Genet. Res., Camb. (2000), **76**, *pp.* 87–104. *With* 1 *figure*. Printed in the United Kingdom © 2000 Cambridge University Press

Fine-mapping of quantitative trait loci in half-sib families using current recombinations

GEORG THALLER[1]* AND INA HOESCHELE[2]

[1] *Lehrstuhl für Tierzucht, Alte Akademie 12, Technische Universität München-Weihenstephan, D-85350 Freising, Germany*
[2] *Department of Dairy Science and VT Institute for Genomics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0315, USA*

(*Received 9 February 1999 and in revised form 26 July 1999 and 3 January 2000*)

Summary

Two groups of methods are being developed to fine-map quantitative trait loci (QTLs): identity-by-descent methods or methods using historical recombinations, and genetic chromosome dissection methods or methods utilizing current recombinations. Here we propose two methods that fall into the second group: contrast mapping and substitution mapping. A QTL has previously been detected via linkage mapping in a half-sib design (granddaughter or daughter design), and sires (grandsires) likely to be heterozygous at the QTL have been identified. A sire (grandsire) and its recombinant offspring are then genotyped for a series of ordered markers spanning the initial marker interval. Offspring are grouped by paternal multi-marker haplotype with haplotypes differing in the location of the recombination event. In the contrast method, contrasts between the phenotypic averages of haplotypes or offspring groups are calculated which correspond to marker intervals within the original interval. The expected value of the contrast for the true QTL interval is always maximum, hence the interval with maximum observed contrast is assumed to contain the QTL. Alternative statistics for determining the interval most likely to contain a QTL are presented for contrast mapping, as well as a bootstrap estimation of the probability of having identified the correct interval. For an initial marker bracket of 20 cM and 10 additional equidistant markers, the probability of assigning the QTL to the correct 2 cM marker interval or to a combined 4 cM interval was calculated. For substitution effects of 0·093, 0·232, 0·464, 0·696 and 0·928 (in additive genetic SD), power values near 0·14, 0·26, 0·48, 0·67 and 0·80 (0·25, 0·53, 0·86, 0·97 and 0·99) are achieved for a family of 200 (1000) sons, respectively. In substitution mapping, QTL segregation status of recombinant sons must be determined using daughter genotyping. Combinations of two haplotypes with their segregation status are required to assign the QTL to an interval. Probabilities of correct QTL assignment were calculated assuming absence of the mutant QTL allele in dams of sons. For a 2 cM interval and a QTL at the midpoint of an interval, power near 0·95 (0·90) is reached when the number of recombinant sons is 70 (60), or total number of sons is 424 (363). For QTL positions away from the midpoint, power decreases but can be improved by combining marker intervals. For a QTL located halfway to the midpoint, and 182 sons in a family resulting in 30 recombinant sons, probability is 0·94 for assignment to either a 2 cM or a combined 4 cM interval. Effect of type I and type II errors in segregation status determination on power of QTL assignment was found to be small. Errors in segregation status due to QTL segregation in dams have an impact if the frequency of the mutant QTL allele is intermediate to high.

* Corresponding author. e-mail: Georg.Thaller@flg.tum.de

Downloaded from http://www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http://www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

1. Introduction

Linkage mapping of QTLs in half-sib families leads to the assignment of a QTL to a region of 10 to 20 cM or larger (e.g. Zhang *et al.*, 1998), with the smallest region achievable in a large grand-daughter design in dairy cattle. Assignment of a QTL to a smaller region, possibly 1 to 2 cM or less, is desirable and important for several reasons. Fine-mapping of a QTL reduces the problem of establishing marker-QTL phase, which in turn increases the accuracy of QTL parameter estimation and increases the efficiency of marker-assisted selection. Fine-mapping also enables a distinction between pleiotropy and linkage in a multiple trait context. Finally, fine-mapping is a prerequisite for gene isolation via (comparative) positional candidate cloning or pure positional cloning, as a region of 1 to 2 cM still contains about 25 to 50 genes in mammals.

Two groups of methods for the fine-mapping of QTLs can be distinguished: identity-by-descent methods or methods using historical recombinations, and genetic chromosome dissection methods or methods utilizing current recombinations. Both groups of methods rely on prior assignment of the QTL to a confidence region of, say, 20 cM length, and on the saturation of this region with ordered markers. Group 1 methods (for theory see Xiong & Guo, 1997) have been used predominantly for fine-mapping of human disease genes (e.g. Hastbacka *et al.*, 1994), have recently been used to fine-map a gene affecting milk fat percentage in dairy cattle (Riquet *et al.*, 1999), rely on the assumption of a unique ancestral mutation in a defined population, and utilize linkage disequilibrium between the gene and very close markers. Group 2 methods utilize recombinant chromosomes derived from a heterozygous individual (Thoday, 1961; Darvasi, 1997; Soller & Andersson, 1998). Within the two groups, different variants of methods exist. These methods are based on different assumptions, and no method will work in every situation, necessitating the development of alternative approaches. Below we present two methods, referred to as 'contrast mapping' and 'substitution mapping', which both belong to group 2. The two methods are closely related. Both rely on prior QTL detection via linkage mapping in half-sib families (daughter or granddaughter design), and identification of those sires (grandsires) that are heterozygous at the QTL.

These methods are used after a confidence region (say 10–20 cM) obtained from the initial linkage analysis has been saturated with additional markers (at, say, 1–2 cM intervals). To further reduce the initial confidence region using current recombinations, only additional recombinant (for the initial flanking markers) offspring should be genotyped for additional markers in the region. Although a more sophisticated method for linkage analysis such as Composite Interval Mapping (Zeng, 1994) or Bayesian analysis (e.g. Ulimari & Hoeschele, 1997) could in principle be used to fine-map a QTL in this region, it seems appropriate to consider simpler approaches that only assign a QTL to a marker sub-interval within the initial confidence, because there should be little information to further localize the QTL within a sub-interval.

2. Methods

(i) *Contrast mapping*

(a) *Basic contrast mapping*

Basic contrast mapping utilizes current recombinations which occur within the designs used for linkage mapping, e.g. half-sib (daughter or granddaughter) designs in cattle, and is illustrated in Fig. 1*a*. First, information from linkage mapping is used to identify individual sires which are heterozygous at the QTL with high probability. All methods for linkage mapping in granddaughter designs provide information about whether a sire is heterozygous or homozygous at the QTL (for a review of these methods see Hoeschele *et al.*, 1997). Least squares (LS) analysis estimates regression coefficients within sires (a two-sided *t*-test can be used to test whether the regression coefficient for a specific sire is zero indicating homozygosity). Variance components (VC) methods provide estimates of the sire's allelic effects, with similarity of the effects relative to the variance at the QTL indicating homozygosity. Maximum Likelihood (ML) and Bayesian analyses directly provide genotype probabilities for the sires, assuming a bi-allelic QTL. A sire's genotype at the QTL and its closest, informative flanking markers is denoted by M_1-Q-M_2/m_1-q-m_2, with the '/' separating the parental chromosomes in the sire, and with Q (q) representing the 'mutant' ('normal') QTL allele. Based on the large numbers of sons in granddaughter designs (>30), the sire's marker haplotype containing Q can usually be determined with high accuracy. For example, in LS the sign of the regression coefficient indicates the haplotype including Q, or in ML and Bayesian analyses, genotype probabilities are calculated separately for the Qq and qQ heterozygotes. Depending on the distance between M1 and M2, measured by recombination rate *r*, a proportion *r* of the offspring are expected to be recombinant, having received either M_1-m_2 or m_1-M_2 from their sire, while a proportion $(1-r)$ are non-recombinant and supply no information for finer localization of the QTL. The sire and its recombinant offspring are genotyped with additional, ordered markers between M1 and M2. With fully informative markers, $2(m-1)$ different haplotypes from the sire are possible in the offspring,

Downloaded from http://www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http://www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

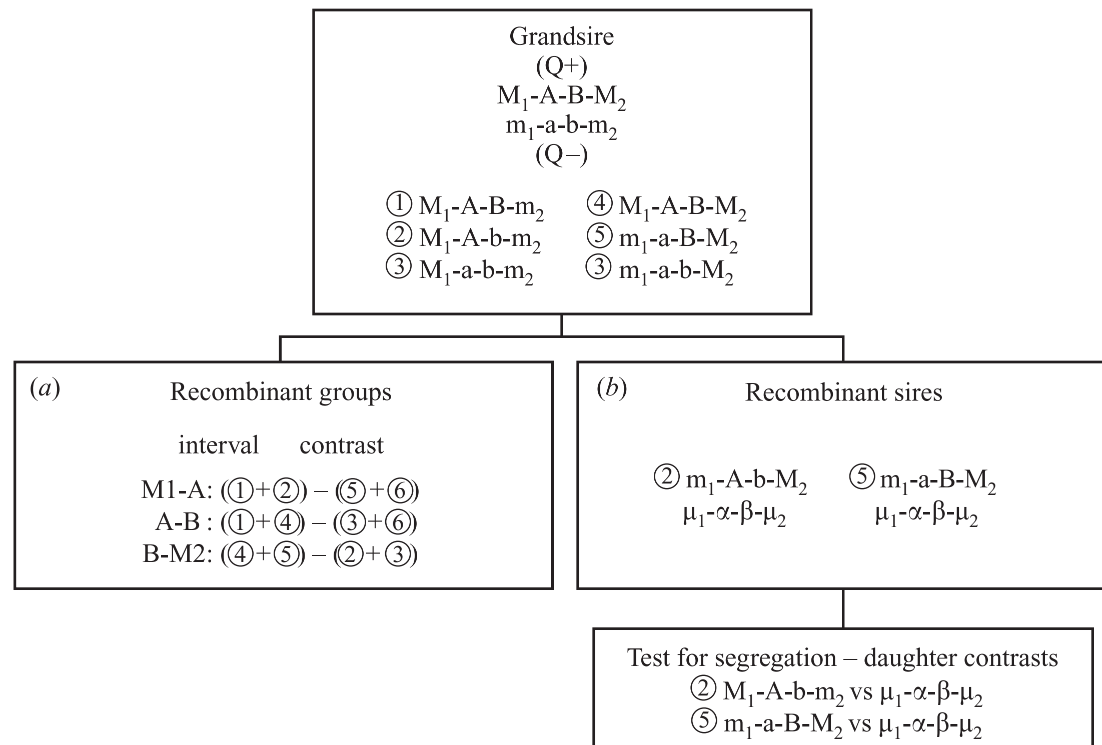

Fig. 1. Contrast and substitution mapping are based on recombinant offspring of known QTL-heterozygous grandsires. Differences between recombinant groups designed for putative QTL intervals are used in contrast mapping to assign the QTL (*a*); single recombinant sires are tested for segregation at the QTL, as certain combinations of recombinant haplotypes and segregation status lead to QTL assignment to a specific marker interval in substitution mapping (*b*).

Table 1. *Offspring groups by paternal haplotypes differing in location of recombination event*

Offspring group	Haplotype from sire[a]
1	M_1-A-B-C-D-m_2
2	M_1-A-B-C-d-m_2
3	M_1-A-B-c-d-m_2
4	M_1-A-b-c-d-m_2
5	M_1-a-b-c-d-m_2
6	M_1-A-B-C-D-M_2
7	M_1-a-B-C-D-M_2
8	M_1-a-b-C-D-M_2
9	M_1-a-b-c-D-M_2
10	M_1-a-b-c-d-M_2

[a] M1 (alleles M_1 and m_1) and M2 (alleles M_2 and m_2) are initial flanking markers; A (alleles A and a) to D (alleles D and d) are additional markers placed between initial markers.

where m is the number of markers including M1 and M2. These haplotypes represent the different marker intervals within the M1–M2 interval, where a recombination event occurred. For $m=6$ and with A, B, C and D denoting additional markers between M1 and M2, the resulting haplotypes or offspring groups are listed in Table 1.

We will temporarily assume that the QTL is always situated at the midpoint of an interval formed by two adjacent markers of the set including the initial markers (M1, M2) and the additional markers (A, …). Let $a(-a)$ be the mean of offspring inheriting Q (q) from the sire. Table 2 contains the means for the 10 offspring groups, defined in Table 1, as a function of the true interval containing the QTL, and lists appropriate phenotypic contrasts (last rows), assuming that the QTL is in a given interval (1–5), and their expected values.

Table 2 shows that the expected value of a contrast (E(c)) is maximum for the true QTL interval, and that contrasts are not independent, because they involve some of the same haplotype groups. Given that the true interval is i, the expected value of contrast i is $2a$. The expected value of contrast $i-k$ or $i+k$ is $2a(m-1-2k)/(m-2)$ for $0<k<i$ and $0<k<(m-1-i)$, which reduces to the expected values of contrasts in Table 2 for $m=6$. For a given true interval i, these expected values from the mean vector of the contrasts, $E(\mathbf{c})=\mu_i$, where $\mathbf{c}=[c_1, c_2, \ldots, c_{m-1}]$. Assuming constant sample size across haplotype groups are setting the variance of a single group mean equal to σ^2, variance of a contrast equals $2\sigma^2/(m-2)$, and covariance between contrasts i and $i+k$ is $2\sigma^2(m-1-2k)/[(m-2)^2]$. These elements form the variance–covariance matrix of the contrasts, $\mathbf{V}$.

In reality, interval i (the true interval containing the QTL) is unknown. Based on $E(\mathbf{c})$, we expect to find

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

Table 2. *Means[a] of the haplotype groups in Table 1 and expected values of contrasts derived from these groups for different true QTL intervals*

Group/True interval	QTL: M1–A	QTL: A–B	QTL: B–C	QTL: C–D	QTL: D–M2
1	a	a	a	a	0
2	a	a	a	0	$-a$
3	a	a	0	$-a$	$-a$
4	a	0	$-a$	$-a$	$-a$
5	0	$-a$	$-a$	$-a$	$-a$
6	0	a	a	a	a
7	$-a$	0	a	a	a
8	$-a$	$-a$	0	a	a
9	$-a$	$-a$	$-a$	0	a
10	$-a$	$-a$	$-a$	$-a$	0
$\frac{1}{4}$(1,2,3,4) – $\frac{1}{4}$(7,8,9,10)[b]	$2a$	$2\frac{3}{4}a$	$2\frac{1}{4}a$	$2(-\frac{1}{4})\,a$	$2(-\frac{3}{4})\,a$
$\frac{1}{4}$(1,2,3,6) – $\frac{1}{4}$(5,8,9,10)	$2\frac{3}{4}a$	$2a$	$2\frac{3}{4}a$	$2\frac{1}{4}a$	$2(-\frac{1}{4})\,a$
$\frac{1}{4}$(1,2,6,7) – $\frac{1}{4}$(4,5,9,10)	$2\frac{1}{4}a$	$2\frac{3}{4}a$	$2a$	$2\frac{3}{4}a$	$2\frac{1}{4}a$
$\frac{1}{4}$(1,6,7,8) – $\frac{1}{4}$(3,4,5,10)	$2(-\frac{1}{4})\,a$	$2\frac{1}{4}a$	$2\frac{3}{4}a$	$2a$	$2\frac{3}{4}a$
$\frac{1}{4}$(6,7,8,9) – $\frac{1}{4}$(2,3,4,5)	$2(-\frac{3}{4})\,a$	$2(-\frac{1}{4})\,a$	$2\frac{1}{4}a$	$2\frac{3}{4}a$	$2a$

[a] $a(-a)$: Mean of offspring inheriting Q (q) from the sire.
[b] Average phenotype of groups 1 through 4 minus average phenotype of groups 7 through 10 (other contrasts defined similarly).

the largest observed contrast in interval i. Hence, one way of identifying the QTL interval is to determine the interval associated with the largest contrast. We want the probability that c_i is larger than all other contrasts, given that i is the true interval, to be at least 90% or 95%. For given family size N, recombination rate r, number of markers m, and parameter a, standard deviation of individual records σ_y, and true interval i, we can calculate $\mathbf{V}$ and μ_i and subsequently the probability that c_i is larger than the other contrasts, which is

$$\mathbf{P}(\mathbf{c_i} > \sup(\mathbf{c_j}; \mathbf{j} = 1, \mathbf{m}-1, \mathbf{j} \neq \mathbf{i} \mid \mathbf{V}, \mu_\mathbf{i}) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{\substack{\sup(c_j)\\ j=1,m-1\\ j\neq i}}^{\infty} \mathbf{P}(\mathbf{c} \mid \mathbf{V}, \mu_\mathbf{i})\,\mathbf{dc}. \quad (1)$$

For calculating this probability at various values of N, r, m, a, σ_y and i, the integration can be avoided by sampling contrasts from $N(\mu_i, \mathbf{V})$, counting the number of samples where c_i is maximum, and dividing by sample size, to obtain a Monte Carlo estimate of (1).

Up to this point we have assumed that the QTL is located in the middle of an interval. Now we are investigating the effect of a QTL position different from the midpoint of an interval. When considering the case of $m = 6$ in Table 2, the means in this table change as follows. For the first 10 rows corresponding to the haplotype groups, values of 0 are replaced by $a(r_2 - r_1)/r$ for groups 1–5 and by $a(r_1 - r_2)/r$ for groups 6–10, where r_1 (r_2) is the recombination rate of the left (right) marker with the QTL. For the contrast rows, $0{\cdot}5a(r_2 - r_1)/r$ is added to all terms above the diagonal (all diagonal elements equal to $2a$ remain unchanged), while $0{\cdot}5a(r_1 - r_2)/r$ is added to all elements below the diagonal. In general, the expected value of contrasts $i-k$ or $i+k$ is now $2a(m-1-2k)/(m-2) + [2/(m-2)]a(r_1 - r_2)/r$. The expected values of the contrasts no longer decline symmetrically around the maximum value. If the QTL is located closer to the left marker in the true interval, then there is a smaller difference between the expected values of contrasts i and $i-1$, and hence the power of assigning the QTL to the correct interval decreases (see Table 3).

The above QTL interval identification relies on equal sample size in all recombinant groups. This is not true in practice, and the effect of unequal sample sizes on the probability of identifying the correct interval could be examined as follows. For given N, r and m and hence a sample size of Nr recombinant offspring, group sample sizes are drawn from the multinomial distribution with equal probabilities of $1/[2(m-1)]$ for the recombinant haplotype groups of offspring. Then we need to calculate $\mathbf{V}$ and μ_i for these data. Vector μ_i, is the same as before for the balanced case, but $\mathbf{V}$ is different. For example, when $m = 6$, then $m-2 = 4$, variance of contrast $c_1 = (y_1 + y_2 + y_3 + y_4)/4 - (y_7 + y_8 + y_9 + y_{10})/4$ is $\sigma_y{}^2/[(m-2)^2] \times$

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

Table 3. *Power of test for symmetry of declining contrast values around the maximum, and power of identifying the true interval and its adjacent intervals*

QTL position[a]	QTL effect[b]	Power *t*-test symmetry	Power max. contrast[c]
Midpoint	1	0·514	1·0 (0·0, 0·0)
0·25	1	0·815	0·994 (0·006, 0·0)
0·05	1	0·999	0·694 (0·306, 0·0)
Midpoint	0·25	0·710	0·777 (0·105, 0·108)
0·25	0·25	0·149	0·697 (0·266, 0·028)
0·05	0·25	0·028	0·544 (0·440, 0·009)

[a] 0·25, 0·05: fraction of distance between left and right markers starting at the left marker.
[b] *t*-test statistic = absolute value of difference between contrast values of intervals left and right to interval with maximum contrast value divided by standard deviation of this difference.
[c] Frequency of samples where the true interval has maximum estimated contrast; frequency of samples where the left or right interval has maximum contrast is given in parentheses.

$(1/n_1+1/n_2+1/n_3+1/n_4+1/n_7+1/n_8+1/n_9+1/n_{10})$, the y_i are averages and the n's are group sample sizes, and the covariance between, e.g., c_1 and c_4 is $\sigma_y{}^2/[(m-2)^2]\times(1/n_1+1/n_{10}-1/n_3-1/n_4-1/n_7-1/n_8)$. With $\mathbf{V}$ and μ_i calculated, we proceed as above for the balanced design.

(b) *Minimum RSS criterion*

Another way of inferring the QTL interval is to determine which model best fits the observed contrasts. Denote by y_i the average phenotype in group i, and by c_j the jth contrast. Then, $m-1$ weighted LS analyses of the contrasts are performed, one for each putative QTL interval. The model is

$$\mathbf{c}=\mathbf{x}_k a+\mathbf{e};\ E(\mathbf{c})=\mathbf{x}_k a=\mu_k,\qquad \mathrm{Var}(\mathbf{c})=\mathbf{V},\ k=1,\ldots,m-1. \quad (2)$$

We solve $m-1$ linear equations corresponding to the $\mathbf{x}_k$ for postulated intervals $k=1,\ldots,m-1$, and calculate the residual sums of squares (RSS) as

$$\mathrm{RSS}_k=\mathbf{c}'\mathbf{V}^{-1}\mathbf{c}-\hat{a}^2\mathbf{x}_k\mathbf{V}^{-1}\mathbf{x}_k. \quad (3)$$

For example, for $m=6$ and interval $k=2$ (A–B interval in Table 2), $\mathbf{x}=[2\tfrac{3}{4},2,2\tfrac{3}{4},2\tfrac{1}{4},-2\tfrac{1}{4}]$.

The model resulting in the smallest RSS determines the QTL interval. The probability of identifying the correct interval is obtained by sampling contrasts as before, determining the minimum RSS for each sample vector of contrasts, and counting the number of samples in which RSS is minimum for the interval.

(c) *Multiple marker regression*

Our basic contrast mapping uses pairs of flanking markers. Although this approach is better than calculating contrasts at individual markers, it is less suitable for detecting the presence of multiple QTLs in different sub-intervals. Detecting multiple QTLs requires simultaneous consideration of more than two markers. Therefore, our third approach for assigning QTLs to sub-intervals consists of regressing either the phenotypic averages of the haplotypes, or the individual phenotypes of all members of the haplotype groups, on the marker alleles (coded '1' for those associated with the mutant QTL allele in the sire and '-1' for others) at all markers in the initial confidence region, or at three consecutive markers at a time. Sliding regression on three markers at a time, described below, was considered after we noticed a substantial reduction in power when multiple marker regression was compared with basic contrast mapping.

Zeng (1993) showed that with multiple marker regression, the partial regression coefficient of marker i has a non-zero expected value if and only if there is at least one QTL located between markers $i-1$ and i, or between $i+1$ and i. Hence, if there is a single QTL in the region being examined, then only the two markers flanking the sub-interval containing the QTL have non-zero expected partial regression coefficients. If there are two QTLs in well-separated sub-intervals between markers 2 and 3 and between 5 and 6, then only those four markers have non-zero expected partial regression coefficients. If two QTLs are in two adjacent sub-intervals between markers $i-1$, i and $i+1$, then only these three markers have non-zero expected partial regression coefficients. Last, if QTLs are in two sub-intervals separated by one empty interval, i.e. between markers $i-2$, $i-1$ and i, $i+1$, then only these four markers have non-zero expected partial regression coefficients. In the last case, more markers must be placed in the region to discriminate

Downloaded from http://www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http://www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

among the three sub-intervals involved. The same results can be found when three consecutive markers are analysed simultaneously (or two markers at the end of the linkage group), but only the estimate or test for the intermediate marker is considered. More specifically, first markers 1 and 2 are fitted jointly to estimate and test the partial regression coefficient for marker 1. Subsequently, markers 1, 2 and 3 are fitted jointly to estimate and test the partial regression coefficient for marker 2, etc. The partial regression coefficient for the intermediate marker has the same expected value as the corresponding regression coefficient in the all-marker model. For sub-interval i containing a QTL of effect a, with flanking markers i and $i-1$, and flanked by empty sub-intervals, expected values of the partial regression coefficients are $(r_2/r)\,a$ and $(r_1/r)\,a$, respectively. We will refer to the two approaches as all-marker regression and (sliding) three-marker regression below.

Obviously, multiple marker analysis requires testing of multiple partial regression coefficients, hence the problem of appropriate significance tests arises. However, here we are conducting fine-mapping in a region already 'known' (from initial linkage analysis) to contain at least one QTL. Hence, we suggest assigning a QTL to the sub-interval flanked by markers i and $i+1$ with the largest partial F- (or equivalently t-) values. This procedure is analogous to basic contrast mapping, where we do not test but rather select the interval with the highest contrast. We will refer to this approach as three- or all-marker regression with the f-max criterion. For comparison, we also present results for the f-sig criterion, where we require both flanking markers of the true interval to be significant (at the 0·05 level) and at least one marker next to the flanking markers to be not significant.

(d) *Impact of dominance*

Up to this point, haplotype means (or deviations) of offspring inheriting Q (q) were arbitrarily set to a ($-a$), and the maximum contrast value was $2a$. Individuals receiving a paternal haplotype containing Q (q) have genotypes QQ and Qq (qQ and qq) with probabilities of p and $(1-p)$, respectively. Therefore, and in the context of average effects of alleles (Falconer & Mackay, 1996), deviations of the Q- and q-haplotypes are $pa+(1-p)\,d$ and $pd-(1-p)\,a$, respectively, where a is half the difference between homozygotes at a biallelic locus, and d is dominance deviation. These average effects are expressed relative to the population mean as $\alpha_1(\alpha_2)$ with a difference of $\alpha = \alpha_1 - \alpha_2$, which is equal to a in the absence of dominance.

Variances due to the QTL among individuals with a Q- or q-haplotype are, respectively, $\sigma_Q{}^2 = p[q(a+d)]^2 + q[p(d-a)]^2$ and $\sigma_q{}^2 = p[q(a+d)]^2 + q[p(d+a)]^2$. These variances are equal in the absence of dominance ($d = 0$). Earlier, we defined σ^2 to be the variance of a single haplotype group mean, which was constant across groups in the balanced case. Since we do not know which haplotypes contain Q or q, variance of the group mean must be allowed to differ among groups. Now we define σ^2 as the average variance of all group means included in a specific contrast. Then, variance of a contrast is still $2\sigma^2/(m-2)$, which differs, however, among contrasts. Covariances among contrasts must also be computed by allowing for different variances of group means. For example, covariance among contrasts 1 and 3 in Table 2 is $(\sigma_1{}^2+\sigma_2{}^2+\sigma_9{}^2+\sigma_{10}{}^2-\sigma_4{}^2-\sigma_7{}^2)\,/\,(m-2)^2$, where $m = 4$, and $\sigma_i{}^2$ is the variance of the mean of group i (estimated from the within-group variance). Unless the QTL is a major gene with dominant gene action, difference in variance due to the QTL between Q- and q-haplotype groups should be small and may be ignored. For multiple marker regression, different variances of individual haplotype means should be incorporated if these appear to differ noticeably.

In Section 3, power values will be interpreted in terms of the substitution effect in additive genetic SD (α/σ_A) and, hence, results will be valid with or without dominant gene action. When considering jointly the impact of QTL position and dominance, values of 0 in Table 2 for groups 1–5 (6–10) are to be replaced by $\alpha_1 r_2 + \alpha_2 r_1)\,/\,r$ instead of $a(r_2-r_1)\,/\,r((\alpha_1 r_1+\alpha_2 r_2)\,/\,r$ instead of $a(r_1-r_2)\,/\,r)$. For contrasts, we add $\frac{1}{4}\alpha(r_2-r_1)\,/\,r$ above and $\frac{1}{4}\alpha(r_1-r_2)\,/\,r$ below the diagonal. For $\alpha = 2a$, changes are identical to those given earlier, and again results interpreted in terms of α are valid with and without dominance.

(e) *Confidence of correct sub-interval identification*

While we can evaluate the power of maximum contrast, minimum RSS and multiple marker regression via data simulation as the frequency of correct assignments across replicates, for real data analysis we can compute the probability that the sub-interval identified actually contains the QTL, via bootstrapping. For minimum RSS and marker regression, prior to bootstrapping regression coefficients are estimated from the real data (contrasts and marker haplotype averages or individual phenotypes, respectively). Then, means of the (n) phenotypes are estimated from these coefficients, and residuals are estimated as the difference between phenotypes and means. Bootstrapping is then performed by sampling n residuals with replacement and adding those to the n means previously estimated. This step is performed repeatedly to obtain the bootstrap samples. Each bootstrap sample is analysed in the same way as the

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

Table 4. *Recombinant haplotypes for $m = 6$ markers and corresponding QTL region depending on segregation status*

Type of recombinant sire	Haplotype of recombinant sire	QTL interval[a]	
		With segregation	Without segregation
1	M_1-A-B-C-D-m_2	[M1–M2[	]D–M2]
2	M_1-A-B-C-d-m_2	[M1–D[	]C–M2]
3	M_1-A-B-c-d-m_2	[M1–C[	]B–M2]
4	M_1-A-b-c-d-m_2	[M1–B[	]A–M2]
5	M_1-a-b-c-d-m_2	[M1–A[	]M1–M2]
6	m_1-A-B-C-D-M_2	]M1–M2]	[M1–A[
7	m_1-a-B-C-D-M_2	]A–M2]	[M1–B[
8	m_1-a-b-C-D-M_2	]B–M2]	[M1–C[
9	m_1-a-b-c-D-M_2	]C–M2]	[M1–D[
10	m_1-a-b-c-d-M_2	]D–M2]	[M1–M2[

[a] [X–Y], region with markers X and Y included;]X–Y], region with markers X and Y excluded and included, respectively; [X–Y[, region with markers X and Y included and excluded, respectively;]X–Y[, region with markers X and Y excluded.

real data, and the number of samples with the same QTL interval identified as for the real data is counted. When working with individual phenotypes, an alternative bootstrap technique of sampling (with replacement) sets of phenotypes and corresponding covariates can be used, and only this approach is suitable for the maximum contrast method, where are no covariates to carry along in the bootstrap, but rather marker haplotypes. Properties and performance of this bootstrap application will be evaluated in Section 3.

(ii) *Substitution mapping*

(a) *Basic substitution mapping*

Basic substitution mapping was first proposed by Paterson *et al.* (1990) for backcross type designs in species where inbred lines are available. It is closely related to contrast mapping, but uses one additional generation as shown in Fig. 1*b*. Here we investigate a potential application of this approach to half-sib families in a segregating population. The starting point is again the granddaughter design where a QTL has been found to be segregating in some grandsires and has been mapped to a region of, say, 20 cM. We again select one or several grandsires from this design, which are very likely to be heterozygous at the QTL. The recombinant sons of these grandsires (subsequently referred to as 'sires') are then genotyped for additional, ordered markers located between the original markers flanking the QTL region. For a QTL segregating in the grandsires, we will distinguish between the mutant and the normal allele, assuming a biallelic QTL. In analogy with the backcross design, we will initially employ the assumption that dams of sires do not carry the mutant allele. Deviation from this assumption will be considered later.

We begin with the same groups of recombinant offspring (here, sires) as for contrast mapping, e.g. the 10 groups listed in Table 1 for $m = 6$ markers. Table 4 contains all types of recombinant sires, with their respective haplotypes and their QTL segregation status, for the case of $m = 6$ markers. We assume that a sufficient number of daughters of each sire have been genotyped, so that segregation status of a sire can be determined with high probability (segregating = heterozygous, not segregating = homozygous for the normal allele). Note that due to our assumption of a zero frequency for the mutant QTL allele in the dams of sires, any sire that is found to be segregating has inherited the mutant allele from the respective grandsire. Then, determining the segregation status at the QTL for an individual of a given recombinant haplotype leads to the assignment of the QTL to a sub-region within the original M1–M2 confidence region. These sub-regions of QTL location are listed in columns 3 and 4 of Table 4.

Table 4 shows that in a few cases the occurrence of a single haplotype and the knowledge of the segregation status of the sire carrying the haplotype can lead to the assignment of the QTL to a specific interval formed by two adjacent markers, e.g. haplotype 5 segregating or haplotype 6 not segregating lead to the assignment of the QTL to the M1–A interval. For the other flanking marker interval (D–M2), a single haplotype and its segregation status are also sufficient for QTL assignment (1 not segregating, 10 segregating). For all other intervals, however, a single haplotype and its segregation status do not contain

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

Table 5. *QTL assignment based on combinations of different recombinant haplotypes together with their segregation status*

Haplotype	Segregation status	1		2		3		4		5	
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
6	Yes	]M1–M2[	]D–M2]	]M1–D[	]C–M2]	]M1–C[	]B–M2]	]M1–B[	]A–M2]	]M1–A[	]M1–M2]
	No	[M1–A[	Error	[M1–A[	Error	[M1–A[	Error	[M1–A[	Error	[M1–A[	]M1–A[
7	Yes	]A–M2[	]D–M2]	]A–D[	]C–M2]	]A–C[	]B–M2]	]A–B[	]A–M2]	Error	]A–M2]
	No	[M1–B[	Error	[M1–B[	Error	[M1–B[	Error	[M1–B[	]A–B[	[M1–A[	]M1–B[
8	Yes	]B–M2[	]D–M2]	]B–D[	]C–M2]	]B–C[	]B–M2]	Error	]B–M2]	Error	]B–M2]
	No	[M1–C[	Error	[M1–C[	Error	[M1–C[	]B–C[	[M1–B[	]A–C[	[M1–A[	]M1–C[
9	Yes	]C–M2[	]D–M2]	]C–D[	]C–M2]	Error	]C–M2]	Error	]C–M2]	Error	]C–M2]
	No	[M1–D[	Error	[M1–D[	]C–D[	[M1–C[	]B–D[	[M1–B[	]A–D[	[M1–A[	]M1–D[
10	Yes	]D–M2[	]D–M2]	Error	]D–M2]	Error	]D–M2]	Error	]D–M2]	Error	]D–M2]
	No	[M1–M2[	]D–M2[	[M1–D[	]C–M2[	[M1–C[	]B–M2[	[M1–B[	]A–M2[	[M1–A[	]M1–M2[

Error: this finding is not possible under the assumptions employed.

sufficient information to assign the QTL to a single marker interval. A QTL can be assigned uniquely to one of these intervals, e.g. to interval A–B, if two recombinant sires of the same type are found, e.g. of type 4 in Table 4, one of which is segregating and the other is not. In addition, Table 5 presents combinations of different haplotypes from the two recombinant haplotype groups (1–5, 6–10) together with their segregation status for assignment of the QTL to a specific marker interval.

From Table 5, using certain combinations of two different haplotypes and their segregation status, the QTL can now be assigned to individual intervals (e.g. A–B) that are not at the ends (M1–A, D–M2). For example, haplotype 4 segregating and haplotype 7 segregating leads to the assignment of the QTL to interval A–B. The same interval is found when haplotypes 4 and 7 are both not segregating. In general, the occurrence of a recombinant event within a specific interval X–Y both for a M1–m2 and a m1–M2 recombinant sire leads to the assignment of a QTL to the X–Y interval if the two sires are found to be both segregating or not segregating. In addition, two individuals of the same recombinant haplotype (either both M1–m2 or both m1–M2), with the recombination event occurring in interval X–Y, allow us to assign the QTL to the X–Y interval if one of the two sires is segregating and the other is not.

Cells which contain the outcome 'error', pertain to combinations of haplotypes with segregation status that are not possible under the assumptions employed. Assumptions are that (i) the segregation status of each sire can be determined with certainty, and (ii) that the QTL is not present in the dams of the sire, so that a sire can inherit the mutant allele only from a grandsire. For example, haplotypes 1 and 6 not segregating leads to an error, which is likely to be caused by a false segregation status. Haplotypes 2 and 10 segregating also leads to an error, which is either due to a false segregation status or inheritance of the mutant QTL allele from a dam. Errors found in real data can therefore be used to verify segregation status and, given correct status, to indicate a deviation from the assumption of a zero frequency of the mutant QTL allele in dams.

In practice, one would start by genotyping recombinant sires for the additional, ordered markers. Once a suitable haplotype (1, 5, 6 or 10) or a pair of haplotypes (e.g. 4 and 4, 4 and 7) is found, the segregation status of the individuals involved will be evaluated. Determining segregation status will proceed as follows. First, the genotype of the grandsire, partitioned into its paternal and maternal contribution, is M_1-A-B-C-D-M_2/m_1-a-b-c-d-m_2, where M1–M2 is known to carry the mutant QTL allele. The genotype of the sire is M_1-A-B-c-d-m_2/μ_1-α-β-χ-δ-μ_2, where μ_1-α-β-χ-δ-μ_2 is the chromosome segment

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

inherited from its dam. To determine segregation status of the sire, one could genotype a sufficient number of daughters of this sire for the M_1 and M_2 loci, and compare the phenotypes of the two non-recombinant daughter groups M_1-m_2 and μ_1-μ_2. Alternatively one could genotype daughters for the M_1 and C loci which flank the segment in the sire derived from the M_1-M_2 chromosome in the grandsire known to contain the mutant QTL allele. Using M_1 and C would somewhat increase the fraction of non-recombinant daughters among all daughters, as M_1 and C have a smaller recombinant fraction than M_1 and M_2. If, based on the resulting haplotype × segregation status combination, the QTL interval can be determined, the process can stop. Otherwise, more recombinant sires need to be genotyped for the additional markers and their segregation status determined.

We would like to obtain an idea about how many recombinant sires (and their daughters) need to be genotyped on average until the QTL is assigned to a marker interval (e.g. A–B). Suppose that interval A–B contains the QTL. Then, for a given number of recombinant sires, we evaluate the probability that at least two desired recombinant types are present (4 and 4, 4 and 7, or 7 and 7), i.e. that there are n_4 and n_7 recombinants of types 4 and 7, respectively, such that $n_4+n_7 \geqslant 2$. This probability is computed using the multinomial distribution as

$$\sum_{n_X+n_Y\geqslant 2}\frac{n!}{n_X!n_Y!(n-n_X-n_Y)!}p^{(n_X+n_Y)}(1-2p)^{(n-n_X-n_Y)}$$
$$\text{with } p = 1/[2(m-1)], \quad (7)$$

where m is the number of markers, and $X = 4$ and $Y = 7$ in our example. The number of recombinant sires (n) can be obtained from the total number of sires times the recombination rate ($r = 0{\cdot}16484$ for a 20 cM M1–M2 interval and Haldane's mapping function). Now given that $n_4+n_7 \geqslant 2$, there are four cases in which assignment of the QTL to a given interval (A–B) is not possible: (1) $n_X = 0, n_Y \geqslant 2$ and all sires of type Y are segregating or not segregating; (2) $n_X \geqslant 2, n_Y = 0$ and all sires of type X are segregating or not segregating; (3) $n_x > 0$ and $n_Y > 0$ with all X sires segregating and all Y sires not segregating; (4) $n_X > 0$ and $n_Y > 0$ with all X sires not segregating and all Y sires segregating. The probabilities attached to these four cases are denoted by $q_i, i = 1, \ldots, 4$. As an example, q_3 is computed as

$$q_3 = \left[\frac{(1-r_A)\,r_B}{r_{AB}}\right]^{n_X+n_Y}, \quad (8)$$

where r_{AB} is the recombination rate for the marker interval A–B, while r_A and r_B are recombination rates between the QTL and the markers A and B, respectively. X is a haplotype of type M_1–m_2($X = 4$), and Y is of type m_1–M_2 ($Y = 7$). The other q probabilities are computed similarly. Each term in the summation in (7) is multiplied by the probability $(1-\Sigma_i q_i)$ for given n_x and n_Y, resulting in the probability of QTL assignment to a single marker interval.

Consider Table 5 again, and suppose that a QTL is located in the interval B–C. In this case, all individuals with haplotypes 1, 2, 6 and 7 will be segregating, and all individuals with haplotypes 4, 5, 9 and 10 will not be segregating. Assignment to interval B–C can be made if one of the following cases is observed: (1) one individual of haplotype 3 is segregating and another individual of haplotype 3 is not (3+, 3−); (2) one individual of haplotype 8 is segregating and another individual of haplotype 8 is not (8+, 8−); (3) one individual of haplotype 3 is segregating and another individual of haplotype 8 is also segregating (3+, 8+); and (4) one individual of haplotype 3 is not segregating and another individual of haplotype 8 is also not segregating (3−, 8−). If none of these cases is observed (e.g. there are no individuals with haplotype 8 and all sires with haplotype 3 segregate), it may still be possible to assign the QTL to either the A–C combined interval or the B–D region.

Assignment to A–C occurs when one sire with haplotype 3 segregates and another sire with haplotype 7 also segregates, or when one sire with haplotype 4 and another sire with haplotype 8 both do not segregate. Similarly, assignment to B–D occurs when one sire with haplotype 2 segregates and another sire with haplotype 8 also segregates, or when one sire with haplotype 3 and another sire with haplotype 9 both do not segregate. Additional assignment to the A–C region is possible if a sire with haplotype 3 segregates and a sire with haplotype 4 does not segregate (note that both haplotypes are of the type M_1–m_2 and have a recombination event in A–C), or if a sire with haplotype 7 segregates and a sire with haplotype 8 does not segregate. Similarly, assignment to the B–D region is possible if a sire with haplotype 2 segregates and a sire with haplotype 3 does not segregate, or if a sire with haplotype 8 segregates and a sire with haplotype 9 does not segregate.

(b) *Testing for segregation status*

Up to this point we have assumed that segregation status of sires is determined without error, i.e. if an individual is found to be segregating or not segregating, it carries one or no mutant (Q) allele from its sire with certainty. In reality, segregation status must be inferred from the daughters of a sire by contrasting daughters which inherited alternative, non-recombinant marker haplotypes from the sire. These marker

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

haplotypes consist of the two markers forming the sub-interval which contains the recombination event in the sire. If either of these two markers is not informative (daughter has the same marker genotype as the sire), the daughter's paternal marker allele may be determined from other, nearby markers. We assume that a one-sided *t*-test can be used, based on the daughters' phenotype contrast, to test for a substitution effect of zero versus a positive effect. This test assumes that if the sire inherited the favourable allele from its grandsire, then it will also be the favourable allele in the sire. The *a priori* probability that the sire inherited Q versus q from the grandsire is 0·5, since we are assigning a QTL to a sub-interval rather than to a particular position within the interval. Hence, a simple *t*-test as described appears to be appropriate for determining sire segregation status. If the *t*-statistic exceeds a certain threshold value (null hypothesis: sire is not segregating), the sire is assumed to be segregating. Consequently, both type I (sire declared as segregating although it is not) and type II (sire declared as not segregating although it is) errors will occur. The influence of such errors on the power of assigning QTLs to specific regions was therefore investigated as follows.

QTL assignment to a single interval (e.g. B–C) occurs when one of the following cases (defined earlier) is observed: (1) 3+ and 3−, (2) 8+ and 8−, (3) 3+ and 8+, and (4) 3− and 8−. When segregation status is not known with certainty, then for example case (1) above must be replaced with 3Q+ and 3q−, where 3Q+ refers to a sire with marker haplotype 3 and QTL allele Q (inherited from its sire), which is also correctly identified as segregating (+). Note that for case 1, QTL assignment occurs if at least one 3Q and one 3q individual occur, and both are correctly identified as segregating and not segregating, respectively, or if at least one 3Q and one 3q individual occur and both are misclassified as not segregating and segregating, respectively. The latter event should have low probability and was not considered here (i.e. was not accounted for as a correct assignment in the power calculations, because it is based on wrong decisions regarding the segregation status). We define $p(-\mid q) = 1-\alpha$ and $p(+\mid Q) = 1-\beta$, where α is the type I error and β the type II error, with α predetermined by the investigator and power $1-\beta$ affected by the size of the experiment.

As α is predetermined by the investigator, β can be controlled via sample size. When testing whether the difference between two group means is zero versus positive, the following equation gives the approximate sample size to control both errors (Neyman *et al.*, 1935, in Snedecor & Cochran, 1980, p. 104):

$$n = 2\left(\frac{\hat{\sigma}}{\delta}\right)^2 (t_{\alpha^{,2n-n}} + t_{\beta^{,2n-2}})^2 \qquad (9)$$

where n is sample size in one group with $n_1 = n_2 = n$, σ^2 is the variance of the individual observations, δ the difference between the two group means and $t_{\alpha,2n-2}$ as well as $t_{\beta,2n-2}$ are values for one-sided *t*-tests. Here, σ^2 corresponds to $\mathrm{Var}(\mathrm{YD}\mid\mathrm{sire}) = (1/h^2 - 0{\cdot}25)\,\sigma_A{}^2$, and δ is the difference between daughter groups, or the gene substitution effect α which is also measured in σ_A.

Finally, we note that Table 5 can serve as a control to verify the power of detecting segregation. For example, it is not possible that haplotypes 3 and 7 both do not segregate, given the knowledge that there is a QTL between markers M1 and M2 (the cell corresponding to this combination in Table 5 is marked 'error'). However, an 'error' is not necessarily due to a misclassification of segregation status (see below).

(c) *QTL segregation in dams*

Another factor reducing the power of QTL assignment to a marker interval is the segregation of the QTL in the dams of the sires. Up to this point we have assumed that the dams are homozygous normal (qq), i.e. that sons found to be segregating (genotype Qq) have inherited the mutant Q allele always from their heterozygous sire and never from their dam. If the QTL is segregating in the dams, an incorrect QTL assignment decision will be made if a sire that inherited Q both from its sire and the dam (genotype QQ) is not found to be segregating and hence not to have inherited Q from its sire, or if a sire that inherited q from its sire and Q from its dam is found to be segregating and hence assumed to have inherited Q from its sire. As an example, case 1 above (3Q+, 3q−) must now be replaced with (3Qq+, 3qq−), and the probability of this case is obtained by multiplying the probability of (3Q+, 3q−) by $(1-p(\mathrm{Q}))^2$, where $p(\mathrm{Q})$ represents the frequency of the Q allele in the dams.

There are two ways of assessing the frequency of Q in the dams. It may be possible to estimate allele frequency in the dams when performing linkage analysis (using Maximum Likelihood or Bayesian methods), or some of the cells in Table 5 containing 'error' can be used again. In the latter approach, for example, haplotypes 4 and 8, or 3 and 7, cannot both be segregating under the assumption that the Q allele can only be inherited from the sire of sons but not from their dams. Potential causes of 'errors' are incorrect assignment of segregation status or homozygosity (QQ instead of Qq).

It is quite likely that a particular dam has a father which has a granddaughter design himself. Then, one should check whether the dam's father is segregating at a QTL located in the same (M1–M2) marker interval as the one being investigated here. If the

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

answer is no, then probably the dam did not transmit the Q allele to her son (unless its frequency is high), and the son's segregation status has probably been determined correctly.

3. Results and discussion

(i) *Power of contrast mapping*

Simulation studies were conducted to compare the statistics maximum contrast and minimum RSS for inferring the correct QTL interval, and to evaluate power as a function of number of offspring (N), effect of the QTL (a), recombination rate between flanking markers, and spacing of markers within the region investigated. As described above, contrasts were sampled from the appropriate distribution, and the two statistics were calculated for each sample. For each situation, 10000 samples were drawn from $N(\mu_i, \mathbf{V})$, and power was determined for both test statistics as the proportion of samples where the true interval was inferred. In general, total number of offspring and QTL effect are the most critical factors for fine-mapping. Therefore, power values in Table 6 were estimated for different values of these parameters, assuming an initial marker bracket of size 20 cM and 10 additional, equidistant, ordered markers. QTL effect (a) is defined according to Table 2 and measured in standard deviations of the observed variable y (σ_y), and the true position of the QTL is assumed to be at the midpoint of interval A–B.

The result that minimum RSS was inferior in power to maximum contrast, in particular for smaller numbers of offspring and smaller QTL effects, was initially surprising, as minimum RSS seems to use more information. However, minimum RSS needs to find the correct order of the contrasts, which is more error prone than just finding the maximum contrast. We replaced the minimum RSS criterion by the maximum a estimate obtained from regression model (2) for all intervals. Within this criterion, virtually the same power was achieved as with the maximum contrast approach. As an example, for a QTL with effect of $0{\cdot}25\sigma_y$ and 500 (1000) offspring, power was 0·3838 (0·5284) for maximum contrast, 0·3837 (0·5284) for maximum a estimate, and only 0·3590 (0·5152) for minimum RSS.

Power of the all-marker and sliding three-marker regression methods was also evaluated by simulating haplotype means using the same parameter values as for the simulation of the contrasts. These results are also included in Table 6. When the *f*-max criterion was used, then three-marker regression had the same power as basic contrast mapping, while the power of all-marker regression was less, in particular for cases with moderate to low power. We initially expected to find the same power for all-marker and three-marker regression when both are implemented with the *f*-max criterion. However, we noticed that regression coefficient estimates from the all-marker analysis (expectedly) had higher SE compared with the three-marker analysis, and that the corresponding F-statistics were more variable across replicates.

When three- or all-marker regression was implemented with the *f*-sig criterion, then power was much less compared with basic contrast mapping. We verified the results for the power of marker regression with the *f*-sig criterion by performing analytical power calculations with the non-centrality parameter evaluated at different expected values for the regression coefficients of the two markers flanking the QTL for various values of QTL effect a and sample size. Generally, the analytical calculations supported the low power values in Table 6.

The results in Table 6 were calculated for parameter definitions given earlier and can be interpreted quite generally. However, for fine-mapping in cattle, the design consists of sons or daughters of QTL-heterozygous sires, and it is necessary to relate the more general parameters to the properties of actual data. First, parameter $2a$ corresponds to the allelic substitution effect α (Falconer & Mackay, 1996). Secondly, α is typically measured in additive genetic standard deviations (σ_{A}) instead of σ_y. The following relationships allow us to relate results in Table 6 to real data.

For a granddaughter design, the phenotypic observations pertaining to sons are Daughter Yield Deviations (VanRaden & Wiggans, 1991), or DYD. Variance of DYD within a sire family can be partitioned as

$$\mathrm{Var}(\mathrm{DYD}\,|\,\mathrm{sire}) = \frac{1}{\mathrm{REL}}0{\cdot}25\sigma^2{}_{\mathrm{A}} - \frac{1}{16}\sigma^2{}_{\mathrm{A}} = \frac{3}{16}\sigma^2{}_{\mathrm{A}} + \frac{1-\mathrm{REL}}{\mathrm{REL}}0{\cdot}25\sigma^2{}_{\mathrm{A}}, \quad (10)$$

where REL is Reliability (VanRaden & Wiggans, 1991), and Var(DYD | sire) corresponds to σ_y used earlier. Table 7 contains the allelic substitution effect in additive genetic standard deviations as a function of parameters a/σ_y and REL.

For reliabilities around 0·9 (achieved by dairy sires with sufficient numbers of daughters), QTL effects in Table 6 correspond quite closely to the allelic substitution effects α (in σ_{A}) in Table 7. Hence, power values for a/σ_y closely approximate power values for $\alpha/\sigma_{\mathrm{A}}$, e.g. for $\alpha/\sigma_{\mathrm{A}}$ equal to 0·0928 (0·232, 0·464, 0·696, 0·928), a power value near 0·14 (0·26, 0·48, 0·67, 0·80) is found in a family of 200 sons, while a power value near 0·25 (0·53, 0·86, 0·97, 0·99) is achieved in a family of 1000 sons. The lower the reliability, the lower is the power to detect a QTL of a given effect: i.e. for reliabilities of 0·7 and 0·5 instead of 0·9, allelic

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

Table 6. *Power of inferring the true QTL interval, as a function of the total number of offspring and QTL effect, for the following statistics: maximum contrast and three-marker regression (f-max criterion[2]) in the first row;[a] minimum RSS in the second row; all-marker regression (f-max/f-sig criterion[2]) in the third row; three-marker regression (f-sig criterion[2]) in the fourth row*

No. of offspring	QTL effect				
	0·1	0·25	0·5	0·75	1·0
100	0·124	0·201	0·353	0·508	0·639
	0·113	0·163	0·318	0·491	0·633
	0·099/0·015	0·104/0·015	0·113/0·013	0·145/0·014	0·196/0·014
	0·009	0·011	0·009	0·008	0·009
200	0·139	0·261	0·045	0·669	0·804
	0·122	0·220	0·458	0·666	0·803
	0·098/0·015	0·114/0·013	0·140/0·013	0·212/0·017	0·331/0·040
	0·009	0·011	0·008	0·009	0·026
300	0·164	0·309	0·568	0·763	0·886
	0·137	0·274	0·559	0·762	0·886
	0·103/0·014	0·116/0·014	0·161/0·014	0·283/0·029	0·464/0·079
	0·011	0·009	0·010	0·017	0·069
400	0·169	0·349	0·639	0·828	0·931
	0·144	0·319	0·637	0·827	0·931
	0·106/0·015	0·121/0·012	0·194/0·018	0·363/0·046	0·576/0·138
	0·012	0·008	0·009	0·036	0·126
500	0·186	0·396	0·694	0·873	0·957
	0·155	0·368	0·691	0·873	0·957
	0·102/0·015	0·119/0·012	0·237/0·022	0·430/0·071	0·666/0·206
	0·010	0·009	0·010	0·061	0·203
1000	0·244	0·528	0·853	0·971	0·995
	0·207	0·514	0·853	0·971	0·995
	0·111/0·016	0·149/0·011	0·396/0·060	0·725/0·256	0·910/0·568
	0·009	0·007	0·043	0·257	0·609
1500	0·285	0·621	0·922	0·991	1·00
	0·250	0·615	0·922	0·991	1·000
	0·108/0·014	0·190/0·016	0·554/0·124	0·864/0·468	0·976/0·808
	0·011	0·008	0·108	0·493	0·851
2000	0·314	0·692	0·957	0·998	1·000
	0·283	0·690	0·957	0·998	1·000
	0·116/0·013	0·233/0·018	0·664/0·209	0·933/0·640	0·992/0·920
	0·010	0·013	0·213	0·689	0·945
2500	0·352	0·747	0·976	0·999	1·000
	0·322	0·745	0·976	0·999	1·000
	0·119/0·014	0·271/0·027	0·763/0·292	0·967/0·776	0·998/0·963
	0·008	0·017	0·313	0·812	0·982
3000	0·388	0·784	0·986	1·000	1·000
	0·362	0·783	0·986	1·000	1·000
	0·124/0·013	0·311/0·034	0·831/0·394	0·982/0·865	1·000/0·981
	0·008	0·024	0·415	0·898	0·993
5000	0·480	0·890	0·998	1·000	1·000
	0·464	0·890	0·998	1·000	1·000
	0·139/0·012	0·478/0·088	0·952/0·706	0·999/0·978	1·000/0·989
	0·009	0·075	0·740	0·992	1·000
10000	0·641	0·978	1·000	1·000	1·000
	0·637	0·978	1·000	1·000	1·000
	0·192/0·014	0·762/0·306	0·998/0·966	1·000/0·990	1·000/0·992
	0·009	0·314	0·982	1·000	1·000
15000	0·734	0·995	1·000	1·000	1·000
	0·733	0·995	1·000	1·000	1·000
	0·265/0·023	0·893/0·523	1·000/0·991	1·000/0·992	1·000/0·991
	0·015	0·562	0·999	1·000	1·000
20000	0·808	0·999	1·000	1·000	1·000
	0·808	0·999	1·000	1·000	1·000
	0·335/0·041	0·951/0·711	1·000/0·993	1·000/0·992	1·000/0·991
	0·027	0·742	1·000	1·000	1·000

[a] These two methods give exactly the same power values.

Downloaded from http://www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http://www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

Table 7. *Allelic substitution effect α/σ_A corresponding to QTL effect a/σ_y for various Reliabilities (REL) of Daughter Yield Deviations (DYD) of sons*

	REL		
QTL effect a/σ_y	0·5	0·7	0·9
0·1	0·1323	0·1086	0·0928
0·25	0·3307	0·2714	0·2320
0·5	0·6614	0·5428	0·4640
0·75	0·9921	0·8142	0·6960
1·0	1·3228	1·0856	0·9280

Table 8. *Allelic substitution effect α/σ_A corresponding to QTL effects a/σ_y (in Table 6) for various heritabilities of Yield Deviations of daughters*

	Heritability		
QTL effect a/σ_y	0·1	0·3	0·5
0·1	0·6245	0·3552	0·2646
0·25	1·5612	0·8780	0·6615
0·5	3·1225	1·7560	1·3229
1·0	6·2450	3·5120	2·6458

substitution effects must be larger (1·0856 and 1·3228 instead of 0·928) to achieve a power value of 0·80.

Similarly, the phenotypic observations of daughters are Yield Deviations (VanRaden & Wiggans, 1991), YD, with variance within sire equal to

$$\mathrm{Var(YD\,|\,sire)} = \frac{3}{4}\sigma^2{}_{\mathrm{A}} + \mathrm{Var(e)} = \left(\frac{1}{h^2} - \frac{1}{4}\right)\sigma^2{}_{\mathrm{A}}, \qquad (11)$$

where h^2 is the narrow-sense heritability of the trait under investigation. Now we can relate the QTL effect a in Table 6 to the allelic substitution effect α (in σ_A) as a function of the narrow-sense heritability in the daughter design. Table 8 shows that QTL effects in Table 6 correspond to much larger allelic substitution effects in the daughter design. For a substitution effect of 0·3552 σ_A (heritability of 0·3 in Table 8) and 5000, 10000, 15000 and 20000 offspring, power values are near 0·48, 0·64, 0·73 and 0·81 (maximum contrast, Table 6), respectively. For a substitution effect of $0{\cdot}878\sigma_A$, power values of 0·89, 0·98, 0·99 and 1·0 are achieved for 5000, 10000, 15000 and 20000 offspring, respectively. For the QTL effect sizes and offspring numbers considered, often a desired level of power (0·9, 0·95) cannot be achieved. Furthermore, the number of large son or daughter half-sib families in real populations is limited, and therefore combining information across families may be necessary.

Darvasi & Soller (1997) proposed a simple method for calculating confidence intervals in backcross and F2 designs. For an 'infinite' number of markers (e.g. markers every 0·1 cM), the confidence interval corresponds to the resolving power of a given design. Resolving power (95% confidence interval) is calculated by the simple expression $\mathrm{CI} = 3000/[Nm\delta^2]$, where N is sample size, $m = 1$ for the backcross design or a half-sib family, and $\delta = \alpha$ is the QTL substitution effect in residual SD. For the half-sib design, residual variance equals $\sigma_p{}^2 - 0{\cdot}25\alpha^2$. To compare their resolving power with our results in Table 6, we define the QTL effect ($a = \alpha$ for an additive QTL) in Table 6 as $f = \alpha/\sigma_p$. In residual SD, this effect is re-scaled using $\delta = f/(1-0{\cdot}25f^2)^{0{\cdot}5}$. Resolving power was calculated for those cases in Table 6 where the power of assigning the QTL to a 2 cM interval was around 0·95, i.e. for $N = 2000$ and $f = 0{\cdot}5$ ($\delta = 0{\cdot}5164$), for $N = 1000$ and $f = 0{\cdot}75$ ($\delta = 0{\cdot}8090$), and for $N = 5000$ and $f = 1{\cdot}0$ ($\delta = 1{\cdot}1547$). Resolving power for these three cases was 5·625 cM, 4·58 cM and 4·50 cM, respectively. Although resolving power assumes a higher marker density than the 2 cM marker spacing used to calculate Table 6, the results in Table 6 are favourable, but this finding might be due to the assumption that the QTL is located at the midpoint of a marker interval. If the QTL is located at positions 0·25 and 0·05 (in units of length of the marker interval), then for $N = 500$ and $f = 1$, the power of locating the QTL in the correct interval (2 cM) is 0·845 for position 0·25 and 0·583 for position 0·05, while the power of locating the QTL either in the correct interval or in the next interval to the left (4 cM interval combined) is 0·999 for both positions (0·25, 0·05). For $N = 2000$ and $f = 0{\cdot}5$, the power of locating the QTL in the correct interval (2 cM) is 0·848 for position 0·25 and 0·580 for position 0·05, while the power of locating the QTL either in the correct interval or in the next interval to the left (4 cM interval combined) is 0·999 for both positions (0·25, 0·05). These results are still favourable when compared with the resolving power for linkage mapping of Darvasi & Soller (1997). When investigating heterogeneity within an interval McMillan & Robertson (1974) also achieved the highest power for QTLs lying in the middle of the interval and a strong decrease in power for QTLs close to the markers, which is in agreement with our results.

(ii) *Bootstrap power estimation for contrast mapping*

An additional simulation study was conducted to evaluate the bootstrap procedures described earlier. Initially we noticed that the bootstrap power estimates did not agree well with the power figures determined by simulation or analytically. To understand the properties of the bootstrap, we first considered a much simpler data structure. Forty data points were

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

generated as a group mean plus a normal deviate. There were two groups containing 20 observations each. The difference between the group means ranged from 0 to 2 within-group SD, and group 1 always had the higher true mean. For each group mean difference, 10 000 data sets were simulated, and the power of identifying group 1 correctly as having the higher mean was estimated as the number of replicates, where group 1 had a higher estimated mean.

For group mean differences of 0, 0·1, 0·2, 0·3, 0·4, 0·5, 1·0 and 2·0, power was 0·497, 0·623, 0·738, 0·827, 0·899, 0·942, 0·999 and 1·0, respectively. For each group mean difference, 1000 data sets were randomly chosen among all replicate data sets for which the correct group 1 had been identified. Bootstrapping was then performed on each of these data sets, by counting the number of bootstrap samples for which group 1 was identified also. This power estimate was averaged across all 1000 data sets. For example, when the group difference was 0·5 (0·1) SD, the average bootstrap power was 0·902 (0·781) and hence lower (higher) than the expected value of 0·942 (0·623) above. Bootstrapping all 10 000 data sets (including those where the wrong group 2 was identified as having a higher mean) did not eliminate the bias of the bootstrap estimates.

Next, bootstrap power estimates from the selected 1000 data sets were averaged within classes formed by the simulated group mean difference (given the same true group mean difference). Then, for a true difference of 0·5 SD and class mean differences (in SD) of 0·1, 0·2, 0·3, 0·4, 0·5, 0·6, 0·7, 0·8, 0·9 and $> 1{\cdot}0$, corresponding frequencies of the classes were 0·060, 0·101, 0·110, 0·104, 0·122, 0·114, 0·104, 0·080, 0·055 and 0·083, and average bootstrap power estimates were 0·643, 0·751, 0·838, 0·904, 0·947, 0·974, 0·986, 0·995, 0·998 and 1·0. Similarly, for a true difference of 0·1 SD and class mean differences (in SD) of $< 0{\cdot}05$, 0·1, 0·2, 0·3, 0·4, 0·5, 0·6, 0·7, 0·8, 0·9 and $> 1{\cdot}0$, corresponding frequencies of the classes were 0·060, 0·142, 0·118, 0·109, 0·085, 0·063, 0·036, 0·020, 0·007, 0·005 and 0·005, and average bootstrap power estimates were 0·540, 0·636, 0·747, 0·837, 0·900, 0·947, 0·972, 0·983, 0·994, 0·998 and 1·0. These results show that irrespective of the true group mean difference (0·5 SD or 0·1 SD), average bootstrap power estimates are the same within the classes of simulated class mean differences. The expected power values (from simulation or analytical calculation) for class mean differences of 0·5 and 0·1 are 0·942 and 0·623, respectively. Irrespective of the true group mean difference, the two classes representing group mean differences of 0·5 and 0·1, respectively, have average bootstrap power estimates of 0·947 and 0·643 (0·636), respectively, which are very close to the expected values. Therefore, the bootstrap provides practically unbiased power estimates when the data closely reflect the underlying true parameter(s), requiring sufficient sample size.

The above property of the bootstrap was also investigated for contrast mapping. The situation considered was 1000 offspring and a QTL effect of 0·75 (in σ_y), where the power for contrast mapping or minimum RSS is near 0·97 according to Table 6. This design was replicated 10 000 times and, for each replicate, bootstrapping was performed. The average bootstrap power, averaged over all data sets or only over data sets where the correct sub-interval was identified, was 0·84 or 0·85, respectively. The true sub-interval was interval 2 with expected contrast of 1·50, and expected contrasts of adjacent intervals 1 and 3 were both 1·33. Then, only those replicates (20 in 10 000) were considered where the differences among contrasts of intervals 1 and 2 and of intervals 2 and 3 were both between 0·1636 and 0·1696, given an expected difference of $0{\cdot}167 = 1{\cdot}50 - 1{\cdot}333$. For those data sets, the average bootstrap power was approximately 0·97, hence unbiased.

(iii) *Substitution mapping*

Probabilities of QTL assignment to a single marker interval were computed using equations (7) and (8) as described earlier, and are given in Table 9 for increasing total number of recombinant sires and different positions of the QTL within the interval (the interval is not at the ends of the region, i.e. not M1–A or D–M2).

Table 9 shows that the numbers of recombinant sires needed to assign a QTL to a single interval are high even for situations where the QTL is located in the middle of the interval (the best case). Only few families in the existing GDDs are sufficiently large to achieve a power value of at least 90 %. For QTL position 1 cM, a power value near 0·95 (0·90) is reached when the number of recombinant sons is 70 (60); hence the total number of sons is $70/(r = 0{\cdot}165) = 424$ (363). Because power decreases substantially when the QTL is located closer to one of the flanking markers, power may be increased by assigning a QTL to a region consisting of two adjacent intervals (4 cM instead of 2 cM in Table 9) rather than to a single interval, similar to what was done to improve the power of contrast mapping.

Probabilities of QTL assignment to A–C or B–D, given that B–C is the true QTL interval and assignment to B–C is not possible, were calculated using simulation with 1 000 000 replications per case (number of recombinant sires and position of QTL in B–C). Results are in Table 10. When adding, for a particular case, both values in Table 10 (for A–C and B–D) to the corresponding figure in Table 9 (for B–C), the total probability of assigning the QTL (with true location in B–C) to either B–C, A–C or B–D, i.e. to a

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

Table 9. *Probabilities for assignment of a QTL to a marker interval of 2 cM in dependency of number of recombinant sires and QTL position within the marker interval*

No. of recombinant sires	QTL position relative to left marker			
	1 cM	0·5 cM	0·25 cM	0·125 cM
10	0·151	0·114	0·067	0·036
15	0·279	0·212	0·126	0·068
20	0·405	0·309	0·185	0·100
25	0·517	0·399	0·242	0·132
30	0·613	0·479	0·295	0·163
35	0·693	0·548	0·343	0·191
40	0·758	0·608	0·387	0·218
45	0·810	0·660	0·428	0·244
50	0·851	0·704	0·465	0·268
55	0·884	0·742	0·499	0·292
60	0·910	0·774	0·531	0·314
65	0·930	0·803	0·560	0·336
70	0·946	0·827	0·588	0·357
75	0·958	0·848	0·613	0·377
80	0·967	0·867	0·637	0·396

Table 10. *Probabilities for additional assignment of a QTL to region A–C (roman) or B–D (italic) given that the QTL cannot be assigned to the true interval B–C, in dependency of number of recombinant sires and position of the QTL in B–C*

No. of recombinant sires	QTL position relative to left marker (B)			
	1 cM	0·5 cM	0·25 cM	0·125 cM
10	0·161/*0·161*	0·272/*0·071*	0·338/*0·034*	0·373/*0·016*
15	0·206/*0·206*	0·378/*0·084*	0·490/*0·038*	0·554/*0·018*
20	0·209/*0·209*	0·424/*0·079*	0·575/*0·035*	0·665/*0·016*
25	0·192/*0·192*	0·427/*0·006*	0·611/*0·028*	0·726/*0·013*
30	0·166/*0·166*	0·410/*0·052*	0·615/*0·021*	0·752/*0·010*
35	0·138/*0·138*	0·378/*0·039*	0·603/*0·015*	0·758/*0·007*
40	0·112/*0·112*	0·343/*0·029*	0·579/*0·011*	0·751/*0·005*
45	0·090/*0·090*	0·310/*0·021*	0·554/*0·008*	0·739/*0·003*
50	0·072/*0·072*	0·276/*0·015*	0·525/*0·005*	0·722/*0·002*
55	0·055/*0·055*	0·246/*0·010*	0·496/*0·003*	0·703/*0·001*
60	0·044/*0·044*	0·217/*0·008*	0·466/*0·002*	0·681/*0·001*
65	0·034/*0·034*	0·192/*0·005*	0·439/*0·002*	0·663/*0·001*
70	0·027/*0·027*	0·170/*0·004*	0·413/*0·001*	0·643/*0·000*
75	0·021/*0·021*	0·150/*0·003*	0·386/*0·001*	0·623/*0·000*
80	0·016/*0·016*	0·132/*0·002*	0·364/*0·000*	0·604/*0·000*

region of 4 cM or less containing the true location, is obtained. For example, for 30 recombinant sires and QTL position 0·5 cM, the total probability is $0·410+0·052+0·479=0·941$, which is a much more favourable figure than the value of 0·479 in Table 9.

Simulations with 1 000 000 replicates were used to determine the influence of different type II error rates (β), given a type I error rate of $\alpha=0·05$, on the power of QTL assignment to a single marker interval (these values should be compared with Table 9, column for QTL position 1 cM). Results in Table 11 indicate that the influence of error rates on power of QTL assignment is quite small.

The formula for n in equation (9) can be partitioned into (σ/α)**2, which is due to the QTL effect and the variance of YD, and $(t_\alpha+t_\beta)$**2, which results from the error rates. Some realistic values for the components (assuming $h^2=0·3$) are given in Table 12 (t values for DF = infinity) as well as the required sample sizes for various scenarios.

The above calculations show that power can be increased by increasing the number of sires per family

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

Table 11. *Influence of type I and type II error rates for QTL segregation status on power of QTL assignment to a single marker interval*

No. of recombinant sires	$\alpha = 0{\cdot}05$			
	$\beta = 0{\cdot}0$	$\beta = 0{\cdot}10$	$\beta = 0{\cdot}20$	$\beta = 0{\cdot}30$
10	0·146	0·136	0·121	0·110
20	0·391	0·365	0·341	0·317
30	0·600	0·571	0·541	0·511
40	0·745	0·719	0·690	0·661
50	0·840	0·818	0·795	0·770
60	0·903	0·887	0·868	0·846
70	0·940	0·926	0·914	0·898
80	0·964	0·955	0·945	0·932

Table 12. *Contribution of QTL substitution effect in additive genetic SD* (α_S/σ_A)*, variance of observations* (σ) *and type I* (α) *and type II* (β) *error rates to sample size of daughter groups due to* (9)

			σ_S/σ_A		
			0·25	0·5	1·0
			$(\sigma/\alpha_S)^2$		
α	β	$(t_\alpha + t_\beta)^2$	49	12·25	3·06
0·05	0·2	6·15	602	150	38
	0·1	8·53	836	208	52
0·01	0·2	9·99	980	244	60
	0·1	12·96	1270	318	80

(hence the number of recombinant sires) and by typing additional daughters of sires to increase the accuracy of declaring segregation status. The former factor seems more important and more limiting in the success of fine-mapping using substitution mapping.

Finally, simulations with 1 000 000 replicates were used to determine the influence of segregation of the mutant QTL allele in dams of sires, for given type I and type II error rates, on the power of QTL assignment to a single marker interval. The results in Table 13 show that power is little affected when the frequency of the mutant allele is low (0·2 or less), while power is reduced substantially when the frequency is intermediate to high.

(iv) *General considerations*

All the results presented above are based on the assumption that markers are fully informative in the sons of grandsires and daughters of sires. A marker is not informative when an offspring has the same genotype as its parent. For contrast mapping, only those offspring (sons) which are informative at both markers forming a sub-interval should contribute to the calculation of the contrast for that sub-interval. Similarly, for substitution mapping only those sons which are informative at both markers of a sub-interval should be classified as having or not having a recombination event in that sub-interval. Suppose that the genotype of the grandsire at the markers forming a sub-interval is A-B/a-b. Given that a son inherits the A-B haplotype, inheritance at the first marker will not be known if the son inherits allele '*a*' from the dam, which occurs with probability p_a. Similar for the other marker. Hence, a fraction of

Table 13. *Influence of frequency of the mutant allele in dams of sires* (p_Q) *on power of QTL assignment to a single marker interval for given type I and type II errors in segregation status*

No. of recombinant sires	$\alpha = 0{\cdot}05/\beta = 0{\cdot}10$				
	p_Q = 0·0	p_Q = 0·1	p_Q = 0·2	p_Q = 0·3	p_Q = 0·5
10	0·136	0·112	0·091	0·073	0·040
20	0·365	0·321	0·273	0·226	0·135
30	0·571	0·515	0·454	0·390	0·250
40	0·719	0·666	0·603	0·534	0·365
50	0·818	0·774	0·718	0·649	0·473
60	0·887	0·849	0·802	0·740	0·567
70	0·926	0·900	0·862	0·809	0·647
80	0·955	0·934	0·905	0·861	0·716

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

$(1-p_a)(1-p_b)$ of the sons will be informative for this interval. Consequently, the number of sons needed has to be increased by a factor of $1/[(1-p_a)(1-p_b)]$ relative to the numbers given in Tables 6, 9, 10 and 13. The number of daughters needed to determine the segregation status of sires in substitution mapping is less affected, because if a flanking marker is not informative, the next marker (further away from the QTL) can be used in many cases.

The impact of multiple linked QTLs on the methods proposed depends mainly on the tightness of the linkage. In contrast to genome-wide or chromosome-wide linkage mapping, here only a small chromosomal region is being examined. The smaller the region, the less likely the existence of multiple QTLs that are not so tightly linked that they act as one QTL. Clusters of very tightly linked QTLs, with hardly any recombination between them, are considered as one QTL (see McMillan & Robertson, 1974). If several distinct QTLs existed in the original (e.g. 20 cM) interval, then contrast mapping, especially three-marker regression, should be able to fine-map multiple QTL with the existing marker data or indicate where additional markers are needed.

To obtain an assessment of the probability of having identified the correct sub-interval in contrast mapping, we propose (i) performing data simulation, using the same design as the actual design, to evaluate the power of correct assignment, and (ii) performing bootstrapping as described earlier. We recommend use of both methods, because both have potential disadvantages. With data simulation using the same design, one can capture sample size but not other (unknown) features of the data. For the same reason, data permutation is preferred over data simulation for determination of significance thresholds in linkage mapping (e.g. Churchill & Doerge, 1994). The bootstrap gives accurate power estimates only if the data closely reflect the underlying true parameters.

To investigate limits to fine-mapping based on current recombinations, we could have performed linkage mapping using LS, VC or Bayesian analysis. However, for a simple design such as the half-sib design considered here, linkage mapping has at most two potential advantages: (i) an estimate of the QTL position within a sub-interval is obtained, but this estimate will probably be rather inaccurate given the small size (2 cM) of the sub-intervals and hence not useful; (ii) a confidence interval is obtained for the QTL position, but these confidence intervals are often not very accurately determined (asymptotic conditions do not hold; different bootstrap techniques may yield different results; e.g. see Zhang *et al.*, 1998). In practice, for a given size of the actual design and estimate of QTL effect, we suggest conducting a simulation similar to those performed here to evaluate the power of correct QTL assignment to the sub-interval identified. A potential advantage of substitution mapping is that genotyping can be terminated if, after evaluation of only a few sires (two in the most favourable case), a combination of haplotypes and segregation states leads to QTL assignment to a sub-interval.

4. Conclusions

Methods for the fine-mapping of QTLs can be assigned to two groups: identity-by-descent methods or methods using historical recombinations, and genetic chromosome dissection methods or methods utilizing current recombinations. Up to now, methods using historical recombinations (or linkage disequilibrium, LD) have been successfully applied to the fine-mapping of Mendelian disease genes. Whether similar methodology can be developed and applied successfully to quantitative traits is still uncertain. Here, we have described two methods for fine-mapping using current recombinations: contrast mapping and substitution mapping. These methods may in some cases produce a sufficiently small region for a QTL, but in most situations should probably be used as an intermediate step between initial linkage analysis and LD mapping, in populations with large half-sib families.

While contrast mapping requires the identification of sires which are heterozygous at a QTL (information available either in a daughter or a granddaughter design), substitution mapping requires identifying both sires and sons which are heterozygous at the QTL (information available in a granddaughter design). Substitution mapping also works best when the mutant QTL allele is not carried by the dams of the sons, an assumption likely to be violated if the design derives from a single, segregating population. Both methods require, on average, large sire families, limiting their application to a few families in Holstein dairy cattle. Genotyping large numbers of individuals, needed especially for daughter contrasts, is a major effort but should become feasible from the standpoint of technology and costs (SNPs and DNA-chips, recent advances in DNA sampling in connection with tagging animals). However, we have shown that with both methods it is feasible to map a QTL to a region of 2–4 cM, a result that, if achieved in practice, represents a large step from linkage mapping towards positional cloning or (comparative) positional candidate cloning. A method based on historical recombinations has been used recently with success to map a QTL to a 5 cM region in Holsten cattle (Riquet *et al.*, 1999). The assumptions of this method, such as QTL allelic homogeneity, will not always be met. Consequently, several methods employing different assumptions are needed, and here we have described two alternative methods that should be considered

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf

when attempting to fine-map QTLs previously identified via linkage mapping.

This work was supported by EU-grant BIO4-98-0471 (Euribdis) to G.T., US National Science Foundation grant DBI-9723022 to I.H., US Department of Agriculture NRICGP grant 96-35205-3662 to I.H., and grants from Infigen Inc. and US Holstein Association to I.H. I.H. also acknowledges financial support from NIH grant GM45344 (PI: Bruce Weir) while on sabbatical at North Carolina State University. Constructive comments by a reviewer are appreciated.

References

Churchill, G. A. & Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963–971.

Darvasi, A. (1997). Interval-specific congenic strains (ISCS): an experimental design for mapping a QTL into a 1-centimorgan interval. *Mammalian Genome* **8**, 163–167.

Darvasi, A. & Soller, M. (1997). A simple method to calculate resolving power and confidence interval of QTL map location. *Behavior Genetics* **27**, 125–132.

Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*, 4th edn. London: Longman.

Hastbacka, J., de la Chapelle, A., Mahtani, M. M., Clines, G., Reeve-Daly, M. P., Daly, M., Hamilton, B. A., Kusumi, K., Trivedi, B., Weaver, A., *et al*. (1994). The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. *Cell* **78**, 1073–1087.

Hoeschele, I., Uimari, P., Grignola, F. E., Zhang, Q. & Gage, K. M. (1997). Advances in statistical methods to map quantitative trait loci in outbred populations. *Genetics* **147**, 1445–1457.

McMillan, I. & Robertson, A. (1974). The power of methods for the detection of major genes affecting quantitative characters. *Heredity* **32**, 349–356.

Paterson, A. H., DeVerna, J. W., Lanini, B. & Tanksley, S. D. (1990). Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. *Genetics* **124**, 735–742.

Riquet, J., Coppieters, W., Cambisano, N., Arranz, J.-J., Berzi, P., Davis, S. K., Grisart, B., Farnir, F., Karim, L., Mni, M., Simon, P., Taylor, J. F., Vanmanshoven, P., Wagenaar, D., Womack, J. E. & Georges, M. (1999). Fine-mapping of quantitative trait loci by identity by descent mapping in outbred populations: application to milk production in dairy cattle. *Proceedings of the National Academy of Sciences of the USA* **96**, 9252–9257.

Snedecor, G. W. & Cochran, W. G. (1980). *Statistical Methods*. Ames, Iowa: Iowa State University Press.

Soller, M. & Andersson, L. (1998). Genomic approaches to the improvement of disease resistance in farm animals. *Révue Scientifique et Technique* **17**, 329–345.

Thoday, J. M. (1961). Location of polygenes. *Nature* **191**, 368–370.

Uimari, P. & Hoeschele, I. (1997). Mapping-linked quantitative trait loci using Bayesian analysis and Markov chain Monte Carlo algorithms. *Genetics* **146**, 735–743.

VanRaden, P. M. & Wiggans, G. R. (1991). Derivation, calculation, and use of national animal model information. *Journal of Dairy Science* **74**, 2737–2746.

Xiong, M. & Guo, S. W. (1997). Fine-scale genetic mapping based on linkage disequilibrium: theory and applications. *American Journal of Human Genetics* **60**, 1513–1531.

Zeng, Z.-B. (1993). Theoretical basis for separation of multiple linked gene effects on mapping quantitative trait loci. *Proceedings of the National Academy of Sciences of the USA* **90**, 10972–10976.

Zeng, Z.-B. (1994). Precision mapping of quantitative trait loci. *Genetics* **136**, 1457–1468.

Zhang, Q., Boichard, D., Hoeschele, I., Ernst, C., Eggen, A., Murkve, B., Pfister-Genskow, M., Witte, L. A., Grignola, F. E., Uimari, P., Thaller, G. & Bishop, M. D. (1998). Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. *Genetics* **149**, 1959–1973.

Downloaded from http:/www.cambridge.org/core. Technical University of Munich University Library, on 15 Sep 2016 at 11:38:12, subject to the Cambridge Core terms of use, available at http:/www.cambridge.org/core/terms. http://content-service:5050/content/id/urn:cambridge.org:id:article:S0016672300004638/resource/name/S0016672300004638a.pdf