Mapping Quantitative Trait Loci in the Case of a Spike in the Phenotype Distribution

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ABSTRACT

A common departure from the usual normality assumption in QTL mapping concerns a spike in the phenotype distribution. For example, in measurements of tumor mass, some individuals may exhibit no tumors; in measurements of time to death after a bacterial infection, some individuals may recover from the infection and fail to die. If an appreciable portion of individuals share a common phenotype value (generally either the minimum or the maximum observed phenotype), the standard approach to QTL mapping can behave poorly. We describe several alternative approaches for QTL mapping in the case of such a spike in the phenotype distribution, including the use of a two-part parametric model and a nonparametric approach based on the Kruskal-Wallis test. The performance of the proposed procedures is assessed via computer simulation. The procedures are further illustrated with data from an intercross experiment to identify QTL contributing to variation in survival of mice following infection with *Listeria monocytogenes*.

THE standard approach for mapping the genetic loci (quantitative trait loci, QTL) contributing to variation in a quantitative trait makes use of the assumption that the residual environmental variation follows a normal distribution (Lander and Botstein 1989). A common departure from this assumption is to observe a spike in the phenotype distribution. For example, in Figure 1, the survival time (in hours) following infection with *Listeria monocytogenes* is displayed, for 116 female intercross mice (from Boyartchuk *et al.* 2001). Approximately 30% of the mice recovered from the infection and survived to the end of the study (264 hr). Other examples include the density of metastatic tumors, with some individuals exhibiting no metastasis (Hunter *et al.* 2001), and gallstone weight, with some individuals having no gallstones (Wittenburg *et al.* 2002).

Let us assume, without loss of generality, that the spike in the distribution is at 0 (which we call the *null* phenotype) and that all other phenotype values are strictly positive. QTL mapping under a normal model can work reasonably well in this situation if the proportion of individuals with the null phenotype is not large and the remainder of the phenotype distribution is not far above 0. However, when this is not the case, maximum-likelihood estimation under a normal mixture model can occasionally produce spurious LOD peaks in regions of low genotype information (*e.g.*, widely spaced markers).

A simple method of analysis is to consider separately the binary trait, defined by whether or not an individual has the null phenotype, and the quantitative trait, for those individuals having a strictly positive phenotype. We develop a parametric, two-part model that allows us to combine these two analyses. In this single-QTL model, an individual with QTL genotype g has probability π_g of having a nonzero phenotype; if its phenotype is nonzero, the value is assumed to follow a normal distribution with mean μ_g and standard deviation (SD) σ.

We also describe an extension of the Kruskal-Wallis test statistic for nonparametric interval mapping in an intercross (exactly analogous to the extension of the rank-sum test described in Kruglyak and Lander 1995, which was suitable for a backcross). While Kruglyak and Lander (1995) had randomized the rank of any tied phenotypes, we assign the average rank to tied phenotypes and apply a standard correction factor. A possible advantage of the nonparametric approach is that the statistical test concerns 2 d.f., while the test derived from use of the two-part model concerns 4 d.f. and so has a larger null threshold.

We illustrate the use of these procedures with data on survival time of mice, following infection with *Listeria monocytogenes* (Boyartchuk *et al.* 2001). We further study their performance via computer simulations.

METHODS

Consider n F_2 progeny from an intercross between two inbred strains. Let y_i denote the quantitative phenotype for individual i. We assume, without loss of generality, that the spike in the phenotype distribution is at 0. Let $z_i = 0$ if $y_i = 0$ and $z_i = 1$ if $y_i > 0$. Consider data on

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a set of genetic markers, with a known genetic map. Let $\boldsymbol{m}_i$ denote the multipoint marker data for individual i.

Conditional and binary trait analyses: A simple approach for QTL mapping in this situation is to first analyze the quantitative phenotype, y_i, using only the individuals for which $y_i > 0$, by standard interval mapping using a normal model (Lander and Botstein 1989), and then separately analyze the binary trait z_i.

The analysis of the binary trait deserves further explanation. Xu and Atchley (1996) described maximum-likelihood estimation for a binary trait in the context of composite interval mapping (Zeng 1993, 1994). Visscher *et al.* (1996) and McIntyre *et al.* (2001) described approximate methods for analysis of binary traits. We prefer the approach of Xu and Atchley (1996). We briefly describe the special case of no marker covariates.

We consider some fixed position in the genome as the location of a putative QTL and let $g_i = 1$, 2, or 3, according to whether individual i has genotype AA, AB, or BB, respectively, at the QTL. Let us assume that the binary phenotypes, z_i, are independent, and let $\pi_j = \Pr(z_i = 1|g_i = j)$. Given the marker data, $\boldsymbol{m}_i$, but not knowing the QTL genotypes g_i, the z_i follow mixtures of Bernoulli distributions (analogous to the mixtures of normals that arise in standard interval mapping).

We assume that we may calculate $p_{ij} = \Pr(g_i = j|\boldsymbol{m}_i)$, the QTL genotype probabilities, given the observed multipoint marker data. Under no crossover interference and no genotyping errors, the distribution depends only on the nearest flanking typed markers, but one may also use the approach of Lincoln and Lander (1992) to take account of the presence of genotyping errors.

The likelihood for the parameters $\boldsymbol{\pi} = (\pi_j)$, given the observed data $\{(\boldsymbol{m}_i, z_i)\}$, is then

$$L(\boldsymbol{\pi}) = \prod_i \sum_j p_{ij}(\pi_j)^{z_i}(1 - \pi_j)^{(1-z_i)}.$$

We obtain maximum-likelihood estimates (MLEs), $\hat{\pi}_j$, using a form of the expectation-maximization (EM) algorithm (Dempster *et al.* 1977). At iteration $s + 1$, we have estimates of the parameters, $\hat{\boldsymbol{\pi}}^{(s)}$. In the E-step, we calculate weights for each individual and for each genotype:

$$w_{ij}^{(s+1)} = \Pr(g_i = j|z_i, \boldsymbol{m}_i, \hat{\boldsymbol{\pi}}^{(s)}) = \frac{p_{ij}(\hat{\pi}_j^{(s)})^{z_i}(1 - \hat{\pi}_j^{(s)})^{(1-z_i)}}{\sum_k p_{ik}(\hat{\pi}_k^{(s)})^{z_i}(1 - \hat{\pi}_k^{(s)})^{(1-z_i)}}.$$

In the M-step, we reestimate the probabilities π_j as weighted proportions using the weights, $w_{ij}^{(s+1)}$:

$$\hat{\pi}_j^{(s+1)} = \frac{\sum_i z_i w_{ij}^{(s+1)}}{\sum_i w_{ij}^{(s+1)}}.$$

We begin the algorithm by taking $w_{ij}^{(0)} = p_{ij}$ and iterate until the estimates converge, giving the MLE, $\hat{\boldsymbol{\pi}}$.

We next calculate a LOD score for the test of H_0: $\pi_j \equiv \pi$. First note that the MLE, under H_0, of the common probability π is the overall proportion, $\hat{\pi}_0 = \sum_i z_i / n$. Letting $\hat{\boldsymbol{\pi}}_0 = (\hat{\pi}_0, \hat{\pi}_0, \hat{\pi}_0)$, the LOD score is $\text{LOD} = \log_{10}\{L(\hat{\boldsymbol{\pi}})/L(\hat{\boldsymbol{\pi}}_0)\}$.

As with standard interval mapping, the likelihood under H_0 is calculated once, while the EM algorithm is performed at each position in the genome (in practice, at 1-cM steps), producing a LOD curve for each chromosome.

Two-part model: The two separate analyses described above suggest the following two-part, single-QTL model. We again consider n F_2 progeny and some fixed position in the genome as the location of a putative QTL. Let y_i, z_i, g_i, and $\boldsymbol{m}_i$ be defined as above, and again let $p_{ij} = \Pr(g_i = j|\boldsymbol{m}_i)$.

We assume that the $(\boldsymbol{m}_i, y_i, z_i)$ are mutually independent, that $\Pr(z_i = 1|g_i = j) = \pi_j$, and that $y_i|(g_i = j, z_i = 1) \sim \text{normal}(\mu_j, \sigma^2)$. In other words, the probability that an individual with QTL genotype j has the null phenotype is $1 - \pi_j$; if this individual's phenotype is nonnull, it follows a normal distribution with mean μ_j, depending on the QTL genotype, and with SD σ, independent of genotype.

This model contains seven parameters, $\boldsymbol{\theta} = (\pi_1, \pi_2, \pi_3, \mu_1, \mu_2, \mu_3, \sigma)$. The likelihood function is

$$L(\boldsymbol{\theta}) = \prod_i \sum_j p_{ij}(1 - \pi_j)^{1-z_i}\{\pi_j f(y_i; \mu_j, \sigma)\}^{z_i},$$

where $f(y; \mu, \sigma)$ is the density function for a normal distribution with mean μ and SD σ.

We may again obtain MLEs with a form of the EM algorithm. Assume at iteration $s + 1$ we have estimates $\hat{\boldsymbol{\theta}}^{(s)}$. In the E-step, we calculate weights for each individual and each genotype:

$$w_{ij}^{(s+1)} = \Pr(g_i = j|y_i, z_i, \boldsymbol{m}_i, \hat{\boldsymbol{\theta}}^{(s)}) = \begin{cases} \dfrac{p_{ij}(1 - \hat{\pi}_j^{(s)})}{\sum_k p_{ik}(1 - \hat{\pi}_k^{(s)})} & \text{if } z_i = 0 \\ \dfrac{p_{ij}\hat{\pi}_j^{(s)} f(y_i; \hat{\mu}_j^{(s)}, \hat{\sigma}^{(s)})}{\sum_k p_{ik}\hat{\pi}_k^{(s)} f(y_i; \hat{\mu}_k^{(s)}, \hat{\sigma}^{(s)})} & \text{if } z_i = 1. \end{cases}$$

In the M-step, we obtain revised estimates of the parameters according to the following equations:

$$\hat{\pi}_j^{(s+1)} = \frac{\sum_i w_{ij}^{(s+1)} z_i}{\sum_i w_{ij}^{(s+1)}}$$

$$\hat{\mu}_j^{(s+1)} = \frac{\sum_i y_i w_{ij}^{(s+1)} z_i}{\sum_i w_{ij}^{(s+1)} z_i}$$

$$\hat{\sigma}^{(s+1)} = \sqrt{\frac{\sum_i \sum_j (y_i - \hat{\mu}_j^{(s+1)})^2 w_{ij}^{(s+1)} z_i}{\sum_i z_i}}.$$

We again start the algorithm by taking $w_{ij}^{(0)} = p_{ij}$ and iterate until the estimates converge, producing the MLEs, $\hat{\boldsymbol{\theta}}$.

We may calculate a LOD score for the test of H_0: $\pi_j \equiv$

π, $\mu_j \equiv \mu$. We first note that, under H_0, the MLEs of the three parameters, π, μ, and σ, are

$$\hat{\pi}_0 = \frac{\sum_i z_i}{n}$$

$$\hat{\mu}_0 = \frac{\sum_i z_i y_i}{\sum_i z_i}$$

$$\hat{\sigma}_0 = \sqrt{\frac{\sum_i (y_i - \hat{\mu}_0)^2 z_i}{\sum_i z_i}}.$$

In other words, $\hat{\pi}_0$ is the proportion of individuals with a positive phenotype, and $\hat{\mu}_0$ and $\hat{\sigma}_0$ are the sample mean and SD, among individuals with positive phenotypes. Letting $\hat{\boldsymbol{\theta}}_0 = (\hat{\pi}_0, \hat{\pi}_0, \hat{\pi}_0, \hat{\mu}_0, \hat{\mu}_0, \hat{\mu}_0, \hat{\sigma}_0)$, the LOD score is LOD $= \log_{10}\{L(\hat{\boldsymbol{\theta}})/L(\hat{\boldsymbol{\theta}}_0)\}$.

Note that in the case of complete QTL genotype information (*i.e.*, when the putative QTL is at a marker that has been fully typed), the p_{ij} are all either 1 or 0, and the two parts of the model separate fully. As a result, the MLEs under the two-part model are exactly those obtained by the two separate analyses (the analysis of the binary trait and the conditional analysis of the quantitative trait, for those individuals with nonzero phenotype). Further, the LOD score for the two-part model is simply the sum of the LOD scores from the two separate analyses.

Nonparametric analysis: Kruglyak and Lander (1995) described an extension of the Wilcoxon rank-sum test for nonparametric interval mapping in a backcross. The rank-sum test is a nonparametric version of the two-sample *t*-test. In the case of an intercross, they suggested tests for the additive or dominant effects at a putative QTL. An alternative approach is to extend the Kruskal-Wallis test statistic, a nonparametric version of a one-way analysis of variance, for the comparison of two or more samples (*e.g.*, see Lehmann 1975). We describe such an extension below.

Rank the phenotypes, y_i, from 1, . . . , n, and let R_i denote the rank for individual i. In the case of ties, use the average rank within each group of ties. We again consider some fixed position in the genome as the location of a putative QTL and let $p_{ij} = \Pr(g_i = j|\boldsymbol{m}_i)$, the QTL genotype probabilities for individual i, given the available multipoint marker data. Whereas, in the Kruskal-Wallis test statistic, one considers the sum of the ranks within each group, here the exact assignment of individuals to QTL genotype groups is not known; rather, individual i has prior probability p_{ij} of belonging to group j. We follow the approach of Kruglyak and Lander (1995) and consider the expected rank sum, $S_j = \Sigma_i p_{ij} R_i$. We then consider the statistic

$$H = \sum_j \left(\frac{n - \sum_i p_{ij}}{n}\right)\left[\frac{(S_j - E_{0j})^2}{V_{0j}}\right],$$

where E_{0j} and V_{0j} are the mean and variance of S_j under the null hypothesis of no linkage, considering the p_{ij} as fixed. After some algebra, we obtain the formula

$$H = \frac{12}{n(n+1)} \sum_j \frac{(n - \sum_i p_{ij})(\sum_i p_{ij})^2}{n\sum_i p_{ij}^2 - (\sum_i p_{ij})^2}\left[\frac{\sum_i p_{ij} R_i}{\sum_i p_{ij}} - \frac{n+1}{2}\right]^2.$$

In the case that the putative QTL is at a fully typed genetic marker, the p_{ij} will all be 0 or 1, and the above statistic reduces to the Kruskal-Wallis test statistic.

Kruglyak and Lander (1995) had randomized any tied phenotypes, a reasonable approach in the case of very few ties. In our application, however, a large proportion of the individuals share a common phenotype. Thus, rather than randomizing ties, we assign the average rank to each individual within a set of tied phenotypes. A standard correction for the case of ties is to use the statistic $H' = H/D$, where $D = 1 - \Sigma_k(t_k^3 - t_k)/(n^3 - n)$, with t_k being the number of values in the kth group of ties. Note that if there are no ties, $D = 1$ and so $H' = H$. [Of course, if one uses a permutation test (Churchill and Doerge 1994) to obtain the genome-wide significance threshold, as we recommend, the correction factor is unnecessary.] As the nonparametric statistic H' follows, approximately, a χ^2 distribution under the null hypothesis of no linkage, we convert the statistic to the LOD scale by taking LOD $= H'/(2 \ln 10)$.

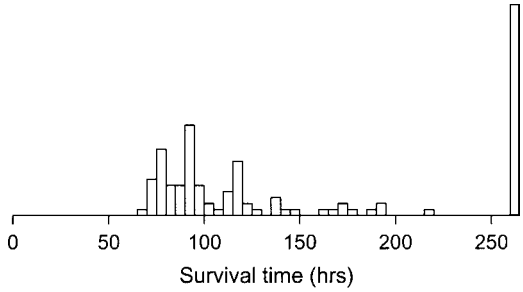

Figure 1.—Histogram of survival time, following infection with *Listeria monocytogenes*, of 116 intercross mice. Approximately 30% of the mice recovered from the infection and survived to the end of the experiment (264 hr).

EXAMPLE

To illustrate our methods, we consider the data of Boyartchuk *et al.* (2001), on the time to death following infection with *L. monocytogenes* in 116 F_2 mice from an intercross between the BALB/cByJ and C57BL/6ByJ strains. The mice were typed at 133 markers, including 2 on the X chromosome. A histogram of the survival times (in hours) appears in Figure 1. Note that ~30% of the mice recovered from the infection and survived to 264 hr.

We applied each of the four methods described above to these data: analysis of the binary trait, survived/died ("binary"); standard interval mapping with the log time to death, with only those mice that died ("QT"); use of

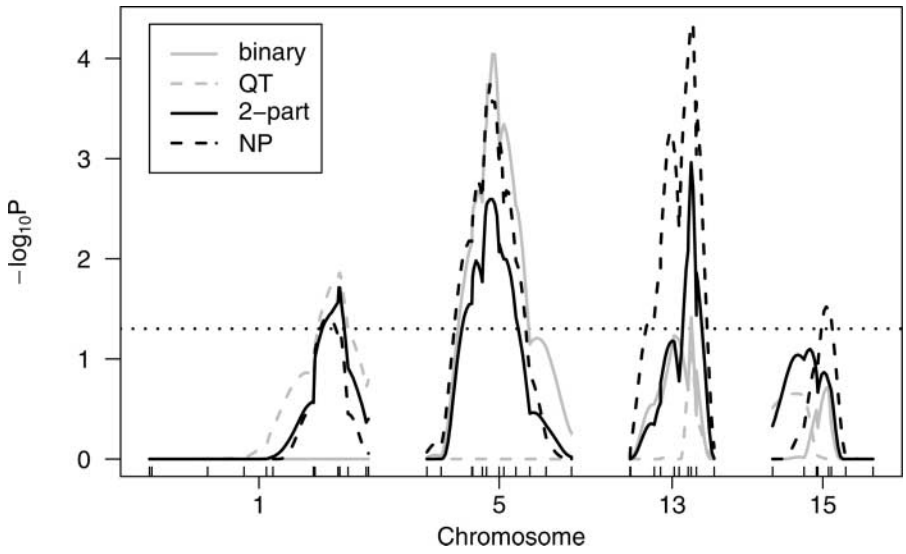

FIGURE 2.—Results of four QTL mapping methods for data on survival time following infection with *Listeria monocytogenes* in 116 intercross mice. The four methods are analysis of the binary trait, survived/died (binary); standard interval mapping using the log time to death for the nonsurviving individuals (QT); use of the two-part model (2-part); and nonparametric interval mapping (NP). LOD scores were converted to experiment-wise P values derived from permutation tests; $-\log_{10}P$ is plotted as a function of genomic position. A horizontal line is plotted at $-\log_{10}(0.05)$.

our two-part model ("two-part"); and the nonparametric interval-mapping method based on the Kruskal-Wallis test statistic ("NP").

Genome-wide LOD thresholds were obtained by permutation tests (CHURCHILL and DOERGE 1994), using 11,000 permutation replicates. The estimated 95% genome-wide LOD thresholds for the four methods, binary, QT, two-part, and NP, were 3.54, 3.96, 4.91, and 3.27, respectively. The estimated standard errors (SEs) for these thresholds were $\sim$0.02.

Because of the large differences in the LOD thresholds for the four methods, we converted the LOD curves to a common scale, the estimated experiment-wise P values derived from the permutation tests. The results indicated evidence for QTL on chromosomes 1, 5, 13, and 15. In Figure 2, the statistic $-\log_{10}P$ for each method is displayed for these selected chromosomes.

The locus on chromosome 1 appears to have an effect only on the average time to death among the nonsurvivors. The locus on chromosome 5 appears to have an effect only on the chance of survival. The loci on chromosomes 13 and 15 have an effect on both the chance of survival and the average time to death among nonsurvivors. Note that the locus on chromosome 15 achieved the 5% genome-wide significance level only with the nonparametric interval-mapping method.

SIMULATIONS

To better understand the relative performance of these approaches for QTL mapping in the case of a spike in the phenotype distribution, we performed a small simulation study. We first estimated the 95% genome-wide LOD threshold for each method, in the case of 250 intercross individuals with 25% having the null phenotype and an autosomal genome modeled after the genetic map for the mouse described in ROWE *et al.* (1994), consisting of 19 autosomes with total length 1300 cM. Genetic markers were equally spaced on each chromosome, with a marker spacing of 10–12 cM. (The intermarker spacing was slightly different for each chromosome, so that the chromosomal lengths could match those in the genetic maps of ROWE *et al.* 1994.) A random 10% of the marker genotype data was missing. We simulated a phenotype that was independent of the marker data. Each individual had probability 25% of having a null phenotype; otherwise their phenotype was drawn from a normal distribution with mean 10 and SD 1.

For each of 10,000 replicates, we simulated such data under the null hypothesis of no QTL, applied each of the four methods, and recorded the maximum LOD score, genome-wide, for each method. The 95th percentiles of the maximum LOD score, for the four methods, binary, QT, two-part, and NP, were 3.55, 3.53, 4.64, and 3.41, respectively. Note that the binary, QT, and NP methods have similar LOD thresholds. The LOD threshold for the two-part model is much higher, due to the fact that the corresponding statistical test concerns four free parameters, rather than two.

We also considered a fifth approach, in which one takes the maximum of the LOD scores from the binary and conditional quantitative trait analyses. For this approach, we used a Bonferroni correction and declared significant linkage if the LOD scores for either the binary trait analysis or the conditional quantitative trait analysis exceeded the corresponding 97.5% genome-wide LOD thresholds, which were estimated to be 3.88 and 3.86, respectively.

To investigate the power and precision of each of these methods, we simulated data under the two-part model described above, with a single QTL located between two markers near the center of chromosome 1 (of length 103 cM). The QTL was taken to have multiplicative effect ϕ_π on the probabilities π_j and additive effect Δ_μ on the conditional means μ_j. The probabilities, π_j, were chosen so that $\pi_2 = \phi_\pi\pi_1$ and $\pi_3 = \phi_\pi^2\pi_1$ and so

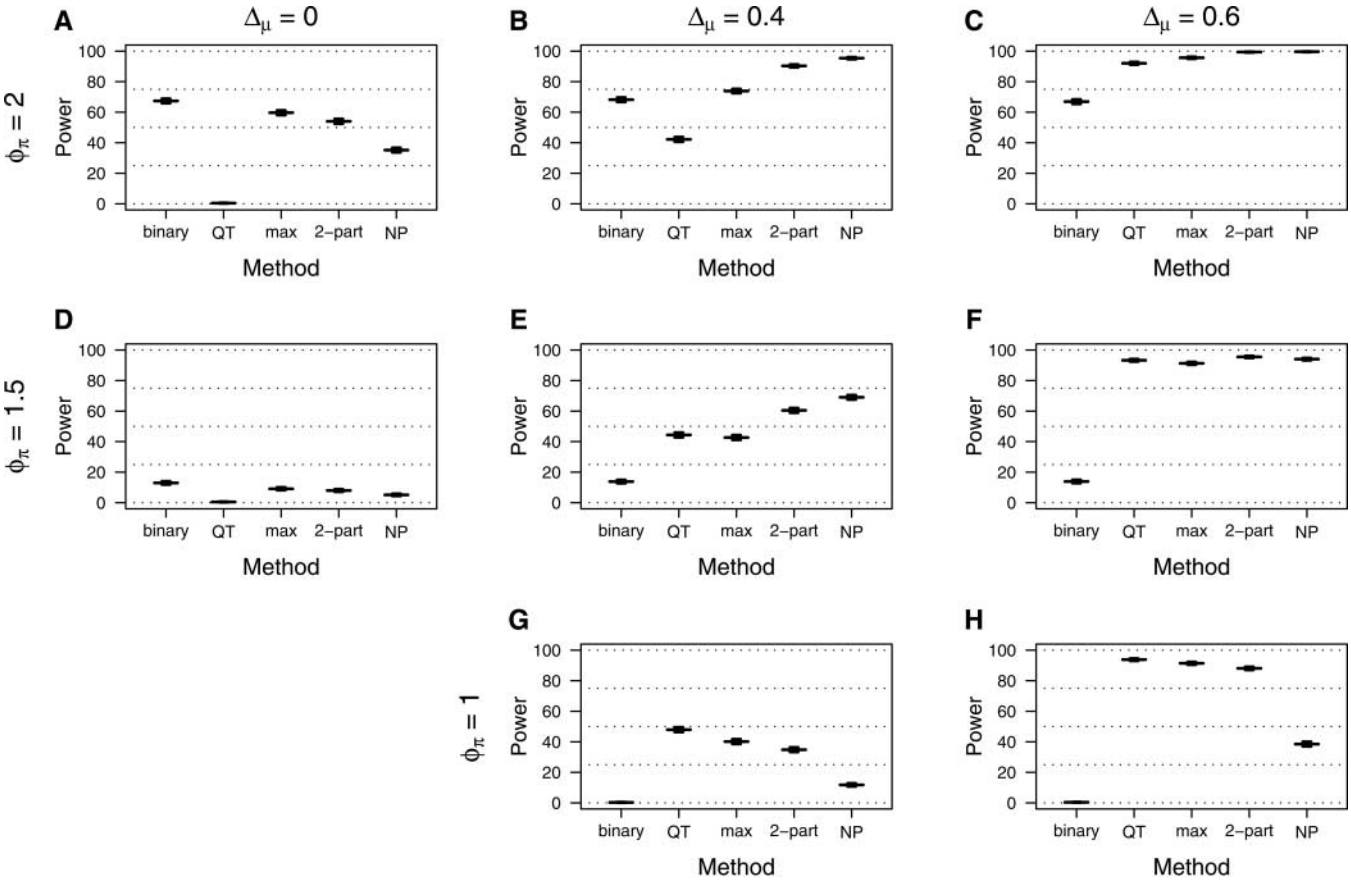

FIGURE 3.—Estimated power (±2 SE) to detect a QTL, based on 4000 simulation replicates. An intercross with 250 individuals was simulated, with an average of 25% of individuals having the null phenotype. The alleles at the QTL have an additive effect, Δ_μ, on the phenotype means, and a multiplicative effect, ϕ_π, on the probability of having a positive phenotype. Five analysis methods were studied: analysis of the binary phenotype (binary), standard interval mapping using only those individuals with a positive phenotype (QT), the maximum of the binary and conditional quantitative trait analyses (max), use of the two-part model (2-part), and a nonparametric analysis (NP).

that the overall proportion of individuals with positive phenotypes was $\pi_1/4 + \pi_2/2 + \pi_3/4 = 75\%$. The means were chosen so that $\mu_1 = \mu_2 - \Delta_\mu$ and $\mu_3 = \mu_2 + \Delta_\mu$, with $\mu_2 = 10$. The residual SD was $\sigma = 1$. We considered the values $\phi_\pi = 1$, 1.5, and 2 and $\Delta_\mu = 0$, 0.4, and 0.6. (Note that $\phi_\pi = 1$ and $\Delta_\mu = 0$ correspond to no QTL effect.)

We performed 4000 simulations of 250 intercross individuals, for all pairs of effects (ϕ_π, Δ_μ), except for the case $\phi_\pi = 1$, $\Delta_\mu = 0$. The latter corresponds to the null hypothesis of no QTL; simulations for this case were used to estimate the LOD thresholds (see above). In each case, we applied the four methods to the simulated data on chromosome 1 (containing the QTL), calculated the maximum LOD score on that chromosome, and finally calculated the power of each test, as the proportion of the simulation replicates for which the maximum LOD score exceeded the corresponding 95% genome-wide LOD threshold. The power of the fifth procedure, taking the maximum of the binary and conditional quantitative trait LOD scores, was estimated as the proportion of the 4000 replicates in which either the binary or the conditional quantitative trait LOD score exceeded its corresponding 97.5% genome-wide LOD threshold.

The estimated power of the procedures appears in Figure 3. In Figure 3, A and D, the QTL had effect only on the probabilities, π_j. In these cases, the conditional analysis of the quantitative trait had no power, and the analysis of the binary trait had the greatest power. The two-part model was somewhat inferior to the binary trait analysis, but had greater power than the nonparametric method. Use of the maximum of the binary and conditional quantitative trait LOD scores (with correction for the use of two tests) had somewhat greater power than the two-part model.

In Figure 3, G and H, the QTL had effect only on the conditional means, μ_j. In these cases, analysis of the binary trait had no power, and the conditional analysis of the quantitative trait had the greatest power. The results for the other methods were similar to the results in Figure 3, A and D: the two-part model was superior to the nonparametric method, but inferior to either the conditional quantitative trait analysis on its own or the

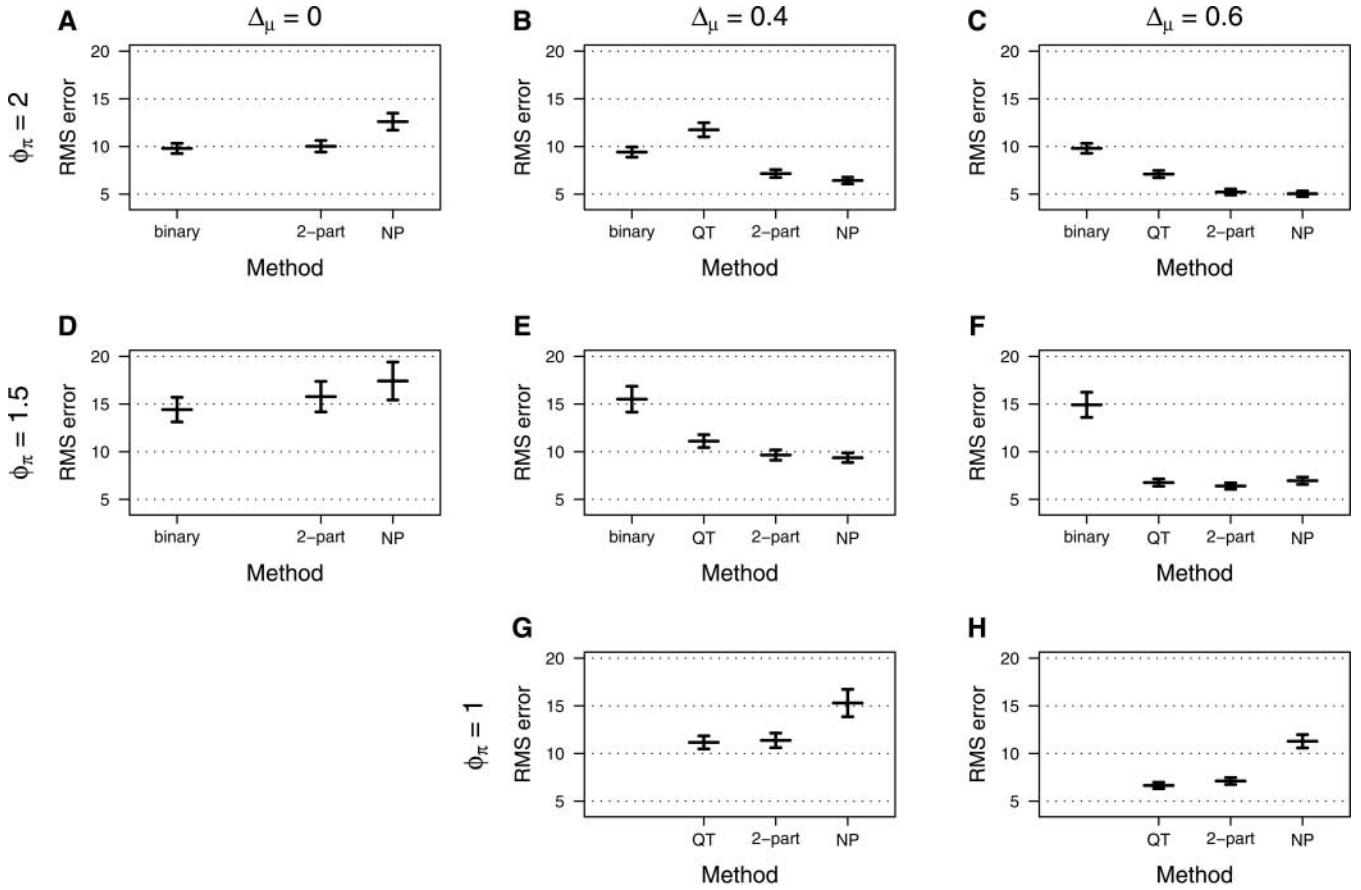

Figure 4.—Estimated root-mean-square (RMS) error (±2 SE) of the estimated QTL location, among simulation replicates showing significant evidence for the presence of a QTL.

maximum of the binary and conditional quantitative trait analyses.

In Figure 3, B, C, E, and F, the QTL had effect on both the probabilities, π_j, and the conditional means, μ_j. In these cases, the nonparametric method was best, although the use of the two-part model was competitive; both of these approaches showed considerable gains over either of the two separate analyses and over the maximum of the two separate analyses.

Figure 4 contains the results on the precision of QTL localization for the four basic methods. For each method and for each setting of the parameter values (ϕ_π, Δ_μ), the root-mean-square (RMS) of the error in the estimated QTL location, among simulation replicates in which there was significant evidence for the presence of a QTL (*i.e.*, in which the maximum LOD score exceeded the corresponding 95% genome-wide LOD threshold), was calculated. Results for the conditional quantitative trait analysis (QT) for Figure 4, A and D, and for the binary trait analysis for Figure 4, G and H, are not shown, since these methods have no power to detect a QTL with the corresponding parameter settings. The results in Figure 4 mirror those in Figure 3. The methods with the highest power have the greatest precision of QTL localization (*i.e.*, the smallest RMS error), while those with the lowest power have the lowest precision.

In summary, if a QTL has an effect only on the probabilities, π_j, or the conditional means, μ_j, greatest power to detect the QTL is obtained with the separate analysis of that aspect of the data. If a QTL has an effect on both the probabilities, π_j, and the conditional means, μ_j, the nonparametric method performed best. In all cases, analysis under the two-part model (with which the data were simulated) was second place, in terms of power. Note that further simulations, with 100 rather than 250 intercross individuals and with the proportion of individuals with the null phenotype taken to be 15 or 35% rather than 25%, gave qualitatively similar results (data not shown).

DISCUSSION

We have considered the problem of QTL mapping in the case of a spike in the phenotype distribution, a common departure from the usual normality assumption in standard interval mapping. Standard interval mapping works reasonably well when the spike is not too far from the rest of the phenotype distribution and contains only a small proportion of the individuals.

When the spike is well separated and contains an appreciable proportion of the data, maximum-likelihood estimation under a normal mixture model has a tendency to produce spurious LOD score peaks in regions of low genotype information (*e.g.*, widely spaced markers).

We developed a parametric, two-part model for QTL mapping in this situation and have described an extension of the Kruskal-Wallis test statistic for nonparametric interval mapping in the case of an intercross. These approaches serve to combine the analysis of the binary trait with the conditional analysis of the quantitative trait among individuals with positive phenotype.

The interpretation of the results of analysis with the two-part model may deserve further explanation. A QTL identified through the two-part model may influence the probability of having a nonnull phenotype or the average phenotype among individuals with positive phenotypic values or both. Inspection of the estimated QTL effects (the $\hat{\pi}_j$ and $\hat{\mu}_j$) or of the results of the separate binary and conditional quantitative trait analyses should assist in discriminating between these cases.

In our simulation results, most interesting was the comparison among the two-part model, the nonparametric method, and the maximum of the binary and conditional quantitative trait analyses. In the case that QTL have an effect on both the parameters π_j (the probability that an individual with QTL genotype j will have a positive phenotype) and μ_j (the conditional mean phenotype, among individuals with positive phenotype and QTL genotype j), the nonparametric approach was seen to have greater power than analysis under the two-part model; this is largely due to the fact that the genome-wide LOD threshold is considerably larger for the latter method. In the case that QTL have an effect on only the π_j or only the μ_j, the maximum of the separate analyses will have greatest power, and the nonparametric method will have the least power. Thus, analysis under the two-part model is always second best. On the other hand, the overall average power, across the eight parameter settings considered herein, was greatest for the two-part model. Further, the parametric, two-part model may be more useful in consideration of multiple-QTL models.

Thus, while nonparametric interval mapping is a valuable general method, analysis under the two-part model may be preferred for the situation considered here. The extensions of the two-part model for use with multiple QTL (for example, by combining a logistic model for the probabilities with a linear model for the conditional means) deserve exploration.

The methods described in this article have been implemented in the QTL mapping software, R/qtl (http://www.biostat.jhsph.edu/~kbroman/qtl), an add-on package for the general statistical software, R (Ihaka and Gentleman 1996).

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