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ORIGINAL PAPER

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Power of mixed-model QTL mapping from phenotypic, pedigree and marker data in self-pollinated crops

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Abstract The power of QTL mapping by a mixed-model approach has been studied for hybrid crops but remains unknown in self-pollinated crops. Our objective was to evaluate the usefulness of mixed-model QTL mapping in the context of a breeding program for a self-pollinated crop. Specifically, we simulated a soybean (*Glycine max* L. Merr.) breeding program and applied a mixed-model approach that comprised three steps: variance component estimation, single-marker analyses, and multiple-marker analysis. Average power to detect QTL ranged from <1 to 47% depending on the significance level (0.01 or 0.0001), number of QTL (20 or 80), heritability of the trait (0.40 or 0.70), population size (600 or 1,200 inbreds), and number of markers (300 or 600). The corresponding false discovery rate ranged from 2 to 43%. Larger populations, higher heritability, and fewer QTL controlling the trait led to a substantial increase in power and to a reduction in the false discovery rate and bias. A stringent significance level reduced both the power and false discovery rate. There was greater power to detect major QTL than minor QTL. Power was higher and the false discovery rate was lower in hybrid crops than in self-pollinated crops. We conclude that mixed-model QTL mapping is useful for gene discovery in plant breeding programs of self-pollinated crops.

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Introduction

Current QTL mapping studies have relied on designed populations such as F_2 or backcross populations developed from crossing two inbreds. The resulting progenies, which have a simple pedigree, are then evaluated at several environments for one or more quantitative traits (Beavis 1994, 1998; Kearsey and Farquhar 1998). Although this approach yields balanced data and allows a relatively simple statistical analysis, it has important limitations. First, most QTL studies have used small population sizes (100–250 progenies) that limit the power to detect and correctly estimate the location and magnitude of QTL effects (van Ooijen 1992; Beavis 1994, 1998). Second, progenies are usually evaluated in only two to ten environments, thus sampling a limited set of QTL x environment interactions and preventing results from being applicable to a wider range of environments. Small population sizes and limited phenotypic evaluation together constitute insufficient sampling, which can cause lack of repeatability of QTL mapping results (Beavis 1994). Third, designed populations represent a rather narrow germplasm base, and mapping results may not apply to other genetic backgrounds (Parisseaux and Bernardo 2004).

In silico mapping is an alternative approach that circumvents the need for designed populations by exploiting existing phenotypic and genomic databases (Grupe et al. 2001). In silico mapping of quantitative traits can be applied in plant breeding programs to potentially overcome some of the limitations of current QTL mapping experiments (Parisseaux and Bernardo 2004). Every year plant breeding programs generate vast numbers of progenies that are evaluated at multiple environments. The progenies represent a more diverse sample of genetic backgrounds than designed mapping populations, and inferences would apply to a wider germplasm base. In advanced plant breeding programs, elite inbreds are routinely genotyped with a random set of markers for the purposes of germplasm organization

and variety protection (Smith et al. 1995; Smith and Beavis 1996). Such marker data can also be used in gene discovery. A disadvantage, however, is that plant breeding data are highly unbalanced, as inbreds or hybrids are evaluated in different sets of environments. A second disadvantage is that the inbreds, which are developed at different stages of a plant breeding program, do not represent a single population but rather a mixture of breeding populations composed of related individuals. The inbreds cannot be assumed to be random members of a homogeneous population. In this context current QTL mapping methods are not applicable, and in silico mapping in plant breeding would require accounting for unbalanced data and for pedigree relationships among inbreds.

These limitations can be overcome by a mixed-model approach to QTL mapping. The mixed-model approach, developed initially for animal breeding, has proved useful for predicting breeding values while managing unbalanced phenotypic data and accounting for pedigree relationships among individuals (Henderson 1984). The mixed-model approach can readily be extended to include genomic data to map QTL (Kennedy et al. 1992; Parisseaux and Bernardo 2004). In two previous studies (Parisseaux and Bernardo 2004; Yu et al. 2005) we called this approach in silico QTL mapping. The term ''in silico mapping'' has also been used in more general bioinformatics applications, e.g., in silico mapping of ESTs. To avoid confusion with other in silico procedures, we now refer to this approach as mixed-model QTL mapping.

Our previous studies of mixed-model QTL mapping (Parisseaux and Bernardo 2004; Yu et al. 2005) were for hybrid crops. Genotypic effects of hybrids comprise three components: the additive effect (or general combining ability effect) of the first inbred parent, the additive effect of the second inbred parent, and the dominance effect (or specific combining ability effect) between the two inbred parents. Mixed models in cross-pollinated crops need to account for these three genetic effects. In contrast, self-pollinated crops comprise only inbreds. This simplifies the mixed model to only one genetic effect (i.e., additive).

No studies have been conducted on the power and limitations of this methodology for self-pollinated crops. Our objective was to evaluate the usefulness of mixed-model QTL mapping in the context of a breeding program for a self-pollinated crop. We considered soybean (*Glycine max* L. Merr.) as a model species but our results should be generally applicable to other self-pollinated crops

Materials and methods

We conducted a simulation study to mimic the two main stages in a soybean breeding program: inbred development and performance testing. During inbred development, crosses among selected parents were made, recombinant inbreds were developed, and the best recombinant inbreds were selected based on phenotypic data. During performance testing, inbreds developed from different crosses were tested more extensively in performance trials. Only data from performance trials were used in QTL mapping.

The breeding process was simulated for different levels of population size ($N = 600$ and 1,200), number of QTL ($l = 20$ or 80), heritability ($H = 0.40$ or 0.70), number of markers ($m = 300$ or 600), generations of random mating ($t = 10$ or 20) and levels of significance ($\alpha = 0.01$ or 0.0001). The two levels of each of these six factors resulted in $2^6 = 64$ simulation experiments. Forty-eight of these simulation experiments were repeated 50 times. Due to computer-time limitations, the remaining 16 experiments (which were the larger experiments) were repeated between 30 to 49 times. Simulations with large population sizes and large number of markers took about 4–5 months to complete. In each repeat, QTL and marker locations were different, leading to different breeding populations and inbreds. Each repeat was analyzed separately and the results were summarized for each of the 64 experiments. Simulations and mixed-model analysis were performed with a C++ computer program written by M. Arbelbide. Simulations were conducted on an IBM Power4 supercomputer cluster at the University of Minnesota Supercomputing Institute for Digital Simulation and Advanced Computation.

Inbred development

The following groups of inbreds were simulated: ancestral inbreds, founder inbreds, first-cycle inbreds, second-cycle inbreds, and third-cycle inbreds. Four ancestral inbreds, each with different QTL and marker allele genotypes, were random mated for $t = 10$ or 20 generations to generate 20 founder inbreds. Current soybean cultivars, which have a narrow genetic base (Delannay et al. 1983; Gizlice et al. 1994), have complex pedigrees and are different numbers of generations removed from the founder cultivars. Thus, actual patterns of linkage disequilibrium among soybean cultivars vary widely across populations (Zhu et al. 2003), and for simplicity and convenience we used different numbers of generations of random mating to generate high ($t = 10$) and low ($t = 20$) levels of initial linkage disequilibrium. The development of first, second, and third-cycle inbreds as described below led to further variation in linkage disequilibrium. Linkage disequilibrium was calculated for multi-allelic loci as described by Farnir et al. (2000).

The 20 founder inbreds comprised random recombinant inbreds derived from the random mated ancestral population. First-cycle inbreds were developed from random crosses among the 20 founder inbreds. Similarly, second-cycle inbreds were developed from random crosses among the first-cycle inbreds, and third-cycle inbreds were developed from random crosses among the second-cycle inbreds. The first, second, and third-cycle inbreds were developed by single seed descent

from the F_2 population between two parents. A total of 100 recombinant inbreds were developed per cross. The best 1% of recombinant inbreds were selected after phenotypic evaluation, and these selected inbreds were designated as the first, second, or third-cycle inbreds. Equal numbers (200 or 400) of first, second and third-cycle inbreds were developed for a total of $N = 600$ or 1,200 inbreds. Heritability on an entry-mean basis was $H = 0.4$ or 0.7 during inbred development. Genotypes, genotypic values, and phenotypic values of inbreds were obtained as described in later sections.

Genotypes and genotypic values of inbreds

Each QTL had four alleles (each from a different ancestral inbred), Q_i^1, Q_i^2, Q_i^3, and Q_i^4, for $i = 1, ..., l$ QTL. Similarly, each marker had four alleles, M_j^1, M_j^2, M_j^3, and M_j^4, for $j = 1, ..., m$ markers. Therefore, genotypes of the four ancestral inbreds for the ith QTL were QQ_i^1, QQ_i^2, QQ_i^3, and QQ_i^4, and genotypes for the jth marker were MM_j^1, MM_j^2, MM_j^3, and MM_j^4. The Kosambi mapping function was used to relate map distance and recombination frequency. Genotypes of inbreds were simulated according to their respective parental QTL and marker genotypes, allowing for recombination as a function of map distance.

A soybean linkage map of 2,524 cM comprising 20 linkage groups (Song et al. 2004) was considered. An additive genetic model with no dominance or epistasis was considered for a quantitative trait controlled by $l = 20$ or 80 QTL. Markers and QTL were randomly located in the genome. The effects of QTL followed a geometric series. Specifically, the effect of the ith QTL was a function of a^i, where $a = (l-1)/(l+1)$ and $i = 1, ..., l$ QTL (Lande and Thompson 1990). Genotypic values at each ith QTL were a^i for QQ_i^1, $0.5(a^i)$ for QQ_i^2, $-0.5(a^i)$ for QQ_i^3, and $-(a^i)$ for QQ_i^4. Under this model, all QTL genotypes had a mean of zero, and locus 1 had the largest effect while locus l had the smallest effect. The overall genotypic value of an inbred was obtained as the sum of genotypic values across all QTL.

We considered a total of $m = 300$ or 600 markers distributed at random across the genome. For $m = 300$ markers the average distance between markers was approximately 8.3 cM, and for $m = 600$ markers the average distance between markers was approximately 4.2 cM.

Performance trials

The first, second, and third-cycle inbreds were evaluated in different performance trials. The mean effect of each performance trial was drawn from a normal distribution with zero mean and variance scaled such that it explained 70% of the total variation (Delacy and Cooper 1990). A total of 30 different inbreds were evaluated in a performance trial. The total number of inbreds, including the 20 founder inbreds, was $N_T = N + 20$. The total number of performance trials was $E = N_T/30$. A residual effect due to random error was added. These residuals were drawn from a normal distribution with zero mean and variance scaled so that the heritability on an entry-mean basis was (as mentioned previously) $H = 0.40$ or 0.70 during inbred development. During performance trials heritability was adjusted from 0.40 to 0.67, and from 0.70 to 0.88, given that the number of environments per performance trial is about three times greater than that during inbred development (Smith et al. 1999).

Mixed model for marker effects

The data available for the mixed-model analysis consisted of: i) phenotypic data for N_T inbreds, ii) marker information on m markers for each inbred, and iii) pedigree records that described the relationships among inbreds. We used the following mixed model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{W}\mathbf{m} + \mathbf{Z}\mathbf{u} + \mathbf{e},$$

where $\mathbf{y}$ = $N_T \times 1$ vector of simulated phenotypic observations of inbreds; $\boldsymbol{\beta}$ = $E \times 1$ vector of fixed effects associated with performance trials plus the overall mean; $\mathbf{m}$ = $4m' \times 1$ vector of fixed effects associated with the four alleles at each marker locus for a subset of m' markers; $\mathbf{u}$ = $N_T \times 1$ vector of additive polygenic effects not accounted for by the m' markers; $\mathbf{e}$ = $N_T \times 1$ vector of residual effects; and $\mathbf{X}$, $\mathbf{W}$, and $\mathbf{Z}$ were incidence matrices of ones and zeros relating $\mathbf{y}$ to $\boldsymbol{\beta}$, $\mathbf{m}$, and $\mathbf{u}$, respectively. The means of the random vectors $\mathbf{u}$ and $\mathbf{e}$ were zero, and variances were $\mathrm{Var}(\mathbf{u}) = \mathbf{A}V_A$, and $\mathrm{Var}(\mathbf{e}) = \mathbf{I}V_R$. $\mathbf{A}$ was the additive relationship matrix, V_A was the additive genetic variance due to polygenic effects, $\mathbf{I}$ was an identity matrix, and V_R was the residual variance on an entry-mean basis. The relationship matrix $\mathbf{A}$ was composed of twice the coefficient of coancestry among inbreds. The coefficients of coancestry were calculated based on pedigree records via the tabular method (Emik and Terrill 1949). Marker effects were considered fixed as proposed by Kennedy et al. (1992).

Mixed-model equations (Henderson 1984) were used to obtain best linear unbiased estimates (BLUE) of fixed effects $\boldsymbol{\beta}$ and $\mathbf{m}$, and best linear unbiased predictions (BLUP) of random effects $\mathbf{u}$. The following mixed-model equations were considered:

$$\begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{m}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{W} & \mathbf{X}'\mathbf{Z} \\ \mathbf{W}'\mathbf{X} & \mathbf{W}'\mathbf{W} & \mathbf{W}'\mathbf{Z} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{W} & \mathbf{Z}'\mathbf{Z} + \boldsymbol{\theta} \end{bmatrix} \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{W}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \end{bmatrix} = \begin{bmatrix} \mathbf{C}_{11} & \mathbf{C}_{12} & \mathbf{C}_{13} \\ \mathbf{C}_{21} & \mathbf{C}_{22} & \mathbf{C}_{23} \\ \mathbf{C}_{31} & \mathbf{C}_{32} & \mathbf{C}_{33} \end{bmatrix} \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{W}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \end{bmatrix}$$

where $\boldsymbol{\theta} = \mathbf{A}^{-1}(V_R/V_A)$. Restricted maximum likelihood (REML) estimates of V_R and V_A were obtained by iterating on the following equations (Henderson 1984, p. 200):

V_R: $=[\mathbf{y}'\mathbf{y}$ – (solution vector)′(right-hand side vector)] / $[N_T$ – rank(**X**) – rank(**W**)]V_A: $= [\mathbf{u}'\mathbf{A}^{-1}\mathbf{u} + V_R \text{ trace}(\mathbf{A}^{-1}\mathbf{C}_{33})] / N_T$

Data analysis

Data analysis consisted of three steps. First, a mixed model excluding marker information was used to obtain REML estimates of V_R and V_A. These estimates were used in a second step where the mixed-model included a single marker, and equations were solved to obtain BLUE values of marker effects assuming of V_R and V_A were known. This process was repeated for all markers on a single-marker basis. For each marker, an F test was constructed to test its significance at two threshold levels ($\alpha = 0.01$ or 0.0001), as described by Kennedy et al. (1992).

Once all markers had been tested on a single-marker basis, significant markers were selected for the third and final step. To reduce multicollinearity of marker effects, only the marker with the smallest P value was selected whenever adjacent markers were significant. In the third step, all selected markers were fitted using a multiple marker mixed model. The final estimates of marker effects and of V_R and V_A were obtained from this final step. Specifically, marker effects were expressed as the maximum difference between marker allele effects. We considered this criterion as meaningful to plant breeders, who are most interested in the extremes in a given population.

Power of mixed-model QTL mapping

We calculated the average power, false discovery rate (FDR), and bias in each experiment. A true positive was declared if a marker had a significant effect and had at least one immediately adjacent QTL (Whittaker et al. 1996). Otherwise, a significant marker was declared a false positive. False positives were considered to have an effect of zero. Average power was equal to the number of true positives divided by the number of QTL, averaged across the number of repeats. The FDR was estimated as the number of false positives divided by the number of markers declared significant, averaged across the number of repeats. The ratio between average power and FDR was calculated for each experiment, and a linear regression line through the origin was fitted to the resulting power to FDR ratios.

For the markers declared significant, bias was estimated as the percentage of deviation of an estimated effect from the true effect of the nearest QTL, averaged across the number of repeats. This estimator of bias was expected to be negative because it did not account for the recombination frequency between the significant marker and the linked QTL. A positive value, however, indicated an upwards bias. Specifically, the expected bias (expressed as a proportion) was (1–2R) where R was the recombination frequency among inbreds (Haldane and Waddington 1931). Based on the marker density, the maximum amount of bias purely due to recombination between a marker and QTL was −28% when 300 markers were used and −15% when 600 markers were used.

We defined major QTL as the top 25% of the QTL with the largest effects (upper quartile), and minor QTL as the bottom 25% of the QTL with the smallest effects (lower quartile). We calculated average power and bias for all QTL as well as for major QTL and minor QTL.

Results

The factors with the largest effects on average power to detect QTL were the number of QTL controlling the trait, the significance level used to detect QTL, and the size of the population (Table 1). The average power across experiments (i.e., averaged across experiments at a given level of a factor) decreased from 0.20 when 20 QTL controlled the trait, to only 0.04 when 80 QTL controlled the trait. Average power was consistently higher when the trait was controlled by 20 QTL regardless of the levels of other factors. When the number of QTL increased from 20 to 80, average power to detect major QTL decreased from 0.39 to 0.10 whereas power to detect minor QTL decreased from 0.04 to 0.01. FDR, however, remained constant at 0.22 regardless of whether 20 or 80 QTL controlled the trait. Average bias across detected QTL decreased from 38% when 80 QTL controlled the trait, to −6% when 20 QTL controlled the trait. This decrease in bias was mostly due to a decrease in bias at the minor QTL (Table 1). On average, the effects of major QTL were overestimated when 80 QTL controlled the trait but were underestimated when 20 QTL controlled the trait. In contrast, the effects of minor QTL were always overestimated and amount of upward bias increased as the number of QTL increased from 20 to 80. The amount of bias had a wide range across experiments: average bias ranged from −547 to 784% (results not shown) with a mean across experiments of 16%.

A less stringent significance level led to higher power (Table 1). Across experiments, a decrease in the significance threshold level from $\alpha = 0.01$ to 0.0001 reduced average power from 0.17 to 0.07. Most of this reduction in power was due to a loss in the power to detect minor QTL (Table 1). In contrast, a more stringent significance level of $\alpha = 0.0001$ reduced FDR from 0.32 to 0.11 across experiments. Bias increased from 13 to 20% when the significance level decreased from $\alpha = 0.01$ to 0.001. This increase was mostly due to an increase in bias at the minor QTL (Table 1).

Larger population sizes led to higher power (Table 1). Across experiments, when population size increased from $N = 600$ to 1,200 inbreds, average power increased two-fold, while FDR decreased from 0.24 to

0.20. A larger population size of 1,200 resulted in a reduction in average bias from 25 to 8%. Bias at the major QTL decreased from 1 to −25% when the population size increased from 600 to 1,200 inbreds.

The number of markers, the heritability of the trait, and the amount of initial linkage disequilibrium had only minor effects on the power to detect QTL. Across experiments, average power increased from 0.10 to 0.15 when the number of markers increased from 300 to 600. This increase was largely due to an increase in power (from 0.20 to 0.30) to detect major QTL. FDR increased only slightly, from 0.20 to 0.24 across experiments, when 600 markers were used. The gain in power outweighed the loss in FDR. Average bias decreased only slightly, from 18 to 15%, when the number of marker increased from 300 to 600. Most of this change in average bias was at the minor QTL.

When heritability increased from 0.40 to 0.70, average power across experiments increased from 0.11 to 0.14. The corresponding FDR increased from 0.21 to 0.23. Average bias decreased from 28 to 4%, with this decrease being mostly due to reduced bias at the minor QTL. Bias at major QTL changed from slight overestimation (0.3%) to underestimation of (−25%).

Among all the factors studied, the amount of initial linkage disequilibrium had the smallest effect on average power. Linkage disequilibrium decreased from 0.66–0.78 after 10 generations of random mating, to 0.57–0.69 after 20 generations of random mating. Across experiments, this decay in linkage disequilibrium decreased power from 0.13 to 0.12 and increased FDR from 0.22 to 0.23. However, average bias decreased from 26 to 6%. This change mostly occurred at the major QTL.

Discussion

Both precision and accuracy are important in QTL mapping. Power and FDR were functions of the precision in QTL mapping in this study, whereas bias measured the accuracy in QTL mapping. Power to detect QTL and FDR were affected by the genetic architecture of the trait and resources available for mixed-model QTL mapping. Power was maximized when the trait was controlled by fewer QTL and a large population was used for detecting QTL. These results agreed with those reported for designed mapping populations (van Ooijen 1992; Beavis 1994; Bernardo 2004). Beavis (1994) found that power decreased from 0.67 to 0.29 when the number of QTL in an F2 population increased from 10 to 40. van Ooijen (1992) found that increasing the size of an F_2 population from 200 to 400 individuals led to an almost two-fold increase in the probability of detecting a single QTL. Beavis (1994) found that increasing the population size from 500 to 1,000 individuals increased power to detect QTL by 60% on average. However, the increase in power due to larger population size varied from 6 to 127%, depending on number of QTL and heritability of the trait.

A stringent significance level of $\alpha = 0.0001$ instead of 0.01 led to fewer QTL declared significant, but also to fewer QTL being falsely declared significant. This result was consistent with previous results for an F_2 mapping population (Bernardo 2004). Our results showed that although fewer QTL would be reported at high levels of significance, these significant markers have a much higher probability of being truly linked to a QTL. Having fewer declared QTL with high confidence is crucial in gene discovery. Therefore, minimizing false leads through the use of stringent significant levels is recommended. Given that power was higher for major QTL than for minor QTL, the QTL detected under stringent significance levels are likely to be major QTLs regardless of the magnitude of their estimated effects.

In this study, the number of markers had only a minor effect on the power to detect QTL. This result was likely due to the marker density already being high when 300 markers were used. The average distance between markers was about 8.3 cM when 300 markers were used and 4.2 cM when 600 markers were used. Our results agreed with those of Darvasi et al. (1993), who reported little gain in power for marker distances less than 10 cM. Similarly, heritability also had a minor effect on power and FDR. Estimation of marker effects by the mixed-model approach utilizes information from relatives. The use of information from relatives leads to better estimates of genotypic values, which in effect causes heritability to be higher. We infer that exploiting information from relatives made heritability sufficiently high regardless of the simulated base values of 0.40 and 0.70.

Bias of effect estimates was affected by genetic architecture of the trait as well as resources available. Beavis (1998) found that the phenotypic variance associated with a correctly identified QTL became overestimated as the population size decreased. In addition, the effects of minor QTL were greatly overestimated when the population size was small. Our results showed a similar trend; a small population size resulted in a large bias, and the effects of major QTL were generally underestimated while the effects of minor QTL were generally overestimated (Table 1). These results agree with those of Kennedy et al. (1992), who found that bias tended to be proportionately larger for QTL with small effect than for QTL with large effect. Furthermore, the underestimation of effects at major QTL could have been partly due to the failure of the bias estimator to account for recombination between a significant marker and a linked QTL. Overall, our results imply that although it would be difficult to predict whether an effect is over- or underestimated in a particular experiment, any bias is reduced as more resources become available for mapping. This gain in accuracy is further leveraged by an increase in power and a reduction in FDR.

In a previous study (Yu et al. 2005), we examined the power of mixed-model QTL mapping via a mixed model in hybrid crops under similar experimental conditions. A comparison of our results in the present study with those

Table 1 Average power, false discovery rate (FDR), and average bias for mixed-model QTL mapping in a self-pollinated crop

α	Linkage disequilibrium[a]	Number of QTL	Number of markers	*H*	Population size	Power			FDR	Bias (%)[c]		
						All QTL	Major QTL[b]	Minor QTL		All QTL	Major QTL	Minor QTL
0.01	High	20	300	0.40	600	0.17	0.40	0.02	0.39	42	−30	257
					1,200	0.29	0.53	0.08	0.29	16	−50	135
				0.70	600	0.19	0.37	0.06	0.36	−75	−47	−6
					1,200	0.33	0.51	0.12	0.30	−32	−64	13
			600	0.40	600	0.19	0.47	0.02	0.38	−22	−32	−81
					1,200	0.38	0.75	0.08	0.29	−1	−35	73
				0.70	600	0.25	0.54	0.05	0.35	−18	−37	15
					1,200	0.47	0.76	0.12	0.29	−20	−54	50
		80	300	0.40	600	0.05	0.10	0.03	0.30	53	52	17
					1,200	0.10	0.19	0.04	0.20	83	−1	240
				0.70	600	0.05	0.11	0.03	0.30	67	−13	353
					1,200	0.12	0.22	0.05	0.20	49	−25	144
			600	0.40	600	0.05	0.12	0.02	0.38	103	−1	168
					1,200	0.11	0.21	0.04	0.30	62	−1	114
				0.70	600	0.06	0.11	0.02	0.41	131	−16	412
					1,200	0.15	0.31	0.05	0.24	29	−20	184
	Low	20	300	0.40	600	0.15	0.34	0.03	0.38	−27	−35	103
					1,200	0.23	0.36	0.06	0.36	−35	−54	8
				0.70	600	0.20	0.32	0.10	0.35	−17	−45	64
					1,200	0.28	0.42	0.10	0.33	−39	−67	16
			600	0.40	600	0.20	0.44	0.02	0.36	36	−33	247
					1,200	0.36	0.65	0.02	0.34	−7	−37	73
				0.70	600	0.23	0.45	0.04	0.33	−20	−50	51
					1,200	0.42	0.69	0.07	0.31	−29	−49	24
		80	300	0.40	600	0.03	0.07	0.02	0.39	134	−9	346
					1,200	0.08	0.18	0.02	0.26	19	−14	47
				0.70	600	0.04	0.08	0.01	0.32	27	1	164
					1,200	0.09	0.17	0.02	0.26	−14	−33	−116
			600	0.40	600	0.04	0.10	0.02	0.40	37	8	130
					1,200	0.11	0.28	0.01	0.32	18	−17	97
				0.70	600	0.05	0.11	0.02	0.43	−136	−20	260
					1,200	0.12	0.27	0.02	0.30	−4	−32	25
0.001	High	20	300	0.40	600	0.06	0.19	0.00	0.09	23	−6	564
					1,200	0.14	0.32	0.02	0.07	33	−38	169
				0.70	600	0.07	0.16	0.01	0.19	−2	−35	94
					1,200	0.16	0.32	0.04	0.11	−21	−52	27
			600	0.40	600	0.08	0.23	0.00	0.07	−43	−18	−112
					1,200	0.21	0.58	0.00	0.09	−5	−30	
				0.70	600	0.10	0.23	0.01	0.14	−7	−21	−111
					1,200	0.31	0.59	0.03	0.06	−31	−47	−59
		80	300	0.40	600	0.01	0.02	0.00	0.04	57	87	838
					1,200	0.03	0.06	0.00	0.04	44	35	63
				0.70	600	0.01	0.02	0.00	0.08	22	10	257
					1,200	0.03	0.08	0.01	0.11	29	−18	202
			600	0.40	600	0.01	0.02	0.00	0.20	91	143	−328
					1,200	0.03	0.09	0.01	0.21	45	5	392
				0.70	600	0.01	0.03	0.00	0.17	95	74	538
					1,200	0.05	0.11	0.01	0.13	38	−2	396
	Low	20	300	0.40	600	0.05	0.16	0.01	0.05	20	−8	412
					1,200	0.10	0.20	0.03	0.09	−12	−42	84
				0.70	600	0.07	0.15	0.02	0.10	129	−32	903
					1,200	0.14	0.24	0.03	0.08	−41	−55	−71
			600	0.40	600	0.07	0.20	0.00	0.16	14	−29	298
					1,200	0.21	0.49	0.02	0.04	−11	−31	52
				0.70	600	0.10	0.22	0.01	0.15	−4	−30	94
					1,200	0.24	0.52	0.03	0.10	7	−39	201
		80	300	0.40	600	0.00	0.01	0.00	0.07	24	93	719
					1,200	0.02	0.05	0.00	0.02	20	13	383
				0.70	600	0.01	0.02	0.00	0.15	4	7	
					1,200	0.02	0.06	0.01	0.15	−8	−15	−117
			600	0.40	600	0.01	0.03	0.00	0.12	44	51	710
					1,200	0.03	0.08	0.01	0.12	48	76	438
				0.70	600	0.01	0.02	0.00	0.21	10	55	−102
					1,200	0.03	0.07	0.01	0.26	17	−25	231

[a]High linkage disequilibrium was achieved by 10 generations of random mating; low linkage disequilibrium was achieved by 20 generations of random mating

[b]Major QTL were the top 25% of QTL with the largest effects; minor QTL were the bottom 25% of QTL with the smallest effects

[c]Percentage deviation of the estimated marker effect from the true QTL effect

of Yu et al. (2005) indicated that average power was higher for hybrid crops than for self-pollinated crops (Fig. 1). In hybrid crops, average power to detect QTL ranged from 0.11 to 0.59 for a significance level of $\alpha = 0.01$, and from 0.01 to 0.47 for $\alpha = 0.0001$ (Yu et al. 2005). The FDR ranged from 0.22 to 0.74 for $\alpha = 0.01$, and from 0.05 to 0.46 for $\alpha = 0.0001$. In the present study, we found for self-pollinated crops that the average power ranged from 0.03 to 0.47 for $\alpha = 0.01$, and from 0.01 to 0.31 for $\alpha = 0.0001$ (Table 1, Fig. 1). The FDR ranged from 0.20 to 0.43 for $\alpha = 0.01$, and from 0.03 to 0.33 for $\alpha = 0.0001$. The average ratio of power to FDR was 0.75 for hybrid crops and 0.51 for self-pollinated crops (Fig. 1). These comparisons suggested that, in addition to higher power of mixed-model QTL mapping in hybrid crops than in self-pollinated crops, the FDR at a given level of power is lower in hybrid crops than in self-pollinated crops. In both cases, however, the power to FDR ratios were lower than 1. This result should be interpreted with caution for two reasons. First, average power was roughly twice as high among the major QTL than among all the QTL. The power to FDR ratios would therefore increase when only the major QTL, which have the largest effects and would be the prime targets for gene discovery, are considered. Second, the low power to FDR ratios could be partially explained by the very strict criteria we used for defining power and FDR. Suppose M_1, M_2, and M_3 are closely adjacent markers, and a QTL is found between M_2 and M_3. Further suppose that only M_1 is found significant, perhaps due to other QTL in the vicinity. In this situation, power was declared equal to zero whereas the FDR was declared equal to 1 even though M_1 could have been only a few centimorgan away from the QTL.

The reasons are unclear for the higher power to FDR ratio in hybrid crops than in self-pollinated crops. Similar population sizes ($N = 600$), numbers of QTL controlling the trait, significance levels for detecting QTL, heritabilities, and marker densities were used in the current study and by Yu et al. (2005). In this study we considered four alleles per locus (QTL or marker) in contrast to two alleles per locus considered by Yu et al. (2005). It seems unlikely this difference in the number of alleles per locus would explain the difference in power between the two studies. Xu and Atchley (1995) used a mixed model with random marker effects and reported little change in power to detect QTL when the number of QTL alleles increased from two to six. A possible reason, however, is that the estimation of marker effects among hybrids capitalizes on information from relatives from the two pedigrees corresponding to the two parental inbreds of a hybrid. In contrast, the estimation of marker effects among inbreds exploits only one pedigree. We speculate that this higher degree of interconnectedness in hybrid crops led to a better estimation of marker effects and prediction of genotypic effects and, consequently, higher power of QTL detection.

How much power is enough power? The answer probably depends on whether the objective is gene discovery or marker-assisted selection. The precision and accuracy of the analysis for gene discovery would require larger populations and availability of markers to pinpoint specific candidate loci. In this context, minimizing FDR would be more critical than maximizing power. In contrast, marker-assisted selection requires that the phenotype can be predicted from the marker profile of an individual. Therefore, markers need not need to be as close as possible to the underlying QTL but rather be as highly associated with the trait of interest as possible.

In this study and in those of Parisseaux and Bernardo (2004), Yu et al. (2005), and M. Arbelbide and R. Bernardo (2005), mixed-model QTL mapping via a mixed model was applied considering markers as fixed effects.

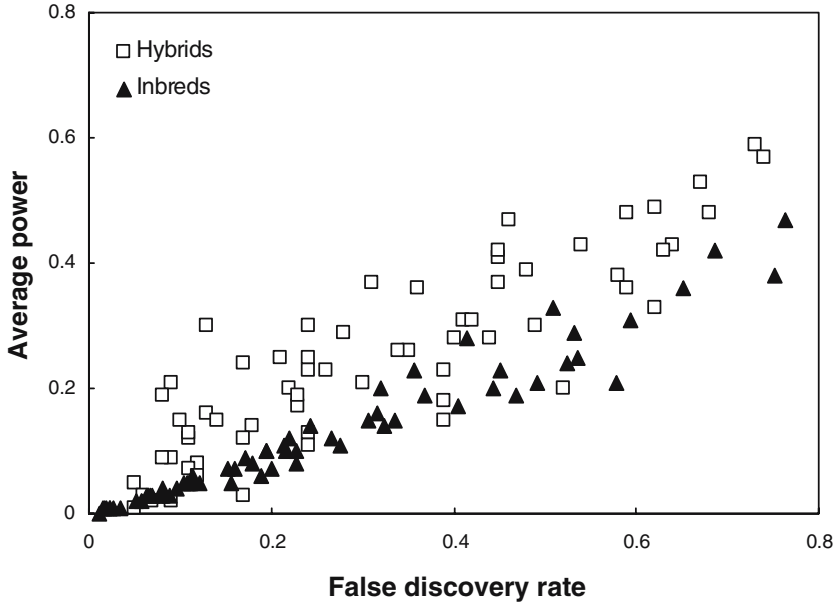

Fig. 1 Average power versus false discovery rate for self-pollinated and hybrid crops. Information on power and FDR for hybrid crops was obtained from Yu et al. (2005). On average, the power to FDR ratio was 0.75 for hybrids and 0.51 for inbreds

In contrast, Crepieux et al. (2004), Crepieux et al. (2005), and Zhang et al. (2005) proposed similar methods but considered markers as random effects and adapted the two-step variance component method of George et al. (2000) to inbred lines in complex pedigrees. Considering marker information as random effects allows the identification of marker intervals with putative QTLs and allows the prediction of overall breeding values for inbreds based on putative QTL alleles. Although this information can be used for selection, it does not provide estimates of mean effects associated with specific marker alleles linked to QTLs, preventing direct identification of favorable alleles.

In contrast, a fixed-effect approach allows the estimation of an effect for each marker allele. This approach inherently identifies the favorable marker alleles and the inbreds that most likely carry favorable alleles at specific QTL. As genetic maps become more dense, differences become negligible between interval mapping versus estimating the mean effect of a marker allele (Rebaï et al. 1995). If the markers are not candidate genes, then the fixed-effects approach is a first step towards gene discovery. If the marker loci are candidate genes themselves (e.g. single nucleotide polymorphisms or haplotypes within coding regions) or functional sequences, the analysis provides direct information on the genes or regulatory elements affecting the trait. In either case marker-assisted selection can still be practiced.

A possible drawback, however, of considering marker effects as fixed instead of random is overparameterization in the model (Crepieux et al. 2005). Overparameterization occurs in a system of equations when the number of unknowns (i.e., marker effects to be estimated) exceeds the number of equations. In our study overparameterization was avoided by three procedures. First, single-marker analysis was conducted prior to multiple-marker analysis. Considering a marker at a time allowed the preliminary identification of putative marker-QTL associations. Second, only the marker with the smallest P value was selected whenever adjacent markers were significant in the single-marker analysis. Third, marker effects within a locus were expressed as orthogonal contrasts among alleles.

In summary, our results indicate that QTL mapping via a mixed model approach is useful for gene discovery in the context of a breeding program for a self-pollinated crop. The method is most useful when few QTL control the trait and a large population is used to detect the QTL. In a companion study (Arbelbide and Bernardo 2006), we validated this mixed-model QTL mapping methodology by detecting previously reported QTL and known candidate genes for kernel hardness and dough strength in a bread wheat (*Triticum aestivum* L.) breeding program.

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