

Method

Model for mapping imprinted quantitative trait loci in an inbred F₂ design

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Abstract

The role of imprinting in shaping development has been ubiquitously observed in plants, animals, and humans. However, a statistical method that can detect and estimate the effects of imprinted quantitative trait loci (iQTL) over the genome has not been extensively developed. In this article, we propose a maximum likelihood approach for testing and estimating the imprinted effects of iQTL that contribute to variation in a quantitative trait. This approach, implemented with the EM algorithm, allows for a genome-wide scan for the existence of iQTL. This approach was used to reanalyze published data in an F₂ family derived from the LG/S and SM/S mouse strains. Several iQTL that regulate the growth of body weight by expressing paternally inherited alleles were identified. Our approach provides a standard procedure for testing the statistical significance of iQTL involved in the genetic control of complex traits.

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Genomic imprinting refers to a phenomenon in which the same genes are expressed differently, depending on their parental origin [1]. A locus with two alleles *A* and *a* is thought to be imprinted if genotype *Aa* that inherits *A* from the maternal parent and *a* from the paternal parent is different from genotype *aA* that inherits the two alleles the other way around. Since its first discovery in the middle 1980s [2,3], such imprinted inheritance of a gene that obviously violates traditional Mendelian inheritance has been unequivocally demonstrated in an incredible range of species spanning from plants to animals to humans [1,4–7]. Some authors suggest that genomic imprinting, also called parent-of-origin effects, might be more common than previously thought [8].

As a multistep developmental process, the parent-of-origin effects start to emerge either during gametogenesis or in the zygote, prior to fusion of the two gametes, and are subsequently recognized by a transcriptional machinery. Several biochemical and genetic approaches including positional cloning and candidate gene testing have been exploited to detect and isolate imprinted genes with monoallelic expression [6]. It

has become clear that genetic mapping based on molecular linkage maps can be a powerful approach for genome-wide identification of imprinted quantitative trait loci (iQTL) that are expressed from only one chromosome depending on the epigenetic modification of their maternal and paternal alleles. The imprinted effects of iQTL can be tested and estimated using identical-by-descent-based models for a complex pedigree [9–12] or by genomic mapping in controlled crosses between outbred parents [8,13–15]. A cross between outbred lines is used to study genomic imprinting because parental origins of alleles can be traced from the offspring to the parents [13]. Significant evidence for iQTL has been detected for many traits, such as body composition and body weight, in outbred crosses [8,15–19].

Strictly speaking, a QTL identified with outbred crosses is not necessarily an iQTL. Because of a high heterozygosity, alleles at a given QTL can be different between two outbred parents. As a result, paternally and maternally expressed genetic differences detected may be simply due to different alleles [20] rather than the imprinted effect of the same alleles. The F₂ population, which ensures the transmission of the same alleles from the F₁ parents, provides an ideal design for studying the imprinting inheritance of QTL in the genetic control of a

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quantitative trait. However, an important limitation of the F_2 used to examine genomic imprinting is that it has not an adequate degree of freedom to estimate the difference between two parent-of-origin-dependent formations of the heterozygote Aa and aA .

In this article, we develop a statistical framework for interval mapping of iQTL in an F_2 family, initiated with two inbred lines, using codominant molecular markers. As a most commonly used mapping design, the use of the F_2 will enormously contribute to the understanding of the role of genomic imprinting in creating quantitative genetic variation. Our modeling here will capitalize on the information about sex differences in recombination fractions first discovered as early as the 1920s [21,22] and later observed in many species. In many mammals, males show reduced recombination frequency compared to females. Averaged over the entire genome, female-to-male recombination rates are 1.6:1.0 for humans [23], 1.4:1.0 for dogs [24], 1.4:1.0 for pigs [25], and 1.25:1.0 for mice [26]. In plants, sex-specific recombination rates have also been found to be common [27,28]. Wu et al. [29] derived the EM algorithm to estimate the sex-specific linkage between different markers for various genetic designs including the F_2 . They showed that when sex difference in recombination rates is incorporated the linkage phase between partially informative markers can be characterized.

The principle for interval mapping of QTL is based on cosegregation of different loci that allows for the derivation of the conditional probabilities of QTL genotypes given marker genotypes. The incorporation of sex-specific linkage into the procedure for mapping iQTL makes it possible to distinguish between two different formations of the heterozygote Aa and aA based on the linked markers. Our statistical model of iQTL mapping is constructed within the context of maximum likelihood approaches, implemented with the EM algorithm [30]. Our model has been used to scan genome-wide for iQTL that affect body weight in an F_2 family derived from two different mouse strains. A simulation study was performed to examine the statistical behavior of our model.

Quantitative genetics models

Consider an F_1 family derived from two inbred parents as well as an F_2 family generated by crossing the F_1 individuals. Suppose there is a putative QTL that is segregating with two alleles A and a in this pedigree. Based on traditional Mendelian inheritance, we have three distinguishable QTL genotypes, AA , Aa , and aa , whose values depend only on the specific alleles they carry, irrespective of the parental origin of the alleles. Different from this theory, however, genetic imprinting suggests that the alleles inherited from one parent are not completely expressed. Here, we provide a general theory for modeling the genetic effects of imprinted QTL on a quantitative trait.

Let A_M and a_M be two alleles from the maternal F_1 parent and A_P and a_P be two alleles from the paternal F_1 parent. The

values of four possible imprinted genotypes formed by different allelic combinations from the two F_1 parents are expressed as

$$\begin{aligned}\mu_1 &= \mu + \frac{1}{2}\alpha_M + \frac{1}{2}\alpha_P + \frac{1}{4}\gamma, \text{ for } A_M A_P, \\ \mu_2 &= \mu + \frac{1}{2}\alpha_M - \frac{1}{2}\alpha_P - \frac{1}{4}\gamma, \text{ for } A_M a_P, \\ \mu_3 &= \mu - \frac{1}{2}\alpha_M + \frac{1}{2}\alpha_P - \frac{1}{4}\gamma, \text{ for } a_M A_P, \\ \mu_4 &= \mu - \frac{1}{2}\alpha_M - \frac{1}{2}\alpha_P + \frac{1}{4}\gamma, \text{ for } a_M a_P,\end{aligned}\quad (1)$$

where μ is the overall mean, α_M is the QTL effect of the allele inherited from the maternal parent (or the maternally inherited QTL effect), α_P is the QTL effect of the allele inherited from the paternal parent (or the paternally inherited QTL effect), and γ is the dominant effect due to different alleles at the QTL. The genetic parameters (μ , α_M , α_P , γ) at the imprinted QTL can be estimated from the estimates of the four genotypic values.

Eq. (1) can quantify the effects of imprinted QTL. If a_M equals a_P , this implies that the alleles inherited from the two parents have the same effect, i.e., no parent-of-origin effect. In this case, Eq. (1) is reduced to the Mendelian inheritance. We thus use the difference,

$$\tau = \alpha_M - \alpha_P \quad (2)$$

as the measure for the magnitude of the imprinted effect. The complete paternal imprinting, complete maternal imprinting, and no imprinting correspond to $\tau = \alpha_M$, $\tau = -\alpha_P$, and $\tau = 0$, respectively.

Statistical methods

Finite mixture model

Statistical methods for mapping QTL based on a mixture model have been previously developed [31]. In the mixture model, each observation y is assumed to have arisen from one and only one of J (possibly unknown but finite) components, each component being modeled by a density from the parametric family f_j , which is expressed as

$$y \sim p(y|\vec{\omega}, \vec{\varphi}, \eta) = \omega_1 f_1(y; \varphi_1, \eta) + \dots + \omega_J f_J(y; \varphi_J, \eta), \quad (3)$$

where $\omega = (\omega_1, \dots, \omega_J)$ is a vector for the mixture proportions, which are constrained to be nonnegative and sum to unity; $\varphi = (\varphi_1, \dots, \varphi_J)$ is a vector for the component-specific parameters, with φ_j being specific to component J ; and η is a parameter (i.e., residual variance) that is common to all components.

The likelihood function

An imprinted F_2 family is genotyped by a set of codominant molecular markers with which a genetic linkage map is constructed as well as phenotyped for a quantitative trait. In such an imprinted family, the same allele A or a at a QTL is expressed differently, depending on its parental origin, maternal or paternal. For this reason, we should consider four QTL genotypes ($A_M A_P$, $A_M a_P$, $a_M A_P$, and $a_M a_P$), rather than three (AA , Aa , and aa) as for Mendelian inheritance. Traditional interval mapping approaches attempting to identify the three QTL genotypes need be modified to accommodate the imprinted property of QTL inheritance.

Based on the mixture model (Eq. (3)), we formulate the likelihood of the marker data (\mathbf{M}) and trait phenotypes (y) controlled by the putative QTL that is located between two flanking markers in the imprinted F_2 family as

$$L(\Theta|\mathbf{M}, y) = \prod_{i=1}^n [\omega_{1|i}f_1(y_i) + \omega_{2|i}f_2(y_i) + \omega_{3|i}f_3(y_i) + \omega_{4|i}f_4(y_i)] \quad (4)$$

where the unknown vector Θ contains the QTL position, QTL effects, and residual variance. The QTL position is measured in terms of the recombination fractions between the QTL and the two flanking markers that bracket it. The mixture proportions in Eq. (4), $\omega_{j|i}$, are the frequencies of QTL genotypes in the F_2 family. When these mixture proportions are described in terms of the overall distribution of QTL genotypes in the F_2 , they should each be 1/4. However, in our case for interval mapping, the mixture proportions are marker genotype dependent and, therefore, they become the conditional probabilities of QTL genotypes given known marker genotypes. General genetic literature has provided the conditional probabilities of QTL genotypes in a nonimprinted F_2 family as a function of the recombination fractions among the QTL and the two markers [32].

In the imprinted F_2 family, the heterozygote, Aa , has two different forms, A_Ma_P and a_Ma_P , which are assigned by different genotypic values, μ_2 and μ_3 (see Eq. (1)). Consider a pair of flanking markers \mathbf{M}_1 with two alleles M_1 and m_1 and \mathbf{M}_2 with two alleles M_2 and m_2 . These two markers, linked with the recombination fraction r , have the recombination fractions r_1 and r_2 with the QTL, respectively. The position of the QTL within the marker interval is thus described by $\Theta_p = \{r_1 \text{ or } r_2\}$. As can be seen, the conditional probabilities of the two different forms, A_Ma_P and a_Ma_P , given marker genotypes have the same expression (Table 1) and, thus, it is impossible to distinguish between μ_2 and μ_3 based on the conditional probabilities of A_Ma_P and a_Ma_P because fitting A_Ma_P with μ_2 and a_Ma_P with μ_3 has the same likelihood value as fitting A_Ma_P with μ_3 and a_Ma_P with μ_2 . But the absolute difference between μ_2 and μ_3 is an exact measure of the size of imprinted effect, i.e.,

$$\log L(\Theta|\mathbf{M}, y) = \sum_{i=1}^n \log \left[\sum_{j=1}^4 \omega_{j|i} f_j(y_i) \right]. \quad (5)$$

Eq. (5) provides the estimate of the degree of the QTL being expressed in a parent-of-origin effect manner, but it cannot determine the direction of QTL imprinting. To resolve this issue, we need additional information about gene inheritance and transmission. Here, we implement sex difference in the recom-

ination fraction into the mixture-based likelihood function. Let r_M and r_P be the recombination fractions between two flanking markers for the maternal and paternal parent, respectively. A putative QTL is located between these two markers, with respective recombination fractions r_{M1} and r_{M2} in the maternal parent and r_{P1} and r_{P2} in the paternal parent. We derive and tabulate the conditional (prior) probabilities of different QTL genotypes, conditional upon flanking marker genotypes, expressed as functions of sex-specific recombination fractions arrayed by $\Theta_p = \{r_{M1} \text{ or } r_{M2}, r_{P1} \text{ or } r_{P2}\}$ (Table 1). As shown by Eq. (1), the imprinted effects of QTL are reflected within the expected values, μ_1, \dots, μ_4 , for QTL genotypes in the normal distribution. Let σ^2 be the residual variance within QTL genotypes. These mean and variance parameters are arrayed by $\Theta_q = \{\mu_j, \sigma^2\}_{j=1}^4$.

Computational algorithm

All unknown parameters $\Theta = \{\Theta_p, \Theta_q\}$ can be estimated by a standard maximum likelihood approach implemented with the EM algorithm. This includes differentiating the log-likelihood function (Eq. (4)) with respect to each unknown, setting the derivatives equal to 0, and solving the derived log-likelihood equations. The log-likelihood function of Eq. (4) is given by

$$\begin{aligned} \frac{\partial}{\partial \Theta_\ell} \log L(\Theta|\mathbf{M}, y) &= \sum_{i=1}^n \left\{ \frac{f_i(y_i) \frac{\partial \omega_{j|i}}{\partial \Theta_p} + \omega_{j|i} \frac{\partial}{\partial \Theta_q} f_j(y_i)}{\sum_{j'=1}^n \omega_{j'|i} f_{j'}(y_i)} + \frac{\omega_{j|i} \frac{\partial}{\partial \Theta_q} f_j(y_i)}{\sum_{j'=1}^4 \omega_{j'|i} f_{j'}(y_i)} \right\} \\ &= \sum_{i=1}^n \frac{\omega_{j|i} f_j(y_i)}{\sum_{j'=1}^4 [\omega_{j'|i} f_{j'}(y_i)]} \left\{ \frac{1}{\omega_{j|i}} \frac{\partial \omega_{j|i}}{\partial \Theta_p} + \frac{\partial}{\partial \Theta_q} \log f_j(y_i) \right\} \\ &= \sum_{i=1}^n \Pi_{j|i} \left\{ \frac{1}{\omega_{j|i}} \frac{\partial \omega_{j|i}}{\partial \Theta_p} + \frac{\partial}{\partial \Theta_q} \log f_j(y_i) \right\} \end{aligned} \quad (6)$$

with a derivative for a particular element Θ_ℓ ,

where we define

$$\Pi_{j|i} = \frac{\omega_{j|i} f_j(y_i)}{\sum_{j'=1}^4 \omega_{j'|i} f_{j'}(y_i)} \quad (7)$$

as the posterior probability of QTL genotype j for individual i that carries a particular marker genotype.

Table 1
Conditional probabilities of QTL genotypes given marker genotypes in terms of sex-specific recombination fractions for F_2 design

Marker	A_Ma_P	A_Ma_P	a_Ma_P	a_Ma_P
$M_1M_1M_2M_2$	$\frac{\theta_{M00}\theta_{P00}}{(1-r_M)(1-r_P)}$	$\frac{\theta_{M00}\theta_{P11}}{(1-r_M)(1-r_P)}$	$\frac{\theta_{M11}\theta_{P00}}{(1-r_M)(1-r_P)}$	$\frac{\theta_{M11}\theta_{P11}}{(1-r_M)(1-r_P)}$
$M_1M_1M_2m_2$	$\frac{\theta_{M01}\theta_{P00} + \theta_{M00}\theta_{P01}}{r_M(1-r_P) + (1-r_M)r_P}$	$\frac{\theta_{M01}\theta_{P11} + \theta_{M00}\theta_{P10}}{r_M(1-r_P) + (1-r_M)r_P}$	$\frac{\theta_{M10}\theta_{P00} + \theta_{M11}\theta_{P01}}{r_M(1-r_P) + (1-r_M)r_P}$	$\frac{\theta_{M10}\theta_{P01} + \theta_{M11}\theta_{P01}}{r_M(1-r_P) + (1-r_M)r_P}$
$M_1M_1m_2m_2$	$\frac{\theta_{M01}\theta_{P01}}{r_M r_P}$	$\frac{\theta_{M01}\theta_{P10}}{r_M r_P}$	$\frac{\theta_{M10}\theta_{P01}}{r_M r_P}$	$\frac{\theta_{M10}\theta_{P10}}{r_M r_P}$
$M_1m_1M_2M_2$	$\frac{\theta_{M00}\theta_{P10} + \theta_{M10}\theta_{P00}}{(1-r_M)r_P + r_M(1-r_P)}$	$\frac{\theta_{M00}\theta_{P01} + \theta_{M10}\theta_{P11}}{(1-r_M)r_P + r_M(1-r_P)}$	$\frac{\theta_{M11}\theta_{P10} + \theta_{M01}\theta_{P00}}{(1-r_M)r_P + r_M(1-r_P)}$	$\frac{\theta_{M11}\theta_{P01} + \theta_{M01}\theta_{P11}}{(1-r_M)r_P + r_M(1-r_P)}$
$M_1m_1M_2m_2$	$\frac{z_{11}}{2(1-r_M)(1-r_P) + 2r_M r_P}$	$\frac{z_{10}}{2(1-r_M)(1-r_P) + 2r_M r_P}$	$\frac{z_{01}}{2(1-r_M)(1-r_P) + 2r_M r_P}$	$\frac{z_{00}}{2(1-r_M)(1-r_P) + 2r_M r_P}$
$M_1m_1m_2m_2$	$\frac{\theta_{M01}\theta_{P11} + \theta_{M11}\theta_{P01}}{(1-r_M)r_P + r_M(1-r_P)}$	$\frac{\theta_{M01}\theta_{P00} + \theta_{M11}\theta_{P10}}{(1-r_M)r_P + r_M(1-r_P)}$	$\frac{\theta_{M00}\theta_{P01} + \theta_{M10}\theta_{P11}}{(1-r_M)r_P + r_M(1-r_P)}$	$\frac{\theta_{M10}\theta_{P00} + \theta_{M00}\theta_{P10}}{(1-r_M)r_P + r_M(1-r_P)}$
$m_1m_1M_2M_2$	$\frac{\theta_{M10}\theta_{P10}}{r_M r_P}$	$\frac{\theta_{M10}\theta_{P01}}{r_M r_P}$	$\frac{\theta_{M01}\theta_{P10}}{r_M r_P}$	$\frac{\theta_{M01}\theta_{P01}}{r_M r_P}$
$m_1m_1M_2m_2$	$\frac{\theta_{M10}\theta_{P11} + \theta_{M11}\theta_{P10}}{r_M(1-r_P) + (1-r_M)r_P}$	$\frac{\theta_{M10}\theta_{P00} + \theta_{M11}\theta_{P01}}{r_M(1-r_P) + (1-r_M)r_P}$	$\frac{\theta_{M01}\theta_{P11} + \theta_{M00}\theta_{P10}}{r_M(1-r_P) + (1-r_M)r_P}$	$\frac{\theta_{M01}\theta_{P00} + \theta_{M00}\theta_{P01}}{r_M(1-r_P) + (1-r_M)r_P}$
$m_1m_1m_2m_2$	$\frac{\theta_{M11}\theta_{P11}}{(1-r_M)(1-r_P)}$	$\frac{\theta_{M11}\theta_{P00}}{(1-r_M)(1-r_P)}$	$\frac{\theta_{M00}\theta_{P11}}{(1-r_M)(1-r_P)}$	$\frac{\theta_{M00}\theta_{P00}}{(1-r_M)(1-r_P)}$

$\theta_{M00} = (1-r_{M1})(1-r_{M2})$, $\theta_{M01} = (1-r_{M1})r_{M2}$, $\theta_{M10} = r_{M1}(1-r_{M2})$, $\theta_{M11} = r_{M1}r_{M2}$, $\theta_{P00} = (1-r_{P1})(1-r_{P2})$, $\theta_{P01} = (1-r_{P1})r_{P2}$, $\theta_{P10} = r_{P1}(1-r_{P2})$, $\theta_{P11} = r_{P1}r_{P2}$, $z_{11} = \theta_{M00}\theta_{P11} + \theta_{M01}\theta_{P10} + \theta_{M10}\theta_{P01} + \theta_{M11}\theta_{P00}$, $z_{10} = \theta_{M00}\theta_{P00} + \theta_{M01}\theta_{P01} + \theta_{M10}\theta_{P10} + \theta_{M11}\theta_{P11}$, $z_{01} = \theta_{M11}\theta_{P11} + \theta_{M10}\theta_{P10} + \theta_{M01}\theta_{P01} + \theta_{M00}\theta_{P00}$, $z_{00} = \theta_{M11}\theta_{P00} + \theta_{M10}\theta_{P01} + \theta_{M01}\theta_{P10} + \theta_{M00}\theta_{P11}$.

Letting the log-likelihood function equal 0, we derive the closed form expressions of the estimates of the genotypic values and residual variance in terms of the posterior probabilities, expressed as

$$\hat{\mu}_j = \frac{\sum_{i=1}^n \Pi_{ji} y_i}{\sum_{i=1}^n \Pi_{ji}}$$

$$\hat{\sigma}^2 = \frac{1}{n} \sum_{i=1}^n \left[\sum_{j=1}^4 \Pi_{ji} (y_i - \hat{\mu}_j)^2 \right]. \quad (8)$$

It is possible to derive the log-likelihood equation of sex-specific recombination fractions between the QTL and the markers, but these expressions will be complex unless a further assumption is made, for example, $r_M = r_{M1} + r_{M2}$ and $r_P = r_{P1} + r_{P2}$ for a dense map. In practice, we usually do not estimate the location of QTL based on a log-likelihood equation. Instead, the QTL position parameter can be viewed as a fixed parameter by searching for a putative QTL at every 1 or 2 cM on a map interval bracketed by two markers throughout the entire linkage map. Unlike a traditional search approach, our search will be performed on sex-specific linkage maps. The log-likelihood ratio test statistic for a QTL at a sex-specific map position is displayed graphically to generate a likelihood map or profile. The genomic position that corresponds to a peak of the profile is the maximum likelihood estimate (MLE) of the QTL location.

For the genetic effect and residual variance, the EM algorithm is implemented. In the M step, the posterior probabilities are calculated using Eq. (7), whereas in the E step, these calculated posterior probabilities are used to solve the effect and variance parameters using Eq. (8). Iterations are repeated between Eqs. (7) and (8) until convergence. The values at convergence are the MLEs. With the MLEs of μ_j 's, the MLEs of the additive, dominant, and imprinted effects of the QTL, as indicated in Eq. (1), can be obtained by solving a system of regular equations.

After the point estimates of parameters are obtained by the EM algorithm, we derive the asymptotic variance-covariance matrix and evaluate the sampling errors of the estimates Θ . The techniques for so doing involve calculation of the incomplete-data information matrix, which is the negative second-order derivative of the incomplete-data log-likelihood. The incomplete-data information can be calculated by extracting the information for the missing data from the information for the complete data [33]. A different so-called supplemented EM algorithm or SEM algorithm was proposed by Meng and Rubin [34] to estimate the asymptotic variance-covariance matrices, which can also be used for the calculations of the sampling errors for the MLEs of the parameters (Θ).

Hypothesis tests

The existence of a QTL can be tested on the basis of the log-likelihood ratio (LR) test statistics between the full (there is a QTL) and the reduced (there is no QTL) model. These two models correspond to two alternative hypotheses, expressed as

$$H_0 : \mu_j \equiv \mu (j = 1, \dots, 4) \text{ vs } H_1 : \mu_j \neq \mu (j = 1, \dots, 4).$$

At least one of these equalities does not hold.

Letting ω and ω be the MLEs of the unknown parameters under H_0 and H_1 , respectively, we calculate the LR test statistic as

$$\text{LR} = -2[\log L(\Omega|y) - \log L(\Omega|\mathbf{M}, y)], \quad (9)$$

which is asymptotically χ^2 distributed with 4 degrees of freedom. An empirical approach for determining the critical threshold is based on permutation tests. By repeatedly shuffling the relationship between marker genotypes and phenotypes, series of the maximum log-likelihood ratios are calculated from the distribution of which the critical threshold is determined.

If the detected QTL is significant, we need to test whether it is an imprinted or Mendelian QTL. De Koning et al. [14] gave a procedure for making such a test. Here, we provide two approaches for testing the imprinting effect of the detected QTL.

Test 1—the existence of the imprinted effect

Because of the imprinted effect, two parent-of-origin-dependent genotypes, $A_M a_P$ and $a_M A_P$, will have different values, μ_2 and μ_3 . However, a general mapping model cannot discern the identification of these two genotypes based on the assigned genotypic values, but it provides the possibility of testing for the difference between the two genotypic values, which is just the reflection of the imprinted effect (see Eq. (5)). The test for the existence of the imprinted effect can be based on the null hypothesis formulated by Eq. (5).

Test 2—the direction of the imprinted effect

Which parent contributes to the imprinted effect can be tested through joint modeling of sex-specific recombination events. When sex-specific recombination fractions are incorporated, we can distinguish between two genotypic values, μ_2 and μ_3 , based on the parent-of-origin-dependent genotypes. By setting α_P or α_M equal to 0, we can test whether this imprinted QTL is paternally or maternally imprinted. The sequence for performing the tests of the imprinted direction is given below:

$$H_0 : \alpha_M = 0 \text{ vs } H_1 : \alpha_M \neq 0, \quad (10)$$

$$H_0 : \alpha_P = 0 \text{ vs } H_1 : \alpha_P \neq 0, \quad (11)$$

and

$$H_0 : \alpha_M = \alpha_P \text{ vs } H_1 : \alpha_M \neq \alpha_P. \quad (12)$$

The rejection of the null hypothesis in Eq. (10) implies that only the paternally inherited allele is expressed so that the maternally inherited allele is completely imprinted. By contrast, the rejection of the null hypothesis in Eq. (11) corresponds to the inverse direction of allelic expression or imprinting. The null hypothesis in Eq. (12) is the traditional Mendelian model and its rejection indicates that both alleles are expressed at different levels in the progeny. If the two alleles are expressed at different levels, they can be said to be partially imprinted.

The critical thresholds for both the tests are determined by simulation studies. Samples of size n are simulated under the absence of imprinted effect and are analyzed to calculate the LR value by the model developed. The percentile of the LR values from many simulation replicates is used as the threshold value.

Results

Our statistical model is employed to map iQTL that contribute to variation in growth trajectories in an animal model system—mouse. Vaughn et al. [35] constructed a linkage map with 96 microsatellite markers for 502 F_2 mice derived from two strains, the Large (LG/J) and the Small (SM/J).

This map has a total map distance of ~ 1780 cM (in Haldane's units) and an average interval length of ~ 23 cM. The F_2 progeny were measured for their body mass at 10 weekly intervals starting at age 7 days. The raw weights were corrected for the effects of each covariate due to dam, litter size at birth and parity, and sex [35].

The growth data measured at each time point were subjected to three types of statistical analyses, traditional interval mapping (Mendelian model), iQTL interval mapping assuming no sex-specific discrepancy in recombination events (imprinting-existence model), and iQTL interval mapping incorporated by sex-specific recombination events (imprinting-direction model). Although the three analyses lead to the same profile of the log-likelihood test statistics throughout the genome (Fig. 1), they provide different information about the inheritance of QTL. The first analysis based on Mendelian inheritance assumes no difference between two parent-of-origin-dependent genotypes, $A_M a_P$ and $a_M A_P$. The other two analyses with iQTL assume that these two genotypes are different due to different expressions of maternal and paternal alleles.

It was found that different QTL were involved in the control of growth during different stages of mouse development (Fig. 1). A QTL detected on chromosome 15 is obviously an early locus that affects body mass growth only during the first 4 weeks. As this QTL is switched off, some other QTL were activated to regulate mouse development. For example, a QTL on chromosome 6 became operational at week 4, a QTL on chromosome 7 operational at week 5, and a QTL on chromosome 10 operational at week 7. These three QTL have triggered continuous effects on growth since they were activated.

Table 2 gives the MLEs of the QTL positions and effects and the LR values for the tests of the statistical significance of the detected QTL. The QTL effect parameters are maternally and paternally derived allelic effects, the dominant effect and the imprinted effect. The maternally and paternally derived allelic effects are distinguished by incorporating sex-specific recombination fractions into the mapping model. In mice, the female is, on average, longer by 25% in genetic distances between homologous loci than the male [26]. This sex-specific discrepancy, expressed as $r_M = 1.25r_P$, was used to recalculate the marker distances for each sex according to the marker order estimated for the F_2 mouse progeny in Vaughn et al. [35]. If no information is available about sex-specific difference in recombination fractions, our model can still test the significance of the imprinted QTL effect, although it is not possible to discern the direction of the imprinted effect.

We have detected four genome-wide significant QTL distributed on different chromosomes, 6, 7, 10, and 15 (Fig. 1; Table 2). It appears that these QTL are expressed differently during development. Early growth of body mass from age 2 to 5 weeks was determined by the QTL on chromosome 15. Other QTL on chromosomes 6 and 7 became operational after the early QTL was switched off. The QTL on chromosome 10 was involved in controlling late growth after age 7 weeks. It is interesting to note that the detected QTL display different imprinted effects in their size and direction. The early QTL on chromosome 15 is not imprinted at the beginning of its

expression because the difference (lil) between the maternally and the paternally derived allelic effects is not significant (Table 2). But this QTL is subject to partial paternal imprinting at the late stage of its expression. For the QTL that were expressed after a particular time point of development, some displayed strong imprinted effects, while others did not. The QTL on chromosomes 6 and 10 were partially maternally imprinted during the entire activated period because the additive effects were significantly larger ($p < 0.05$) due to the paternal rather than the maternal alleles (Table 2). The QTL on chromosome 7 had similar maternally and paternally derived allelic effects and, therefore, was not imprinted.

Monte Carlo simulation

We performed simulation studies to investigate the statistical properties of our imprinting model. Consider a genome composed of four small linkage groups each with six evenly spaced markers. An F_2 family of sample 200 or 400 is simulated with these marker genotypes and a normally distributed quantitative trait. The marker genotypes in the F_2 family are simulated by mimicking sex-specific recombination fractions in mice, i.e., $r_M = 1.25r_P$. Three different QTL that display different imprinted features are assumed on different linkage groups, with the locations and effect values given in Tables 3–5. To examine the impacts of parameter spaces on parameter estimation, we simulated the data under different heritability (H^2) levels from 0.10 to 0.40.

Completely imprinting iQTL

Our iQTL interval mapping model can provide accurate estimates of QTL location, QTL effects, and residual variance for a completely imprinted QTL (Table 3). The precision of parameter estimation is quite low, but it can be remarkably increased by increasing the heritability and sample size. A more favorable effect on the estimation precision can be obtained by increased heritability rather than increased sample size. There is adequate power to detect a significant imprinting effect of QTL based on Eq. (12), increasing with heritability and sample size. The simulated data with a completely imprinted QTL were subjected to analyses of a Mendelian model (assuming $\alpha_M = \alpha_P$). The results suggest that the Mendelian model provides slightly larger standard errors for the MLE of the QTL location, and it estimates the additive effect of the iQTL as half the effect of the expressed allele (maternal allele in this case), but strikingly overestimates the dominant effect of the iQTL (Table 3). Also, the estimation precision of the dominant effect is very poor for the imprinted data using the Mendelian model, irrespective of increases of heritability and sample size.

Partially imprinting iQTL

The imprinting model can provide reasonable estimates of the effects of a partially imprinted QTL (Table 4). The estimation precision and power can increase with heritability

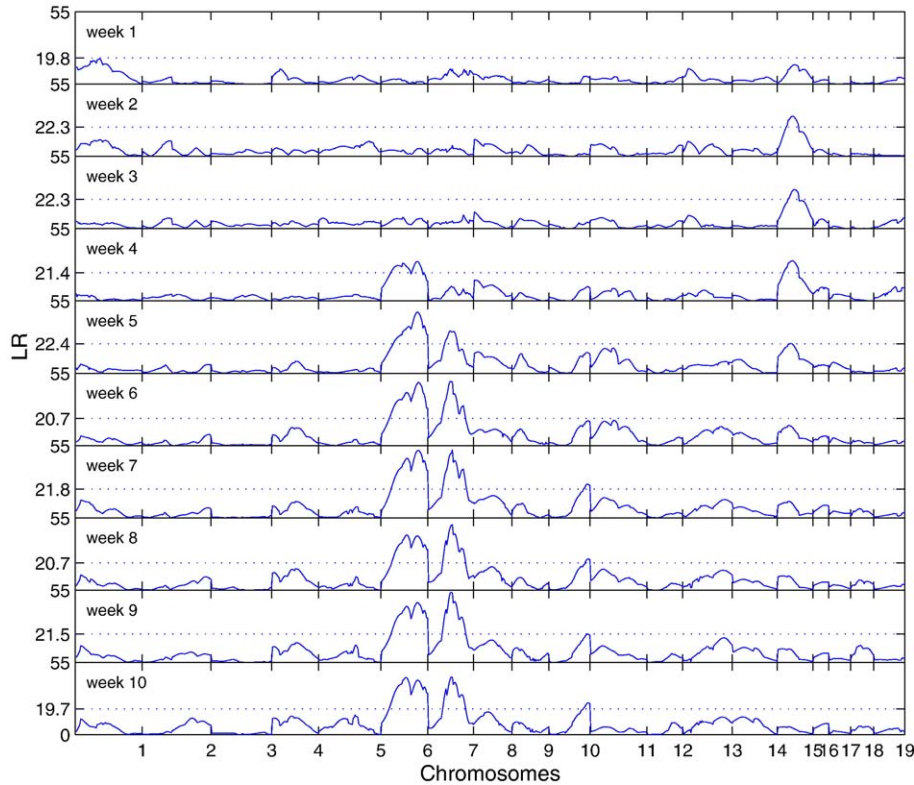


Fig. 1. The profiles of the log-likelihood ratios (LR) between the full and the reduced (no QTL) model estimated from the imprinting interval model for body mass growth trajectories across the entire genome from chromosome 1 to 19 using the linkage map constructed from microsatellite markers [35]. The genomic positions corresponding to the peaks of the curves are the MLEs of the QTL positions. The genome-wide threshold values for claiming the existence of QTL are given as the dotted horizontal lines.

and sample size. As for the completely imprinting case, the Mendelian model provides a biased estimate of the QTL effect when the underlying QTL is partially imprinted (results not shown).

No imprinting QTL

For a dataset containing no iQTL, the imprinting model can provide reasonably accurate estimates of the QTL effect

Table 2
The MLEs of the QTL position and effect parameters for body weight at different ages in the F₂ progeny derived from the LG/J and SM/J strains estimated by the imprinting model

Chromo-some	Marker interval	Age	μ	α_M	α_P	γ	lil	Direction	σ^2	LR
6	D6Mit58–D6Mit15	4	19.16	-0.35	1.92	0.98	2.27	Maternal	2.015	30.00
		5	24.73	-0.23	2.30	-1.02	2.33	Maternal	2.033	46.48
		6	27.31	0.35	2.02	0.56	1.67	Maternal	2.290	47.97
		7	29.67	0.65	2.04	0.77	1.39	Maternal	2.532	51.37
		8	31.44	0.93	1.75	-1.08	0.83	Maternal	2.939	42.04
		9	33.55	0.77	2.48	-0.33	1.70	Maternal	3.039	45.60
7	D7Mit17–D7Mit9	10	35.53	1.10	2.05	-0.99	0.95	Maternal	3.401	43.55
		5	24.76	1.34	0.41	-0.63	0.93	—	2.283	32.22
		6	27.33	1.31	1.03	-0.27	0.28	—	2.408	48.87
		7	29.72	1.25	1.29	-0.52	0.04	—	2.633	51.80
		8	31.40	1.36	1.41	-0.65	0.05	—	2.940	49.75
10	D10Mit133–D10Mit14	9	33.57	1.43	1.55	-1.03	0.12	—	3.158	53.33
		10	35.48	1.39	1.57	-1.13	0.18	—	3.478	43.96
		7	29.73	0.15	1.62	0.09	1.47	Maternal	2.633	25.73
15	D15Mit5–D15Mit3	8	31.45	-0.03	1.88	-0.25	1.94	Maternal	2.912	23.82
		2	8.25	0.32	0.31	-0.71	0.01	—	0.965	30.57
		3	12.55	0.53	0.49	-1.03	0.03	—	1.521	29.76
		4	19.20	1.18	0.57	-0.46	0.63	Paternal	2.176	30.52
		5	24.81	1.19	0.41	0.00	0.77	Paternal	2.280	22.57

“—” denotes no imprinted effect detected.

Table 3

The MLEs of the QTL position and effect parameters for the simulated completely imprinted iQTL obtained from the imprinting (upper) and Mendelian (lower) model

H^2	n	Position at 14 cM	$\mu = 10$	$a_M = 1$	$a_P = 0$	$\gamma = 0$	σ^2	Power
0.10	200	15.30 (9.70)	9.98 (0.11)	0.98 (0.53)	0.02 (0.54)	-0.02 (0.58)	2.07 (0.34)	61
		15.83 (10.67)	9.99 (0.12)	0.51 (0.17)	0.98 (1.09)	2.32 (0.24)		
	400	14.11 (5.87)	9.98 (0.08)	0.93 (0.47)	0.08 (0.47)	-0.02 (0.39)	2.15 (0.24)	84
		12.69 (5.66)	9.99 (0.08)	0.49 (0.11)	0.94 (0.99)	2.35 (0.20)		
0.25	200	14.27 (5.58)	9.99 (0.07)	0.96 (0.29)	0.04 (0.29)	-0.02 (0.33)	0.71 (0.11)	89
		13.61 (6.01)	9.99 (0.07)	0.50 (0.10)	0.99 (1.04)	0.87 (0.14)		
	400	14.26 (3.18)	9.99 (0.05)	0.99 (0.20)	0.02 (0.19)	-0.01 (0.21)	0.73 (0.08)	96
		13.92 (3.15)	10.00 (0.04)	0.49 (0.07)	0.98 (0.99)	0.88 (0.14)		
0.40	200	13.71 (2.59)	9.99 (0.05)	0.99 (0.16)	0.02 (0.16)	-0.01 (0.24)	0.36 (0.05)	96
		13.75 (4.16)	9.99 (0.05)	0.50 (0.07)	0.99 (1.01)	0.490 (0.13)		
	400	14.41 (1.73)	9.99 (0.04)	1.01 (0.08)	0.01 (0.08)	-0.01 (0.16)	0.36 (0.04)	100
		14.02 (2.12)	10.00 (0.04)	0.49 (0.05)	0.98 (0.99)	0.50 (0.13)		

The data were simulated for an iQTL with a complete paternal imprinting effect under different heritabilities ($H^2 = 0.10, 0.25, 0.40$) and sample sizes ($n = 200, 300$). The square roots of the mean squared errors of the MLEs, given in parentheses, were estimated from 200 simulation replicates. The maternal marker distance is 1.25 times the paternal marker distance. The locations of the QTL are described by the map distances (in cM) from the first marker of the linkage group (100 cM long). The hypothesized σ^2 value is 2.25 for $H^2 = 0.10$, 0.75 for $H^2 = 0.25$, and 0.38 for $H^2 = 0.40$. Power is calculated as the percentage of the number of those simulations in which significant iQTL is detected.

(Table 5), but the estimation precision is lower compared with that from the Mendelian model. In general, the estimation precision increases with heritability and sample size, but with increasing rate not being comparable to that for the Mendelian model. There is a small Type I error rate (2–5%) in detecting a significant iQTL for the simulated data that contain no iQTL using the imprinting model (Table 5).

In sum, for all the iQTL imprinted at different levels, the correct models, i.e., imprinting model for imprinted data and Mendelian model for no imprinting data, can always provide better estimates of QTL effects than the incorrect models, i.e., imprinting model for nonimprinted data and Mendelian model for imprinting data. Of the two incorrect models, the imprinting model for nonimprinted data performs better does the Mendelian model for imprinting data. Both the incorrect models are less sensitive in their precision of parameter estimation to increased heritability and sample size than are the correct models.

We performed additional simulation studies to investigate the statistical behavior of our model by increasing the sex-specific difference in recombination fractions to 1.6-fold as a case for humans. The results consistently support the capacity of our model to estimate precisely the imprinted effects of QTL (results not shown).

Discussion

The discovery of genomic imprinting [2,3] has led to the emergence of a new genetic discipline, epigenetics—the study of heritable changes in gene function that occur without a change in the DNA sequence [36]—that is changing the way we think about heredity. Because of such a pivotal role in reshaping our understanding of phenotype transmission and development, genomic imprinting that results in the expression of genes from only one of the two parental chromosomes, or parent-of-origin effects [1,7], has been incorporated into linkage analysis [8–15]. However, a general approach that can exploit available mapping materials to estimate genome-wide the imprinted effects based on molecular linkage maps has not been well developed [14].

In this article, we present a new statistical framework to search for the existence and distribution of quantitative trait loci with imprinted effects throughout the entire genome. An imprinted quantitative trait locus is defined as a QTL at which both maternal and paternal alleles are present, but only one allele will be expressed, with the other remaining inactive. Unlike traditional interval mapping models based on Mendelian inheritance [31], our model allows for the estimation and test of the difference between the expression of maternally and paternally inherited alleles at iQTL in the offspring. The prime idea

Table 4

The MLEs of the QTL position and effect parameters for the simulated partially paternally imprinted iQTL obtained from the imprinted model

H^2	n	Position at 24 cM	$\mu = 10$	$a_M = 1$	$a_P = 0.5$	$\gamma = 0.2$	σ^2	Power
0.10	200	21.48 (9.23)	9.98 (0.12)	1.10 (0.53)	0.43 (0.57)	0.14 (0.62)	2.61 (0.39)	81
	400	22.76 (5.87)	9.99 (0.08)	0.97 (0.50)	0.53 (0.46)	0.18 (0.43)	2.70 (0.27)	92
0.25	200	22.95 (5.15)	9.99 (0.07)	1.00 (0.30)	0.51 (0.32)	0.18 (0.34)	0.89 (0.13)	96
	400	23.90 (2.48)	9.99 (0.05)	0.98 (0.27)	0.53 (0.25)	0.20 (0.24)	0.91 (0.09)	99
0.40	200	23.82 (3.16)	9.99 (0.05)	0.99 (0.21)	0.52 (0.23)	0.20 (0.23)	0.45 (0.06)	97
	400	24.09 (1.19)	9.99 (0.03)	0.98 (0.19)	0.53 (0.18)	0.21 (0.17)	0.46 (0.05)	100

The hypothesized σ^2 value is 2.84 for $H^2 = 0.10$, 0.95 for $H^2 = 0.25$, and 0.47 for $H^2 = 0.40$. See Table 3 for the other explanations.

Table 5

The MLEs of the QTL position and effect parameters for the simulated QTL with no imprinting effect obtained from the imprinted model

H^2	n	Position at 34 cM	$\mu = 10$	$a_M = 1$	$a_P = 1$	$\gamma = 0.5$	σ^2	Type I error rate
0.10	200	30.91 (9.67)	9.96 (0.16)	1.10 (0.83)	0.93 (0.74)	0.43 (0.84)	4.25 (0.70)	5
	400	32.58 (6.21)	9.98 (0.12)	1.15 (0.67)	0.79 (0.64)	0.58 (0.53)	4.38 (0.49)	5
0.25	200	33.44 (3.70)	9.98 (0.09)	1.08 (0.48)	0.93 (0.42)	0.54 (0.38)	1.43 (0.21)	3
	400	33.93 (2.02)	9.98 (0.07)	1.14 (0.37)	0.86 (0.37)	0.52 (0.26)	1.48 (0.13)	2
0.40	200	33.79 (2.94)	9.98 (0.07)	1.07 (0.32)	0.93 (0.31)	0.54 (0.27)	0.71 (0.11)	2
	400	34.32 (1.22)	9.98 (0.05)	1.09 (0.26)	0.92 (0.25)	0.53 (0.18)	0.74 (0.08)	2

The hypothesized σ^2 value is 4.64 for $H^2 = 0.10$, 1.55 for $H^2 = 0.25$, and 0.77 for $H^2 = 0.40$. Type I error rate is calculated as the percentage of the number of those simulations in which false positive iQTL are detected. See Table 3 for the other explanations.

used to derive the interval model for mapping iQTL is the existence of differential recombination events between homologous loci across two different sexes. Many comparative studies in mammals using molecular markers have suggested that the female displays longer genetic distances between syntenic loci than the male; for example such a ratio is 1.60:1 in humans [23] and 1.25:1 in mice [26].

Beyond current iQTL mapping models based on controlled crosses derived from outbred parents [8,13,14], our model that was constructed for an F_2 population has two advantages. In principle, an F_2 initiated with two contrasting inbred lines provides ideal material to study the imprinting effect of a QTL because the same alleles inherited from different parents can be traced. In an outbred cross, the genetic effect of QTL can be due to different alleles rather than to the parent-dependent expression of the same allele. Given the commonality of the F_2 as a mapping population, our model can be useful practically for extracting additional information about iQTL from available datasets.

Our model has been employed to reanalyze the growth data collected in an F_2 progeny derived from two different mouse strains [35], which has led to the identification of iQTL that are responsible for body mass growth. The QTL detected by our imprinted model are broadly consistent with those found by traditional interval mapping approaches [35]. Different types of QTL expressed in different stages of development have been identified in the same F_2 progeny by these authors. Of the four QTL detected for body mass growth (Fig. 1) from our imprinting model, three on chromosomes 6, 10, and 15, respectively, are subject to imprinting. Yet, imprinting seems to be under developmental control. For example, the QTL on chromosome 15 starts to be imprinted after it has been expressed for a particular period. Imprinted QTL have been observed in other animals, such as pig [14,16–19], sheep [37], and chicken [15]. In particular, an imprinted QTL with major effect on muscle growth and fat composition in pigs has mapped to the insulin-like growth factor 2 (*IGF2*) locus, which regulates growth hormone action, stimulates the growth of cultured cells, stimulates the action of insulin, and is involved in development and growth. More recently, this imprinted QTL has been fine-mapped using association studies to a 250-kb chromosome segment containing the insulin gene and *IGF2* as the only known paternally expressed genes [18].

One important imprinting phenomenon identified in mammals is that the imprinted genes are not distributed uniformly

throughout the genome, but tend to cluster together [6]. One of the largest clusters is found at the distal end of mouse chromosome 7 and at the proximal end of human chromosome 11p15.5 [38]. This information, incorporated into our mapping model, will help to narrow down search intervals for important imprinted genes.

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