Quantitative trait locus mapping analysis of multiple traits when using genotype data with potential errors

Liang Tong[1,2]**, Ying Zhou**[3,*]**, Yixing Guo**[4]**, Hui Ding**[2]**, and Donghai Ji**[1,*]

[1]**School of Science, Harbin University of Science and Technology, Harbin 150080, P. R. China**
[2]**School of Information Engineering, Suihua University, Suihua 152061, P. R. China**
[3]**School of Mathematical Sciences, Heilongjiang University and Heilongjiang Provincial Key Laboratory of the Theory and Computation of Complex Systems, Harbin 150080, P. R. China**
[4]**Dalian University of Science and Technology, Dalian 116052, P. R. China**

Corresponding authors:
Ying Zhou, Donghai Ji

Email addresses: zhouying@hlju.edu.cn, jidonghai@hrbust.edu.cn

ABSTRACT

Background. Quantitative trait locus (QTL) analysis aims to locate and estimate the effects of the genes influencing quantitative traits and infer the relationship between gene variants and changes in phenotypic characteristics using statistical methods. Some methods have been developed to map QTLs of multiple traits in the case of no genotype error in a given dataset. However, practical genetic data that people use may contain some potential errors because of the limitations of biotechnology. Common genetic data correction methods can only reduce errors, but cannot calculate the degree of error. In this paper, we propose a QTL mapping strategy for multiple traits in the presence of genotype errors. Methods. The additive effect, dominant effect, recombination rate, error rate, and other parameters of QTLs can be simultaneously obtained using this new method in the framework of multiple-interval mapping. Results. Our simulation results show that the accuracy of parameter estimation can be improved by considering the errors of marker genotypes during the analysis of genetic data. Real data analysis also shows that the new method proposed in this paper can map the QTLs of multiple traits more accurately.

INTRODUCTION

Recently, multiple-trait gene mapping has been widely studied, which refers to analyzing multiple quantitative/qualitative traits simultaneously, considering the correlations among multiple traits. It is necessary to consider the correlations among multiple traits in the process of quantitative trait locus (QTL) mapping because the joint information of multiple traits can improve the accuracy of detecting QTLs that influence the traits of interest. Multiple-trait methods have been validated to be more powerful than single-trait methods (Zhu and Zhang, 2009), and they are being used in many fields such as complex trait analysis and animal or plant genetic breeding.

Genome-wide association studies (GWAS) have become a powerful strategy for exploring candidate genes for important traits, including human complex diseases (Li and Leal, 2008; Maity et al., 2012; Sainani, 2015; Shi et al., 2017; Storey and Tibshirani, 2003; Wang et al., 2016; Zhang et al., 2017). Currently, several methods for detecting associations between multiple traits and common/rare variants have been proposed. For instance, Zhu et al. (2012) presented a covariate-adjusted association method based on generalized Kendall' tau, Zhu and Zhang (2013) proposed a nonparametric regression method for multiple longitudinal phenotypes using multivariate adaptive splines, Zhou et al. (2016) developed a nonparametric method to test for associations between rare variants and multiple traits, Kwak and Pan (2017) proposed gene- and pathway-based association tests for multiple traits with GWAS summary statistics, and Gai and Eskin (2018) presented a meta-analysis method for multiple-trait association

46 analysis.

47   In addition, linkage analysis methods, such as interval mapping (IM), composite interval mapping
48 (CIM), and multiple-interval mapping (MIM), have also been used to detect QTLs that control sin-
49 gle/multiple traits. Linkage mapping methods have their own advantages in cases of both single and
50 multiple traits and different mapping methods depend on their own statistical models. Unfortunately,
51 however, the linkage analysis method for a single trait cannot accurately map the genes that have multi-
52 ple effects. Jiang and Zeng (1995) proposed the MT-CIM method based on a mixed linear model and
53 maximum likelihood method. This method enables the simultaneous mapping of multiple traits using
54 composite interval mapping. Compared with the single-trait QTL mapping method, it can improve the
55 accuracy and efficiency of QTL mapping. Based on the idea of MIM, using the MT-CIM model, Joehanes
56 (2009) proposed a multi-trait and multi-interval mapping method abbreviated MTMIM and showed that
57 the accuracy of MTMIM is higher than that of MIM and MT-CIM. Recently, Tong et al. (2018) proposed
58 a method (MTMIM-NEW) for simultaneously estimating QTL parameters for mapping multiple traits,
59 which showed higher precision.

60   All the above-mentioned methods are applicable to cases where there are no genotype errors included
61 in the practical data set. However, due to the constraints in genotype scoring software and biochemical
62 anomalies, most of the data that researchers have used may contain certain potential genotype errors. The
63 genotype errors cannot be neglected arbitrarily in statistical analysis and may have a significant impact on
64 the study of genetic linkage analysis (Ronin et al., 2015; Tong et al., 2015; Yan et al., 2016).

65   To circumvent this difficulty, in this paper, we proposed a multi-trait multi-interval mapping method
66 in the presence of genotype errors in the genetic data. The closed iteration formulas of QTL effects, QTL
67 recombination rates, the covariance matrix, and the genotype error rate are given in the new method,
68 which guarantees the precision of parameter estimation. To validate the feasibility of the new method,
69 we analyzed mouse high-density lipoprotein cholesterol data with missing genotypes. The results show
70 that the method can effectively solve the problem of incomplete marker genotypes caused by biological
71 or physical deficiencies. Simulation results also show that the new method has advantages in estimating
72 all parameters of interest when marker genotypes contain errors. In particular, compared with existing
73 methods, the new method can provide higher precision in estimating QTL positions (recombination rates).

THEORY AND METHODS

75 We consider the data of n individuals from the F_2 population with t phenotypic traits; $q+1$ marker loci
76 are closely linked to form q marker intervals. Assuming that Y_{jl} $(j = 1,...,n, l = 1,...,t)$ represents the
77 l^{th} phenotypic trait value of the jth individual, X_{ji} $(j = 1,...,n, i = 1,...,q+1)$ and $\widetilde{X}_{ji}$ $(j = 1,...,n, i =$
78 $1,...,q+1)$ denote the true marker genotype and the observed marker genotype, possibly with error of the
79 i^{th} marker of the jth individual, respectively, X^*_{ji} $(j = 1,...,n, i = 1,...,q)$ denotes the latent QTL genotype
80 within the ith marker interval of the jth individual. Let $Y_j = (Y_{j1}, Y_{j2},...,Y_{jt})'$, $X_j = (X_{j1}, X_{j2},...,X_{j(q+1)})'$,
81 $\widetilde{X}_j = (\widetilde{X}_{j1}, \widetilde{X}_{j2},...,\widetilde{X}_{j(q+1)})'$ and $X^*_j = (X^*_{j1}, X^*_{j2},...,X^*_{jq})'$. γ_i and γ_{i1} respectively denote recombination rate
82 of the ith marker interval which is known and the recombination rate between the ith marker and the latent
83 QTL in the marker interval. Assuming that each marker interval of the F_2 group has at most one QTL
84 (Zhou, 2010), and when the genotype combination X^M_{ji} of the ith marker interval of the jth individual is
85 known, the conditional probabilities $P(X^*_{ji}|X^M_{ji})$ of QTL genotype $X^*_{ji}(Q_iQ_i, Q_iq_i, q_iq_i)$ are presented in
86 Table 1.

Here, we assume that parameter $\theta = P(\widetilde{X}_{ji} \neq x|X_{ji} = x)$ denotes the parameter of the genotype error rate for any genotype x, and further assume that φ^j denotes the joint error rate of the jth individual. During each step of the iteration computation in our new method, when the true marker genotype X_j is given, we can calculate the number k_j of false genes coded at the $q+1$ marker loci. Assuming that whether a marker genotype has a genotyping error or not is independent of other marker genotypes, we can obtain

$$\varphi^j = P(\widetilde{X}_j|X_j) = \prod_{i=1}^{q+1} P(\widetilde{X}_{ji}|X_{ji}) = (\theta/2)^{k_j} \cdot (1-\theta)^{(q+1)-k_j}.$$

87 The joint error rates $\varphi^j (j = 1,...,n)$ can be used to infer the parameter θ of the genotype error rate in the
88 new method.

Table 1. The conditional probabilities of QTL genotypes given the marker genotypes

		QTL genotype		
Code	Marker genotype	QQ	Qq	qq
1	$M_iM_iM_{i+1}M_{i+1}$	1	0	0
2	$M_iM_iM_{i+1}m_{i+1}$	$1-r_i$	r_i	0
3	$M_iM_im_{i+1}m_{i+1}$	$(1-r_i)^2$	$2r_i(1-r_i)$	r_i^2
4	$M_im_iM_{i+1}M_{i+1}$	r_i	$1-r_i$	0
5	$M_im_iM_{i+1}m_{i+1}$	0	1	0
6	$M_im_im_{i+1}m_{i+1}$	0	$1-r_i$	r_i
7	$m_im_iM_{i+1}M_{i+1}$	r_i^2	$2r_i(1-r_i)$	$(1-r_i)^2$
8	$m_im_iM_{i+1}m_{i+1}$	0	r_i	$1-r_i$
9	$m_im_im_{i+1}m_{i+1}$	0	0	1

$r_i = \gamma_{i1}/\gamma_i$

Statistical model

In the framework of multiple-interval mapping, to solve the problem of multiple-trait analysis with genotype errors, we consider building a statistical model as follows:

$$\underset{n\times t}{Y} = \sum_{i=1}^{q}\left[\underset{n\times 1}{u_i}\,\underset{1\times t}{a_i} + \underset{n\times 1}{v_i}\,\underset{1\times t}{d_i}\right] + \underset{n\times t}{e}, \tag{1}$$

where $\underset{n\times t}{Y}$ is the phenotype value matrix with element Y_{ji}, $\underset{n\times 1}{u_i} = [u_{1i},u_{2i},...,u_{ni}]'$ and $\underset{n\times 1}{v_i} = [v_{1i},v_{2i},...,v_{ni}]'$ denotes the indicator vector of the ith QTL genotypes of n individuals, respectively. In detail,

$$u_{ji} = 1,\ v_{ji} = -0.5,\ if\ X_{ji}^* = Q_iQ_i;$$

$$u_{ji} = 0,\ v_{ji} = 0.5,\ if\ X_{ji}^* = Q_iq_i;$$

$$u_{ji} = -1,\ v_{ji} = -0.5,\ if\ X_{ji}^* = q_iq_i.$$

$a_i = (a_{i1},a_{i2},...,a_{it})$ and $d_i = (d_{i1},d_{i2},...,d_{it})$ denote the additive effect vector and dominant effect vector of the ith QTL on t traits, respectively.

Furthermore, let the QTL effect matrix be

$$C = \begin{pmatrix} a_{11} & a_{12} & \cdots & a_{1t} \\ d_{11} & d_{12} & \cdots & d_{1t} \\ \vdots & \vdots & & \vdots \\ a_{q1} & a_{q2} & \cdots & a_{qt} \\ d_{q1} & d_{q2} & \cdots & d_{qt} \end{pmatrix},$$

$e = \{e_{ji}\}_{n\times t}$ represents the residual matrix, $e_j = [e_{j1},e_{j2}...,e_{jt}]'$, and $E(e_j) = 0$, $cov(e_j) = \Sigma_e$. For convenience, we denote the covariance matrix as:

$$\Sigma_e = \begin{pmatrix} \sigma_{11} & \sigma_{12} & \cdots & \sigma_{1t} \\ \sigma_{21} & \sigma_{22} & \cdots & \sigma_{2t} \\ \vdots & \vdots & & \vdots \\ \sigma_{t1} & \sigma_{t2} & \cdots & \sigma_{tt} \end{pmatrix},$$

where $\sigma_{il} = cov(e_{ji},e_{jl}) = \rho\sqrt{\sigma_{ii}\sigma_{ll}}$, $i,\ l = 1,...,t$, for any individual, j.

To solve the problem of multiple-trait, multiple-interval mapping with genotype errors, based on Model (1), we next provide a detailed derivation process of the new method via the classical expectation–maximization (EM) algorithm (Dempster et al., 1977). Let $\Omega = (C, \Sigma_e, \gamma, \theta)$ denote all parameters of interest, where $\gamma = (\gamma_{11},\gamma_{21},...,\gamma_{q1})$ is the parameter vector of QTL positions. The new method has the advantage of simultaneously estimating all model parameters in the framework of multiple-interval mapping.

Parameter estimation via the EM algorithm

In our new method, we focus on the observed data $(\widetilde{\mathbf{X}}, \mathbf{Y})$, where $\widetilde{\mathbf{X}} = (\widetilde{X}_1, \widetilde{X}_2, ..., \widetilde{X}_n)'$, $\mathbf{Y} = (Y_1, Y_2, ..., Y_n)'$, and deeply mine the generating mechanism of $\widetilde{\mathbf{X}}$. At the same time, we need to comply with the statistical model consisting of multiple traits and the latent QTLs given in Model (1). Therefore, based on the logistic structure of all random variables, as well as the background of genetic linkage, the EM algorithm can be applied to infer all parameters of interest here.

The complete log-likelihood function of the parameter matrix Ω can be expressed as follows:

$$l(\Omega) = \log[\prod_{j=1}^{n} P(Y_j|X_j^*, \Omega) \cdot P(X_j^*|X_j, \Omega) \cdot P(\widetilde{X}_j|X_j, \Omega)]$$

$$= \sum_{j=1}^{n} [\log P(Y_j|X_j^*, \Omega) + \log P(X_j^*|X_j, \Omega) + \log P(\widetilde{X}_j|X_j, \Omega)].$$

$\mathbf{E - Step}$: Given the observed data $(\widetilde{\mathbf{X}}, \mathbf{Y})$ and the sth-step parameter values $\Omega^{(s)}$, we calculate the conditional expectation $Q(\Omega|\widetilde{\mathbf{X}}, \mathbf{Y}, \Omega^{(s)})$ of $l(\Omega)$.

$$Q(\Omega|\widetilde{\mathbf{X}}, \mathbf{Y}, \Omega^{(s)}) = \sum_{j=1}^{n}\sum_{X_j^*} \log P(Y_j|X_j^*, \Omega) \cdot \tau_{X_j^*}^{(s)} + \sum_{j=1}^{n}\sum_{X_j X_j^*} \log P(X_j^*|X_j, \Omega) \cdot \tau_{X_j X_j^*}^{(s)}$$

$$+ \sum_{j=1}^{n}\sum_{X_j} \log P(\widetilde{X}_j|X_j, \Omega) \cdot \tau_{X_j}^{(s)}. \tag{2}$$

Here, $\tau_{x_j x_j^*}^{(s)} = P(X_j^* = x_j^*, X_j = x_j|\widetilde{X}_j, Y_j, \Omega^{(s)})$, $\tau_{x_j^*}^{(s)} = P(X_j^* = x_j^*|\widetilde{X}_j, Y_j, \Omega^{(s)})$, and $\tau_{x_j}^{(s)} = P(X_j = x_j|\widetilde{X}_j, Y_j, \Omega^{(s)})$. According to the Bayes formula, $\tau_{x_j x_j^*}^{(s)}$ can be calculated and expressed in the following form:

$$\tau_{x_j x_j^*}^{(s)} = \frac{P(Y_j|x_j^*, \Omega^{(s)}) \cdot P(x_j^*|x_j, \Omega^{(s)}) \cdot P(\widetilde{X}_j|x_j, \Omega^{(s)})}{\sum_{x_j}\sum_{x_j^*} P(Y_j|x_j^*, \Omega^{(s)}) \cdot P(x_j^*|x_j, \Omega^{(s)}) \cdot P(\widetilde{X}_j|x_j, \Omega^{(s)})}.$$

Further, we let

$$\tau_{x_{jk}^*}^{(s)} = P(X_j^* = x_j^{*k}|\widetilde{X}_j, Y_j, \Omega^{(s)})$$

$$= \sum_{x_j} \frac{P(Y_j|x_j^{*k}, \Omega^{(s)}) \cdot P(x_j^{*k}|X_j, \Omega^{(s)}) \cdot P(\widetilde{X}_j|X_j, \Omega^{(s)})}{\sum_{k=1}^{3^q} P(Y_j|x_j^{*k}, \Omega^{(s)}) \cdot P(x_j^{*k}|X_j, \Omega^{(s)}) \cdot P(\widetilde{X}_j|X_j, \Omega^{(s)})}, \quad \forall k \in \{1, ..., 3^q\}. \tag{3}$$

where $\tau_{x_{jk}^*}^{(s)}$ represents the conditional probability that x_j^* takes the kth value x_j^* under the given condition of $\widetilde{X}_j, Y_j, \Omega^{(s)}$. In a detailed analysis, $P(Y_j|x_j^{*k}, \Omega^{(s)}) = f(Y_j; \mu_{jk}, \Sigma_\mathbf{e})$, which is a multivariate normal density with mean μ_{jk} and covariance matrix $\Sigma_\mathbf{e}$. $P(x_j^{*k}|X_j, \Omega^{(s)})$ can be expressed by a function of conditional probabilities listed in Table 1, because each QTL genotype is conditionally independent, given the condition of marker genotypes. In detail, $P(x_j^{*k}|X_j, \Omega^{(s)})$ is the function of recombination rates $\gamma_{11}, \gamma_{21}, ...,$ and γ_{q1}.

$\mathbf{M - Step}$: Calculate the maximum value of the conditional expectation $Q(\Omega|\widetilde{\mathbf{X}}, \mathbf{Y}, \Omega^{(s)})$, in order to obtain $\Omega^{(s+1)}$.

Updating C and $\Sigma_\mathbf{e}$

Based on the first part of $Q(\Omega|\widetilde{\mathbf{X}}, \mathbf{Y}, \Omega^{(s)})$ in Equation (2), the iteration expression of the QTL effect matrix C and covariance matrix $\Sigma_\mathbf{e}$ can be obtained as follows:

$$C_{cq \times t}^{(s+1)} = R^{(s)} - M^{(s)}C^{(s)},$$

$$\Sigma_{\mathbf{e}}^{(s+1)} = \frac{1}{n}(\mathbf{Y} - W^{(s)}DC^{(s+1)})'(\mathbf{Y} - W^{(s)}DC^{(s+1)}),$$

where $W = [\tau_{x_{jk}^*}]$ is a $n \times 3^q$ matrix with element $\tau_{x_{jk}^*}$ given in Equation (3), G is the QTL genotype sequence, and $D = (D_1, D_2, D_3, ..., D_{cq})$ is a $3^q \times cq$ matrix with expression

$$D = \begin{bmatrix} 1 & -1/2 & 1 & -1/2 & \cdots & 1 & -1/2 & 1 & -1/2 \\ 1 & -1/2 & 1 & -1/2 & \cdots & 1 & -1/2 & 0 & 1/2 \\ & & & & \vdots & & & & \\ -1 & -1/2 & -1 & -1/2 & \cdots & -1 & -1/2 & 0 & 1/2 \\ -1 & -1/2 & -1 & -1/2 & \cdots & -1 & -1/2 & -1 & -1/2 \end{bmatrix} = \begin{bmatrix} G & 1 & 1\cdots & 1 & 1 \\ G & 1 & 1\cdots & 1 & 0 \\ & & \vdots & & \\ G_{-1} & -1\cdots & -1 & 0 \\ G_{-1} & -1\cdots & -1 & -1 \end{bmatrix};$$

The formulae of $R^{(s)}$ and $M^{(s)}$ can be expressed as

$$R^{(s)} = \begin{bmatrix} \dfrac{D_1'W'^{(s)}Y}{\mathbf{1}'W^{(s)}(D_1\#D_1)} \\ \vdots \\ \dfrac{D_{cq}'W'^{(s)}Y}{\mathbf{1}'W^{(s)}(D_{cq}\#D_{cq})} \end{bmatrix}_{cq \times t}, \quad M^{(s)} = \begin{bmatrix} 0 & \dfrac{\mathbf{1}'W^{(s)}(D_1\#D_2)}{\mathbf{1}'W^{(s)}(D_1\#D_1)} & \cdots & \dfrac{\mathbf{1}'W^{(s)}(D_1\#D_{cq})}{\mathbf{1}'W^{(s)}(D_1\#D_1)} \\ \dfrac{\mathbf{1}'W^{(s)}(D_2\#D_1)}{\mathbf{1}'W^{(s)}(D_2\#D_2)} & 0 & \cdots & \dfrac{\mathbf{1}'W^{(s)}(D_2\#D_{cq})}{\mathbf{1}'W^{(s)}(D_2\#D_2)} \\ \vdots & \vdots & \ddots & \vdots \\ \dfrac{\mathbf{1}'W^{(s)}(D_{cq}\#D_1)}{\mathbf{1}'W^{(s)}(D_{cq}\#D_{cq})} & \dfrac{\mathbf{1}'W^{(s)}(D_{cq}\#D_2)}{\mathbf{1}'W^{(s)}(D_{cq}\#D_{cq})} & \cdots & 0 \end{bmatrix}_{cq \times cq},$$

where notation "#" in expressions $R^{(s)}$ and $M^{(s)}$ denotes the Hadamard product of two vectors and $\mathbf{1}$ is an $n \times 1$ column vector of ones.

Updating γ

We define that the indicator function, I_{st}^{ji}, of the QTL genotype and the marker-interval genotype have the following form:

$$I_{(ji)}^{st} = \begin{cases} 1, & X_{ji}^* = s, X_{ji}^M = t; \ s = 0, \ 1, \ 2; \ t = 1, \ldots, 9, \\ 0, & \text{else.} \end{cases}$$

Here, $j = 1, \ldots, n$, $i = 1, \ldots, q$. According to the conditional independence assumption of QTL genotypes, given marker-interval genotypes, the second part of $Q(\Omega|\widetilde{\mathbf{X}}, \mathbf{Y}, \Omega^{(s)})$ in Equation (2) can be transformed into the following form:

$$\sum_{j=1}^{n} \sum_{X_j} \sum_{X_j^*} \log P(X_j^*|X_j, \Omega) \cdot \tau_{X_jX_j^*}^{(s)} = \sum_{j=1}^{n} \sum_{X_j} \sum_{X_j^*} \tau_{X_jX_j^*}^{(s)} \cdot \sum_{i=1}^{q} \log P(X_{ji}^*|X_{ji}^M, \Omega). \tag{4}$$

Hereinto, $P(X_{ji}^*|X_{ji}^M, \Omega)$ can be expressed as a function of the probabilities listed in Table 1 and the indicator function $I_{(ji)}^{st}$s. When we maximize the above Equation (4), an explicit closed expression of the recombination rate γ_{i1} can be obtained:

$$\gamma_{i1}^{(s+1)} = $$

$$\frac{\gamma_i \cdot \sum_{j=1}^{n} \sum_{x_j} \sum_{x_j^*} \tau_{x_jx_j^*}^{(s)} \cdot (I_{(04)}^{ji} + 2I_{(07)}^{ji} + I_{(12)}^{ji} + I_{(18)}^{ji} + 2I_{(23)}^{ji} + I_{(26)}^{ji} + I_{(13)}^{ji} + I_{(17)}^{ji})}{\sum_{j=1}^{n} \sum_{x_j} \sum_{x_j^*} \tau_{x_jx_j^*}^{(s)} \cdot (I_{(02)}^{ji} + 2I_{(03)}^{ji} + I_{(04)}^{ji} + 2I_{(07)}^{ji} + I_{(12)}^{ji} + I_{(14)}^{ji} + I_{(16)}^{ji} + I_{(18)}^{ji} + I_{(26)}^{ji} + 2I_{(23)}^{ji} + I_{(28)}^{ji} + 2I_{(27)}^{ji} + 2 \cdot I_{(13)}^{ji} + 2 \cdot I_{(17)}^{ji})}.$$

Updating θ

As for the third part of $Q(\Omega|\widetilde{\mathbf{X}}, \mathbf{Y}, \Omega^{(s)})$ in Equation (2),

$$\sum_{j=1}^{n} \sum_{X_j} \log P(\widetilde{X}_j|X_j, \Omega) \cdot \tau_{X_j}^{(s)} = \sum_{j=1}^{n} \sum_{X_j} \log P(\widetilde{X}_j|X_j, \Omega) \cdot P(X_j = x_j|\widetilde{X}_j, Y_j, \Omega^{(s)}),$$

and maximization of this function could lead to an explicit expression of the error rate, θ:

$$\theta^{(s+1)} = \frac{\sum_{j=1}^{n} \sum_{x_j} k_j \cdot P(X_j = x_j|\widetilde{X}_j, Y_j, \Omega^{(s)})}{n \cdot (q+1)}.$$

119 By repeating the above entire updating process for C, Σ_e, γ, and θ until convergence, the parameter
120 estimate of Ω can eventually be obtained. For convenience, this proposed method for mapping QTLs of
121 multiple traits is referred to as MTMIM_e.
122   In our study, we suppose that the overall genotype error rate is in a given data set, which means that
123 the genotype error rates for each locus are same. When considering multiple datasets with same error
124 rate (e.g., the genotype data in each dataset were obtained under the same environment), we can combine
125 these datasets into one big dataset. At this time, the total error rate is still the fixed parameter, so the
126 combined dataset can be regarded as a new dataset, and then can be analyzed by the method provided
127 in this paper. All parameters including the genotype error rate can be estimated, and moreover, the
128 accuracy of parameter estimation will be improved due to the increase of sample size. If these datasets
129 have different genotype error rates (e.g., $\theta_1, \theta_2, \theta_3$), we can also combine these data sets into one big
130 dataset, but the estimating strategy in our method need to be adjusted. The parameters C, Σ_e, and γ can be
131 estimated by the whole data, but the genotype error rates need to be estimated by their respective dataset.

SIMULATION STUDIES

133 To evaluate the performance of the proposed MTMIM_e method in this study and objectively compare it
134 with the existing methods, MTMIM (Joehanes, 2009) and MTMIM-NEW (Tong et al., 2018), extensive
135 simulation studies were conducted in this section.
136   In our simulation design, two quantitative traits and two latent causal QTLs were considered. The two
137 QTLs were respectively located in two equally spaced marker intervals that consisted of three markers on
138 a chromosome. We randomly set certain genotype errors at the first marker in error rate θ. The length
139 of the marker interval was taken as 10 cM, and the sample size was $N = 500$. A map distance of 10
140 cM corresponds to a recombination rate of 0.0906 according to the Haldane map function (Ott, 1999).
141 To study the impact of genotype errors on the estimates of the recombination rate γ, the effect matrix
142 C, and the covariance matrix Σ_e, simulated data were generated under different error rates ($\theta =0$, 0.05,
143 and 0.1); the true values of recombination rates were taken as $\gamma_{11} = 0.03$ and $\gamma_{21} = 0.04$, and the effect
144 matrix C was selected to make the heritability h^2 close to 0.05 and 0.2, respectively. For each group
145 of parameters, we computed the estimates of all parameters using the above three methods. The entire
146 process was repeated 1000 times and the mean of the estimates for each parameter was computed. To
147 evaluate the accuracy of the estimates obtained by the different methods, the mean square error (MSE) of
148 each parameter estimate and the total mean (TM) of MSEs of all parameter estimates (except θ) were
149 also calculated (Tables 2 to 4; Figures 1 to 2).
150   Table 2 provides the estimates of all parameters (except θ) and the MSEs with different error rates
151 when heritability $h^2 = 0.2$. Simulation results show that genotype error is an important factor affecting
152 parameter estimation; larger genotype error rates would result in lower estimation accuracy. To see the
153 performance of the three methods more intuitively, we present the TM histograms of the three methods
154 (Fig. 1). The gray bar graph shows the TM values of the newly proposed method under different error
155 rates. In the case of the same genotype error rate ($\theta =0.05$, 0.1), it can be seen from Figure 1 that each
156 value of the TM of the proposed method (MTMIM_e) is uniformly lower than the corresponding values of
157 MTMIM-NEW and MTMIM. When the genotype error rate $\theta = 0$ (i.e., no genotype error in the simulated
158 data set), the performance of the new method is also comparable to that of the MTMIM-NEW method
159 and better than that of the original MTMIM method. That is, the new method outperforms the other two
160 methods in terms of estimating accuracy, and it can effectively overcome the influence of the genotype
161 errors on gene mapping.
162   We also consider the impact of different heritabilities on the estimation accuracy. Table 3 gives the
163 estimates of all parameters (except θ) and their MSEs, with different heritability values when the genotype
164 error rate $\theta =0.05$. The simulation results showed that, with an increase in heritability, the accuracy
165 of these three methods also increases as expected; however, among all these methods, the new method
166 still has the highest estimation accuracy. Figure 2 shows the TM histogram of the three methods under
167 different heritability ($h^2 = 0.05, 0.2$) conditions. The TM value of the new method (MTMIM_e) is smaller
168 than that of the other two methods; in other words, the new method can weaken the influence of genotype
169 error on parameter estimation (Fig. 2).
170   In addition to the estimates of QTL effects, QTL positions, and the covariance matrix of multiple traits,
171 the estimate of the genotype error rate can also be obtained simultaneously using the new method. Table
172 4 shows the estimates of error parameter θ and the corresponding MSEs with true values of genotype

Table 2. The estimates of all parameters (except θ) and the corresponding mean square errors (MSEs) with different error rates when $h^2 = 0.2$

	Parameter	True value	$\theta=0$ MTMIM	MTMIM-NEW	MTMIM_e	$\theta=0.05$ MTMIM	MTMIM-NEW	MTMIM_e	$\theta=0.1$ MTMIM	MTMIM-NEW	MTMIM_e
	γ_{11}	0.03	0.0437	0.0318	0.0292	0.0554	0.0308	0.0241	0.0573	0.0297	0.0254
			$(0.0228)^c$	(0.0117)	(0.0067)	(0.0334)	(0.0193)	(0.0071)	(0.0240)	(0.0176)	(0.0070)
	γ_{21}	0.04	0.0351	0.0455	0.0402	0.0360	0.0362	0.0384	0.0343	0.0347	0.0386
			(0.0193)	(0.0113)	(0.0133)	(0.0184)	(0.0193)	(0.0127)	(0.0196)	(0.0185)	(0.0138)
	ρ	0.9	0.8778	0.8866	0.8824	0.8730	0.8821	0.8744	0.8570	0.8819	0.8574
			(0.0689)	(0.0622)	(0.0675)	(0.0710)	(0.0669)	(0.1053)	(0.0673)	(0.1669)	(0.1402)
	σ_{11}	1	0.9648	0.9846	0.9805	0.9779	0.9796	0.9720	0.9773	0.9794	0.9559
			(0.0716)	(0.0656)	(0.0690)	(0.0703)	(0.0704)	(0.1055)	(0.0688)	(0.1679)	(0.1355)
	σ_{22}	1	0.9606	0.9839	0.9799	0.9636	0.9790	0.9709	0.9690	0.9796	0.9518
			(0.0746)	(0.0657)	(0.0714)	(0.0784)	(0.0710)	(0.1137)	(0.0742)	(0.1719)	(0.1578)
T1	Q1	-0.1478^a	-0.1780	-0.1427	-0.1440	-0.2121	-0.1396	-0.1322	-0.2009	-0.1288	-0.1459
			(0.1528)	(0.1292)	(0.1305)	(0.2169)	(0.2139)	(0.1389)	(0.2012)	(0.1946)	(0.1773)
		0.1182^b	0.1230	0.1082	0.1159	0.1664	0.1026	0.1019	0.1596	0.0775	0.0966
			(0.1539)	(0.1527)	(0.1588)	(0.2424)	(0.2226)	(0.1939)	(0.2719)	(0.1949)	(0.2229)
	Q2	0.1288	0.1608	0.1207	0.1251	0.1905	0.1224	0.0998	0.1783	0.1113	0.0971
			(0.1678)	(0.1296)	(0.1258)	(0.2008)	(0.2104)	(0.1083)	(0.2160)	(0.1911)	(0.1108)
		0.1492	0.1218	0.1570	0.1564	0.1128	0.1592	0.1764	0.1101	0.1848	0.1881
			(0.1667)	(0.1538)	(0.1441)	(0.2479)	(0.2247)	(0.1383)	(0.2620)	(0.1945)	(0.1403)
T2	Q1	-0.1478	-0.1700	-0.1473	-0.1425	-0.2148	-0.1466	-0.1354	-0.1945	-0.1328	-0.1476
			(0.1404)	(0.1292)	(0.1269)	(0.2251)	(0.2263)	(0.1487)	(0.2005)	(0.1930)	(0.1931)
		0.1135	0.1200	0.1084	0.1117	0.1838	0.1060	0.1000	0.1589	0.0820	0.0978
			(0.1555)	(0.1521)	(0.1609)	(0.2477)	(0.2259)	(0.1914)	(0.2753)	(0.1993)	(0.2369)
	Q2	0.2593	0.2794	0.2578	0.2541	0.3195	0.2582	0.2312	0.2996	0.2454	0.2271
			(0.1450)	(0.1289)	(0.1223)	(0.2107)	(0.2233)	(0.1116)	(0.2058)	(0.1876)	(0.1120)
		0	-0.0241	-0.0049	0.0036	-0.0484	0.0046	0.0259	-0.0312	0.0265	0.0353
			(0.1611)	(0.1561)	(0.1457)	(0.2557)	(0.2281)	(0.1383)	(0.2612)	(0.1971)	(0.1377)

[a] Additive effect of QTL.
[b] Dominant effect of QTL.
[c] MSE of the estimates for each parameter.
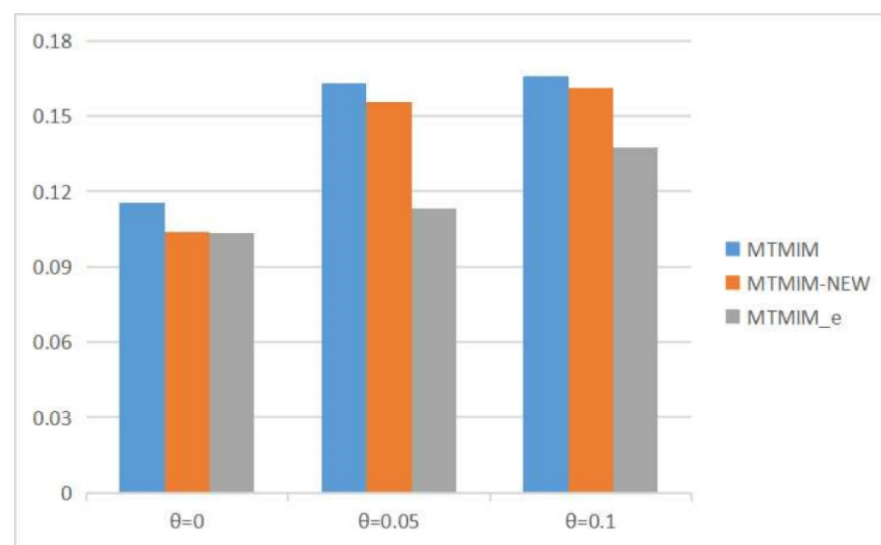
Figure 1. The total means (TMs) of MSEs of all parameter estimates (except θ) in Table 2

Table 3. The estimates of all parameters (except θ) and the corresponding MSEs with different heritabilities when $\theta = 0.05$

	Parameter	True value $h^2 = 0.05$	True value $h^2 = 0.2$	$h^2 = 0.05$ MTMIM	$h^2 = 0.05$ MTMIM-NEW	$h^2 = 0.05$ MTMIM_e	$h^2 = 0.2$ MTMIM	$h^2 = 0.2$ MTMIM-NEW	$h^2 = 0.2$ MTMIM_e
	γ_{11}	0.03	0.03	0.0560	0.0256	0.0191	0.0554	0.0308	0.0241
				$(0.0340)^c$	(0.0250)	(0.0129)	(0.0334)	(0.0193)	(0.0071)
	γ_{21}	0.04	0.04	0.0364	0.0308	0.0343	0.0360	0.0362	0.0384
				(0.0239)	(0.0292)	(0.0188)	(0.0184)	(0.0193)	(0.0127)
	ρ	0.9	0.9	0.8762	0.8828	0.8714	0.8730	0.8821	0.8744
				(0.0773)	(0.0761)	(0.1128)	(0.0710)	(0.0669)	(0.1053)
	σ_{11}	1	1	0.9760	0.9824	0.9704	0.9779	0.9796	0.9720
				(0.0822)	(0.0700)	(0.1177)	(0.0703)	(0.0704)	(0.1055)
	σ_{22}	1	1	0.9722	0.9793	0.9663	0.9636	0.9790	0.9709
				(0.0831)	(0.0804)	(0.1226)	(0.0784)	(0.0710)	(0.1137)
T1	Q1	-0.0678^a	-0.1478	-0.0377	-0.0676	-0.0698	-0.2121	-0.1396	-0.1322
				(0.2273)	(0.2166)	(0.1847)	(0.2169)	(0.2139)	(0.1389)
		0.0542	0.1182^b	0.0799	0.0616	0.0549	0.1664	0.1026	0.1019
				(0.2499)	(0.2349)	(0.1899)	(0.2424)	(0.2226)	(0.1939)
	Q2	0.0932	0.1288	0.0873	0.0946	0.0801	0.1905	0.1224	0.0998
				(0.2178)	(0.2120)	(0.1267)	(0.2008)	(0.2104)	(0.1083)
		-0.0510	0.1492	-0.0578	-0.0524	-0.0353	0.1128	0.1592	0.1764
				(0.2367)	(0.2291)	(0.1443)	(0.2479)	(0.2247)	(0.1383)
T2	Q1	-0.0678	-0.1478	-0.0307	-0.0661	-0.0700	-0.2148	-0.1466	-0.1354
				(0.2164)	(0.2085)	(0.1883)	(0.2251)	(0.2263)	(0.1487)
		0.0520	0.1135	0.0792	0.0573	0.0492	0.1838	0.1060	0.1000
				(0.2490)	(0.2340)	(0.1908)	(0.2477)	(0.2259)	(0.1914)
	Q2	0.1089	0.2593	0.0895	0.1085	0.0953	0.3195	0.2582	0.2312
				(0.2079)	(0.2026)	(0.1261)	(0.2107)	(0.2233)	(0.1116)
		0	0	-0.0122	0.0014	0.0168	-0.0484	0.0046	0.0259
				(0.2405)	(0.2296)	(0.1463)	(0.2557)	(0.2281)	(0.1383)

[a]Additive effect of QTL when heritability $h^2 = 0.05$.
[b]Dominant effect of QTL when heritability $h^2 = 0.2$.
[c]MSE of the estimate for each parameter.
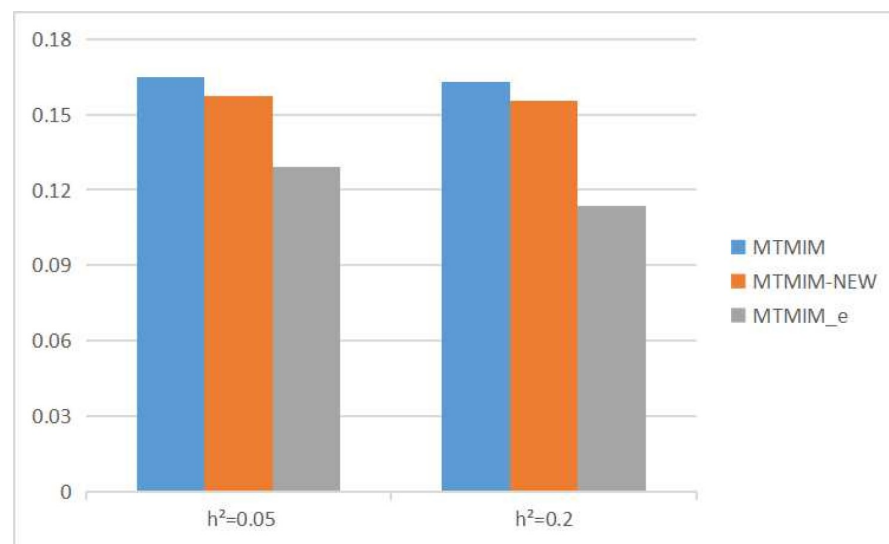
Figure 2. The total means (TMs) of MSEs of all parameter estimates (except θ) in Table 3

Table 4. The estimates of error rate θ with different heritabilities and QTL effects

Heritability	Trait	QTL	Effect parameter Additive	Effect parameter Dominant	True value of error rate θ 0	True value of error rate θ 0.05	True value of error rate θ 0.1
$h^2 = 0.2$	T1	Q1	-0.1478	0.1182	0.0106	0.0585	0.1013
		Q2	0.1288	0.1492	(0.0225)	(0.0703)	(0.1051)
	T2	Q1	-0.1478	0.1135			
		Q2	0.2593	0			
$h^2 = 0.05$	T1	Q1	-0.0678	0.0542	0.0306	0.0580	0.1019
		Q2	0.0932	-0.0510	(0.1296)	(0.1319)	(0.1369)
	T2	Q1	-0.0678	0.0520			
		Q2	0.1089	0			

173 error rate θ =0, 0.05, 0.1, when the heritability is h^2 =0.05, 0.2. The estimation accuracy of error rate θ
174 increases with an increase in heritability h^2, as expected, and the estimation accuracy decreases with an
175 increase in the genotype error rate (Table 4). This is because simulated data with higher genotype error
176 rates provide more uncertainty to the practical inference, relative to the cases with lower genotype errors
177 or no genotype error.

178 In order to further evaluate the performance of the proposed method on analyzing multiple datasets, we
179 conducted another simulation study. Three datasets with sample sizes $N_1 = 200$, $N_2 = 300$, and $N_3 = 500$,
180 and genotype error rates $\theta_1 = 0.01$, $\theta_2 = 0.02$, and $\theta_3 = 0.05$ were simulated under two heritabilities (see
181 Table 5 for the true values of parameters), and the MTMIM_e method was used to estimate all parameters.
182 The means of the estimates for each parameter over 1000 replications and the corresponding MSEs were
183 provided in Table 5. From the results, we conclude that the new method can effectively and simultaneously
184 analyze multiple datasets with potential genotype errors.

Table 5. The estimates of all parameters and the corresponding MSEs from 3 datasets

Heritability	Trait	QTL	Effect parameter Additive	Dominant	Estimates Additive	Dominant	γ_{11} 0.03	γ_{21} 0.04	ρ 0.9	σ_{11} 1	σ_{21} 1	Error rate θ $\theta_1 = 0.01$	$\theta_2 = 0.02$	$\theta_3 = 0.05$
$h^2 = 0.2$	T1	Q1	-0.1478	0.1182	-0.1457	0.1013	0.0269	0.0379	0.8844	0.9822	0.9822	0.0189	0.0301	0.0397
					(0.1244)	(0.0929)	(0.0039)	(0.0076)	(0.0894)	(0.0944)	(0.0907)	(0.0751)	(0.0823)	(0.0814)
		Q2	0.1288	0.1492	0.1120	0.1646								
					(0.0700)	(0.0972)								
	T2	Q1	-0.1478	0.1135	-0.1473	0.0977								
					(0.1253)	(0.0917)								
		Q2	0.2593	0	0.2436	0.0175								
					(0.0723)	(0.0985)								
$h^2 = 0.05$	T1	Q1	-0.0678	0.0542	-0.0725	0.0492	0.0300	0.0413	0.8798	0.9779	0.9788	0.0263	0.0378	0.0374
					(0.1350)	(0.1748)	(0.0040)	(0.0064)	(0.0962)	(0.1088)	(0.0926)	(0.0861)	(0.0932)	(0.0898)
		Q2	0.0932	-0.0510	0.0828	-0.0465								
					(0.0774)	(0.0948)								
	T2	Q1	-0.0678	0.0520	-0.0699	0.0433								
					(0.1276)	(0.1521)								
		Q2	0.1089	0	0.0969	0.0064								
					(0.0782)	(0.0941)								

STATISTICAL ANALYSIS OF REAL DATA

186 In this section, we further verified the feasibility of the proposed method in the mapping of multiple-
187 trait loci, and an experimental data set on high-density lipoprotein cholesterol levels in mice in the
188 published literature was used for the analysis (Stylianou et al., 2006). High-density lipoprotein (HDL) and
189 body weight (BW) traits were selected for data analysis. We chose three candidate markers (D08.0989,
190 D08.1099, and D08.1238) to perform multiple-interval mapping, which were located at 49.225 cM, 54.685
191 cM, and 61.605 cM of chromosome 8, respectively. These markers construct two marker intervals. In fact,
192 there are missing values in the genotype data of the first marker of the considered dataset. For illustration,
193 the missing values were imputed by the corresponding modes of genotypes on the locus, and then the new
194 genotype data became observed data but with genotype errors.

Table 6. The QTLs identified in the high-density lipoprotein data of mice

Trait	Chr.	QTL	MTMIM Position	Additive	Dominant	MTMIM-NEW Position	Additive	Dominant	MTMIM_e Position	Additive	Dominant
BW^a	8	QTL1	49.7^c	-0.0260	0.0037	50.2	-0.0138	-0.0089	50.4	-0.0120	-0.0087
		QTL2	60	0.0349	-0.0172	56	0.0088	-0.0042	56.8	0.0086	-0.0093
HDL^b	8	QTL1	49.7	0.0262	0.0037	50.2	0.0190	-0.0087	50.4	0.0203	-0.0092
		QTL2	60	0.0394	-0.0119	56	0.0139	-0.0105	56.8	0.0110	-0.0109

^{a}Body weight.
^{b}High-density lipoprotein.
^{c}The unit of QTL position is cM.

195 The proposed method, MTMIM_e, in which genotype errors are considered, and the other two methods,
196 MTMIM and MTMIM-NEW, in which genotype errors are neglected, were all used to deal with the
197 imputed data set (Table 6). All the estimated results of QTL positions and effects obtained from the
198 three methods show that QTL 1 located at ∼50 cM and QTL 2 located at ∼57 cM of chromosome 8 had
199 significant effects on the two traits, HDL and BW. Moreover, QTL 1 and QTL 2 showed additive/dominant
200 effects on the two traits in the same direction for all three methods. A small difference is that the MTMIM
201 method gave a positive dominant effect estimate on QTL 1, but the other two methods obtained a negative
202 estimate value of the dominant effect of this QTL. These conclusions are similar to those drawn by

²⁰³ Stylianou et al. (2006); however, the estimated results of MTMIM-NEW and MTMIM_e seem much
²⁰⁴ closer to those obtained by Stylianou et al. (2006). In addition, the proposed MTMIM_e can simultaneously
²⁰⁵ estimate the genotype error rate θ of the imputed data set, and the estimated value is $\hat{\theta} = 0.0252$, which
²⁰⁶ is close to the missing proportion of genotypes on the first marker locus.

DISCUSSION

²⁰⁸ In this paper, a new multiple-trait, multiple-interval mapping method (MTMIM_e) was proposed to deal
²⁰⁹ with multiple-trait genetic data with potential genotype errors, owing to the fact that most genetic datasets
²¹⁰ may contain certain genotype measurement errors. In fact, the proposed method can also be used to
²¹¹ analyze a dataset that contains missing values. In this case, one needs to impute the missing values by
²¹² statistical methods first, and then consider the imputed missing values as data with genotyping errors,
²¹³ so that the MTMIM_e can be applied, as shown in our analysis of a real example. Extensive simulation
²¹⁴ studies have validated that the new method is advantageous for parameter estimation in the QTL mapping
²¹⁵ of multiple traits and it can effectively overcome the impact of genotype errors/loss and estimate all
²¹⁶ parameters simultaneously, compared with existing methods. The proposed method can also be used to
²¹⁷ deal with the problems of other cases, for example, other experimental populations, or different error rates
²¹⁸ on different loci, in which we only need to change the conditional probabilities provided in Table 1, or
²¹⁹ adjust the presentation of the joint error rate φ^j (Tong et al., 2015), respectively. The statistical Model (1)
²²⁰ considered in this study can also be further generalized; for example, genotype information of markers can
²²¹ be added and considered simultaneously, which will lead to a composite multi-interval mapping model.

²²² After obtaining an estimate of Ω, we can further consider whether there are significant QTLs in the
²²³ considered marker intervals that control multiple traits. A global test using the likelihood-ratio (or log10
²²⁴ of odds; LOD score) statistic can be performed first to test two hypotheses of the effect matrix C, i.e.,
²²⁵ $H_0: C = 0$ vs $H_1: C \neq 0$; and furthermore, a local test can be conducted to test two hypotheses about the
²²⁶ specific QTL effect, i.e., $H_0^{il}: C_{il} = 0$; $H_1^{il}: C_{il} \neq 0$, if the global test is significant. It is considered that
²²⁷ there exists a significant QTL effect on trait l in the considered marker interval if $H_0^{il}: C_{il} = 0$ is rejected.

²²⁸ Although the new method has several advantages, there are still aspects that can be improved. One
²²⁹ disadvantage of the new method is that the amount of computation increases as the number of markers
²³⁰ (intervals) or traits increases. For the computation of one simulated data containing 500 individuals
²³¹ (considering three maker loci and two phenotypes), the EM algorithm takes about 300 iterations to
²³² converge in the Windows System with Intel Core i5-10210U 2.11 GHz processor and 16 GB memory, and
²³³ the memory consumption is about 500MB. Phenotype amount has a little impact on memory consumption,
²³⁴ but the most significant impact factor is the number of marker loci. When dealing with case of multiple
²³⁵ real QTLs (or multiple markers), we suggest that two marker intervals constructed by three markers are
²³⁶ analyzed by our method at one time. Performing this operation until all markers are completely analyzed
²³⁷ will save much running time of the algorithm. Alternatively, we suggest using the idea of a two-step
²³⁸ mapping method (Tong et al., 2015), i.e., first detect and retain the markers with larger effects of all
²³⁹ the markers. Marker intervals can then be constructed using the selected markers so that the proposed
²⁴⁰ method in this paper can be used with less computational burden. The problem of gene mapping is
²⁴¹ very important for human disease research, as well as for animal and plant genetic breeding; however,
²⁴² incomplete marker genotypes caused by biological or physical deletions are inevitable. In the future, we
²⁴³ will focus on improving the performance of the proposed method in this paper, so that it can adapt better
²⁴⁴ to more complex cases.

ACKNOWLEDGMENTS

²⁴⁶ The authors would like to thank the joint editor and referees for their helpful comments that greatly
²⁴⁷ improved the presentation of the paper.

Funding

²⁵⁰ This research was supported by the National Natural Science Foundation of China (Grant No. 12071114),
²⁵¹ the Natural Science Foundation of Heilongjiang Province of China (LH2019A020 and LH2019D018),
²⁵² the Fundamental Research Funds on Basic Research Project of Heilongjiang Provincial Colleges and

Universities (KYYWF10236190103), and the Innovation Project for University Students of Heilongjiang Province (201910236004).

Competing Interests

The authors declare there are no competing interests.

Author Contributions

Liang Tong and Ying Zhou conceived the experiments and performed the data analysis. Yixing Guo and Hui Ding conducted the simulations. Donghai Ji performed the theoretical analysis. All authors reviewed drafts of the paper and approved the final version of the manuscript.

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