation rates FixM$^+$ and FixM$^-$ for the markers submitted to selection, and the percentage FixN of 'neutral' markers fixed for either one of the two alleles.

3. Results

(i) *Truncation selection (MTS)*

The results for truncation selection with the base parameter set are presented in Fig. 2 and Table 2 for $N'(g > 0) = 2, 4, 10, 20, 30, 40$ or 50. Larger numbers of individuals selected were also investigated, but led to lower selection efficiencies (results not shown). The first conclusion that must be drawn from Fig. 2 and Table 2 is that all $f(Q^+)$ values at 10 generations of selection are remarkably high, the highest being $f(Q^+) = 94.2\%$ for $N' = 10$. Generations at which fixation at all flanking markers is reached (FixM$^+$ + FixM$^-$ = 100%) are indicated by tilded g values in Table 2. Results for $g > \tilde{g}$ are not given because, once the flanking markers are completely fixed, it is no longer possible to select for the favourable QTL alleles, and QTL allele frequencies then evolve under pure genetic drift, unless new marker–QTL associations are found and/or phenotypic selection is applied. At the limit, the strategies with $N' \geqslant 20$ provide a perfect 100% rate of favourable fixations at the flanking markers,

but none of the strategies was able to exceed a maximal value of $f(Q^+) = 96\%$ at the QTLs, due to recombination events between QTLs and flanking markers.

As expected, it can be seen from Fig. 2 that the strategies with highest selection intensities ($N' = 2$ or 4) give the best selection responses in early generations ($g = 5$) but then reach a plateau faster than the strategies with lower selection intensities. At generation $g = 10$, $N' = 2$ is clearly not the best strategy; the mean favourable allele frequency for $N' = 4$ is only slightly lower than that for $N' = 10$ or 20, but it is clear that the $N' = 4$ strategy has reached its maximum efficiency, while $N' = 10$ or 20 have not. This is supported by the results for fixations (Table 2). At generation 10, fixation of all QTL alleles (FixQ$^+$ + FixQ$^-$) is close to 100% for $N' = 2$ and $N' = 4$, and much lower for $N' = 10$ and 20. More importantly, fixations for the unfavourable alleles are close to zero for $N' = 10$ and 20, while they are 11% and 5% for $N' = 2$ and 4, respectively.

For $N' = 2$, fixations of unfavourable alleles occur very rapidly ($g = 2$, data not shown). At short and mid term ($g = 5$), $N' = 4$ could be chosen for rapid fixation of the favourable allele (60%), but at the expense of 3% fixations of unfavourable alleles; while for $N' = 10$, almost 62% of favourable fixations is achieved at generation 8 with less than 1% of unfavourable fixations. $N' = 10$ appeared to provide the highest $f(Q^+)$ at generation 10 as well as at the limit. Moreover, the variance of the response for $N' = 10$ (SD = 1.8) is about the same as for lower
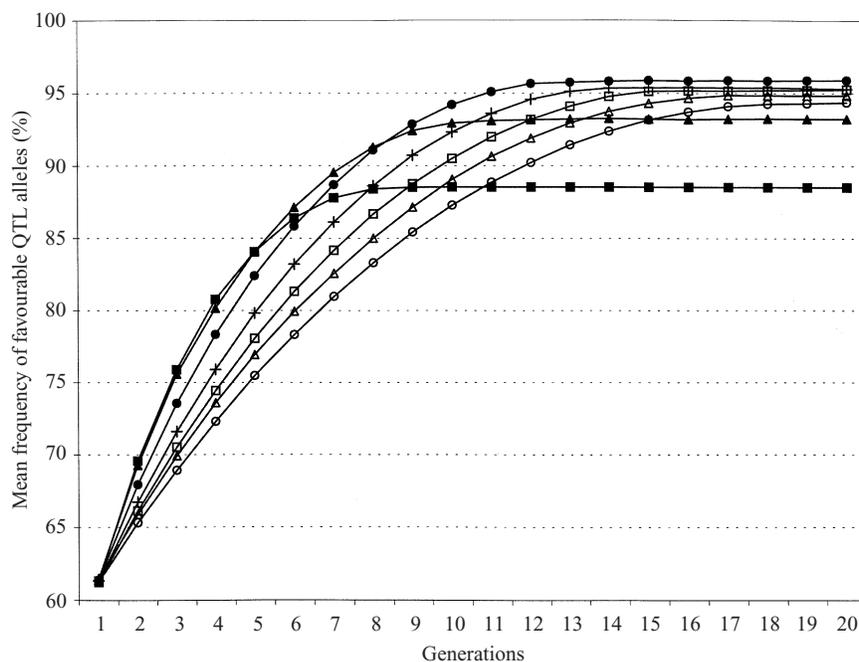

Fig. 2. Efficiency of truncation selection (MTS) for different selection intensities. Symbols indicate the number N' of individuals selected at each generation ($g > 0$) among a total of 200: ■, 2; ▲, 4; ●, 10; +, 20; □, 30; △, 40; ○, 50. Abscissa, generation; ordinate, mean frequency of favourable QTL allele (%).

Table 2. *Efficiency of truncation selection (MTS) for different selection intensities*

N'[a]	g[b]	$f(Q^+)$[c]	FixQ^{+}[d]	FixQ^{-}[e]	FixN[f]	FixM^{+}[g]	FixM^{-}[h]
2	5	84·1 ± 0·5	74·9	9·3	81·6	76·8	9·0
	1̃0	88·5 ± 0·8	88·1	11·0	98·1	90·4	9·6
4	5	84·0 ± 0·5	59·9	2·9	58·4	62·0	3·1
	10	92·9 ± 0·5	89·7	5·0	89·8	95·1	3·9
	1̃5	93·2 ± 0·6	92·2	5·9	95·3	96·1	3·9
10	5	82·4 ± 0·4	31·4	0·2	25·8	32·6	0·2
	10	94·2 ± 0·4	76·5	0·7	63·0	84·9	0·5
	1̃5	95·9 ± 0·4	89·6	1·0	78·4	99·5	0·5
20	5	79·8 ± 0·4	10·9	0·0	8·9	12·3	0
	10	92·3 ± 0·4	51·2	0·0	38·1	60·8	0
	1̃5	95·4 ± 0·3	78·9	0·1	59·3	100·0	0
30	5	78·1 ± 0·4	4·0	0	3·1	4·4	0
	10	90·5 ± 0·3	31·9	0	22·3	39·0	0
	1̃7	95·2 ± 0·3	70·8	0	49·4	100·0	0
40	5	76·9 ± 0·4	1·7	0	1·5	2·1	0
	10	89·1 ± 0·3	20·4	0	14·3	25·3	0
	1̃9	94·8 ± 0·3	65·9	0	44·0	100·0	0
50	5	75·5 ± 0·3	0·6	0	0·4	0·8	0
	10	87·3 ± 0·3	11·7	0	8·6	15·1	0
	2̃0	94·4 ± 0·3	57·6	0	37·6	100·0	0

[a] Number of individuals selected at each generation ($g > 0$) among a total of 200.
[b] Generation; values with tilde indicate generation at which complete marker fixation is reached.
[c] Average over 100 simulations of mean frequency of favourable QTL alleles (in per cent), and associated 95 % confidence interval (average ± 1·96 SE).
[d–h] Averages over 100 simulations of percentages of loci fixed for: [d] favourable or [e] unfavourable QTL allele; [f] either of neutral alleles; [g] favourable or [h] unfavourable selected marker allele.

selection intensities (SD $\simeq$ 1·7 for $N' \geqslant 20$), while for higher selection intensities the variance is much higher (SD = 2·8 for $N' = 4$ and SD = 4·0 for $N' = 2$). The more important hazards associated with the latter methods could make them less attractive to breeders. Further conclusions that can be drawn from the results in Fig. 2 and Table 2 depend on the aims of the breeding programme.

If only the MBRS step is considered, and the aim is to provide improved genetic material that is homozygous at QTLs, then one must take into account the possibility that the favourable QTL alleles, which were not fixed at $\tilde{g}$, might be lost afterwards due to genetic drift. Hence, the choice of the most efficient strategy must be based not only on $f(Q^+)$ but also on the percentage of favourable fixations at QTLs. The choice of an optimal selection intensity would then depend on the number of generations of MBRS that the breeder wishes to perform. For five generations, $N' = 2$ produces the most homozygous material with the highest favourable fixations at QTLs. For 10 generations, the maximum FixQ$^+$ is provided by $N' = 4$. Note that if MBRS is to be followed by some generations of continued fixation with no selection (e.g. repeated selfing), then $f(Q^+)$ at the time selection was interrupted estimates the final percentage of QTLs fixed for the favourable allele at the time of complete fixation. In that case, $N' = 10$ would also be a good choice.

If MBRS is only an intermediate step in a breeding programme aimed at improving genetic value not only for the traits controlled by the QTLs considered here but also for other QTLs and/or other traits, then one should favour strategies that increase favourable QTL allele frequencies while maintaining genetic variability outside the QTL segments. This can be estimated from the results for FixN. Fixations of neutral markers at generation 10 for $N' = 2$ and 4 are very high, indicating that these strategies do not maintain genetic variability outside the QTL segments. Conversely, $N' = 20$ or 30 could be a good solution to maintain genetic variability and still increase mean favourable allele frequency at QTLs above 90 % in the population.

(ii) *QTL complementation selection (QCS)*

QCS efficiency is controlled by three parameters: θ_T, n_T and N'_0. Various trials were performed to investigate the effects of these parameters, and identify their optimal values. We will first discuss the effects of the parameters (results not shown), then describe the results for some relevant values (Table 3).

In most cases, the best value of θ_T is 1 (i.e. selection for individuals expected to be either heterozygous or homozygous for the favourable allele at QTLs). Compared with $\theta_T > 1$, the individuals selected at the beginning are possibly heterozygous at the QTLs, but they carry favourable alleles at a greater number of

Table 3. *Efficiency of QTL complementation selection (QCS) for $\theta_T = 1$, $n_T = 3$ and $N'_0 = 3$*

g	N^a	$f(Q^+)$	FixQ$^+$	FixQ$^-$	FixN	FixM$^+$	FixM$^-$	N'	LRS^b
0	1000	50·0±0·0	0·0	0·0	0·0	0·0	0·0	5·4	166·4
1	200	61·6±0·3	0·8	0·0	0·6	0·7	0·0	5·6	68·3
2	200	68·2±0·4	7·2	0·0	7·0	7·7	0·0	5·6	57·2
3	200	73·9±0·5	18·2	0·0	17·0	19·3	0·0	5·4	36·8
4	200	79·1±0·6	32·7	0·1	29·5	34·0	0·0	5·0	17·4
5	200	83·6±0·6	47·0	0·3	42·0	48·8	0·2	4·5	9·4
6	200	87·3±0·6	60·2	0·4	53·8	62·3	0·4	3·9	6·2
7	200	90·6±0·6	72·5	0·9	64·8	75·1	0·6	3·5	3·7
8	200	92·9±0·6	81·6	1·3	74·5	85·3	0·8	3·2	3·3
9	200	94·6±0·5	88·0	1·7	82·3	92·6	0·9	3·0	3·0
10	200	95·4±0·5	91·7	2·1	87·2	96·6	0·9	—	—

[a] Total number of individuals genotyped at each generation.
[b] Lowest Ranked Selection, rank of the individual selected with lowest MS value.

QTLs. There are two main advantages in selecting possibly heterozygous individuals rather than only homozygous ones. First, when individuals complementary for their QTLs are mated, their offspring carrying favourable transgressions are most likely to be heterozygous. These interesting individuals have a chance to be selected only if selection of heterozygotes is allowed. Secondly, selecting individuals heterozygous at QTLs will reduce the negative correlations induced by selection between alleles carried at different QTLs (Bulmer effect: Bulmer, 1971; Hospital & Chevalet, 1996). This will increase the overall MS value of the individuals selected, and reduce the chances of negative fixations. Though $\theta_T = 0.5$ provided higher FixQ$^+$ values than $\theta_T = 1$ for some particular parameter sets, it always led to lower $f(Q^+)$ values and resulted in higher fixations of unfavourable QTL alleles and neutral markers.

When n_T is set greater than one, several individuals in the selected group carry a given favourable QTL allele, which may reduce fixations of unfavourable QTL alleles as well as fixations of neutral alleles. For QCS with $\theta_T = 1$, an n_T value of at least two is needed in order to ensure a high $f(Q^+)$ value. If the aim is to provide a high favourable QTL allele fixation rate,then $n_T = 2$ is preferable. If the aim is to maintain FixQ$^-$ and FixN at values as low as possible, while not reducing $f(Q^+)$, then n_T should be increased up to five or six. An n_T value of three is a good compromise.

Some of the individuals selected by the QCS procedure can have low MS values, which reduces the mean value of the selected group. To avoid this, a kernel of individuals with high MS values should be included in the selected group using parameter N'_0. For QCS with $\theta_T = 1$, an N'_0 value of at least 3 is needed to ensure a high $f(Q^+)$ value. In general, N'_0 has smaller impact on QCS efficiency than n_T.

Looking at results for QCS(1,3,3) (Table 3), it is seen that very few individuals are selected at each generation (ninth column). Note that the number of individuals selected cannot be lower than n_T (three in Table 3). Numbers of individuals selected are even lower with a lower n_T value, but this does not provide the best QCS efficiency. In the conditions of Table 3, the complementation criterion (for each QTL, at least three different individuals carrying at least one favourable QTL allele) was fulfilled in each replicate, at each generation and for all 50 QTLs. This is not always the case for other θ_T values.

Compared with MTS, QCS is most useful for increasing the genetic quality of individuals selected, while reducing their number. In early generations (0 to 4), many of the individuals selected by QCS are not the individuals with best MS values, as indicated by the 'Lowest Ranked Selection' (LRS; last column in Table 3). In later generations LRS decreases rapidly, indicating that most of the individuals in the population are then of good genetic quality, so that they are mostly selected based on their MS values.

At 10 generations, QCS(1,2,3) and QCS(1,3,3) were more efficient for $f(Q^+)$ than the best MTS strategy ($N' = 10$ in Table 2). Though the difference in $f(Q^+)$ is rather small, it must be noted that QCS efficiency at generation 10 is close to the highest MTS efficiency at complete fixation ($N' = 10$ at generation 15 in Table 2). QCS is especially more efficient than MTS for QTL fixations. Compared with the best MTS strategy at generation 10 ($N' = 4$ in Table 2), the rate of favourable QTL allele fixations for QCS(1,3,3) was higher and the rates of fixation of unfavourable QTL alleles *and* of neutral markers were lower.

For intermediate generations, QCS($\theta_T = 1$) can be more efficient than MTS($N' = 4$), but with a different set of parameters than the one of Table 3: for example at generation 5, QCS(1,1,3) provides $f(Q^+) = 85.1\%$, FixQ$^+ = 65.1\%$, FixQ$^- = 1.7\%$ and FixN $= 62.9\%$, to be compared with the results shown in Table 2. QCS($\theta_T = 1$) was never found to be more efficient for
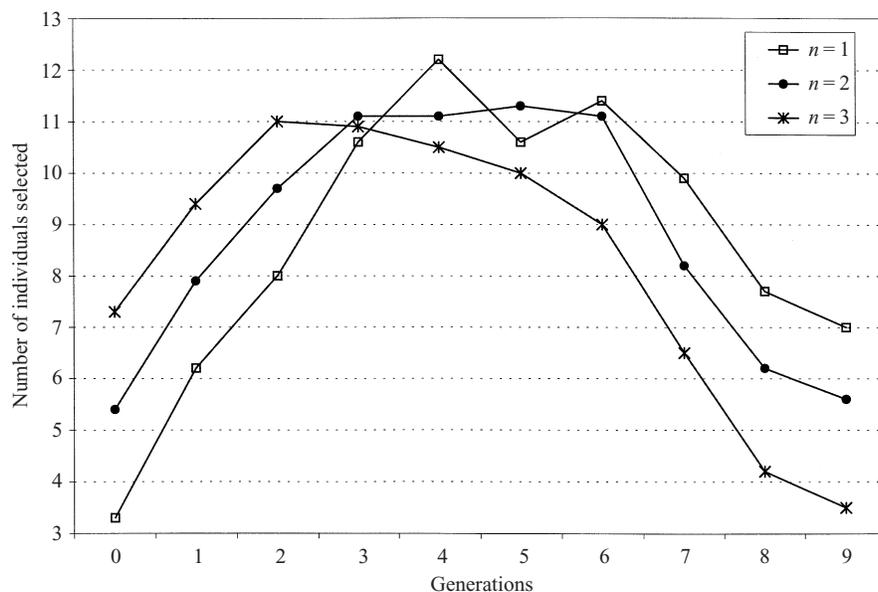
Fig. 3. Optimal intensity of truncation selection. Abscissa, generation; ordinate, number of individuals selected at each generation by the MTSC procedure for $\theta_T = 1$ and n_T values indicated by symbols: □, 1; ●, 2; ✳, 3.

favourable QTL allele fixations at generation 5 than the most efficient MTS strategy at the same generation ($N' = 2$ in Table 2). However, the efficiency of the latter is obtained at the expense of high FixQ⁻ and FixN. Efficiency for $f(Q^+)$ comparable to that of MTS($N' = 2$) can be obtained with QCS($\theta_T = 0.5$).

(iii) *Searching for optimal intensity of truncation selection*

Reducing the number of individuals selected increases short-term response to truncation selection, but also increases negative fixations, which reduces long-term response (see Fig. 2, Table 2, and also Hospital & Chevalet, 1993). Hence, a compromise must be found, which depends on the time objective. QCS provides an objective criterion for doing so. Beyond MBRS optimization, this is of general interest for the methodology of recurrent selection, with or without markers.

To investigate further, we performed simulations with a modified method of truncation selection (MTSC), in which the number of individuals selected is not fixed *a priori* but determined at each generation: ranked individuals are selected until the QTL complementation criterion is fulfilled (this criterion being defined as in QCS). Results for the threshold $\theta_T = 1$ and $n_T = 1$, 2, or 3 are given in Fig. 3.

Compared with MTS for $N' = 4$ or 10 (same magnitude of selection intensity), MTSC with $n_T = 3$ is more efficient ($f(Q^+) = 94.9\%$ and FixQ⁺ = 89.3% at generation 10), indicating that an optimal selection intensity should vary according to the generation. The

number of individuals selected increases in early generations, then decreases in later generations. This shows that, at the beginning, it is easy to find positive alleles at all QTLs within the first best individuals, but then strong selection quickly generates negative correlations between QTL alleles (Bulmer effect) and a larger number of individuals becomes necessary to fulfil the complementation criterion. Recombination and fixation of a large part of the QTLs finally restores the efficiency of selection, so that very few individuals are selected at the end. Note that, as was also the case for QCS, the complementation criterion is fulfilled at each generation and for all 50 QTLs. As expected, with $\theta_T = 1$ and $n_T = 1$, the number of individuals required to fulfil the condition is lower in early generations (five in $g = 0$), but then the dynamics of N' is quite similar to that for $n_T = 3$. The efficiency of MTSC was not improved by setting an upper limit to the number of individuals selected.

In MTSC, the rank of the individual selected with the lowest MS value is simply equal to N' in Fig. 3. This compares with LRS in Table 3, with two main differences: LRS decreases monotonically with time, and is much greater than N' in Fig. 3 in early generations. (Note, however, that the large LRS at generation 0 in Table 3 is due to the particular population size, 1000, at that generation.) The difference in rank dynamics is due to the search for the best complementary individual in QCS, while two individuals with higher MS values combined together might have provided the same level of complementation. However, QCS tries to minimize N' as can be seen from Table 3 (N' is kept between 5 and 6 until $g = 4$ and is lower afterwards), whereas in MTSC (Fig.

Table 4. *Effect of population size: efficiencies of different MTS and QCS strategies at generation 10*

Method	Parameters	N = 100				N = 50			
		$f(Q^+)$	FixQ$^+$	FixQ$^-$	FixN	$f(Q^+)$	FixQ$^+$	FixQ$^-$	FixN
MTS	$N' = 20$	88·1	37·5	0·1	28·7	82·0	25·9	0·1	20·5
MTS	$N' = 10$	91·6	66·9	0·8	56·1	87·0	55·7	1·1	49·2
MTS	$N' = 4$	92·0	87·1	5·2	86·8	88·3	81·0	7·2	84·1
MTS	$N' = 2$	87·3	86·8	12·2	98·0	85·8	85·2	13·6	97·7
QCS	$\theta_T = 0·5\ n_T = 1\ N'_0 = 2$	91·8	91·2	7·6	98·3	89·2	88·3	9·9	97·2
QCS	$\theta_T = 1\ n_T = 1\ N'_0 = 3$	92·4	89·6	5·6	93·6	91·1	86·7	5·5	90·0
QCS	$\theta_T = 1\ n_T = 2\ N'_0 = 3$	93·8	90·2	3·5	89·5	90·9	82·0	3·4	81·7
QCS	$\theta_T = 1\ n_T = 3\ N'_0 = 3$	93·5	87·0	2·6	83·3	90·5	77·1	2·2	73·5

3) N' increases up to 11 and becomes lower than 6 only after generation 7. Consequently, selection intensity is higher in QCS, leading to greater efficiency.

(iv) *Effects of population size*

A major advantage of MBRS is that the genotyping at generations $g > 0$ is done on a rather small-sized population in order to limit the cost of molecular work. Up to now we have considered a population size of 200. Here, we investigate the effect of a reduced population size.

It seems valuable to reduce population size, since it leads to rather small losses of efficiency (Table 4). Fixation rates at QTLs are more affected than allele frequencies. Rankings of methods for $N = 100$ and $N = 50$ are very similar to those described for $N = 200$. When reducing $N(g > 0)$ from 200 to 100, QCS(1,2,3) followed by QCS(1,3,3) results in the best values for $f(Q^+)$ at generation 10; and QCS(1,1,3) is best for $N = 50$, followed by QCS(1,2,3) and QCS(1,3,3). The QCS methods have highest FixQ$^+$ values for $N = 100$; for $N = 50$, QCS(0·5,1,2) has the highest FixQ$^+$ value, but at expense of high FixQ$^-$ and FixN. For the MTS methods, reduced N resulted in lower FixN values for a given N', presumably because of the lower selection pressure on QTL regions.

(v) *Effects of QTL–QTL distance and type of initial population*

Reducing the distance between two adjacent QTL segments (d_{MM}) from 20 to 10 cM has little impact on the efficiency of the methods investigated, so that the ranking is not much modified. It is remarkable that negative fixations at QTLs for QCS stay at rather low levels (for example 2·8% for QCS(1,3,3)) whereas they are strongly increased with MTS (7·7% for $N' = 4$). Neutral fixations are also lower for QCS than for MTS.

Using an F_4 population rather than an F_2 to initiate marker-based selection leads to a lowering of the mean frequency and the fixation rate of positive alleles at QTLs, and an increase in fixations of the negative alleles for MTS with N' values smaller than 20. Fixations of positive alleles at QTLs in $g = 5$ are increased with an F_4 population especially for low selection intensities ($N' = 10$ and 20). However, this does not modify conclusions: at $g = 10$, selection with $N' = 10$ provides the highest mean favourable allele frequency and selection with $N' = 4$ provides the highest fixation rate for positive alleles at QTLs. Hence, an F_2 population should be preferred to an F_4 as a starting point for MBRS except that the F_4 population is expected to give more precision for assessing QTL locations.

(vi) *Effects of marker–QTL distance*

When marker–QTL distance (d_{MQ}) is increased from 5 to 10 cM, the efficiencies of MTS and QCS methods are strongly reduced. $f(Q^+)$ is decreased by 6–8%, FixQ$^+$ is decreased by at least 10%, and FixQ$^-$ is increased. However, FixN is hardly affected. Optimal N' values in MTS are a little lower for $d_{MQ} = 10$ than for $d_{MQ} = 5$. For $d_{MQ} = 10$, $N' = 10$ and $N' = 4$ both provide greatest $f(Q^+)$ values (87%) and $N' = 2$ provides the highest positive fixations at QTLs (83%). Two mechanisms explain these patterns: selection pressure for pairs of marker alleles is lower because of recombination between the two markers, and fixations at the QTLs are also reduced because of recombination between QTLs and flanking markers. Hence, selection intensity in MTS should be a little stronger with $d_{MQ} = 10$, but would be accompanied by high negative fixations at QTLs. Even though the efficiencies of QCS methods are lower for $d_{MQ} = 10$, optimal values of selection parameters are not very different from those for $d_{MQ} = 5$. QCS(1,3,3) still provides the highest $f(Q^+)$ (89%) and QCS(1,2,3) provides one of the highest FixQ$^+$ (85%). Again, QCS methods were more efficient than MTS.

Some simulations were performed with marker–QTL distance set to zero ($d_{MQ} = 0$). Selection directly

on QTLs has been investigated previously by de Koning & Weller (1994) but in a different situation (two-traits index selection, pleiotropic QTLs, disassortative mating). Here, selection directly on QTLs is used as an idealized situation to investigate the effect of marker–QTL distance on MBRS efficiency (i.e. to provide an upper limit of what might be expected when the effects of recombinations between QTLs and flanking markers are removed).

For $d_{MQ} = 0$ and $d_{MM} = 30$, i.e. the same distance between QTLs as in the 'base' parameter set, the efficiencies of all selection methods are improved. For MTS, this improvement is higher for $N' \geqslant 20$ than for stronger selection intensities. MTS methods with $N' \geqslant 20$ even reach a complete 100 % rate of fixation of favourable alleles at all QTLs (e.g. at generation 11 for MTS with $N' = 20$). Note, however, that complete fixation of favourable alleles was already achieved in MTS($N' \geqslant 20$) with the 'base' parameter set, but at later generations and for the flanking markers, not the QTLs (Table 2).

Hence, optimal selection intensity for MTS is weaker than when markers and QTLs are distinct loci. The efficiency of QCS(1,3,3) is also improved. Moreover, with $d_{MQ} = 0$, QCS provides approximately the same efficiency as the best MTS methods ($N' \leqslant 4$) before generation 5, and is superior to any MTS method after generation 5, achieving a complete 100 % rate of fixation of favourable QTL alleles at generation 9 only. Hence, QCS becomes the best overall method. This indicates that the limitations of QCS efficiency are mainly due to recombinations between QTLs and flanking markers.

For $d_{MW} = 0$ and a shorter distance between QTLs ($d_{MM} = 20$), discrepancies are even more marked: optimal selection intensity for MTS is even weaker, and the superiority of QCS over any MTS method is even increased.

4. Discussion

This paper has been devoted to the study of the efficiency of selection based solely on marker genotypes (MBRS) aimed at increasing favourable allele frequency at a large number of previously detected QTLs. Our results indicate that MBRS is remarkably efficient, and can increase favourable QTL alleles frequencies above 90 % in fewer than 10 generations in most cases. Obviously, efficiencies are bounded by 100 %.

It is known from the theory of selection that there is a conflict between short- and long-term response to directional recurrent selection. In finite populations, strong selection intensities are expected to provide the best short-term response but to limit future genetic gains, due to negative fixations. Conversely, Robertson (1970) has shown that maximal response at

the limit is obtained by medium selection intensities (selection rate = 50 %), and Hospital & Chevalet (1993) showed that in the case of tight linkage, optimal selection intensities should be even lower (selection rate = 80 %). Our results for truncation selection (MTS) indicate that maximal response on the markers is achieved relatively quickly (10 to 15 generations; see Table 2) with much stronger selection intensities. This is due to two main reasons. First, heritability on the markers is one here, while heritability for the trait considered by Hospital & Chevalet (1993) was 0·5. Secondly, linkage between markers (and between QTLs) is much looser here. Moreover, MBRS efficiency is evaluated not on the selected markers but on the QTLs. Effective selection must increase favourable QTL allele frequencies *before* marker–QTL linkage disequilibrium is reduced by recombination events. Again, this shifts the optimum towards stronger selection intensities, as clearly indicated by our results with direct selection on QTLs ($d_{MQ} = 0$).

While avoidance of unfavourable fixations is only a wish in classical phenotypic selection, an interesting advantage of marker-based selection (besides increasing the apparent heritability of the trait) is that this can be attempted with a greater probability of success, because the genotypes of the selected individuals are known, at least for the markers. To do so, we applied QTL complementation selection (QCS). The efficiency of QCS after 10 generations was always superior to that of truncation selection (MTS). Though the superiority of QCS with the base parameter set was often not very great, the important result is that one single QCS strategy provides the best efficiency for different selection criteria (*both* $f(Q^+)$ and FixQ$^+$), while different MTS strategies had to be chosen in order to maximize either criterion, so that QCS is more robust than MTS. Moreover, QCS appears more robust than MTS to a reduction in total population size, and to a variation in marker–QTL or QTL–QTL distance. Hence, QCS should be recommended to reduce experimental costs, and in the general case of arbitrary QTL and marker locations.

If it is desired to maintain genetic variation for loci affecting the trait but not identified as QTLs, or for loci affecting other traits of interest, then attention should be paid to neutral fixations (FixN) as well as to fixations of unfavourable QTL alleles (FixQ$^-$). Valid comparisons between methods for FixQ$^-$ and FixN are difficult because both tend to increase as $f(Q^+)$ and FixQ$^+$ increase. For example, comparing MTS($N' = 10$) at $g = 10$ with QCS(1,3,3) at $g = 8$ and 9, $f(Q^+)$ values are about the same, FixQ$^+$ is much higher for QCS, but FixQ$^-$ and FixN are also somewhat higher.

Although the QCS methods investigated showed promising results, the implementations described here are not yet perfect. In particular, negative fixations are

not reduced to zero as could have been expected. Our results with marker–QTL distance set to zero indicate that, when markers and QTLs are distinct loci, these negative fixations are due to recombination between each QTL and its flanking markers, not to the selection method. Hence, the only way to reduce negative fixations is to speed up the response to selection. However, QCS does not provide the best efficiency in the very short term (five generations). Improving QCS efficiency could be achieved by increasing selection on the markers. To do so, rather than mating selected individuals at random, one could think of using a mating scheme that would favour transgressions between individuals carrying interesting genotypes at different loci. Also, one may think of taking account of the genetic distance between QTLs with 'present' alleles in the QCS method in order to improve its efficiency. These are certainly promising avenues of research in marker-based selection programmes, but the theory in this domain remains unexplored and deserves more work. Finally, QCS efficiency could be improved by improving its selection efficiency on the QTLs, given the markers. The bracket scores used in the present paper (1) were based on conditional probabilities in the initial population (Table 1) and are supposed to provide the best selection response in the first generation. However, these scores may not be optimal when response to several successive generations of selection is considered. Using more 'conservative' scores, such as the ones used by van Berloo & Stam (1998) (i.e. attributing a greater score to marker genotypes $(\theta_1, \theta_2) = (1, 1)$ than to genotypes $(0, 2)$ or $(2, 0)$) could be useful in order to reduce negative fixations and increase selection response in later generations. Again, the computation of optimal marker scores under selection is a complex problem which deserves more theoretical work. An approximation for the computation of conditional probabilities of QTL genotypes given marker genotypes over successive generations was provided by Whittaker *et al.* (1995, appendix 1) in the context of selection on marker–phenotype index, and might be applied to the present case. However, such improvements might not increase MBRS efficiency very much, since this efficiency is already high in the present context.

In this paper we have chosen to focus on marker-based selection on previously detected QTLs, and to disregard problems related to the accuracy of QTL detection (effects and location). In this context we have made some assumptions that are favourable from the standpoint of QTL detection but rather unfavourable (or conservative) as far as marker-based selection only is considered: the assumption that 50 QTLs have been detected is favourable for QTL detection (though realistic provided experiment size is large and multi-trait selection is considered), but

unfavourable for MBRS, since selection efficiency should be higher for fewer QTLs; and the assumption that QTLs are located precisely in the middle of the corresponding marker brackets is also unfavourable for MBRS, because with the marker bracket scores considered here, efficiency would be higher if the QTL were closer to either of the markers. In fact, the only assumption favourable to MBRS is that each QTL is actually somewhere in the assigned marker bracket, and not in another bracket.

Obviously, the efficiency of the marker-assisted selection scheme described in Section 1 should depend not only on the efficiency of MBRS but also on the efficiency of the QTL detection phase, as well as the interactions between QTL detection and selection. The problems related to QTL detection (effects of environment, variable gene effects, power of QTL detection) should then be included in future work, in order to evaluate the efficiency of marker-based selection in realistic conditions. On the basis of the present results, a direct comparison of the respective efficiencies of MAS and purely phenotypic selection is not possible, and was not our purpose. However, we believe our methodological study should contribute to the optimization of such a scheme.

In any case, if phenotypic evaluation and QTL detection are performed in the initial population, then this information should be used (for example by selecting on the index proposed by Lande & Thompson, 1990) instead of selecting solely on marker genotypes in the first generation as done here. Such a study was performed by Hospital *et al.* (1997), who concluded that an optimal marker-assisted selection scheme should alternate generations of selection with and without evaluation of effects attributed to markers. Here, we confirm the statement of Hospital *et al.* that, if a large number of QTLs have been detected with large experiment size, then the frequencies of favourable alleles at those QTLs can subsequently be rapidly increased by MBRS with reduced population size.

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