A Random Model Approach to QTL Mapping in Multi-parent Advanced Generation Inter-cross (MAGIC) Populations

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Most standard quantitative trait locus (QTL) mapping procedures apply to populations derived from the cross of two parents. QTL detected from such bi-parental populations are rarely relevant to breeding programs due to the narrow genetic basis; only two alleles are involved per locus. To improve the generality and applicability of mapping results, QTL should be detected using populations initiated from multiple parents, such as the multi-parent advanced generation inter-cross (MAGIC) populations. The greatest challenges of QTL mapping in MAGIC populations come from multiple founder alleles and control of the genetic background information. We developed a random model methodology by treating the founder effects of each locus as random effects following a normal distribution with a locus specific variance. We also fit a polygenic effect to the model to control the genetic background. To improve the statistical power for a scanned marker, we release the marker effect absorbed by the polygene back to the model. In contrast to the fixed model approach, we estimate and test the variance of each locus and scan the entire genome one locus at a time using the likelihood ratio test statistics. Simulation studies showed that the new method can increase statistical power and reduce Type I error compared with composite interval mapping (CIM) and multi-parent whole-genome average interval mapping (MPWGAIM). We demonstrated the method using a public *Arabidopsis thaliana* MAGIC population and a mouse MAGIC population.
INTRODUCTION

There is an urgent need to develop and study multi-parent advanced generation inter-cross (MAGIC) populations (RAKSHIT et al. 2012). Along with the nested association mapping populations (YU et al. 2008), the MAGIC population is called a “second generation mapping resource” (RAKSHIT et al. 2012). Using MAGIC populations to perform QTL mapping was first proposed for mouse by THREADGILL et al. (2002). Such a population is called the collaborative cross (CC) population (CHURCHILL et al. 2004; CONSORTIUM 2012). Simulation studies showed that an 8-parent CC population with 1000 progenies is capable of increasing mapping resolution to the sub-centiMorgan range (VALDAR et al. 2006). MAGIC populations in Drosophila melanogaster are called Drosophila Synthetic Population Resources (DSPR) (MACDONALD and LONG 2007; KING et al. 2012a; KING et al. 2012b). A review of MAGIC populations in crops can be found in HUANG et al (2015). The first plant MAGIC population was developed in Arabidopsis thaliana by KOVER et al. (2009). The population will be described later.

Subsequently, MAGIC populations have been developed in wheat (HUANG et al. 2012; MACKAY et al. 2014), rice (BANDILLO et al. 2013) and other crop species (GAUR et al. 2012; PASCUAL et al. 2015; SANNEMANN et al. 2015). One key difference between MAGIC populations and other multi-parent populations is that all MAGIC lines have experienced multiple generations of inbreeding and thus all are inbred lines. As a result, they are also considered genetic reference populations whose particular genome arrangement can be replicated indefinitely. MAGIC populations in plants will undoubtedly become more popular in the future of plant genetics and breeding (VARSHNEY and DUBEY 2009; RAKSHIT et al. 2012; HUANG et al. 2015), which calls attention to the need for improvements in statistical methods to analyze and interpret data derived from these populations. A recent call for papers on QTL mapping in MAGIC populations by GENETICS and G3 (http://www.genetics.org/) further indicates the urgent need of new technologies in MAGIC population QTL mapping.

Current methods of QTL mapping for MAGIC populations are primarily adopted from methods used in bi-parental populations. For example, composite interval mapping (ZENG 1994) originally developed for bi-parental populations has been used in QTL mapping for MAGIC populations to control genomic background. Other methods and programs of QTL mapping in MAGIC populations include MCQTL (JOURJON et al. 2005), R/qtl (BROMAN et al. 2003), R/happy (MOTT et al. 2000), R/mpMap (HUANG and GEORGE 2011), most of which have an option to perform composite interval mapping (CIM). However, there is an intrinsic limitation in cofactor selection, which is more problematic in MAGIC populations than in bi-parental populations. In an 8-parent initiated MAGIC population, each marker has \( 8 - 1 = 7 \) founder effects to estimate. The total number of effects will soon saturate the linear model as the number of cofactors increases. For example, a MAGIC population of size 500 will only allow less than \( 500 / 7 \approx 71 \) cofactors to be included in the model. When the number of cofactors is small, the CIM procedure is sensitive to the selection of cofactors. Ideally, a model should include all markers in a single model. However, when the marker density is high, genome scanning (a single QTL model) provides a better alternative method for QTL mapping, but the cofactors should be replaced by a polygenic effect, as done in genome-wide association studies (YU et al. 2006). We recently developed a QTL mapping procedure by fitting a polygene using a marker-inferred
relationship matrix (replacing cofactors) and demonstrated the robustness of the new method (Xu 2013b).

Recently, GATTI et al. (2014) developed a mixed model for QTL mapping in Diversity Outbred (DO) mice by treating the effects of scanned markers as fixed and a polygenic effect as random. The polygenic effect essentially replaced co-factors to control the genetic background. The method tends to have a low power because part of the effect of the marker currently scanned is absorbed by the polygene. Our simulation studies showed that dramatic improvement can be achieved in terms of resolution and statistical power of mapped QTL if the effect of the current QTL captured by the polygene is taken into account. VERBYLA et al. (2014) developed a multiple QTL model for QTL mapping in MAGIC populations. The method is called multi-parent whole-genome QTL analysis (MPWGAIM), where several steps are involved in selecting markers for inclusion in the model. First, a polygenic base model is implemented to detect the whole-genome effect on the traits of interest. If the polygenic variance is significantly larger than zero, then markers are subject to selection under a random model approach, i.e., the founder allelic effects of a marker are treated as random effects and the variance of these founder effects are estimated and the marker is then selected if the variance is sufficiently large. The final model will include all markers selected (forward selection). This is a variable selection approach and may be costly if the number of markers and the number of QTL found are large. We will treat this model as the gold standard for simulation and comparison. Another recent study of QTL mapping in MAGIC populations is the Bayesian modeling of haplotype effects (ZHANG et al. 2014), where the founder haplotype effects are estimated via Markov chain Monte Carlo (MCMC) sampling or importance sampling (IS). One important feature of the Bayesian method is the ability to handle uncertainty of the founder allelic inheritance. The only concern of the Bayesian method is the high computational cost when the sample size and the number of markers are very large because Monte Carlo sampling is involved. It is recommended to use the Bayesian method to fine-tune the model after markers are selected using some simple methods such as interval mapping and composite interval mapping.

In this study, we extended the mixed model methodology of QTL mapping in MAGIC populations by fitting a polygenic effect as random and a scanned marker effect either as fixed or random. Furthermore, we released the polygenic counterpart of a scanned marker effect back to the model to avoid competition between the marker effect and its polygenic counterpart. This improved mixed model methodology has significantly improved statistical powers of QTL detection. We used a CC mouse population (CONSORTIUM 2012) to perform simulations to examine the properties of the new methods (there are no phenotypic values available for the CC mouse population). The Arabidopsis MAGIC population of KOVER et al. (2009) and the preCC mouse population of RUTLEDGE et al. (2014) were reanalyzed using the new methods to demonstrate the differences of the new methods from existing methods.

MATERIALS AND METHODS

MAGIC populations

Three MAGIC populations were used in this study to demonstrate the new methods of QTL mapping, two populations in mouse and one in Arabidopsis thaliana. The first MAGIC
population in mouse does not have phenotypes available in the website (http://www.csbio.unc.edu/CCstatus) and was only used for simulation studies. The second MAGIC population of mouse has both genotype and phenotype information and was used as a real example of application. The MAGIC population in Arabidopsis thaliana also has both genotype and phenotype information and was reanalyzed to compare the results of different methods.

**First MAGIC population of mouse:** This MAGIC population is called the Collaborative Cross (CC) population (Churchill et al. 2004). The genotype data were published by the CC Consortium (Consortium 2012). No phenotype information is available in the 458 CC mice and thus the data were only used for simulation study. The CC population is an 8-parent MAGIC population derived from a funnel mating design. We downloaded the recombination breakpoint data of 19 autosomes from 458 CC mice posted in the UNC System Genetics website (http://www.csbio.unc.edu/CCstatus). Using the breakpoint information, we inferred 6683 bins (intact chromosome segments). A bin is defined as a segment that contains no breakpoints across all lines within the segment. Within a bin, all markers segregate in exactly the same pattern across lines (perfect LD). Therefore, a single marker can represent the whole bin. For detailed information of bin data analysis, please refer to Xu (2013a). The bin data are available in File S1.

**Second MAGIC population of mouse:** The second MAGIC population was derived from the same eight parents as the first CC population but the CC mice were not fully inbred, called preCC population. The data were obtained from Rutledge et al. (2014) and consist of 151 individuals. This data set includes 27,039 SNPs evenly distributed among the 20 chromosomes (including the X chromosome). Probabilities of the parental origins of the SNPs were calculated using the HAPPY program based on the hidden Markov model (Mott et al. 2000). In the original study of this population, the authors focused on two traits associated with severe asthma and decrements in lung functions, including airway polymorphonuclear neutrophil (PMN) recruitment and the concentration of CXCL1 in lung lavage fluid. Here, we reanalyzed the first trait PMN.

**MAGIC population of Arabidopsis:** The MAGIC population of Arabidopsis thaliana (Kover et al. 2009) consists of 527 lines descended from a heterogeneous stock of 19 inter-mated parents. These lines and the 19 founders were genotyped with 1260 SNP markers (MAF>5%) and phenotyped for two development-related traits, the number of days between bolting and flowering (DBF) and growth rate (GR), where GR was measured as the residual of regression by fitting the number of leaves to the number of days to germination. The 527 lines were derived from the 19 founder accessions of Arabidopsis thaliana, inter-mating for four generations and then inbreeding for six additional generations, forming nearly homozygous lines. The authors further updated the database after the initial publication. We downloaded the updated genotypes and phenotypes in the following website (http://mus.well.ox.ac.uk/magic/). There were only 426 lines having both the genotype and phenotype information. In this analysis, we included the 426 lines and 1254 markers distributed among five chromosomes (a total length of the genome is 118 Mb). The founder strain probabilities for all loci were calculated using the HAPPY program. We analyzed both DBF and GR.
Statistical methods

Polygenic model: The polygenic model is the null model used to scan the entire genome for QTL identification. We now use an 8-parent MAGIC population as an example to demonstrate the model. The method holds for any p-parent MAGIC populations. Let \( y \) be an \( n \times 1 \) vector of phenotypic values for \( n \) individuals. Define \( Z_k \) as an \( n \times 8 \) matrix of founder allele inheritance indicators for locus \( k \). The \( j \)-th row of matrix \( Z_k \) is defined as an \( 1 \times 8 \) vector. If this individual is a heterozygote carrying the first and second founder alleles, then we define
\[
Z_{jk} = [1 \ 1 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0]
\]
If the individual is a homozygote inheriting both alleles from the fifth founder, then \( Z_{jk} \) is defined as
\[
Z_{jk} = [0 \ 0 \ 0 \ 2 \ 0 \ 0 \ 0]
\]
The general rule for defining \( Z_{jk} \) is that there are at most two non-zero elements and the sum of all the eight elements equals two. We then define the following polygenic model, which is the null model used to test significance of an individual marker. The polygenic model is
\[
y = X \beta + \xi + \epsilon
\] (1)
where \( X \) is an \( n \times r \) design matrix for \( r \) fixed effects, \( \beta \) is an \( r \times 1 \) vector for the \( r \) fixed effects, \( \xi \) is an \( n \times 1 \) vector of polygenic effects with an assumed multivariate normal distribution \( \xi \sim N(0,K\phi^2) \) where \( K \) is a marker derived kinship matrix and \( \phi^2 \) is a polygenic variance, \( \epsilon \sim N(0,I\sigma^2) \) is a vector of residual errors with an unknown error variance \( \sigma^2 \). The marker inferred kinship matrix is defined as
\[
K = \frac{1}{d} \sum_{k=1}^{m} Z_k Z_k^T
\] (2)
where \( d = \frac{1}{m} \text{tr} \left( \sum_{k=1}^{m} Z_k Z_k^T \right) \) is a normalization factor. The expectation of \( y \) is \( \text{E}(y) = X\beta \) and the variance-covariance matrix is
\[
\text{var}(y) = K\phi^2 + I\sigma^2 = (K\lambda + I)\sigma^2 = H\sigma^2
\] (3)
where \( \lambda = \phi^2 / \sigma^2 \) is the variance ratio and \( H = K\lambda + I \). After absorbing \( \beta \) and \( \sigma^2 \), the restricted maximum likelihood is only a function of \( \lambda \), which is
\[
L(\lambda) = -\frac{1}{2} \ln |H| - \frac{1}{2} \ln |X^T H^{-1} X| - \frac{n - r}{2} \ln(y^T P y)
\] (4)
where
\[
P = H^{-1} - H^{-1} X (X^T H^{-1} X)^{-1} X^T H^{-1}
\] (5)
The restricted maximum likelihood solution of \( \hat{\lambda} \) was obtained by maximizing the above likelihood function using the Newton iteration algorithm. The eigen-decomposition algorithm proposed by Kang et al. (2008) was used to evaluate the likelihood function for fast computation. The estimated variance ratio is denoted by \( \hat{\lambda} \) and will be used as a known constant in the genomic scanning model that follows. In File S2 we described the method of
estimating $\lambda$ along with the effect of the marker scanned, the so called exact method (ZHOU and STEPHENS 2012).

**Fixed model:** To test the significance of the $k$th marker, we first used the fixed model approach proposed by GATTI et al. (2014). The model is

$$y = X\beta + Z_k\gamma_k + \xi + \varepsilon$$  \hspace{1cm} (6)

where $Z_k$ is the allelic inheritance matrix for marker $k$ as defined earlier and

$$\gamma_k = [\gamma_{1k} \; \gamma_{2k} \; \gamma_{3k} \; \gamma_{4k} \; \gamma_{5k} \; \gamma_{6k} \; \gamma_{7k} \; \gamma_{8k}]^T$$  \hspace{1cm} (7)

is an 8×1 vector for the eight founder allelic effects. Under this model, the $\gamma_k$ vector is assumed to be fixed effects. The model is in fact a mixed model because it contains both fixed and random effects. We call it the fixed model because later on we will treat $\gamma_k$ as random effects. Under the fixed model, we can only estimate and test seven (8 – 1 = 7) effects by deleting the last founder allele from the model. The maximum likelihood method was used to estimate $\gamma_k$ and the result turned out to be identical to the weighted least squares estimate after pre-multiplying all variables ($y, X$ and $Z_k$) by the eigenvectors of the $K$ matrix and the weight for the $j$th individual being $W_j = 1/ (\delta_j + 1)$, where $\delta_j$ is the $j$th eigenvalue of the $K$ matrix (XU 2013b). Note that $\hat{\lambda}$ is the estimated variance ratio under the polygenic model, as described earlier. This method is called the approximate method (ZHOU and STEPHENS 2012). The likelihood ratio test was used as the test statistic, which is defined as

$$\Gamma_k = -2 \left[ L_0(\hat{\beta}, \hat{\sigma}^2) - L_1(\hat{\beta}, \hat{\gamma}_k, \hat{\sigma}^2) \right]$$  \hspace{1cm} (8)

where $L_0$ is the log likelihood function under the null model ($\gamma_k = 0$) and $L_1$ is the log likelihood function under the alternative model. Note that the estimated $\beta$ and $\sigma^2$ under the two models are different. The p-value was calculated from the Chi-square distribution with seven degrees of freedom. This method is called “FIXED-A” when compared with other methods.

Recall that $Z_k$ contributes to the calculation of the kinship matrix $K$, as shown in equation (2). Although not explicitly estimated in the polygene, the effect of marker $k$ has a polygenic counterpart, which may compete with the estimated $\gamma_k$ when marker $k$ is scanned. Let $\hat{\xi}_k = Z_k \hat{a}_k$ be the estimated polygenic effect contributed by marker $k$ and $\hat{a}_k$ is the estimated effect for this marker under the polygenic model. We can release this effect from the polygene back to the model to avoid this competition. The revised model is

$$y = X\beta + Z_k\gamma_k + \xi - \hat{\xi}_k + \varepsilon$$  \hspace{1cm} (9)

Rearranging this equation leads to

$$y + \hat{\xi}_k = X\beta + Z_k\gamma_k + \xi + \varepsilon$$  \hspace{1cm} (10)

Further defining $y_k = y + \hat{\xi}_k$, we now have a new model

$$y_k = X\beta + Z_k\gamma_k + \xi + \varepsilon$$  \hspace{1cm} (11)
which is the same as equation (6) except that the \( y \) vector changes every time when a marker is
scanned. Note that \( \hat{\xi}_k \), the polygenic component from marker \( k \), is only calculated once under
the null model. Therefore, this revised method does not present much additional computational
burden. The method to obtain \( \hat{\xi}_k \) is called the best linear unbiased prediction (BLUP) and is
described in File S2. This revised method is called “FIXED-B” when compared
with other methods.

Random model: The fixed model approach may not be stable when the number of founders is
large (GATTI et al. 2014) and the design matrix \( Z_k \) may have variable ranks across different
markers. Under the null model, the likelihood ratio test statistic follows a Chi-square distribution
with degrees of freedom dependent on the number of founders. We propose to treat the eight
founder effects as random variables following a normal distribution with mean zero and a
common variance. Although it is still a mixed model, we call it random model to distinguish it
from the fixed model described earlier. The linear model remains the same as equation (6), but
\( \gamma_k \sim N(0, I_8 \phi_k^2) \) is assumed where \( \phi_k^2 \) is a locus specific variance. The expectation of \( y \) remains
\( E(y) = X\beta \) and the variance-covariance matrix is
\[
\text{var}(y) = Z_i Z_i' \phi_k^2 + K \sigma^2 + I \sigma^2 = Z_i Z_i' \phi_k^2 + (K\lambda + I) \sigma^2 = Z_i Z_i' \phi_k^2 + H \sigma^2
\]  
(12)
where \( \lambda \) in \( H \) is replaced by the estimated value under the polygenic model. Restricted
maximum likelihood (REML) estimate of \( \phi_k^2 \) is obtained via maximizing the restricted
likelihood function. Woodbury matrix identities (GOLUB and VAN LOAN 1996) are applied along
with the eigen-decomposition to ease the computational burden (File S2). The
null hypothesis for marker \( k \) is \( \phi_k^2 = 0 \), which is tested using the likelihood ratio test,
\[
\Gamma_k = -2\left[ L_0(\hat{\beta}, \hat{\sigma}^2) - L_1(\hat{\beta}, \hat{\phi}_k^2, \hat{\sigma}^2) \right]
\]  
(13)
Under the null model, this test statistic follows approximately a mixture of \( \chi_0^2 \) and \( \chi_1^2 \)
distributions with an equal weight (CHERNOFF 1954; VISSCHER 2006). This method is called
“RANDOM-A” when compared with other methods.

We also developed a revised version of the random model by avoiding competition between the
current marker scanned and its polygenic counterpart using model (11) as we did for the fixed
model. This revised random model is called “RANDOM-B” to distinguish it from other methods.

Multi-parent whole genome average interval mapping (MPWGAIM): Here we also
performed the analysis using the MPWGAIM approach proposed by VERBYLA et al. (2014) for
comparison using their R package “mpwgaim”. In the mpwgaim package, only detected markers
are reported without test statistics attached. For comparison with our methods, we calculated the
Wald test statistics of detected markers based on their estimated effects and variances, and then
obtained the p-value from the Chi-square distribution with \( 8 - 1 = 7 \) degrees of freedom. For
the simulated data analysis, we also applied the MPWGAIM method. The empirical critical
value for hypothesis test was inferred from multiple (1000) simulations under the null model.
The 95 percentile of the highest Wald test from each of the multiple simulations was chosen as
the empirical critical value. The p-value was transformed by $-\log_{10}$ and used to determine
whether or not a marker exceeds the empirical critical value.

**Interval mapping and composite interval mapping:** Interval mapping (Lander and Botstein 1989) and composite interval mapping (Zeng 1994) were also used to analyze the data to compare the results with the new methods. These two methods are called “IM” and “CIM-$x$”, respectively, where $x$ indicates the number of co-factors included in the model for background control. The statistical model for IM differs from model (6) by ignoring the polygenic effect. The model for CIM differs from model (6) by replacing the polygenic effect with selected co-factors. The IM method was implemented in the HAPPY program (Mott et al. 2000). The CIM method was implemented using our own R program. For the CIM-$x$ method, the number of cofactors ($x$) was set at the following levels for the first MAGIC population of mouse: 65, 50 and 30. For a sample size of 458, the maximum number of cofactors cannot be higher than $458/7 \approx 65$; otherwise, there will not be any degrees of freedom left to estimate the residual error variance. For the second MAGIC population of mouse (the preCC population), the number of cofactors was set at 20, 10 and 5. The population size is 151, and thus the number of cofactors cannot be higher than $151/7 \approx 20$. For the Arabidopsis population, the number of cofactors was set at the following levels: 20, 15 and 10. The maximum number of possible cofactors cannot be greater than $426/18 \approx 23$. The likelihood ratio test statistic was also used for the IM and CIM methods.

**p-value and permutation:** We now have a total of seven methods to compare. FIXED-A, MPWGAIM, IM and CIM are existing methods. FIXED-B, RANDOM-A and RANDOM-B are new methods proposed in this study. The p-value of a marker was calculated from the central Chi-square distribution with $8 - 1 = 7$ degrees of freedom for the two mouse populations and $19 - 1 = 18$ degrees of freedom for the Arabidopsis population under the FIXED-A, FIXED-B, MPWGAIM, IM and CIM methods. For methods of RANDOM-A and RANDOM-B, the p-value for each marker was calculated from a mixture of two Chi-square distributions, denoted by

$$\frac{1}{2} \chi^2_0 + \frac{1}{2} \chi^2_1,$$

where $\chi^2_0$ is just a fixed number of 0 (Chernoff 1954; Visscher 2006). Let $p_k$ be the p-value for marker $k$, it was calculated using

$$p_k = \begin{cases} 1 & \text{for } \Gamma_k = 0 \\ \frac{1}{2} \Pr(\chi^2_1 > \Gamma_k) & \text{for } \Gamma_k > 0 \end{cases}$$

(14)

where $\Gamma_k$ is the likelihood ratio test statistic calculated using equation (13) and $\chi^2_1$ is a Chi-square variable with one degree of freedom. In the real data analysis, we permuted the data 1000 times to generate a null distribution of the test statistics ($-\log_{10}(p)$). From this null distribution, we determined the 95% quantile and used it as an empirical critical value of a test statistic. A marker with the test statistic ($-\log_{10}(p)$) greater than this critical value is claimed to be significant at the 0.05 genome-wide Type I error rate. For the IM and CIM methods, a permuted sample was generated by randomly shuffling the phenotypes and keeping the genotypes intact. For the four methods with polygenic background control, the labels of the kinship matrix go with the reshuffled phenotypes so that the polygenic covariance structure remains the same as that in the original dataset. This kind of permutation will not destroy the polygenic variance (Cheng and Palmer 2013). Note that permutation was only used in real data analysis to generate empirical critical values for significance tests. In power calculation of the simulated data analysis, empirical critical values were generated from multiple simulations under the null model.
**Simulation experiment**

The simulation experiment was conducted based on the genotypic data of the first MAGIC population of mouse (the CC population). As a result, the sample size was fixed at 458. We used genotypes of the first five chromosomes as the true genotypes to conduct the simulation experiment. The five chromosomes contain 490, 503, 428, 423 and 406 bins, respectively, leading to a total of 2250 bins. The design of the simulated QTL mimicked closely to that of Verbyla et al. (2014). We simulated a total of seven QTL distributed on the five chromosomes. Information about the seven simulated QTL is shown in Table 1. The simulated allelic effects of the eight founders are given in Table 2. The polygenic and residual error variances were set at $\phi^2 = 0.5$ and $\sigma^2 = 0.5$, respectively. The seven QTLs collectively have a total variance of 1.1752, which is partitioned into the sum of variances for all seven QTLs (1.80) plus twice the sum of all covariances -0.6248 (1.80 - 0.6248 = 1.1752). The total phenotypic variance is 1.1752 + 0.5 + 0.5 = 2.1752. Therefore, the proportion contributed to the phenotypic variance by all the seven QTLs is 1.1752/2.1752 = 0.5403. The proportion of the polygenic variance contributed to the phenotypic variance is 0.5/2.1752 = 0.2298. The total genetic contribution (QTL + polygene) is 0.5403 + 0.2298 = 0.7701. QTLs 1 and 7 are small in terms of the proportions contributed to the trait phenotypic variance. The remaining four QTL are relatively large.

Under the previous setups of parameters, we generated 1000 independent data sets to evaluate the empirical powers under a 0.05 Type I error. We also generated 1000 additional datasets under the null model (no QTL were simulated but the polygene). Results of the data analysis from the null model were used to generate the empirical distribution of the test statistics ($-\log_{10}(p)$) and draw the empirical thresholds of the test statistics for hypothesis tests. The statistical powers from the 1000 replicated simulation experiments were reported by comparing the results with the empirically drawn thresholds of the test statistics. For each simulated QTL, a 5 cM window around the true position was reserved for power calculation, as done by Verbyla et al. (2014). If any bin within this window was detected, the QTL covered by this window was claimed to be detected. Any detected bins beyond that window were counted as false positives. All seven methods mentioned above were used to analyze the simulated data. The empirical powers were compared for the seven methods.

**Computational resources**

The new methods of QTL mapping for MAGIC populations were implemented in an R package called MagicQTL, which is provided in Supplemental Information and downloadable from the journal article website. R codes for data simulation, data preparation and data analysis are downloadable from the following website: [https://github.com/JulongWei/MagicQTL](https://github.com/JulongWei/MagicQTL). This website also provides the R code for calling the MPWGAIM package.

**RESULTS**

**Simulation studies**

**Statistical powers and false discovery rate (FDR):** The empirical statistical powers drawn from 1000 replicated simulations are given in Table 3. In general, RANDOM-A and
RANDOM-B have slightly higher powers than FIXED-A and FIXED-B for the five large simulated QTLs. FIXED-B and RANDOM-B have substantially higher powers than FIXED-A and RANDOM-A. The MPWGAIM approach has lower power for the first four large QTLs (QTL-2 to QTL-5) than that of FIXED-A, FIXED-B, RANDOM-A, and RANDOM-B. The MPWGAIM method has an advantage over the other methods for detecting the following three QTLs: QTL-1, QTL-6, and QTL-7. Except for MPWGAIM, no methods have sufficient powers to detect the two small QTLs (QTL-1 and QTL-7). Overall, the new methods (FIXED-B, RANDOM-A, and RANDOM-B) are more powerful than the existing methods (FIXED-A, MPWGAIM, IM, and CIM) for large QTLs.

We also compared the false discovery rate (FDR) for the seven methods (see last column of Table 3). Here we defined the FDR as the proportion of detected QTL that are not true (±5.00 cM away from a simulated QTL). Clearly FIXED-A, FIXED-B, RANDOM-A, and RANDOM-B achieve better control of the FDR than MPWGAIM, which in general is better than IM and CIM.

**Behaviors of the methods:** We first demonstrate the difference between the random model and the fixed model in terms of the test statistic expressed as \(-\log_{10}(p)\) of scanned markers using a single simulated dataset (Figure 1). The top panel (Figure 1a) shows the difference between RANDOM-A and FIXED-A. Clearly, the test statistic of FIXED-A is slightly higher than that of RANDOM-A. We also noticed that the statistic of the \(-\log_{10}(p)\) for RANDOM-A is very close to zero in regions where no QTL was simulated. This demonstrates the shrinkage property of the RANDOM method. In either case, the test statistic profiles show clear peaks at positions where simulated QTL reside and the heights of the peaks are proportional to the sizes of the simulated QTLs. The panel in the middle (Figure 1b) compares FIXED-A and FIXED-B, where the \(-\log_{10}(p)\) profile of FIXED-B shows higher peaks than that of FIXED-A. This implies that FIXED-B may have a higher power than FIXED-A. The panel at the bottom (Figure 1c) compares RANDOM-A and RANDOM-B. This also implies that releasing the polygenic counterpart of a marker back to the model may help increase the power of detecting this marker. These types of behaviors are expected to be observed in data analyses of real experiments.

**Average test statistic profiles:** We replicated the simulation experiment 1000 times under both the null model (without QTL effects) and the alternative model (with simulated QTL). The average test statistic profiles (\(-\log_{10}(p)\)) over the 1000 replicates and the 95% threshold values are illustrated in Figure 2. Comparing the fixed models (Figures 2a and 2b) with the random models (Figures 2c and 2d), we found that the test statistics are slightly higher for the fixed models than for the random models, but the former are also associated with higher threshold values in the test statistics. Comparing models with –A (Figures 2a and 2c) and models with –B (Figures 2b and 2d), the latter have higher peaks at positions where simulated QTLs reside. For the four models, peaks corresponding to the five large QTLs are higher than the threshold values, but peaks corresponding to the two small QTLs are below the thresholds. The peaks for the second QTL barely touch the thresholds for models with –A (Figure 2a and 2c), indicating that the modified models (releasing the polygenic effect back to the model) helps boost the power. None of the peaks in interval mapping reaches the threshold value (Figure 2e). Composite interval mapping with 30 co-factors only detected four of the five large QTLs (Figure 2f). When we increased the
number of co-factors to 50 and 65, the CIM method behaved very badly (Figure S1). For the MPWGAIM approach, due to lack of the test statistics in the package, we only reported the power and false discovery rate (FDR).

**Average estimated founder effects:** We also estimated the founder effects for the seven simulated QTLs based on all simulations and they are illustrated in Figure 3 for the fixed and random models and in Figure 4 for the interval and composite interval mapping procedures. The true effects were also plotted along with the estimated effects. All methods provided good estimates of the founder effects. The random models tend to shrink the estimated effects towards zero when the simulated QTL sizes are small (Figures 3a and 3g). Although IM and CIM are not as good as the other methods in terms of statistical power, both gave very good estimated founder effects. Figure S2 shows the average estimated effects of the founders when 50 and 65 markers were used as co-factors for the CIM method.

**Results of experimental data analyses**

**MAGIC population in Arabidopsis thaliana:** Under the polygenic model, we estimated the variance and heritability for each of the two traits, the days between bolting and flowering (DBF) and growth rate (GR). The results are shown in Table 4. The heritability of the two traits are 0.342 and 0.473 respectively. The variance ratios for DBF and GR are $\hat{\lambda}_{DBF} = \hat{\phi}^2 / \hat{\sigma}^2 = 0.5203$ and $\hat{\lambda}_{GR} = \hat{\phi}^2 / \hat{\sigma}^2 = 0.8980$, respectively, which were used as known values and incorporated into the covariance structures for genomic scanning of all markers. Figure 5 illustrates the test statistic profiles ($-\log_{10}(p)$) along with the 95% thresholds generated from 1000 permuted samples for DBF. Markers with test statistic values greater than the thresholds were claimed to be statistically significant. There are two peaks standing out on chromosomes 4 and 5, respectively, for all methods except CIM-10. These two regions also showed up in the original analysis of KOVER et al. (2009). However, the only method that detected both peaks is RANDOM-B, implying that this method may be the most powerful one. The detected QTL on chromosome 4 is located nearby a known gene called FRIGIDA. No related genes were found nearby the detected QTL on chromosome 5. The MPWGAIM method also detected the two QTLs in the same regions (Table 5). In addition, MPWGAIM detected three more QTLs, one on chromosome 1 (PERL0236029) and two on chromosome 3 (MASC00175 and MN3_22843506). Figure S3 (panels a and b) shows the results of this data analysis using CIM when 15 and 20 markers were used as co-factors.

The test statistic profiles along with permutation-generated thresholds are illustrated in Figure 6 for growth rate (GR). There are many bumps of the test statistic profiles below the thresholds, indicating that this trait is mostly polygenic. One peak in the beginning of chromosome 4 appears to be common to all six methods. Except FIXED-A and RANDOM-A, all other methods have detected the peak as statistically significant. We listed the SNPs exceeding the threshold values in Table 5. Three candidate genes are found in this area (about ±200 kb around the detected marker), FRIGIDA, and AT4G02990 (RUG2) and AT4G02780 (GA1). The first candidate gene (FRIGIDA) is known to affect flowering time. This gene is also related to growth rate in the original study (KOVER et al. 2009), who pointed out that this gene not only plays an important role in the reproduction of plant but also is a major determinant of the plant developmental
process. The second candidate gene (AT4G02990) is important for leaf development in *Arabidopsis thaliana* and its loss-of-function leads to a pleiotropic phenotype, including leaf variegation, reduced growth and perturbed mitochondrial and chloroplastic gene expression and development (QUESADA et al. 2011). The third candidate gene (GA1) codes for the enzyme ent-kaurene synthase A. In the GA1 mutants, the gibberellin biosynthesis pathway is inactivated. As a result, these mutants are deficient in bioactive GAs (SUN and KAMIYA 1994). There are some additional markers detected by the IM and MPWGAIM and they are listed in Table S1. The markers on chromosomes 2 and 5 detected by the IM method overlap with the additional markers detected by the MPWGAIM method. The other methods also show some bumps in regions nearby the additional markers detected by IM and MPWGAIM. These regions (about ± 200 kb around the peaked markers) harbored several candidate genes, which are not related to growth rate in terms of gene functions. Figure S3 (panels c and d) shows the results when 15 and 20 markers were used as co-factors for the CIM method.

**preCC population of mouse:** We analyzed a trait named polymorphonuclear neutrophil (PMN) from this population. The phenotypic values were log transformed prior to the analysis, as done in the original study. We estimated the genetic variance and heritability of the trait, which are presented in Table 3. The trait is highly heritable with a heritability of 0.55. The variance ratio is \( \hat{\lambda} = \frac{\hat{\phi}^2}{\hat{\sigma}^2} = \frac{1.373}{1.127} = 1.2183 \), which was used along with the kinship matrix to control the polygenic effect in QTL mapping. We scanned the entire genome using all the seven methods. The test statistic profiles are illustrated in Figure 7. Except FIXED-A and RANDOM-A, all other methods detected a marker on chromosome 2 (see Table 5). This marker was also detected by RUTLEDGE et al. (2014) in the original study. They found a candidate gene (Dpn1) nearby this marker. No other candidate genes were found in the neighborhood of this marker. Figure S3 (panels e and f) shows the results when 10 and 20 markers were used as co-factors for the CIM method.

**DISCUSSION**

A key difference between QTL mapping in MAGIC and bi-parental populations is the difference in the number of effects to be estimated and tested per locus. Under the fixed model framework, for an 8-parent MAGIC population, the number of effects per locus is 8 – 1 = 7, while it is always 2 – 1 = 1 for a bi-parental population. Under the null model, the LRT follows a Chi-square distribution with 7 degrees of freedom. In a \( p \)-parent MAGIC population, \( p – 1 \) is the degrees of freedom. When \( p \) is large, this test is not convenient and sometime can be unstable (GATTI et al. 2014). For example, if some founder alleles fail to appear in the progeny for some loci, the Z matrices for these loci will not have the same rank as those loci with full representation of all founders. This variable rank situation will cause some difficulty in programming. More importantly, the degree of freedom will vary across loci so that the LRT test statistic will not be comparable across loci. We developed a random model approach to estimate and test the variance among all founder effects per locus. As a result, we only need to estimate and test a single parameter (the variance), regardless how large the number of founders is in a MAGIC population. Simulation studies showed that the random model approach is slightly more powerful than the fixed model approach.
Some investigators also considered founder allelic effects as random in MAGIC population QTL mapping (Verbyla et al. 2014; Zhang et al. 2014). The MPWGAIM procedure of Verbyla et al. (2014) assumes that all founder allelic effects of the same locus share a common variance and this variance varies across loci. A forward variable selection approach was adopted by adding one locus to the model at a time until no further improvement was achieved. For consistency of comparison, we adopted the critical value generated from the null model, similar to the other methods, to evaluate the power of QTL detection from the MPWGAIM method using the same criterion of test. We demonstrated lower powers (for large QTL) and higher FDR for MPWGAIM. The MPWGAIM method can be time consuming if the numbers of markers and QTLs included in the model are large. Table 6 compares the computational time of our methods with MPWGAIM under several different scenarios. Clearly, the new genome scanning approaches proposed in this study are substantially faster than the MPWGAIM method. The Bayesian method of Zhang et al. (2014) also treats founder allelic effects as random and it is a multiple QTL model. Since the method is implemented via an MCMC sampling scheme, it is also computationally expensive. The authors suggested that the method is better used to fine-tune the results after an initial genome scan of all markers.

When cofactors are replaced by the polygene for background control, there is a potential competition between a currently scanned marker and its counterpart in the polygene, which has a detrimental consequence to the power. The competition can be very serious when the number of markers used to calculate the kinship matrix is small, although it may be negligible when a very large number of markers are used to calculate the kinship matrix. To prevent such a competition, we proposed to release the polygenic component corresponding to the scanned marker back to the model. This has dramatically increased the statistical power of QTL detection. The BLUP estimate of a marker effect in the polygene is only calculated once prior to the marker scanning step and thus little additional computational cost is present. We could have removed the currently scanned marker from the kinship matrix to avoid the completion. However, this would substantially increase the computational burden because a new kinship matrix had to be provided for each marker scanned. Special algorithms, such as the spectrally transformed linear mixed model (FaST-LMM) proposed by Lippert et al. (2011), may be used to ease the computational intensity. However, the fast speed is not achieved without a cost. One has to use markers with a number substantially smaller than the sample size to gain the fast speed. When the number of markers used to construct the kinship matrix is too small, optimal control of polygene may not be guaranteed (Zhou and Stephens 2012).

The genotype coding system of QTL mapping in MAGIC populations is different from that in bi-parental populations. We used the $Z_k$ variable (an $n \times 8$ matrix) to indicate the founder allelic inheritances for the $k$th marker. This variable was also used to calculate the marker-inferred kinship matrix $K$. The kinship matrix was eventually rescaled by a normalization factor, which is the average of the diagonal elements of the original unnormalized kinship matrix. After normalization, the diagonal elements of the kinship matrix are all around unity. Such a normalization will bring the estimated polygenic variance in the same scale as the residual error variance. Our normalization factor is different from that proposed by VanRaden (2008), which is the sum of heterozygosity across all loci. The normalization factor only changes the scale of the estimated polygenic variance; it affects neither the hypothesis tests nor the results of QTL mapping. In the genome-wide association studies (GWAS) where the $Z_k$ variable is simply a
vector, KANG et al. (2008) placed a weight variable for each marker in calculating the kinship matrix to take into account variable information contents (allele frequencies) across different marker loci. It is not obvious on how to evaluate information contents when the genotype indicator variable $Z_k$ for each marker is a matrix. In the CC and preCC mice, all founders contributed equally to the mapping population and thus the weight variable can be safely ignored (e.g., taking the default value of 1 from all markers). In the 19-parent MAGIC population of Arabidopsis, the parental contribution varies across founders, a weighted kinship matrix may be more appropriate. Further study is needed to develop an appropriate weight matrix. Alternatively, GATTI et al. (2014) method for calculating the kinship matrix may be adopted here. The relationship between each pair of individuals is a kind of average “scaled similarity” over all loci. In our notation, the relationship between individuals $i$ and $j$ (the $i$th row and the $j$th column of the kinship matrix) is expressed as

$$K_{ij} = \frac{1}{m} \sum_{k=1}^{m} \frac{Z_{ik}^T Z_{jk}}{\sqrt{Z_{ik}^T Z_{ik}} \sqrt{Z_{jk}^T Z_{jk}}}$$

We did not use this kinship matrix because the polygenic counterpart of marker $k$ (used in FIXED-B and RANDOM-B) would be difficult to interpret when this $K$ matrix is used. Furthermore, whether or not such a kinship matrix can adjust unbalanced contributions from different founders is still questionable.

The random model approach is a kind of Bayesian analysis if the founder effects are considered as parameters and the variance of the founder effects is considered as a prior variance. Since the prior variance is estimated from the data, it is called empirical Bayes (XU 2007). The random model developed for QTL mapping in MAGIC populations can be used in a number of other situations. The method can be extended to QTL mapping in diversity outbred (DO) populations, such as the DO population in mice developed from the same 8 parents as the CC mice (GATTI et al. 2014).

The random model approach is computationally more intensive than the fixed model approach where the QTL effects are treated as fixed effects because it requires estimation of variance component for each marker scanned. We adopted the eigen-decomposition algorithms for the polygenic (null) model and combined it with the Woodbury matrix identity for estimation of QTL variance. It would not be realistic to perform such a random model QTL mapping without resort to these special algorithms. There may be room for further improvement in the computational speed. However, we emphasize the concept and the novelty of the method, which are far more important than technical improvement of computational speed. Finally, all analyses were performed using an R program written by the authors. We developed an R package named MagicQTL, which is provided in the journal website.

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Valdar, W., J. Flint and R. Mott, 2006 Simulating the collaborative cross: power of quantitative trait loci detection and mapping resolution in large sets of recombinant inbred strains of mice. Genetics 172: 1783-1797.


Xu, S., 2013b Mapping quantitative trait loci by controlling polygenic background effects. Genetics 195: 1209-1222.


Figure Legends

**Figure 1.** Test statistic profiles of different methods from a simulated data set. The test statistics are presented as $-\log_{10}(p)$. Locations of the simulated QTLs are represented by the filled triangles on the x-axis. This figure demonstrates the common behaviors of the different methods that are expected to see in a real data analysis.

**Figure 2.** Average test statistic profiles ($-\log_{10}(p)$) of six methods from 1000 replicated simulation experiments. The horizontal dashed lines represent the 95% thresholds drawn from 1000 simulated samples under the null model. The true locations of the seven simulated QTL are represented by the filled triangles on the x-axis.

**Figure 3.** True and estimated allelic effects of eight founders for seven simulated QTL in the simulation experiment. The estimated effects are the average effects of 1000 replicated experiments. Results from four methods are presented in this figure: FIXED-A, FIXED-B, RANDOM-A and RANDOM-B.

**Figure 4.** True and estimated allelic effects of eight founders for seven simulated QTL in the simulation experiment. The estimated effects are the average effects of 1000 replicated experiments. Results from two methods are presented in this figure: IM and CIM-30, where -30 means 30 markers are used as co-factors.

**Figure 5.** Test statistic profiles ($-\log_{10}(p)$) for the days between bolting and flowering (DBF) of the *Arabidopsis thaliana* MAGIC population obtained from six methods. The horizontal dotted lines represent the 95% thresholds generated from 1000 permuted samples.

**Figure 6.** Test statistic profiles ($-\log_{10}(p)$) for growth rate (GR) of the *Arabidopsis thaliana* MAGIC population obtained from six methods. The horizontal dotted lines represent the 95% thresholds generated from 1000 permuted samples.

**Figure 7.** Test statistic profiles ($-\log_{10}(p)$) for PMN of the preCC mouse population obtained from six methods. The horizontal dotted lines represent the 95% thresholds generated from 1000 permuted samples.
Table 1. Information for the seven simulated QTL using genotypes of the first MAGIC population of mouse.

<table>
<thead>
<tr>
<th>QTL</th>
<th>Chromosome</th>
<th>Position (cM)</th>
<th>Bin</th>
<th>Variance&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Proportion&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTL-1</td>
<td>1</td>
<td>41.35</td>
<td>209</td>
<td>0.10</td>
<td>0.046</td>
</tr>
<tr>
<td>QTL-2</td>
<td>2</td>
<td>21.16</td>
<td>602</td>
<td>0.20</td>
<td>0.092</td>
</tr>
<tr>
<td>QTL-3</td>
<td>3</td>
<td>58.79</td>
<td>1313</td>
<td>0.30</td>
<td>0.138</td>
</tr>
<tr>
<td>QTL-4</td>
<td>3</td>
<td>65.18</td>
<td>1348</td>
<td>0.30</td>
<td>0.138</td>
</tr>
<tr>
<td>QTL-5</td>
<td>4</td>
<td>27.42</td>
<td>1564</td>
<td>0.40</td>
<td>0.185</td>
</tr>
<tr>
<td>QTL-6</td>
<td>4</td>
<td>41.19</td>
<td>1641</td>
<td>0.40</td>
<td>0.185</td>
</tr>
<tr>
<td>QTL-7</td>
<td>5</td>
<td>28.65</td>
<td>1994</td>
<td>0.10</td>
<td>0.046</td>
</tr>
</tbody>
</table>

<sup>a</sup> This is the variance of a QTL, which is defined as \( \text{var}(Z_k \gamma_k) \) and the variance is taken across all individuals in the MAGIC population.

<sup>b</sup> This is the proportion of the total phenotypic variance explained by the QTL.
Table 2. Founder effects for the seven simulated QTL using genotypes of the first MAGIC population of mouse.

<table>
<thead>
<tr>
<th>QTL</th>
<th>Chr.</th>
<th>Position</th>
<th>Founder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A/J</td>
<td>C57BL</td>
</tr>
<tr>
<td>QTL-1</td>
<td>1</td>
<td>41.35</td>
<td>-0.174</td>
</tr>
<tr>
<td>QTL-2</td>
<td>2</td>
<td>21.16</td>
<td>-0.473</td>
</tr>
<tr>
<td>QTL-3</td>
<td>3</td>
<td>58.79</td>
<td>0.21</td>
</tr>
<tr>
<td>QTL-4</td>
<td>3</td>
<td>65.18</td>
<td>-0.294</td>
</tr>
<tr>
<td>QTL-5</td>
<td>4</td>
<td>27.42</td>
<td>0.549</td>
</tr>
<tr>
<td>QTL-6</td>
<td>4</td>
<td>41.19</td>
<td>-0.287</td>
</tr>
<tr>
<td>QTL-7</td>
<td>5</td>
<td>28.65</td>
<td>-0.252</td>
</tr>
</tbody>
</table>
Table 3. Statistical powers for the seven simulated QTLs and false discovery rate (FDR) drawn from 1000 replicated simulation experiments.

<table>
<thead>
<tr>
<th>Method</th>
<th>QTL-1</th>
<th>QTL-2</th>
<th>QTL-3</th>
<th>QTL-4</th>
<th>QTL-5</th>
<th>QTL-6</th>
<th>QTL-7</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIXED-A</td>
<td>0.001</td>
<td>0.699</td>
<td>0.827</td>
<td>0.911</td>
<td>0.985</td>
<td>0.628</td>
<td>0.003</td>
<td>0.015</td>
</tr>
<tr>
<td>FIXED-B</td>
<td>0.003</td>
<td>0.868</td>
<td>0.919</td>
<td>0.957</td>
<td>0.993</td>
<td>0.750</td>
<td>0.014</td>
<td>0.0034</td>
</tr>
<tr>
<td>RANDOM-A</td>
<td>0.002</td>
<td>0.704</td>
<td>0.849</td>
<td>0.916</td>
<td>0.985</td>
<td>0.636</td>
<td>0.004</td>
<td>0.0014</td>
</tr>
<tr>
<td>RANDOM-B</td>
<td>0.003</td>
<td>0.868</td>
<td>0.923</td>
<td>0.959</td>
<td>0.993</td>
<td>0.754</td>
<td>0.014</td>
<td>0.0036</td>
</tr>
<tr>
<td>MPWGAIM</td>
<td>0.150</td>
<td>0.500</td>
<td>0.690</td>
<td>0.700</td>
<td>0.670</td>
<td>0.770</td>
<td>0.340</td>
<td>0.0179</td>
</tr>
<tr>
<td>IM</td>
<td>0.003</td>
<td>0.352</td>
<td>0.326</td>
<td>0.355</td>
<td>0.494</td>
<td>0.389</td>
<td>0.001</td>
<td>0.0608</td>
</tr>
<tr>
<td>CIM-30</td>
<td>0.002</td>
<td>0.476</td>
<td>0.620</td>
<td>0.761</td>
<td>0.986</td>
<td>0.754</td>
<td>0.015</td>
<td>0.0113</td>
</tr>
<tr>
<td>CIM-50</td>
<td>0.001</td>
<td>0.136</td>
<td>0.043</td>
<td>0.015</td>
<td>0.512</td>
<td>0.009</td>
<td>0.003</td>
<td>0.0317</td>
</tr>
<tr>
<td>CIM-65</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
<td>0.002</td>
<td>0.000</td>
<td>0.940</td>
</tr>
</tbody>
</table>
Table 4. Estimated variance components and heritability for two traits from the *Arabidopsis thaliana* population and one trait from the mouse population.

<table>
<thead>
<tr>
<th>Population</th>
<th>Trait</th>
<th>Polygenic variance</th>
<th>Residual variance</th>
<th>Heritability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis thaliana</td>
<td>Bolt to flower</td>
<td>2.187</td>
<td>4.203</td>
<td>0.342</td>
</tr>
<tr>
<td></td>
<td>Growth rate</td>
<td>1.989</td>
<td>2.215</td>
<td>0.473</td>
</tr>
<tr>
<td>Mouse</td>
<td>PMN</td>
<td>1.373</td>
<td>1.127</td>
<td>0.549</td>
</tr>
</tbody>
</table>
Table 5. Significant SNPs associated with two traits in the *Arabidopsis thaliana* and one trait in the mouse population.

<table>
<thead>
<tr>
<th>Population</th>
<th>Trait</th>
<th>Method</th>
<th>SNP</th>
<th>Chr</th>
<th>Position (kb)</th>
<th>p-value(^{a})</th>
<th>Variance(^{b})</th>
<th>Candidate gene (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arabidopsis thaliana</em></td>
<td>Bolt to flower</td>
<td>RANDOM-A</td>
<td>MN4_142943</td>
<td>4</td>
<td>143</td>
<td>0.011</td>
<td>0.437</td>
<td>FRIGIDA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RANDOM-B</td>
<td>MN4_142943</td>
<td>4</td>
<td>143</td>
<td>0.005</td>
<td>0.517</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>MN5_2707605</td>
<td>5</td>
<td>2,708</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td>MASC02783</td>
<td>5</td>
<td>2,522</td>
<td>0.041</td>
<td>0.874</td>
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<tr>
<td></td>
<td></td>
<td>MPWGAIM</td>
<td>MN4_142943</td>
<td>4</td>
<td>143</td>
<td>—</td>
<td>0.527</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MN5_1931248</td>
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<td>1,931</td>
<td>—</td>
<td>0.629</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Growth rate</td>
<td>FIXED-B</td>
<td>GA1_3232</td>
<td>4</td>
<td>1,243</td>
<td>0.013</td>
<td>0.626</td>
<td>FRIGIDA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RANDOM-B</td>
<td>GA1_8429</td>
<td>4</td>
<td>1,238</td>
<td>0.010</td>
<td>0.299</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>MPWGAIM</td>
<td>GA1_8429</td>
<td>4</td>
<td>1,238</td>
<td>—</td>
<td>0.253</td>
<td>AT4G02990 (RUG2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td>FRI_1888</td>
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<td>270</td>
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<td>0.493</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIM-10</td>
<td>GA1_7762</td>
<td>4</td>
<td>1,239</td>
<td>0.011</td>
<td>0.853</td>
<td>AT4G02780 (GA1)</td>
</tr>
<tr>
<td>Mouse</td>
<td>PMN</td>
<td>FIXED-B</td>
<td>M2.887</td>
<td>2</td>
<td>87,583</td>
<td>0.040</td>
<td>0.471</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>RANDOM-B</td>
<td>M2.887</td>
<td>2</td>
<td>87,583</td>
<td>0.030</td>
<td>0.292</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MPWGAIM</td>
<td>M2.887</td>
<td>2</td>
<td>87,583</td>
<td>—</td>
<td>0.263</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td>M2.887</td>
<td>2</td>
<td>87,583</td>
<td>0.013</td>
<td>0.450</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIM-5</td>
<td>M2.824</td>
<td>2</td>
<td>80,663</td>
<td>0.028</td>
<td>0.496</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) p-value was obtained from permutation test.

\(^{b}\) denotes the variance of the effect of the detected marker combining the founder allele inheritance indicators.

\(^{c}\) “—” due to the high computational cost, no permutation analysis was conducted.
Table 6. Computational performances of different methods under different sample sizes and different numbers of markers.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mouse-458-2250&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mouse-458-6683&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mouse-151-27309</th>
<th>Arabidopsis-426-1254</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIXED-A</td>
<td>22 sec</td>
<td>53 sec</td>
<td>1 min 43 sec</td>
<td>23 sec</td>
</tr>
<tr>
<td>FIXED-B</td>
<td>41 sec</td>
<td>1 min 44 sec</td>
<td>4 min 25 sec</td>
<td>34 sec</td>
</tr>
<tr>
<td>RANDOM-A</td>
<td>36 sec</td>
<td>1 min 42 sec</td>
<td>5 min 21 sec</td>
<td>27 sec</td>
</tr>
<tr>
<td>RANDOM-B</td>
<td>55 sec</td>
<td>2 min 31 sec</td>
<td>8 min 10 sec</td>
<td>39 sec</td>
</tr>
<tr>
<td>MPWGAIM</td>
<td>32 min 29 sec</td>
<td>2 h 33 min</td>
<td>1 h 33 min</td>
<td>11 min 51 sec</td>
</tr>
</tbody>
</table>

<sup>a</sup> The first number after the species name is the sample size and the second number is the number of markers.

<sup>b</sup> The number of bins is 6683, which is the total number of bins of the entire 19 chromosomes of the mouse genome.
Figure 1

(a) 

(b) 

(c)
Figure 2

(a) FIXED-A

(b) FIXED-B

(c) RANDOM-A

(d) RANDOM-B

(e) IM

(f) CIM-30

Marker position (cM)
Figure 3

(a) QTL-1
(b) QTL-2
(c) QTL-3
(d) QTL-4
(e) QTL-5
(f) QTL-6
(g) QTL-7

Legend:
- TRUE
- FIXED-A
- FIXED-B
- RANDOM-A
- RANDOM-B
Figure 4

Graphs showing the effect of different QTLs (QTL-1 to QTL-7) on founder number 1 to 8. Each graph represents the effect of the corresponding QTL across the founders.

Legend:
- TRUE
- IM
- CIM-30
Figure 5

(a) FIXED-A

(b) FIXED-B

(c) RANDOM-A

(d) RANDOM-B

(e) IM

(f) CIM-10

\[- \log_{10}(\rho) \text{ vs. Chromosome}\]
Figure 6

(a) FIXED-A

(b) FIXED-B

(c) RANDOM-A

(d) RANDOM-B

(e) IM

(f) CIM-10
Figure 7

(a) FIXED-A

(b) FIXED-B

(c) RANDOM-A

(d) RANDOM-B

(e) IM

(f) CIM-5

-Log_{10}(\rho)

Chromosome