Theor Appl Genet (1996) 92:130–139

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Use of deterministic sampling for exploring likelihoods in linkage analysis for quantitative traits

Received: 1 March 1995 / Accepted: 7 July 1995

Abstract Deterministic sampling was used to numerically evaluate the expected log-likelihood surfaces of QTL-marker linkage models in large pedigrees with simple structures. By calculating the expected values of likelihoods, questions of power of experimental designs, bias in parameter estimates, approximate lower-bound standard errors of estimates and correlations among estimates, and suitability of statistical models were addressed. Examples illustrated that bracket markers around the QTL approximately halved the standard error of the recombination fraction between the QTL and the marker, although they did not affect the standard error of the QTL's effect, that overestimation of the distance between the markers caused overestimation of the distance between the QTL and marker, that more parameters in the model did not affect the accuracy of parameter estimates, that there was a moderate positive correlation between the estimates of the QTL effect and its recombination distance from the marker, and that selective genotyping did not introduce bias into the estimates of the parameters. The method is suggested as a useful tool for exploring the power and accuracy of QTL linkage experiments, and the value of alternative statistical models, whenever the likelihood of the model can be written explictly.

Key words QTL · Linkage · Maximum likelihood · Deterministic sampling · Experimental design

Communicated by D. van Vleck

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Introduction

Searching for quantitative trait loci (QTLs) using DNA markers in commercially used animal and plant species is now the focus of many agricultural genetic research programmes (e.g. Paterson et al. 1988; Edwards et al. 1987; Haley et al. 1990; Keim et al. 1990; Georges et al. 1995). Because of the expense in both genotyping and generating the large experimental populations required for such research, effort has been spent in optimizing the design of experiments so that maximum information is yielded at minimum cost. The design process requires consideration of the statistical power of an experiment (i.e. the probability of detecting a QTL if it is segregating in the population), and the accuracy with which the information on the QTL (e.g. size of effect, relative dominance, allele frequency, and location in the genome) is obtained.

The method of maximum likelihood is often used for analyses because of its ability to separate the different factors influencing the observed contrast between alternative marker-genotype means (i.e. additive and dominance components of the gene effect, gene frequency, recombination fraction between the marker and the QTL) (Weller 1986, 1987; Jensen 1989; Lander and Botstein 1989; Luo and Kearsey 1989). However, because the likelihoods are often complex, and theoretical knowledge of the statistical properties of these models is incomplete, it is not always possible to predict the power of such analyses, or the bias and accuracy of maximum-likelihood estimators. Hence, large numbers of replicates of stochastic simulated data have been used to empirically find these properties for given designs (e.g. Weller 1986; Knott and Haley 1992; Van Ooijen 1992; Darvasi et al. 1993). For simpler experiments, such as those used for the estimation of recombination fractions between two markers from data of a given cross, it has been proposed that questions of the power and accuracy of estimates can be addressed by computing expected values of likelihoods under different hypotheses (Ed-

wards 1984; Ott 1991). For example, to predict power, one can compute the expected values of the likelihood under the true model and null hypothesis and calculate the expected likelihood ratio test and thereby the power. To predict accuracy of parameters, one can compute the first and second derivatives and hence the information matrix around the maximum of the likelihood and use its inverse to compute lower-bound standard errors and correlations for the estimated parameters. In this paper it is shown how the same procedure can be used to predict the power and accuracy of linkage experiments involving quantitative traits and we propose it as a more efficient method for designing optimum experiments and exploring alternative statistical models than employing replicated stochastic simulations. By using examples based on the half-sib design typically used in animal and outbred tree species, the utility of the method is demonstrated. In these examples, four issues are addressed: the shape of the likelihood surface with respect to parameters when single and bracket markers are used; bias caused by error in assumed known parameters; accuracy and correlations among parameters estimated by maximum likelihood; and the power, accuracy and bias relative to linear models when selective genotyping is practised, i.e. when only progeny from the two truncated tails of the phenotypic distribution are genotyped.

Theory

Deterministic sampling

Deterministic sampling is performed by systematically drawing values of a random variable X at fixed points, x_i over the entire range of X. The number of observations, n_i with value equal to x_i is equal to the height of the density function of X at the point x_i multiplied by the width of the interval between x_i and x_{i+1} and the total number of observations in the population. For example, if X is a random variable sampled from a population of size 100 and is assumed to be distributed as $N(0,1)$, then values of x_i may be drawn at intervals of 0.1 over the range from -7 to 7. Then, n_i equals $100 \times 0.1 \times h_i$ where h_i is the height of the standard normal density function at point x_i. Thus the method is simply a numerical integration. Here it is called deterministic sampling to emphasize that the groups of observations that are created in the systematic sampling process are then "analysed" under various models just as real data would be, whereas numerical integration is often used to simply obtain a single calculated value from all the data. In this sense the idea of Kinghorn et al. (1993) is followed: they explored the statistical properties of a major gene model and method of analysis using weighted samples from expected values of data given an underlying genetic model.

Maximum-likelihood theory

The general form of the likelihood, L, of a sample of size n of a random variable X which is assumed to be distributed according to a density function $f(x|\Theta)$, where Θ is a set of parameters which describe the population, can be written as:

$$L = \prod_{i=1}^{n} f(X_i|\Theta). \tag{1}$$

If the parameters are unknown and are to be estimated from the data then Θ is replaced by the vector of estimates $\hat{\Theta}$, and the expected log likelihood conditional on these estimates can then be predicted [see Ott (1991) and Edwards (1984) for descriptions of the method for calculating the expected log likelihood in the case of discrete data]. The expected value of the logarithm of the likelihood ($\log L$) for a set of n data points sampled from a distribution with density dictated by the true parameter Θ conditional on a set of estimates of the parameters $\hat{\Theta}$ is given by:

$$\begin{aligned} E(\log L) &= E\left[\log \prod^{n} f(x|\hat{\Theta})\right] \\ &= E\left[\sum^{n} \log\left(f(x|\hat{\Theta})\right]\right. \\ &= \sum^{n} E[\log f(x|\hat{\Theta})] \\ &= n\int f(x|\Theta)\log f(x|\hat{\Theta})dx. \end{aligned} \tag{2}$$

However, (2) is only exact in the special case when a single hypothesis is assumed e.g. if the marker-QTL linkage phase is assumed known. When multiple hypotheses are being tested, calculation of the expected value of the log likelihood requires an approximation because it involves taking the log of a weighted sum of the likelihoods under the different hypotheses. For example, if the QTL genotype of a sire and the linkage phase with the marker is unknown, then the likelihood of the data from his family is computed for each possible sire marker-QTL genotype, weighted by the prior probability of the sire having that genotype and then summed. In such cases the expected log likelihood is derived as follows:

$$\begin{aligned} E(\log L) &= E\left[\log \sum_H P_H \prod^{n} f(x|\hat{\Theta})\right] \\ &\approx \log E\left[\sum_H P_H \prod^{n} f(x|\hat{\Theta})\right] \\ &= \log\left(\sum_H E\left[P_H \prod^{n} f(x|\hat{\Theta})\right]\right) \\ &= \log\left(\sum_H E\left[\exp\left((\log P_H) + \sum^{n} \log f(x|\hat{\Theta})\right)\right]\right) \\ &\approx \left(\log \sum_H \exp\left(E(\log P_H) + E\left[\sum^{n} \log f(x|\hat{\Theta})\right]\right)\right) \\ &= \log\left(\sum_H \exp\left(E[\log P_H] + n\int_{-\infty}^{\infty} f(x|\Theta)\log f(x|\hat{\Theta})dx\right)\right) \\ &\approx \log\left(\sum_H \exp\left(\log P_H + n\int_{-\infty}^{\infty} f(x|\Theta)\log f(x|\hat{\Theta})dx\right)\right) \\ &= \log\left(\sum_H P_H \exp\left(n\int_{-\infty}^{\infty} f(x|\Theta)\log f(x|\hat{\Theta})dx\right)\right) \end{aligned} \tag{3}$$

where P_H is the prior probability that the Hth hypothesis is true. This approximation is expected to be very close for large n as shown below. The likelihoods for the models considered in this study are special cases of this general form.

The deterministic sampling approach to exploring likelihood functions is to compute Equation (3) using numerical integration for different values of $\hat{\Theta}$ given Θ. In this way the behaviour of the likelihood surface with respect to parameter estimates is evaluated. The likelihood surfaces calculated from (3) have the property of maximizing at the true value of Θ as expected from an infinite sample, but with the shape expected from a finite sample. That is, when n is large, they represent the average shape [i.e. $E(\log L)$] that would be

obtained over many replicates of simulated stochastic data. To demonstrate this property, ten replicate populations each with 100 observations sampled from a standard normal distribution were simulated and maximum-likelihood estimates of the means were obtained (assuming the standard deviation was known). The likelihood profiles with respect to the estimated mean were computed and expressed as deviations from their maxima. These profiles are plotted in Fig. 1. The mean likelihood surface over ten such replicates which represents $E(\log L)$, also deviated from its maximum, is shown. The expected log-likelihood profile computed using (3) was also calculated and plotted. Even though there is variation among replicates in the mean due to sampling in finite populations, the average shape of the surfaces is only slightly broader than that predicted deterministically. Because it is the shape of the surface which yields information on parameter accuracy and power, the deterministic approximate approach to evaluating likelihood functions in finite populations of reasonable size is justified.

A property of maximum-likelihood surfaces is that approximate lower-bound standard errors of maximum-likelihood parameter estimates can be obtained from the shape of the surface near the maximum. This requires invoking the large sample theory for maximum-likelihood estimates (Kendall and Stuart 1979) which is that the distribution of the maximum-likelihood estimates is approximately normal with mean Θ and variance-covariance matrix equal to $1/nI(\Theta)$ where $I(\Theta)$ is the expected information matrix calculated from the expected value of the second derivatives of $\log L$ with respect to Θ, viz:

$$I(\Theta) = -E\left[\frac{\partial^2}{\partial\Theta^2}\log[f(X|\Theta)]\right]. \quad (4)$$

In practice it may be difficult to find the explicit forms of the second derivatives of the likelihood function and expectations of these forms. In such cases, as in this study, the expected information matrix is replaced by a numerically derived observed information matrix which is evaluated at some distance, Δ, from each of the true parameter estimates. The standard errors and correlations between the estimates are calculated from the variance-covariance information matrix using the standard formulae. These are lower-bound standard errors for unbiased estimators according to the Cramer-Rao inequality. Standard errors calculated using the full variance-covariance matrix in this way were termed here "multiparameter" standard errors because the standard errors on each estimate allow for covariances among estimates. Standard errors were also computed by taking the inverse of the information matrix in which non-diagonal elements (covariances) were set to zero. These standard errors, called "uniparameter" standard errors, are the same as those that would be obtained if evaluated from the shape of the likelihood surface around the maximum when all other parameter were at their true values, i.e. assumed known.

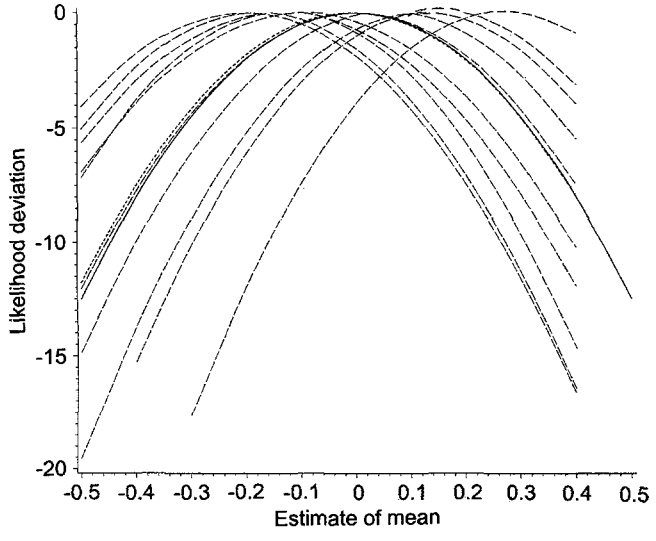
Confidence intervals for a given parameter for a Type-I error A at the $100(1-A)\%$ probability level for these parameters are constructed based on standard normal theory as usual, viz:

$$\hat{\Theta} \pm z(A/2)s_{\hat{\Theta}}, \quad (5)$$

where $s_{\hat{\Theta}}$ is the uniparameter standard error of $\hat{\Theta}$ and $z(A/2)$ is the standard normal deviate corresponding to a probability level of $(1-A/2)$.

The approximate power of the experiment can be found by computing the ratio of the likelihood under the null hypothesis to the likelihood under the alternative hypothesis (the likelihood maximized with respect to all unknown parameters). The natural logarithm of this ratio, called the likelihood ratio test (LRT), is approximately distributed as half a chi-square variable with degrees of freedom equal to the difference in the numbers of parameters estimated in the two alternative hypotheses (Wilks 1938). Power can be calculated as the probability that twice the LRT exceeds the chi-square statistic for a Type-I error of A and 1 degree of freedom from a non-central chi-square distribution. For values of chi-square greater than 3.5 (i.e. for all gene effects which will be detectable with a Type-I error rate of below 6%) the non-centrality parameter in this distribution is very closely approximated by the expected chi-square value. Thus, given the parameters used to generate the data, the expected chi-square value, and hence the non-centrality parameter, can be predicted and the power computed from the non-central distribution. Approximate power can be also be predicted as the probability that the contrast between marker means is greater than the t-statistic for a Type-I error of A and appropriate degrees of freedom from a non-central t-distribution with a non-centrality parameter equal to the expectation of this contrast given the data parameters. This latter method, based on a linear analysis of between-marker means, ignores the effects of recombination which cause each marker group to be a mixture of normals and thus tends to slightly underestimate power (Darvasi et al. 1993). However, it is used here to illustrate the correspondence between the power predicted from the LRT from maximum-likelihood analyses and the power predicted from linear analyses.

Fig. 1 Log likelihoods (expressed as deviations from their maxima) as a function of the maximum-likelihood estimate of the population mean for ten replicate populations (– – –) each of 100 observations sampled from a normal distribution $N(0, 1)$. The average of these ten replicates (also deviated from its maximum) is also shown (·····). The expected log-likelihood profile (———) has a shape similar to the average shape of the replicates



Models

The common model used for the examples in this study is as follows. A sire that is heterozygous for two linked marker loci with alleles M_1 and m_1 at the first locus and M_2 and m_2 at the second locus has many offspring which can be genotyped and sorted according to which paternal alleles they inherited. If the sire is in linkage phase, M_1M_2/m_1m_2, the possible paternal gametes transmitted may be M_1M_2 and m_1m_2 if no recombination or a double recombination between the marker loci occurs, or M_1m_2 and m_1M_2 if a single recombination event occurs. It is assumed that progeny can be assigned to these four possible marker genotypes unambiguously, i.e. the paternal marker alleles can be distinguished from the maternally inherited marker allles. The probability of recombination between the two markers is denoted R. In between these marker loci (called 'bracket' markers) at a recombination fraction of r_1 from the first marker and r_2 from the second marker, is situated a QTL with alleles Q and q. The sire is also heterozygous at the QTL and has a marker-QTL linkage phase of M_1QM_2/m_1qm_2. With respect to the QTL, the transmission of the alleles to the progeny is not certain so that a probability of inheriting alternative QTL alleles is assigned based on the phenotypic value and assumed distributions of the two alternative QTL groups. Assuming that the difference between

phenotypic means of progeny inheriting the Q versus q allele is α, that the overall mean of the population is μ, and that the standard deviation within both these groups is σ, then the respective likelihoods of an animal having a phenotypic value x given that it inherited the sire's Q or q alleles are given by $f[(x-\mu-1/2\alpha)/\sigma)] = f(x|Q)$ and $f[(x-\mu+1/2\alpha)/\sigma)] = f(x|q)$ where $f(Y)$ is the standard normal density of variable Y.

The likelihoods, $\hat{P}_i$, of observing phenotypic value x given that the progeny has marker genotype i ($i = 1,\ldots,4$ for M_1M_2, m_1m_2, M_1m_2 and m_1M_2) are based on the estimates of the unknown parameters $\hat{\Theta} = \{\hat{\alpha}, \hat{\mu}, \hat{\sigma}, \hat{r}_1, \hat{r}_2\}$ as follows:

$$\hat{P}_1 = \frac{(1-\hat{r}_1)(1-\hat{r}_2)}{1-R} f\left(\frac{x-\hat{\mu}-\frac{1}{2}\hat{\alpha}}{\hat{\sigma}}\right) + \frac{\hat{r}_1\hat{r}_2}{1-R} f\left(\frac{x-\hat{\mu}+\frac{1}{2}\hat{\alpha}}{\hat{\sigma}}\right)$$

$$\hat{P}_2 = \frac{\hat{r}_1\hat{r}_2}{1-R} f\left(\frac{x-\hat{\mu}-\frac{1}{2}\hat{\alpha}}{\hat{\sigma}}\right) + \frac{(1-\hat{r}_1)(1-\hat{r}_2)}{1-R} f\left(\frac{x-\hat{\mu}+\frac{1}{2}\hat{\alpha}}{\hat{\sigma}}\right)$$

$$\hat{P}_3 = \frac{(1-\hat{r}_1)\hat{r}_2}{R} f\left(\frac{x-\hat{\mu}-\frac{1}{2}\hat{\alpha}}{\hat{\sigma}}\right) + \frac{\hat{r}_1(1-\hat{r}_2)}{R} f\left(\frac{x-\hat{\mu}+\frac{1}{2}\hat{\alpha}}{\hat{\sigma}}\right)$$

$$\hat{P}_4 = \frac{\hat{r}_1(1-\hat{r}_2)}{R} f\left(\frac{x-\hat{\mu}-\frac{1}{2}\hat{\alpha}}{\hat{\sigma}}\right) + \frac{(1-\hat{r}_1)\hat{r}_2}{R} f\left(\frac{x-\hat{\mu}+\frac{1}{2}\hat{\alpha}}{\hat{\sigma}}\right), \quad (6)$$

where $\hat{r}_2$ is calculated from $\hat{r}_1$ (which is estimated) and R (which is assumed known without error) as:

$$\hat{r}_2 = \frac{R-\hat{r}_1}{1-2\hat{r}_1}. \quad (7)$$

The expected numbers of observations with value x in the data are equal to $1/2n(1-R)P_1$, $1/2n(1-R)P_2$, $1/2nRP_3$ and $1/2nRP_4$ where P_i denotes value of $\hat{P}_i$ when true values are used instead of estimates, i.e. when $\Theta = \hat{\Theta}$. Thus in the terminology of (2), P_i is equivalent to $f(x|\Theta)$ and gives the expected proportion of observations with value x in the ith marker class from a data set with true parameters $\Theta = \{\alpha, \mu, r_1, \sigma\}$ in the model described here. $\hat{P}_i$ is equivalent to $f(x|\hat{\Theta})$ and represents the density for value x conditional on being in the ith marker class and on the parameter estimates $\hat{\Theta}$. The expected value of the log likelihood of the data under this model is then:

$$E(\log L) = n \sum_{i=1}^{4} \int P_i \log \hat{P}_i \cdot dx. \quad (8)$$

Note that (8) takes the form of (2) and not (3) because the sire's marker-QTL genotype is assumed known.

In the following examples (8) was evaluated using numerical integration for sets of Θ and $\hat{\Theta}$ to determine the statistical properties of various models. The integrations were approximated using the trapezoidal rule at 100 points in the range of ± 7 standard deviations.

Numerical examples

Example 1: accuracy of bracket-marker versus single-marker mapping

The aim of this example is to determine the accuracy of locating a QTL when bracket markers versus single markers are used. This was examined by using (8) to calculate likelihoods over a range of $\hat{\alpha}$, $\hat{\mu}$, $\hat{r}_1$ (denoted $\hat{r}$ from here on) and $\hat{\sigma}$ for $n = 200$ progeny from one sire of known marker-QTL genotype and true values of $\alpha = 0.5$, $\mu = 0$, $R = 0.2$, $\sigma = 1$ and $r = 0.05$. For the analysis in which only a single marker is used, R was set to 0.5. The expected values of the likelihood ratio for these parameters against the null hypothesis that $\alpha = 0$ when a single marker and bracket markers are used were found to be 4.89 and 5.06 respectively. These values correspond well with the prediction that the LRT is half the expected chi-square value which in this case is calculated as $(1-2r)^2\alpha^2/(4\sigma^2/n) = 10.13$ (Weller et al. 1990). For a Type-I error rate of 5% and 1 degree of freedom, using 10.13 as the non-centrality parameter, these LRT values correspond to powers of 88% and 89% respectively. The corresponding power using a two-tailed t-test from a distribution with a non-centrality parameter (expected value of t) equal to $(1-2r)\alpha/(2\sigma/\sqrt{n}) = 3.18$, was also 88% for the single-marker case. Thus this set of parameters is typical of an experiment which might be designed in practice. The small difference in power between the single- and bracket-marker cases can be understood by comparing the between-marker variances in the two cases. For the single marker this variance is:

$$V_S = n\frac{\alpha^2}{4}(1-2r_1)^2, \quad (9)$$

and for the bracket marker case is:

$$V_B = n\frac{\alpha^2}{4}\left[\frac{(1-r_1-r_2)^2}{1-R} + \frac{(r_1-r_2)^2}{R}\right]. \quad (10)$$

Using the ratio of V_S to V_B, which is equal to the ratio of the F-statistics for between-marker variances because the residual variance is the same in both cases (ignoring the small contribution of recombination to within-marker variance), gives an approximate relative number of animals required to achieve a given power (ignoring different degrees of freedom in the two cases). This ratio varies from $1-R$ when the QTL is in the middle of the bracket ($r_1 = r_2$) to 1 when the QTL is located at the marker. Thus for a marker spacing of $R = 0.2$, there is very little gain in power by using bracket markers, especially if power is high for the single markers. This conclusion was also reached by Lander and Botstein (1989) and Darvasi et al. (1993).

The plots of $\log L$ versus $\hat{\alpha}$ and $\hat{r}$ are shown in Fig. 2a and b for the bracket- and single-marker cases respectively. The global maximum is reached at the true parameter values showing that the maximum-likelihood estimates are unbiased. The profiles with respect to $\hat{\alpha}$ are symmetric around α and much steeper than those for $\hat{r}$. Standard errors in the single and bracket-marker cases are, respectively, 0.158 and 0.158 for α, and are 0.143 and 0.064 for $\hat{r}$. Thus while $\hat{\alpha}$ is estimated equally accurately in the single- and bracket-marker cases, $\hat{r}$ is estimated less accurately (by a factor of about a half) using single markers than by using bracket markers. This is because most of the information on $\hat{\alpha}$ comes from the between-marker means which are not altered by the use of an extra marker. In contrast, information on $\hat{r}$ comes entirely from within-marker groups when using single markers, but for bracket

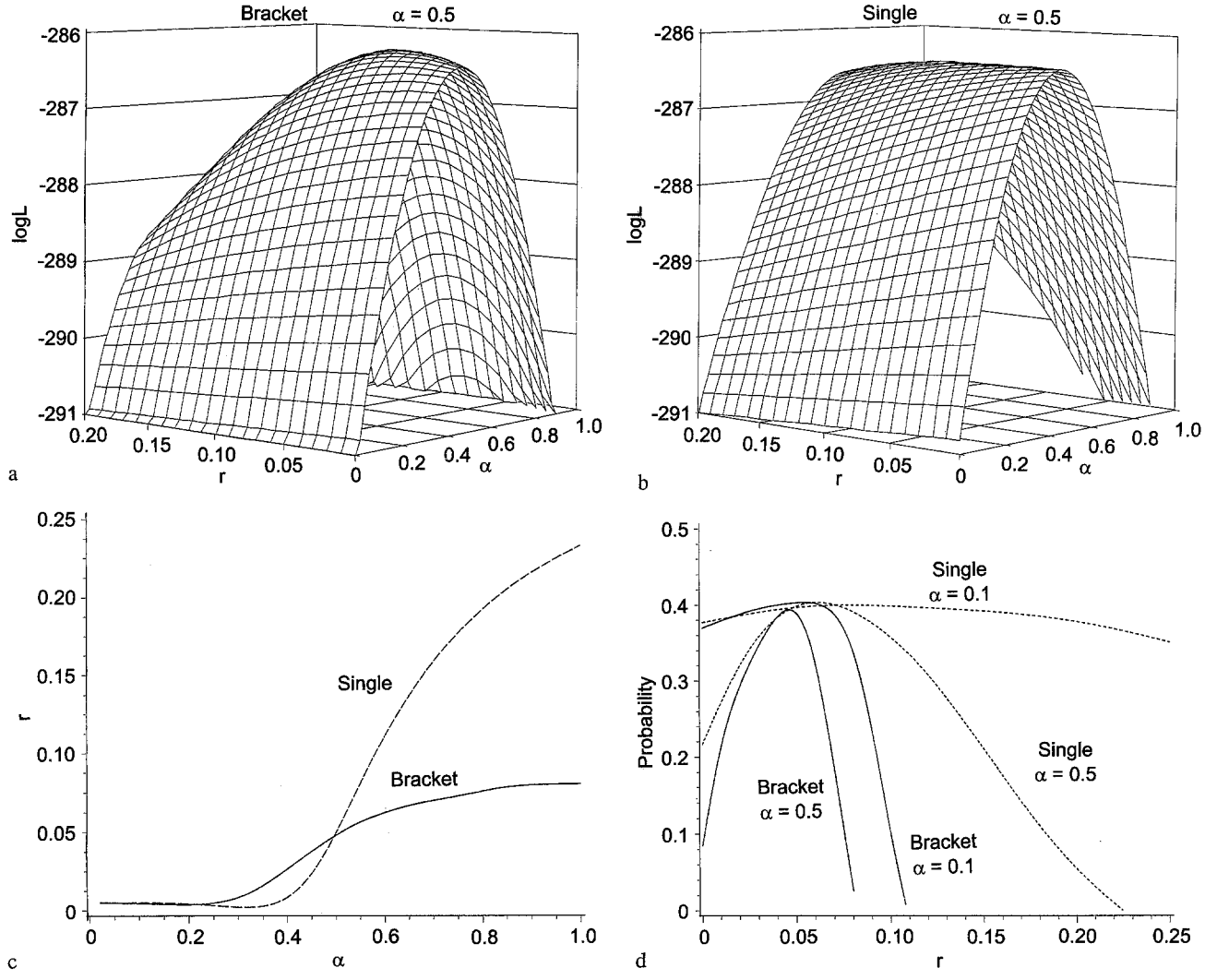


Fig. 2 Expected log-likelihood surfaces with respect to the maximum-likelihood estimates of a QTL effect ($\hat{\alpha}$) and its recombination fraction from a marker ($\hat{r}$) (**a** and **b**), the relationship between $\hat{\alpha}$ and $\hat{r}$ (**c**), and the marginal density of $\hat{r}$ conditional on $\hat{\alpha}$ (**d**) for bracket-marker and single-marker analyses of 200 progeny where true parameters of the QTL are $\alpha = 0.5$ and $r = 0.05$

markers also largely from recombination events between markers. However, twice the number of genotypes have to be performed when using bracket markers versus single markers so that this extra accuracy in $\hat{r}$ does not come without cost.

Relationships between maximum-likelihood estimates of $\hat{\alpha}$ and $\hat{r}$ for bracket- and single-marker analyses are shown in Fig. 2c. These lines are equivalent to joining the maxima of the profiles for $\hat{r}$ over a range of $\hat{\alpha}$ as depicted in Fig. 2a and b. It is clear from these that the dependence of $\hat{r}$ on $\hat{\alpha}$ is far stronger for single markers than for bracket markers. The relevance of this dependence is that variation in $\hat{\alpha}$ caused by sampling is associated with variation in $\hat{r}$, i.e. there is covariation between estimates. This means that inaccuracy in one estimate caused by sampling will introduce a bias in the other estimate. The nature of the joint distribution of these two parameters, represented by the surfaces shown in Figs. 2a and b, is such that the bias will differ depending on whether $\hat{\alpha}$ is overestimated or underestimated. If $\hat{\alpha}$ is underestimated, then $\hat{r}$ also is underestimated in both the bracket and single-marker case. If $\hat{\alpha}$ is overestimated, then the maximum-likelihood estimate of $\hat{r}$ reaches a peak at some intermediate value for $\hat{r}$ in the bracket case but tends towards 0.5 in the single-marker case. The net consequence of the asymmetry in $\hat{r}$ and symmetry in $\hat{\alpha}$ can be described by the marginal distribution of $\hat{r}$, i.e. the probability of getting an estimate of $\hat{r}$ averaged over the distribution of $\hat{\alpha}$. The marginal density for $\hat{r}$ calculated assuming $\hat{\alpha}$ is normally distributed $N(0.5, 0.158)$ is shown in Fig. 2d for the single- and bracket-marker cases. This density shows that most of the mass falls around r in the bracket-marker case but that the distribution is wide for the single-marker case. This means that more accurate localization of a QTL is obtained when bracket markers are used, as pointed out by Lander and Botstein (1989).

Apparent from these marginal densities is the fact that the probability of locating the QTL at the marker is greater than locating it in the middle of the bracket. Thus a disproportionate number of QTLs will be es-

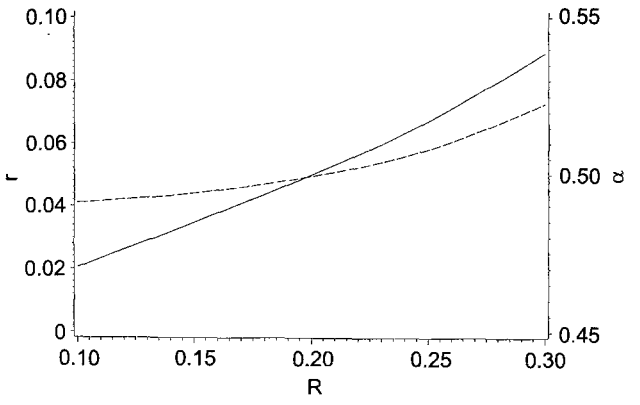


Fig. 3 Bias in estimates of $\hat{r}$ (———) and $\hat{\alpha}$ (– – –) as a function of the estimated recombination fraction between two bracket markers (R) where the true values are $R=0.2$, $\alpha=0.5$ and $r=0.05$ for a population of 200 individuals

timated to be located at the marker itself. This problem will be exacerbated in situations of low power. As an example, the marginal distribution for the case where $\alpha=0.1$ (which was found also to have a standard error of 0.158) was also calculated and plotted (Fig. 2d). In this case the probability of locating the QTL on the marker is almost as high as locating it at its true value. Thus there is a tendency to locate a QTL at the marker when the power to detect the QTL is low. This problem may be partly alleviated by using more than two markers in the analysis if the two closest markers bracketing the QTL are not fully informative. This example illustrates how the joint distribution of parameter estimates as represented by the multidimensional shape of the likelihood surface can dictate the confidence in an estimate when estimates are not independent. The behaviour of one estimate with respect to another could be similarly examined in other models or data structures.

Example 2: bias caused by wrongly specified parameters

The purpose of this example is to examine bias in the estimate of $\hat{r}$ when R is not the true value. This situation would occur if the marker map was in error due to genotyping errors or to the low power in the data used for map construction. To examine this bias, the same situation used in Example 1 is employed ($\alpha=0.5$, $\mu=0$, $r=0.05$, $n=200$, $\sigma=1$, $R=0.2$), except now error is introduced in R to give an estimate, $\hat{R}$, which ranges from 0.1 to 0.3. (The single-marker case is not included in this analysis). Maximum-likelihood estimates of $\hat{\alpha}$ and $\hat{r}$, holding $\hat{\sigma}$ equal to its true value, were computed over the range of $\hat{R}$.

Figure 3 shows the bias in $\hat{\alpha}$ and $\hat{r}$ as a function of $\hat{R}$. The bias in $\hat{r}$ is approximately a multiplicative factor of the error in R. For example, when $\hat{R}$ is 0.25 then $\hat{r}$ is 0.06, i.e., a 20% error in both. This relationship is understood from the form of the likelihood-equation components given in (6). Because $r_1(=r)$ is expressed relative to R, the error in $\hat{r}$ should be relative to the error in $\hat{R}$. Bias in $\hat{\alpha}$ was less than the bias in $\hat{r}$ (relative to the simulated value) but was also positive reflecting the partial dependence between $\hat{\alpha}$ and $\hat{r}$ found in Example 1. This example illustrates that error in the estimates of recombination between markers can produce bias in QTL-parameter estimates. While the bias in $\hat{r}$ is predictable, because r is estimated as a relative position, this can introduce bias in related parameters such as $\hat{\alpha}$. However, the amount of bias is well within the range of standard-error estimates of the parameters for populations of this size (given in Example 1 for this case) and so is unlikely to be of practical significance in experiments of moderate power. Bias would be reduced if information from other markers which are more accurately located is used to locate the QTL when the bracket markers are not fully informative.

Example 3: accuracy of parameter estimates using simple versus complex models

In this example the purpose is to obtain estimates of standard errors and correlations among parameter estimates with two alternative models to ascertain whether one model is advantageous in terms of accuracy of estimation.

The basic model used for the previous example is expanded to include more parameters by writing the likelihood in terms of three genotypes (QQ, Qq, and qq) rather than in terms of the two alleles (Q or q) as before. The three genotype model (called the complex model, C) requires a density function for each of the three possible genotypes. Means of the genotypes are written as $\mu+a$, $\mu+d$ and $\mu-a$ where a is the value of the QQ phenotype, $-a$ the value of the qq phenotype and d the value of the Qq phenotype, all expressed relative to a population mean of μ. Expressing the likelihood in terms of genotypes of the progeny, rather than in terms of the allele (Q or q) inherited from the sire, requires the introduction of another parameter, p, for the frequency of the Q allele in the dam population. Thus there are six unknown parameters, $\hat{\Theta}=\{\hat{a},\hat{d},\hat{r},\hat{p},\hat{\mu},\hat{\sigma}\}$ compared with four ($\hat{\Theta}=\{\hat{\alpha},\hat{\mu},\hat{r},\hat{\sigma}\}$) for the model used previously (called the simple model, S). The α in the S model is equivalent to $a+(1-2p)d$ in the C model.

For a single sire, the C model has likelihood components, $\hat{P}_i$, equal to:

$$\hat{P}_1=\hat{p}\frac{(1-\hat{r}_1)(1-\hat{r}_2)}{1-R}f\left(\frac{x-\hat{\mu}-\hat{a}}{\hat{\sigma}}\right)+\left[\hat{p}\frac{\hat{r}_1\hat{r}_2}{1-R}\right.$$

$$\left.+(1-\hat{p})\frac{(1-\hat{r}_1)(1-\hat{r}_2)}{1-R}\right]f\left(\frac{x-\hat{\mu}-\hat{d}}{\hat{\sigma}}\right)$$

$$+(1-\hat{p})\frac{\hat{r}_1\hat{r}_2}{1-R}f\left(\frac{x-\hat{\mu}+\hat{a}}{\hat{\sigma}}\right)$$

$$\hat{P}_2 = \hat{p}\frac{\hat{r}_1\hat{r}_2}{1-R}f\left(\frac{x-\hat{\mu}-\hat{a}}{\hat{\sigma}}\right)+\left[\hat{p}\frac{(1-\hat{r}_1)(1-\hat{r}_2)}{1-R}\right.$$
$$\left.+(1-\hat{p})\frac{\hat{r}_1\hat{r}_2}{1-R}\right]f\left(\frac{x-\hat{\mu}-\hat{d}}{\hat{\sigma}}\right)$$
$$+(1-\hat{p})\frac{(1-\hat{r}_1)(1-\hat{r}_2)}{1-R}f\left(\frac{x-\hat{\mu}+\hat{a}}{\hat{\sigma}}\right)$$

$$\hat{P}_3 = \hat{p}\frac{(1-\hat{r}_1)\hat{r}_2}{R}f\left(\frac{x-\hat{\mu}-\hat{a}}{\hat{\sigma}}\right)+\left[\hat{p}\frac{\hat{r}_1(1-\hat{r}_2)}{R}\right.$$
$$\left.+(1-\hat{p})\frac{(1-\hat{r}_1)\hat{r}_2}{R}\right]f\left(\frac{x-\hat{\mu}-\hat{d}}{\hat{\sigma}}\right)$$
$$+(1-\hat{p})\frac{\hat{r}_1(1-\hat{r}_2)}{R}f\left(\frac{x-\hat{\mu}+\hat{a}}{\hat{\sigma}}\right)$$

$$\hat{P}_4 = \hat{p}\frac{\hat{r}_1(1-\hat{r}_2)}{R}f\left(\frac{x-\hat{\mu}-\hat{a}}{\hat{\sigma}}\right)+\left[\hat{p}\frac{(1-\hat{r}_1)\hat{r}_2}{R}\right.$$
$$\left.+(1-\hat{p})\frac{\hat{r}_1(1-\hat{r}_2)}{R}\right]f\left(\frac{x-\hat{\mu}-\hat{d}}{\hat{\sigma}}\right)$$
$$+(1-\hat{p})\frac{(1-\hat{r}_1)\hat{r}_2}{R}f\left(\frac{x-\hat{\mu}+\hat{a}}{\hat{\sigma}}\right). \quad (11)$$

So far it has been assumed that the sire is heterozygous at the QTL and that the marker-QTL phase is known. In practice this may not be true, in which case it is necessary to compute the likelihood for a single sire conditional on all possible sire QTL genotypes, and take the sum of these likelihoods weighted by the prior probabilities of the sire having these QTL genotypes. The appropriate expressions for $\hat{P}_i$ for sire genotypes other than M_1QM_2/m_1qm_2 are as follows. When the hypothesized sire is M_1qM_2/m_1Qm_2 then the expressions in (11) for $\hat{P}_1$ and $\hat{P}_2$ are swapped (i.e. $\hat{P}_2$ becomes $\hat{P}_1$ and vice versa), and $\hat{P}_3$ and $\hat{P}_4$ are swapped. If the hypothesized sire is homozygous for the QTL then $\hat{P}_i$ is the same for all i and is equal to

$$\hat{P}_i = \hat{p}f\left(\frac{x-\hat{\mu}-\hat{a}}{\hat{\sigma}}\right)+(1-\hat{p})f\left(\frac{x-\hat{\mu}-\hat{d}}{\sigma}\right) \quad (i=1,..4)$$

and

$$\hat{P}_i = \hat{p}f\left(\frac{x-\hat{\mu}-\hat{d}}{\hat{\sigma}}\right)+(1-\hat{p})f\left(\frac{x-\hat{\mu}+\hat{a}}{\sigma}\right) \quad (i=1,..4) \quad (12)$$

for sires with hypothesized genotypes of M_1QM_2/m_1Qm_2 and M_1qM_2/m_1qm_2 respectively. In practice the analysis will be performed over several sire families to obtain enough power to detect a QTL. The likelihood is therefore summed over all families. A full derivation of the likelihood pooling over families and allowing for the four possible QTL genotypes in the sire for each family is given by Mackinnon and Weller (1995).

Data were deterministically simulated under the C model for a case where $n = 1000$ with 100 progeny in each of ten sire families, and with parameter values of $a = 0.05$, $d = 0$, $r = 0.05$, $p = 0.5$, $\mu = 0$ and $\sigma = 1$. Between-family variation due to genes other than the QTL in question was not incorporated into the simulated data. In this case the values of α and $\hat{\alpha}$ in the S model are equivalent to a and $\hat{a}$ in the C model. The QTL was assumed to be at Hardy-Weinberg equilibrium in the population of sires so that only a fraction $2p(1-p)$ of the sires (5 of 10 in this case) were heterozygous and the rest were homozyzous. The approximate power for such an experiment against the null hypothesis that the QTL

Table 1 Lower-bound estimates of standard errors (multiparameter estimates on diagonals, uniparameter estimates in parentheses below) and correlations among maximum-likelihood estimates of QTL parameters for a population of 1000 individuals in 10 sire families where true values are $a = 0.5$, $d = 0$, $r = 0.05$, $p = 0.5$, $\mu = 0$ and $\sigma = 1$

Model	Parameter	$\hat{a}$	$\hat{d}$	$\hat{r}$	$\hat{p}$	$\hat{\mu}$	$\hat{\sigma}$
Simple	$\hat{a}$	0.057 (0.055)		0.154		0.000	−0.029
	$\hat{r}$			0.047 (0.047)		0.000	−0.053
	$\hat{\mu}$					0.026 (0.026)	0.008
	$\hat{\sigma}$						0.019 (0.019)
Complex	$\hat{a}$	0.055 (0.051)	−0.014	0.152	−0.043	0.035	−0.353
	$\hat{d}$		0.159 (0.051)	−0.000	−0.002	−0.789	−0.010
	$\hat{r}$			0.047 (0.047)	−0.007	0.004	−0.100
	$\hat{p}$				0.111 (0.048)	−0.552	0.015
	$\hat{\mu}$					0.100 (0.027)	0.002
	$\hat{\sigma}$						0.021 (0.020)

effect is zero ($a = 0$ and $d = 0$) is very close to 100%. Likelihoods based on (3) for the C and S models were fitted to these data and their second derivatives evaluated at small distances ($\Delta = 0.01$ or 0.005) from the true values to give the observed information matrix. Multiparameter and uniparameter standard errors, and correlations among estimates were calculated. When the S model was fitted, the maximum-likelihood estimate of σ was found to be inflated ($\hat{\sigma} = 1.025$) because of the assumption that each QTL genotypic group was a single normal population when, in fact, it was a mixture of normals (e.g. QQ and Qq animals are grouped together as Q animals under the S model). $\hat{\sigma}$ was fixed at this maximum-likelihood estimate when the other parameters were being evaluated close to the maximum.

Standard errors and correlations are given in Table 1. (Because the maximum difference in value for when $\Delta = 0.01$ versus $\Delta = 0.005$ was only 0.01, only those for $\Delta = 0.01$ are shown.) Uniparameter and multiparameter standard errors were similar for the S and C models for parameters common to both models (a, μ, r and σ) with the exception of $\hat{\mu}$ which had a considerably higher standard error from the C model than the S model. This was due to the strong correlation between $\hat{\mu}$ and the two extra parameters in the C model, $\hat{p}$ and $\hat{d}$. Correlations among them were also similar except for a stronger correlation between $\hat{a}$ and $\hat{\sigma}$ in the C model than in the S model. While it is not possible to assign a level of significance to these apparent lack of differences in accuracy between the two models, it can be concluded that there is not a cost in terms of accuracy of estimation of the QTL's effect and its location when using models with more unknown parameters. Indeed, the extra information on other parameters, such as population frequency and dominance effects obtained using the more comprehensive model, is advantageous because such information may be important for optimizing the way the QTL is exploited in the population using marker-assisted selection. However, the higher standard errors and correlations for the extra parameters, $\hat{p}$ and $\hat{d}$, somewhat limits confidence in the accuracy of the estimates which must be considered if these parameters are used in the design of breeding programmes.

Both the C and S models have been used to estimate parameters for QTLs affecting milk production in dairy cattle (Bovenhuis and Weller 1994; Georges et al. 1995). This example can be extended to explore alternative data structures and models which could be used to optimize the accuracy of estimating certain parameters.

Example 4: selective genotyping

The purpose of this example is to determine whether, if selective genotyping is practised, the estimates of parameters are altered and whether the gain in power is that predicted by Darvasi and Soller (1992) if a linear analysis is used. Also, it is useful to determine whether the likelihood surface has any special features (e.g. local maxima, asymmetry) which are relevant for analyses on selected data.

The same parameters for Example 1 were used ($\alpha = 1$, $\mu = 0$, $r = 0.05$, $\sigma = 1$) except that now the 200 progeny that were genotyped come from the top 25% and bottom 25% of 400 progeny ranked on phenotype. Letting n now equal the number genotyped (rather than phenotyped) and S the proportion of those phenotyped which are also genotyped, then the likelihood equation (8) can be modified to include those not genotyped to be:

$$E(\log L) \approx \frac{n}{S} \sum_{i=1}^{4} \int_{T}^{\infty} P_i \log \hat{P}_i \, dx + \frac{n}{S} \sum_{i=1}^{4} \int_{-\infty}^{-T} P_i \log \hat{P}_i dx$$

$$+ \frac{n}{S} \sum_{i=1}^{4} \int_{-T}^{T} P_i^* \log \hat{P}_i^* \cdot dx, \qquad (13)$$

where T is the point of truncation above the mean of the phenotypic distribution corresponding to the proportion selected for genotyping in the upper tail (and similarly for $-T$ in the lower tail), and superscript * on P and $\hat{P}$ denotes the portion of the population for which no marker information is available. For this portion we set $P_i^* = \hat{P}_i^* = 1/4$. For the genotyped portions, the values of P_i and $\hat{P}_i$ are as in previous equations.

The likelihood surface with respect to $\hat{\alpha}$ and $\hat{r}$ is shown in Fig. 4. This surface can be compared with Fig. 2a (drawn with the same range of $\hat{\alpha}$ and $\hat{r}$) which is the analogous case for when selective genotyping is not used, i.e. when the 200 progeny which are genotyped comprise the full data set. From these figures it can be

Fig. 4 Expected log-likelihood surface as a function of $\hat{\alpha}$ and $\hat{r}$ when selective genotyping is practised by genotyping only those individuals with phenotypes from the top 25% and bottom 25% of the distribution from a population of 400 progeny in which the true QTL parameters are $\alpha = 0.5$, $r = 0.05$, $\mu = 1$ and $\sigma = 1$

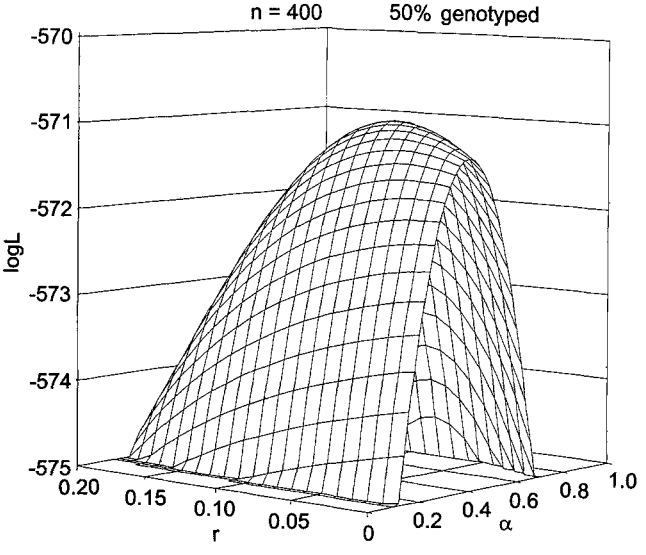


Table 2 Estimates of lower-bound standard errors (multiparameter estimates on diagonals, uniparameter estimates in parentheses below) and correlations among parameter estimates for a population of 200 genotyped individuals representing 50% (selective genotyping) or 100% of the number of phenotyped individuals for a QTL with parameters $\alpha = 0.5$, $\mu = 0$, $r = 0.1$ and $\sigma = 1$

Proportion genotyped	Parameter	$\hat{\alpha}$	$\hat{r}$	$\hat{\mu}$	$\hat{\sigma}$
50%	$\hat{\alpha}$	0.114 (0.109)	0.293	−0.001	−0.084
	$\hat{r}$		0.053 (0.051)	0.000	−0.112
	$\hat{\mu}$			0.050 (0.050)	0.003
	$\hat{\sigma}$				0.037 (0.037)
100%	$\hat{\alpha}$	0.161 (0.154)	0.286	0.000	−0.087
	$\hat{r}$		0.075 (0.072)	0.000	−0.110
	$\hat{\mu}$			0.071 (0.071)	0.007
	$\hat{\sigma}$				0.052 (0.052)

seen that the use of selective genotyping in 50% of the population increases the magnitude of the log likelihood by a factor of almost two. Because the amount of genotyping is the same in both cases, but the surface with respect to $\hat{r}$ is much steeper in the selectively genotyped data, there is more information per genotype in the latter case. The LRT when 50% were genotyped was 8.94 compared with 5.06 when 100% were genotyped, which is interpreted to mean that almost all of the information comes from the 50% of progeny in the tails, as pointed out by Darvasi and Soller (1992). These LRT values correspond to powers to detect a QTL with $\alpha = 0.5$ of 99% and 89% respectively. While in this example the gain in power is not large, because power without selective genotyping is already high, the gain in power when fewer numbers of genotyped progeny are possible would be considerable. This gain in power is in agreement with that predicted by Darvasi and Soller (1992) who computed gains based on the increase in the ratio of between-marker to within-marker variances (i.e. under a linear model).

The similarity between the shape of the likelihood surfaces with and without selective genotyping indicates that the likelihoods should behave the same during maximization (i.e. there are no local maxima) and that parameter estimates also behave similarly. The standard errors and correlations among estimates for the examples with and without selective genotyping are given in Table 2. Standard errors are a factor of $\sqrt{2}$ less when selective genotyping is used, as is expected because the amount of information per progeny is effectively doubled. Correlations among estimates are similar indicating that using information to estimate parameters from opposite tails of the population does not introduce a dependence between parameters.

Discussion

The numerical method of deterministic sampling demonstrated here can be used to gain insight into the behaviour and properties of multi-parameter likelihood functions used in linkage analyses of quantitative traits. The examples illustrate how it can be used to determine the behaviour, power, accuracy and bias in likelihood functions and maximum-likelihood estimators. Such information is useful for not only determining the optimum design of an experiment but also the optimum form of analysis (e.g. the statistical model used). This method has a major advantage in terms of computing costs over the alternative method of performing a large number of stochastic simulations to empirically evaluate these properties (e.g. Knott and Haley 1992; Van Ooijen 1992; Darvasi et al. 1993). This means that deterministic sampling may be able to explore cases that are impossible to explore using stochastic simulation because of computing constraints.

The method could also be used to evaluate the consequences of using inappropriate genetic or statistical models by sampling from a true distribution but writing the likelihood in terms of an assumed distribution. For example, one could explore what happens in cases in which there are multiple QTLs in the region, or multiple alleles in the population, but the likelihood model assumes only a single QTL or only two alleles. The method could also be used to dissect anomalous behaviour in estimates due to incorrect specification of the likelihood function or an inappropriate estimation procedure.

The method is only applicable to situations where the large sample properties of maximum-likelihood estimates hold. For linkage analyses of quantitative traits, many observations are required to detect QTLs so this is not of great concern. Nevertheless, it should be remembered that estimates of the power and accuracy of estimates are lower-bound estimates and therefore should be treated conservatively. It should also be remembered that the method uses an approximation which relies on $E(\log L) \approx \log E(L)$ and $E[\exp(L)] \approx \exp[E(L)]$. In large and symmetric distributions the error in this approximation is small and therefore it is adequate, but for small data sets it may not be. However, in the latter case the accuracy of the estimates would be low and so it is probable that errors due to the approximation are inconsequential.

The deterministic sampling method is limited to cases where the likelihood function can be written explicitly and where the component density functions are of known form. For very complex likelihood functions, such as those for pedigrees involving many types of relationships or for likelihoods involving marginal densities of unknown form, it would be difficult to apply the method of deterministic sampling. In such cases, Gibbs sampling, an alternative stochastic numerical method

for estimating population parameters, can be used (Guo and Thompson 1992; Hoeschele 1994).

Other possible applications not addressed in this paper are to assist in the development of new statistical models (e.g. models which incorporate a term explaining polygenic variation, models explaining multiple QTL effects, or linear models for mixed inheritance), to evaluate alternative experimental designs and mapping methods, and to evaluate marker-assisted selection strategies. However, it should be noted that computational requirements can be high where there is a need to deterministically sample from multivariate distributions rather than from univariate distributions.

In conclusion, deterministic sampling offers a way to predict adequately the asymptotic statistical properties of models and estimators in QTL linkage analyses. This approach was shown to be useful in understanding the behaviour of various models, sources of bias, and relationships among estimates. Other relevant issues to QTL linkage analyses could be investigated using this approach.

Acknowledgements The financial assistance to M. J. M. from the Department of Animal Breeding, University of Wageningen, The Netherlands, which enabled this study to be undertaken, is gratefully acknowledged. Colleagues in this department contributed greatly to the ideas presented.

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