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INTROGRESSING MULTIPLE QTL IN BACKCROSS BREEDING PROGRAMS OF LIMITED SIZE

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ABSTRACT

The ability to enrich a breed with favorable alleles for multiple unlinked QTL from a donor breed in a backcross program of limited size through marker-assisted introgression was evaluated by considering the effects of fraction selected, marker interval, and number of QTL. Informative flanking markers were used to select progeny with the largest expected number of recipient QTL alleles for 5 generations. With less than 5% selected, sufficient progeny were available that were heterozygous for all markers at three QTL and QTL frequencies dropped below 50% only by double recombination. For larger fractions selected, larger marker intervals, and more QTL, reductions from 0.5 were greater and increased over generations. However, even with 20% selected, 3 QTL and marker intervals of 5 or 20 cM, mean QTL frequencies in generation 5 were 0.35 and 0.30, sufficient to allow subsequent selection on QTL.

KEYWORDS: Marker-assisted introgression, backcrossing, QTL

1. INTRODUCTION

Recent breed cross studies have found several quantitative trait loci (QTL) for economic traits that segregate between breeds. For example, an F_2 crosses between Berkshire and Yorkshire grandparents identified several favorable QTL for meat quality in the Berkshire breed, which has undesirable growth performance [1-2]. It is, therefore, of great interest to develop marker-assisted introgression (MAI) strategies to incorporate the desirable QTL allele from a donor breed (Berkshire) into a recipient breed (Yorkshire). Another example of the need for MAI is to improve the quantitative ability of cattle to withstand the effects of trypanosome infections in sub-Saharan Africa. The idea is to introgress alleles at multiple QTL that confer trypanotolerance in breeds such as N'Dama and West African Shorthorn to trypanosusceptible breeds such as Kenyan Boran [3].

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Introgression involves two successive phases: a backcrossing phase and an intercrossing phase. Backcrossing includes foreground selection for carriers of the donor QTL allele(s) and, potentially, background selection for the recipient's background genome. Most studies have considered MAI of single QTL [4-5]. Koudande' *et al*. [6] considered foreground selection on multiple QTL, with alternative strategies based on gene pyramiding [7]. They showed that large population sizes are needed to obtain sufficient individuals that are heterozygous and homozygous for all QTL in the backcrossing and intercrossing phases, respectively. This would make MAI not feasible in livestock breeding programs.

In many cases, however, immediate fixation of introgressed QTL alleles may not be required. Instead, the objective of the backcrossing phase can be to enrich the recipient breed with the favorable donor QTL alleles at high enough frequency such that they can be selected on following backcrossing. Consequently, the objective of this study was to evaluate the efficiency of MAI of multiple QTL in a backcross program of limited size. The impact of selected proportion, size of introgressed regions, and number of QTL was considered.

2. MATERIALS AND METHODS

The F_1 and five backcross (BC) generations from a cross between two inbred lines that were fixed for alternate alleles at QTL and at pairs of flanking markers were simulated stochastically. One, three, or five unlinked QTL were simulated at the center of 0, 5, or 20 cM marker intervals. A total of 500 BC progeny were generated each generation by mating 2, 5, 10, or 20% of BC individuals to the recipient parental line. The BC progeny were selected on the expected number of donor alleles at the *n* introgressed QTL, as determined from marker genotypes: $I = \sum_{i}^{n} P(Q_i)$, where $P(Q_i)$ is the probability that the individual carries the donor allele for QTL *i*. Probabilities $P(Q_i)$ were set equal to 1, ½, and 0 if the individual carried 2, 1, and 0 donor alleles at the two markers that flanked the QTL, respectively, ignoring double recombinants. Efficiency of MAI was evaluated by the frequency of donor QTL alleles, averaged over loci.

3. RESULTS AND DISCUSSION

Table 1 shows the average and standard deviation of QTL frequencies for five backcross generations with introgression of three QTL. Results were based on 100 replicates and averaged over the three QTL. The ability to maintain a frequency of 0.5 for the donor QTL alleles depended on the fraction selected and marker distance. With a selected fraction of 2 or 5%, sufficient BC individuals could be identified that were heterozygous at all flanking markers and reductions in frequencies from 0.5 were the result of double recombinants. Since double recombinants are more frequent with larger marker intervals, a slight reduction in frequency was observed for the 20 cM interval. This was also the case for 10% selected when marker intervals were 0 and 5 cM, but for a 20 cM interval with 10% selected and for all intervals with 20% selected, some selected individuals were not heterozygous for all flanking markers. The number of such individuals increased with selected proportion and marker interval and resulted in greater reductions in allele frequencies. Nevertheless, even with a 20 cM interval, mean frequencies were 0.40 and 0.30 in generation five for 10 and 20% selected, respectively.

Table 1 Average and standard deviation of QTL frequencies in five backcross generations for various selected proportions (SP) and marker interval distances (*d*) for introgression of three unlinked QTL.

SP	*d*	QTL frequency in backcross generation				
(%)	(cM)	1	2	3	4	5
2	0	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
	5	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.01
	20	0.50 ± 0.02	0.49 ± 0.03	0.49 ± 0.06[A]	0.48 ± 0.07 [A]	0.48 ± 0.7[A]
5	0	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
	5	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.01
	20	0.50 ± 0.01	0.49 ± 0.02	0.49 ± 0.02	0.49 ± 0.03	0.48 ± 0.04
10	0	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
	5	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
	20	0.48 ± 0.02	0.46 ± 0.03	0.43 ± 0.03	0.42 ± 0.04	0.40 ± 0.04
20	0	0.44 ± 0.02	0.40 ± 0.02	0.37 ± 0.02	0.36 ± 0.03	0.35 ± 0.03
	5	0.44 ± 0.02	0.40 ± 0.03	0.37 ± 0.03	0.36 ± 0.03	0.35 ± 0.04
	20	0.43 ± 0.02	0.38 ± 0.02	0.35 ± 0.03	0.32 ± 0.03	0.30 ± 0.04

Results are based on 100 replicates. [A] The standard deviation is increased because one QTL was lost in one replicate.

Table 2 shows the effect of the number of QTL that are introgressed for 20% selected. As expected, the reduction in frequency over generations increased with number of QTL and marker distance. Introgression of five QTL resulted in a mean frequency of 0.21 in generation five. Reductions would be smaller for greater selection intensities.

Table 2 Average and standard deviation of QTL frequencies in five backcross generations with introgression of 1, 3, or 5 unlinked QTL (*n*) for different marker intervals (*d*) and 20% selected.

N	*d*	QTL frequency in backcross generation				
	(cM)	1	2	3	4	5
1	0	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
	5	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.01	0.50 ± 0.00	0.50 ± 0.01
	20	0.50 ± 0.01	0.49 ± 0.01	0.48 ± 0.02	0.48 ± 0.03	0.47 ± 0.05
3	0	0.44 ± 0.02	0.40 ± 0.02	0.37 ± 0.02	0.36 ± 0.03	0.35 ± 0.03
	5	0.44 ± 0.02	0.40 ± 0.03	0.37 ± 0.03	0.36 ± 0.03	0.35 ± 0.04
	20	0.43 ± 0.02	0.38 ± 0.02	0.35 ± 0.03	0.32 ± 0.03	0.30 ± 0.04
5	0	0.41 ± 0.02	0.34 ± 0.03	0.31 ± 0.03	0.28 ± 0.04	0.25 ± 0.04
	5	0.41 ± 0.02	0.34 ± 0.03	0.30 ± 0.03	0.27 ± 0.04	0.24 ± 0.04
	20	0.39 ± 0.02	0.32 ± 0.03	0.27 ± 0.03	0.23 ± 0.04	0.21 ± 0.04

Results are based on 100 replicates.

4. CONCLUSIONS

This paper puts forth the concept of the use of MAI to enrich a recipient breed with favorable QTL alleles from a donor breed by introducing donor QTL alleles at a high enough frequency such that they become amenable to subsequent selection. This is in contrast to the traditionally accepted aim of an introgression program, which is to select only individuals that carry the donor alleles at the QTL or its flanking markers during the backcrossing phase and to fix the QTL rapidly during the intercross phase. This, however, requires large numbers of individuals, in particular if multiple QTL are introgressed, which is not feasible in livestock. Immediate fixation of QTL may be required for disease resistance genes that are a prerequisite for survival but this is not necessary for QTL for more general continuous traits, such as growth, yield, and meat quality. Results presented here show that, although it may not be possible to maintain a frequency of 0.5 during backcrossing in populations of limited size, MAI can introduce multiple QTL alleles at frequencies that will enable their selection following backcrossing.

Selection of BC individuals was on the expected number of donor QTL alleles inherited. This is an extension to multiple QTL of selection on the probability of QTL inheritance that was proposed by van Heelsum *et al*. [8] for MAI of a single QTL with incomplete informativeness of markers. Although we assumed fully informative markers, this method can be extended to markers that are not fully informative, following the approach of van Heelsum *et al.* [8-9]. As shown by these authors for a single QTL, use of markers that are not fully informative would result in a further reduction in QTL frequencies. Addition of markers around the QTL would, however, improve the ability to track QTL.

The stochastic simulation used here also allowed assessment of the variance of results. The standard deviation of frequencies tended to be less than 0.04 (Tables 1 and 2). One exception was for 2% selected and the 20 cM interval, which had a standard deviation of 0.07 (Table 1). This was caused by a single replicate in which the one QTL was lost. In practice, this could be prevented by balancing selection of individuals that carry donor alleles for each of the QTL. This would also reduce standard deviations. For a given percentage selected, standard deviations of frequencies were greater with a population size of 300 than the 500 used for Tables 1 and 2, but mean frequencies were similar (results not shown).

Selection during the backcrossing phase capitalizes on the linkage disequilibrium that exists between the donor's marker and QTL alleles, while reducing the contribution of the donor's background genome. The amount of useable linkage disequilibrium decreases over generations, depending on recombination rates between the QTL and markers. The results presented here show that linkage disequilibrium is still substantial after five generations, even for 20 cM intervals.

In this study, a simplified selection criterion was used, which puts equal emphasis on all QTL. If QTL effects are known, differential weights can be applied to QTL to maximize economic response. In addition, no emphasis was put on the background genome. An expanded index could include negative emphasis on the donor's background genome, either based on phenotype, or based on markers spread over the genome. Greater emphasis could be put on markers near the introgressed segment to reduce linkage drag. Any emphasis on the background genome would further reduce frequencies of QTL donor alleles but may be advantageous from an economic perspective. In theory, an optimum selection criterion can be derived given knowledge of QTL effects and background genome differences between the two breeds. Ultimately, the optimal selection strategy, including the number of generations of backcrossing, must be based on an economic analysis that involves the effects of the QTL, the difference in background genome effects, the opportunity cost of potential selection response that is lost for other genes, and the costs that are associated with an introgression program.

5. ACKNOWLEDGEMENT

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