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Limitations of a two-step moment method for mapping linked multiple QTL

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Summary

As an alternative to multiple-interval mapping a two-step moment method was recently proposed to map linked multiple quantitative trait loci (QTLs). The advantage of this moment method was supposed to be its simplicity and computational efficiency, especially in detecting closely linked QTLs within a marker bracket, but also in mapping QTLs in different marker intervals. Using simulations it is shown that the two-step moment method may give poor results compared with multiple-interval mapping, irrespective of whether the QTLs are in the same or in different marker intervals, especially if linked QTLs are in repulsion. The criteria of comparison are number of identified QTLs, likelihood ratio test statistics, means and empirical standard errors of the QTL position and QTL effects estimates, and the accuracy of the residual variance estimates. Further, the joint conditional probabilities of QTL genotypes for two putative QTLs within a marker interval were derived and compared with the unmodified approach ignoring the non-independence of the conditional probabilities.

1. Introduction

Approaches using a multiple-QTL model for simultaneously mapping QTLs should improve the identification of multiple QTLs, allow testing for epistatic interactions between QTLs and be able to improve the precision of QTL position and effect estimates. Kao & Zeng (1997) presented for backcross and F_2 designs a maximum likelihood strategy for mapping multiple QTLs using multiple marker intervals simultaneously. Their approach is based on a finite normal mixture model and the expectation maximization algorithm is used to obtain the QTL estimates. Kao *et al.* (1999) called this method multiple-interval mapping. Nakamichi *et al.* (2001) proposed as an alternative a two-step moment method as a mapping strategy. Although they state they use a multiple-QTL model, their approach actually uses single-interval mapping to obtain crude (biased) estimates of the additive genetic effect, the dominance effect and the population mean and residual variance. In a second step a moment method is applied to these crude estimates to remove the effects of the other QTL. Nakamichi *et al.* (2001) state that the two-step moment method may be less statistically efficient than multiple-interval mapping, but the merit of the moment method is its simplicity and computational efficiency. The differences between true maximum likelihood estimates and the results of the two-step moment method were found to be negligible in several simulated data sets in their study. In this paper it will be shown that although the two-step moment method may approximate multiple-interval mapping in some cases, the differences between the two methods can be very large, irrespective of whether the QTLs are in different marker intervals or closely linked.

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2. Material and methods

(i) *Two-step moment method*

For the analyses using the two-step moment method of Nakamichi *et al.* (2001), the computer program GAQTL (download version 0.2) in C++ language as provided on the worldwide web under http://lbm.ab.a.u-tokyo.ac.jp/~naka/software.html was used. Recently on this web site a second program GAQTL2 (version 0.1b) was provided that is supposed to use

joint conditional probabilities of QTL genotypes for two (or more) putative QTLs within a marker interval. But this program is not recommended by the authors as they describe it as 'unstable'.

The programs of Nakamichi can be used for mapping QTLs in F_2 populations and consider additive and dominance effects, but do not allow the analysis of epistasis. Further, a general mean is estimated. Thus, an observation y_i $(i=1, 2, \ldots, n)$ can be modelled as follows:

$$y_i = \mu + \sum_{k=1}^{m} (a_k x_{ik} + d_k z_{ik}) + e_i \quad (1)$$

where x_{ik} and z_{ik} are the indicator variables of the additive (a_k) and dominance (d_k) effects at putative QTL locus k. The number of QTL loci considered is m. The general mean is μ and the residual effect for observation i is $e_i \sim \text{NID}(0, \sigma^2)$.

In the two-step moment method of Nakamichi *et al.* (2001), crude estimates of the genetic effects (a_k, d_k), the general mean μ, and the residual variance σ^2 are obtained assuming a single QTL and applying single-interval mapping using maximum likelihood methodology. These crude estimates are biased because of the effects of the other QTL. Therefore, in a second step a moment method is applied with the intention of removing the effects of the other QTL from the crude estimates. Therefore, in the approach of Nakamichi *et al.* (2001), the final estimates are obtained by solving the following linear equation system:

$$\tilde{a}_{k'} = \sum_{k=1}^{m} \{(1-2r_{k'k})\hat{a}_k\} \quad (2a)$$

$$\tilde{d}_{k'} = \sum_{k=1}^{m} \{(1-2r_{k'k})^2 \hat{d}_k\} \quad (2b)$$

$$\hat{\mu} = \frac{1}{m} \sum_{k'=1}^{m} \left[\tilde{\mu}_{k'} - 2 \sum_{k=1}^{m} \{r_{k'k}(1-r_{k'k})\tilde{d}_k\}\right] \quad (2c)$$

$$\hat{\sigma}^2 = \frac{1}{m} \sum_{k'=1}^{m} \left[\tilde{\sigma}^2_{k'} - 2 \sum_{k=1}^{m} \{r_{k'k}(1-r_{k'k}) \times [\tilde{a}_k^2 + (1-2r_{k'k}+2r_{k'k}^2)\tilde{d}_k^2]\}\right]. \quad (2d)$$

Here, the tilde $(\sim)$ indicates the single-interval mapping estimates and the hat $(\hat{})$ the estimates of the moment method; $r_{k'k}$ is the recombination rate between QTLs k' and k.

The computer programs compute the 'fitness' of putative QTL locations as a function based on the likelihood plus an Akaike's information criterion-like (Akaike, 1974) penalty function. In the programs of Nakamichi the fitness score is computed as $1/2\times$ (likelihood ratio test statistic using the two-step moment method estimates as described above -6 times number of QTLs) and a genetic algorithm is used to identify the model with the highest 'fitness'.

(ii) *Multiple-interval mapping*

The likelihood of the multiple-interval mapping model is a finite normal mixture. Given trait values $y=\{y_i\}$ and genomic positions and, therefore, the probabilities of QTL genotypes given marker data (M), the likelihood function for parameters $\theta = \{a_k, d_k, \mu, \sigma^2\}$ can be formulated as (Kao & Zeng, 1997; Kao *et al.*, 1999):

$$L(\theta|y, M) = \prod_{i=1}^{n} \left[\sum_{j=1}^{3^m} p_{ij}\phi(\mu_{ij}, \sigma^2)\right] \quad (3)$$

where p_{ij} are the conditional probabilities for the QTL genotypes with the corresponding genotypic values μ_{ij} and ϕ representing the normal density function. Kao & Zeng (1997) proposed general formulas for obtaining the maximum likelihood estimators using an expectation-maximization (EM) algorithm (Dempster *et al.*, 1977; Little & Rubin, 1987). With these general formulas QTL mapping analysis could be extended to using multiple marker intervals simultaneously for mapping multiple QTLs and estimating the QTL effects. This method was called multiple-interval mapping by Kao *et al.* (1999). Further details of the method can be found in Zeng *et al.* (1999).

(iii) *Parameter definition*

To avoid confusion in the comparison and interpretation of QTL estimates, the definition of the QTL parameters in the various computer programs must be observed. Let us assume that (as usual) the homozygous genotypes of the marker and the QTL in the first parental line are coded as 0 and in the second parental line as 2, and further the heterozygous genotypes are coded as 1. Then in the program of Nakamichi *et al.* (2001) the additive genetic effect of a QTL k (a_k) is defined as $+a_k$ if the genotype is coded as 0 and $-a_k$ if the genotype code is 2. The dominance effect is defined as d_k if the genotype is 1 and is 0 otherwise. In the programs of QTL Cartographer (Basten *et al.*, 2001) or in the F_2 QTL analysis servlet in the QTL express package (Seaton *et al.*, 2002), for example, the definition of the additive effects is just the opposite, i.e. $-a_k$ if the genotype coded is 0 and $+a_k$ if the code is 2. As the main purpose of this paper is to study the properties of the two-step moment method, the parameter definition of Nakamichi *et al.* (2001) is used.

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Table 1. *Conditional probabilities for two closely linked QTLs given flanking marker genotypes for an F_2 population*

Marker genotypes	QTL genotypes								
	Q_1Q_1 Q_2Q_2	Q_1Q_1 Q_2q_2	Q_1Q_1 q_2q_2	Q_1q_1 Q_2Q_2	Q_1q_1 Q_2q_2	Q_1q_1 q_2q_2	q_1q_1 Q_2Q_2	q_1q_1 Q_2q_2	q_1q_1 q_2q_2
$M_1M_1M_2M_2$	1	0	0	0	0	0	0	0	0
$M_1M_1M_2m_2$	τ_3	τ_2	0	0	τ_1	0	0	0	0
$M_1M_1m_2m_2$	τ_3^2	$2\,\tau_2\,\tau_3$	τ_2^2	0	$2\,\tau_1\,\tau_3$	$2\,\tau_1\,\tau_2$	0	0	τ_1^2
$M_1m_1M_2M_2$	τ_1	0	0	τ_2	τ_3	0	0	0	0
$M_1m_1M_2m_2$	$\eta\tau_1\,\tau_3$	$\eta\,\tau_1\,\tau_2$	0	$\eta\tau_2\,\tau_3$	$1-2\eta(\tau_1\,\tau_2+\tau_1\,\tau_3+\tau_2\,\tau_3)$	$\eta\tau_2\,\tau_3$	0	$\eta\tau_1\,\tau_2$	$\eta\tau_1\,\tau_3$
$M_1m_1m_2m_2$	0	0	0	0	τ_3	τ_2	0	0	τ_1
$m_1m_1M_2M_2$	τ_1^2	0	0	$2\,\tau_1\,\tau_2$	$2\,\tau_1\,\tau_3$	0	τ_2^2	$2\,\tau_2\,\tau_3$	τ_3^2
$m_1m_1M_2m_2$	0	0	0	0	τ_1	0	0	τ_2	τ_3
$m_1m_1m_2m_2$	0	0	0	0	0	0	0	0	1

Double recombination ignored.
$\tau_1 = r_{M_1Q_1}/r_{M_1M_2}$ (where $r_{M_1Q_1}$ is the recombination rate between marker 1 and QTL 1, etc.).
$\tau_2 = (r_{M_1Q_2} - r_{M_1Q_1})/r_{M_1M_2}$.
$\tau_3 = 1 - (r_{M_1Q_2}/r_{M_1M_2})$.
$\eta = r^2_{M_1M_2}/[r^2_{M_1M_2} + (1 - r_{M_1M_2})^2]$.

(iv) *Models*

Two different models were used to simulate the data. In the first model 100 replicates of an F_2 population with 500 individuals were simulated and analysed. Five QTL at positions 8, 15, 25, 35 and 42 cM on a chromosome of length 50 cM were postulated. The five QTLs had additive genetic effects $a_1 = -0{\cdot}5$, $a_2 = 1{\cdot}0$, $a_3 = -1{\cdot}0$, $a_4 = 1{\cdot}0$ and $a_5 = -0{\cdot}5$, respectively, using the parameterization as described above. There were no dominance effects. The markers were located at the positions 0, 10, 20, 30, 40 and 50 cM, respectively. The residual variance (σ^2) was $0{\cdot}1^2$.

The second model was identical to that used by Nakamichi *et al*. (2001) to demonstrate the behaviour of their method for closely linked QTLs. An F_2 population with sample size 500 was simulated and 100 replicates were analysed. Two QTLs at positions 43 and 47 cM on a chromosome of length 100 cM were assumed. The QTLs had effects $a_1 = 2{\cdot}0$, $a_2 = -2{\cdot}0$ and $d_1 = 1{\cdot}0$, $d_2 = -1{\cdot}0$. Markers were located every 10 cM on the chromosome, i.e. the two QTLs were not separated by a marker. The residual variance was 1·0. In the two-step moment method analyses for both models the default GA-controlling parameters of Nakamichi *et al*. (2001) were used.

To study the influence of the distance between two linked QTLs in a marker interval, two further settings where the QTLs were very close (position 43 and 44 cM for QTL 1 and QTL 2, respectively) and where the QTLs were farther apart (positions 43 and 49 cM) were simulated and analysed.

For QTL detection in multiple-interval mapping a stepwise selection procedure and the likelihood ratio test statistic for adding QTL parameters according to Kao *et al*. (1999) was applied. The Bonferroni argument was used to determine the critical values; thus the critical values for claiming detection of an additional QTL were $\chi^2_{(2,\,0{\cdot}05/5)} = 9{\cdot}2104$ and $\chi^2_{(2,\,0{\cdot}05/10)} = 10{\cdot}5970$ for model 1 and model 2, respectively.

(v) *Extension of multiple-interval mapping to include two QTLs in the same marker interval*

If the QTLs are located in different marker intervals, the probability of the jth ($j = 1, 2, \ldots, 3^m$ for an F_2 population) joint genotype of putative QTLs is the product of the marginal conditional probabilities of each individual QTL. The marginal conditional probabilities for an F_2 scheme can be found, for example, in Kao & Zeng (1997, Table 2) ignoring double recombination. In Table 1 the joint conditional probabilities of QTL genotypes derived for two putative QTLs within a marker interval are shown. Because in the second simulation model two QTLs were located within a marker interval, these conditional probabilities were used to compare the results with the unmodified approach, i.e. ignoring the fact that the conditional probabilities are not independent. I am very grateful to one of the referees who called my attention to the paper of Jiang & Zeng (1995). In Table 2 of their paper the probabilities of QTL genotypes given flanking marker genotype for two QTLs within a marker interval were also derived. By comparing the probabilities in Jiang & Zeng (1995) with the values in Table 1 in this paper it can be seen that there are differences when the marker

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Table 2. *Number of QTLs found for model 1 and range of calculated LRT statistics (in parenthesis) by the two-step moment method and multiple-interval mapping for 100 replicates*

No. of QTLs found	Moment method	Multiple-interval mapping
0	8	0
1	1 (10·3)	0
2	27 (12·5–36·5)	0
3	27 (18·9–43·9)	0
4	26 (29·9–75·8)	0
5	11 (39·0–71·6)	100 (515·5–920·0)
⩾6	0	–

genotype is $m_1m_1M_2M_2$, which may eventually be explained by typographical errors in the paper of Jiang & Zeng (1995).

(vi) *Two-step moment method and multiple QTLs in the same marker interval*

As the use of the 'unstable' program GAQTL2 resulted in some surprising results, a thorough scrutiny showed that there are two programming errors in the source code of the program GAQTL2. Firstly, a wrong residual variance term is used in the computation of the likelihood values for a no-QTL model, resulting in likelihood test statistics or 'fitness' values that are too small. The larger the QTL effects the more are the correct likelihood ratio test statistic values reduced. The second error is in the use of wrong conditional probabilities when the marker constellation is $M_1M_1m_2m_2$ and $m_1m_1M_2M_2$, respectively. But as this is rare in the designs under considerations this aspect did not have a very strong influence on the results. After correcting for these two errors a comparison with own computer programs led to the same results and the modified GAQTL2 program was also used for the analyses of model 2 with linked QTLs to study the effects of using the joint conditional probabilities on the results.

3. Results

(i) *Model 1*

Multiple-interval mapping indubitably identified five QTLs in all the replicates. The partial likelihood ratio test statistic for comparing a five-QTL model with a four-QTL model was $366{\cdot}3 \pm 67{\cdot}6$ (mean $\pm$ standard deviation) and ranged from 188·2 to 547·5. The mean absolute likelihood ratio test statistic was 717·8 and varied between 515·5 and 920·0. In contrast, as can be seen from Table 2, the computer program GAQTL using the two-step moment method found five QTL

Table 3. *Means and empirical standard deviations of QTL position estimates (in cM), effect estimates and the residual variance estimates in the replicates where five QTLs were identified for simulation model 1*

	True values	Moment method ($n=11$)	Multiple-interval mapping ($n=100$)
Positions (cM)			
QTL 1	8	$3{\cdot}18 \pm 7{\cdot}36$	$7{\cdot}82 \pm 0{\cdot}50$
QTL 2	15	$14{\cdot}45 \pm 3{\cdot}67$	$14{\cdot}59 \pm 0{\cdot}68$
QTL 3	25	$27{\cdot}00 \pm 6{\cdot}18$	$24{\cdot}81 \pm 0{\cdot}66$
QTL 4	35	$34{\cdot}36 \pm 5{\cdot}30$	$34{\cdot}64 \pm 0{\cdot}52$
QTL 5	42	$43{\cdot}91 \pm 5{\cdot}34$	$41{\cdot}92 \pm 0{\cdot}50$
Effects			
a_1	−0·5	$-0{\cdot}111 \pm 0{\cdot}035$	$-0{\cdot}506 \pm 0{\cdot}018$
a_2	1·0	$0{\cdot}143 \pm 0{\cdot}077$	$1{\cdot}007 \pm 0{\cdot}018$
a_3	−1·0	$-0{\cdot}029 \pm 0{\cdot}121$	$-1{\cdot}002 \pm 0{\cdot}018$
a_4	1·0	$0{\cdot}075 \pm 0{\cdot}159$	$1{\cdot}004 \pm 0{\cdot}025$
a_5	−0·5	$-0{\cdot}082 \pm 0{\cdot}128$	$-0{\cdot}504 \pm 0{\cdot}021$
d_1	0	$-0{\cdot}005 \pm 0{\cdot}140$	$-0{\cdot}001 \pm 0{\cdot}017$
d_2	0	$-0{\cdot}018 \pm 0{\cdot}173$	$-0{\cdot}000 \pm 0{\cdot}018$
d_3	0	$0{\cdot}066 \pm 0{\cdot}192$	$0{\cdot}002 \pm 0{\cdot}015$
d_4	0	$-0{\cdot}045 \pm 0{\cdot}197$	$-0{\cdot}000 \pm 0{\cdot}020$
d_5	0	$0{\cdot}005 \pm 0{\cdot}089$	$-0{\cdot}002 \pm 0{\cdot}017$
Residual variance	$0{\cdot}1^2$	$0{\cdot}389^2 \pm 0{\cdot}017$	$0{\cdot}119^2 \pm 0{\cdot}003$

in only 11 out of the 100 cases. In 89% of the repetitions between 0 and 4 QTL were 'identified'. It can further be seen from Table 2 that the computed partial likelihood ratio test statistics were rather small as compared to those from multiple-interval mapping.

The relative QTL variance in model 1 was very large, so as expected the position and effect estimates from multiple-interval mapping were very accurate and showed only small empirical standard errors as can be seen from Table 3. It can further be seen that the two-step moment method was not only problematic in identifying the correct number of QTLs, but also resulted in rather inaccurate estimates of the QTL positions, the genetic effects and the variance of the error term. The computer program (GAQTL) of Nakamichi *et al.* (2001) can be forced to identify five QTL. When doing so, the empirical standard deviations (Table 4) ranged from 4·71 to 6·36 cM (QTL positions), from 0·075 to 0·114 (additive genetic effects) and from 0·079 to 0·131 (dominance effects). These values are comparable to those given in Table 3 for the 11 'significant' replicates.

(ii) *Model 2*

When the two QTLs were located at positions 43 and 47 cM, the two-step moment method using independent conditional probabilities found two QTLs in 47 of 100 repetitions, three QTLs in 48 repetitions

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Table 4. *Means and empirical standard deviations of QTL position estimates (in cM), effect estimates and the residual variance estimates in the replicates when the two-step moment method was forced to identify five QTLs for simulation model 1 ($n=100$)*

	QTL 1	QTL 2	QTL 3	QTL 4	QTL 5
Positions (cM)	2·17 ± 5·32	14·92 ± 4·71	25·06 ± 6·36	34·58 ± 5·16	45·28 ± 6·22
Additive effects	−0·072 ± 0·075	0·110 ± 0·110	−0·031 ± 0·114	0·069 ± 0·114	−0·068 ± 0·087
Dominance effects	0·002 ± 0·086	−0·033 ± 0·116	0·052 ± 0·131	−0·021 ± 0·115	0·003 ± 0·079
Residual variance	…	…	$0·394^2 \pm 0·015$	…	…

Table 5. *Comparison of the two-step moment method with multiple-interval mapping for model 2 using independent conditional probabilities. Number of identified QTLs, means and empirical standard deviations of QTL position estimates (in cM), effect estimates and estimates of the residual variance*

True QTL positions (cM):	43/47	43/44	43/49
Moment method			
No. of identified QTLs			
0	0	41	0
1	0	15	0
2	47	32	44
3	48	9	42
4	5	3	14
Estimates (2 QTL results)			
Position (cM)	41·4 ± 1·2, 48·1 ± 1·5	39·7 ± 11·2, 53·1 ± 14·6	41·4 ± 1·0, 48·6 ± 1·2
Additive effects (2·0/−2·0)	0·90 ± 0·27, −0·90 ± 0·28	0·25 ± 0·23, −0·25 ± 0·23	1·43 ± 0·32, −1·43 ± 0·32
Dominance effects (1·0/−1·0)	0·57 ± 0·33, −0·60 ± 0·35	0·24 ± 0·20, −0·24 ± 0·21	0·92 ± 0·32, −0·95 ± 0·33
Residual variance ($1·0^2$)	$1·10^2 \pm 0·080$	$1·04^2 \pm 0·034$	$1·17^2 \pm 0·097$
Multiple-interval mapping			
No. of identified QTLs			
0	0	22	0
1	0	1	0
2	91	74	92
3	9	3	8
4	0	0	0
Estimates (2 QTL results)			
Position (cM)	42·4 ± 2·8, 46·6 ± 3·0	44·2 ± 9·8, 46·5 ± 9·7	42·8 ± 1·8, 48·7 ± 2·0
Additive effects (2·0/−2·0)	1·93 ± 0·29, −1·93 ± 0·29	1·55 ± 0·78, −1·55 ± 0·79	1·95 ± 0·19, −1·95 ± 0·19
Dominance effects (1·0/−1·0)	1·02 ± 0·23, −1·04 ± 0·25	1·09 ± 0·64, −1·10 ± 0·67	1·01 ± 0·18, −1·03 ± 0·19
Residual variance ($1·0^2$)	$1·00^2 \pm 0·066$	$0·99^2 \pm 0·058$	$1·00^2 \pm 0·062$

and four QTLs in 5 repetitions (Table 5). This is in contradiction to the results of Nakamichi *et al.* (2001), who for the same scenario found that the moment method detected two QTLs in 98 of 100 cases. In this simulation study the moment method clearly tended to identify too many QTLs for this scenario. In comparison, multiple-interval mapping, where two QTLs were identified in 91% of the repetitions, was much more accurate in identifying the number of QTLs.

Further, the absolute values of the QTL effect estimates from the moment method were clearly downward-biased. The closer together the QTLs were located the more evident was the downward bias (Table 5). In general, as for model 1 the bias of the effect estimates has the consequence that the residual variance is overestimated. Comparing the two-step moment method with multiple-interval mapping when the QTLs were very close (positions 43/44 cM) the power of detecting two QTLs was much smaller for the moment method, the position estimates were biased and showed higher empirical standard deviations.

The differences between the multiple-interval mapping approach based on the joint conditional probabilities (Table 6) compared with the approach using independent probabilities (Table 5) were only very small. When applying the moment method the use of the joint conditional probabilities resulted in

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Table 6. *Comparison of the two-step moment method with multiple-interval mapping for model 2 using joint conditional probabilities. Number of identified QTLs, means and empirical standard deviations of QTL position estimates (in cM), effect estimates, estimates of the residual variance and means and range of the likelihood ratio test statistics*

True QTL positions (cM):	43/47	43/44	43/49
Moment method			
No. of identified QTLs			
0	0	40	0
1	0	13	0
likelihood ratio test statistics		8·1 (6·2–11·9)	
2	73	37	77
likelihood ratio test statistics	72·3 (24·8–152·9)	18·5 (12·1–38·7)	135·7 (49·2–219·4)
3	22	7	19
likelihood ratio test statistics	88·3 (34·6–137·4)	26·2 (18·1–50·7)	138·0 (76·6–213·7)
4	5	3	4
likelihood ratio test statistics	78·2 (61·7–96·5)	30·1 (27·2–32·9)	168·7 (131·6–201·2)
Estimates (2 QTL results)			
Position (cM)	41·4 ± 1·2, 48·1 ± 1·3	40·2 ± 10·9, 52·9 ± 14·0	41·1 ± 0·9, 48·6 ± 1·0
Additive effects (2·0/−2·0)	0·92 ± 0·23, −0·92 ± 0·23	0·25 ± 0·24, −0·26 ± 0·23	1·34 ± 0·25, −1·34 ± 0·24
Dominance effects (1·0/−1·0)	0·59 ± 0·24, −0·61 ± 0·25	0·22 ± 0·20, −0·24 ± 0·21	0·78 ± 0·27, −0·82 ± 0·26
Residual variance ($1·0^2$)	$1·14^2 \pm 0·095$	$1·04^2 \pm 0·070$	$1·15^2 \pm 0·092$
Multiple-interval mapping			
No. of identified QTLs			
0	0	22	0
1	0	1	0
2	92	74	93
likelihood ratio test statistics	102·7 (45·5–178·7)	30·5 (15·2–83·7)	162·8 (83·1–241·8)
3	8	3	7
4	0	0	0
Estimates (2 QTL results)			
Position (cM)	42·6 ± 2·5, 46·8 ± 2·7	44·1 ± 9·7, 46·4 ± 9·6	42·4 ± 1·7, 48·7 ± 1·9
Additive effects (2·0/−2·0)	1·94 ± 0·29, −1·94 ± 0·29	1·57 ± 0·76, −1·56 ± 0·78	1·95 ± 0·19, −1·95 ± 0·19
Dominance effects (1·0/−1·0)	1·02 ± 0·23, −1·05 ± 0·25	1·10 ± 0·65, −1·11 ± 0·68	1·02 ± 0·18, −1·04 ± 0·19
Residual variance ($1·0^2$)	$1·00^2 \pm 0·065$	$0·99^2 \pm 0·057$	$1·00^2 \pm 0·060$

smaller rates of false discoveries as can be seen from Tables 5 and 6. But these were still quite high and the ability to identify the correct number of QTLs was still far removed from that of the multiple-interval mapping approach. The means and standard deviations of the estimates of the QTL positions, QTL effects and the residual variances for the replicates where two QTL were identified are very close to the values in Table 5 (independent conditional probabilities) and showed similar biases. The likelihood ratio test statistics when applying the two-step moment method were clearly smaller than the values from the multiple-interval mapping approach.

4. Discussion

The computer program of Nakamichi *et al.* (2001) makes use of a penalty function in model selection related to Akaike's information criterion. For a general discussion of information criteria used in QTL mapping see for example Zeng *et al.* (1999). It is well known that Akaike's information criterion is a relatively liberal criterion. It can lead to comparatively higher type I errors, i.e. in our context to false detection of QTLs. Although Nakamichi *et al.* (2001) state that they are using Akaike's information criterion in their computer program, they strictly speaking do not. As can be seen from the likelihood function (3) the parameters of the multiple-QTL model are the additive effects, the dominance effects, the general mean and the residual variance. Thus the number of parameters is $2 \times$ number of QTL $+ 2$ and the penalty function in Akaike's information criterion should be $2 \times$ number of parameters. In the computer program the QTL position is also considered as a parameter. Thus in the program, for an additional QTL to be included the increase in the value of the likelihood function must be 6 and not 4 as would be Akaike's penalty. To get an idea about the magnitude of the type I errors of the two-step moment method in

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the designs of this study, the simulated data were reanalysed setting the QTL effects equal to zero. In the first simulation model (QTL in different marker intervals) the empirical type I error was 23% (in 15 cases one QTL and in 8 cases two QTLs were wrongly identified) and in the second model (closely linked QTL) the error rate was 38% (in 20 cases one QTL and in 18 cases two QTLs were wrongly identified, respectively). As in the first simulation model there were 5 marker intervals and in the second simulation model 10 intervals, the higher type I error for the second design is not surprising. As has been shown above, the critical values in multiple-interval mapping based on the Bonferroni argument are much higher, namely 9·2104 (model 1) and 10·5970 (model 2), respectively.

But despite the large type I errors, when five QTLs with rather large effects were present in simulation model 1 the two-step moment method clearly identified too few QTLs, whereas multiple-interval mapping did not have any problem in correctly identifying the number of QTLs involved. The likelihood ratio test statistics calculated from the score values of the moment method computer program were much smaller than those obtained for the maximum likelihood estimates from the multiple-interval mapping method. In the cases where five QTLs were identified, the empirical standard deviations of the position estimates were about 10 times larger than those from multiple-interval mapping. The mean QTL effect estimates were close to zero, but showed as for the position estimates very large standard deviations. Further, the residual variance estimates were grossly overestimated.

Generating 100 000 repetitions where five QTLs were distributed on a chromosome of length 50 cM using a uniform distribution, the following means $\pm$ standard deviations of the locations were obtained: $7{\cdot}99 \pm 6{\cdot}77$, $16{\cdot}49 \pm 8{\cdot}55$, $25{\cdot}00 \pm 9{\cdot}08$, $33{\cdot}49 \pm 8{\cdot}58$ and $41{\cdot}99 \pm 6{\cdot}77$. Comparing these values with the estimates of the two-step moment method in Table 3 it turns out that distributing the location of QTLs randomly over the chromosome would be almost as accurate as the moment method.

In the scenario of model 2, i.e. when two QTLs were located in one marker interval and when the QTLs were very close together (positions 43/44 cM), the two-step moment method clearly showed more repetitions where no or only one QTL was identified. Thus multiple-interval mapping was again much more accurate in estimating the number of QTLs. The mean position estimates of multiple-interval mapping were closer to the true values and showed smaller standard errors. The effect estimates of the moment method were clearly biased towards zero. Theoretical studies have shown that, when using least squares, it is impossible to map multiple QTLs within the same marker bracket (Whittaker *et al.*, 1996). Maximum likelihood can in principle separate the location and effect although it is known that the amount of information contained in the distribution of the data is small relative to the amount of information contained in the mean marker contrasts. When the QTLs were not so close together (positions 43/47 cM and 43/49 cM, respectively) the two-step moment method overestimated the number of QTLs involved and the QTL parameter estimates (effects, residual variance) were biased. The false discovery rates of multiple-interval mapping were in the expected range.

Nakamichi *et al.* (2001) wrote that in the model 2 scenario their approach detected the two QTLs in 98 cases out of 100 trials. In the other two cases one QTL was detected and no cases of three or more QTLs being detected were observed. These findings cannot be confirmed. In this study, under identical simulation conditions, the two-step moment method of Nakamichi *et al.* (2001) identified two QTLs in less than 50% of the repetitions and three or four QTLs in more than 50%, as can be seen from Table 5. The explanation for this difference may be found in Section 2(vi). On the web site of Nakamichi it is pointed out that Nakamichi *et al.* (2001) did not use the method and computer program for these analyses as indicated in their paper, but their computer program GAQTL2. In agreement with Nakamichi *et al.* (2001), it was found that in the cases where two QTLs were identified the absolute values of the estimates were too small rather than too large. In this downward bias of the absolute effects Nakamichi *et al.* (2001) saw the advantage that these estimates are preventing researchers from being too optimistic.

The likelihood of the multiple-QTL model generally has the form as represented in (3). As the conditional probabilities for the QTL genotypes (p_{ij}) should be the same for the two-step moment method and for the multiple-interval mapping method, the differences between the likelihood values at putative QTL locations using multiple-interval mapping and the respective part of the fitness function using the moment method are due to differences in the estimates for the genotypic values, i.e. differences in the parameter estimates and in the estimates of the residual variance. As already mentioned, Nakamichi *et al.* (2001) also observed that for the model 2 simulations the absolute values of the QTL effect estimates of the two-step moment method were seriously downward-biased. In model 1 situation this had dramatic consequences. For given QTL positions using effect estimates shrunk towards zero and thus some μ_{ij}^* instead of μ_{ij} in (3) in conjunction with σ^* much larger than σ has an impact on the calculation of the fitness and in consequence on model selection. As expected in this situation, even if the computer program was forced to identify the correct number of QTLs ($m = 5$),

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this had very little consequence on the accuracy of the position and effect estimates (Table 4), despite the fact that the true error variance was very small. If three QTLs were located at positions 17, 43 and 85 cM, respectively, with equal additive effects as in the first simulation model of Nakamichi *et al*. (2001), the differences between the two-step moment method and multiple-interval mapping were small (results not shown), although the moment method showed an empirical type I error of 13% and the interval-mapping method still gave somewhat more precise estimates. Similarly if QTL effects were set in coupling in model 1 and model 2, respectively, then the two-step moment method gave fairly good approximate maximum likelihood estimates (results not shown).

In conclusion it was shown that differences between the two-step moment method of Nakamichi *et al*. (2001) and multiple-interval mapping (Kao & Zeng, 1997; Kao *et al*., 1999) can be large, irrespective of whether the QTLs are in the same or in different marker intervals. The reason are differences in the parameter estimates, which can be large, especially if linked QTLs are in repulsion. The bias in the two-step moment method estimates of the QTL effects and of the residual variance directly influences the likelihood ratio test statistics or fitness scores in the sense that it leads to smaller values compared with multiple-interval mapping and thus has a strong influence on model selection. On the other hand the use of Akaike's information criterion in the programs of Nakamichi as a liberal criterion in model selection counteracts this in the way that it increases the number of 'found' QTL. If QTL effects were in coupling then the two-step moment method resulted in fairly good approximate maximum likelihood estimates in the scenarios studied. But even in those cases it would be desirable to have an option in the computer programs by Nakamichi for selecting among various penalty functions.

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