A Unified Semiparametric Framework for Quantitative Trait Loci Analyses, With Application to Spike Phenotypes

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This article proposes a general semiparametric model for multiple quantitative trait loci (QTL) analyses of complex phenotypes in backcross and intercross designs. The model provides tests about genetic hypotheses, such as additivity, dominance, and epistasis, that do not require specifying the form of the phenotypic distribution. This contrasts with previous approaches based on transformations to normality and generalized linear models, which require careful consideration of the phenotypic distribution. Inferences involve extensions of partial and conditional likelihoods developed for single-QTL backcross models. We demonstrate that conditional likelihood is robust to unobserved selective genotyping, whereas partial likelihood and other standard methods are not. To facilitate genome screens, a novel resampling method is proposed that is similar in spirit to the popular permutation tests. Its main advantages are that it is broadly applicable to multiple QTLs with nonnormal phenotypes and achieves a substantial reduction in computational burden. A thorough case study of spike data on the genetic influences to recovery from *Listeria* infection in a mouse intercross experiment is presented. The application reveals that the proposed methods may give substantively different conclusions than those obtained with existing interval mapping methods from parametric models in the presence of unobserved selection.

KEY WORDS: Biased sampling; Epistasis; Exponential tilt model; Genomewide testing; Nonnormal phenotypes; Pseudolikelihood; Resampling.

1. INTRODUCTION

1.1 Overview of Quantitative Trait Loci Analyses

Quantitative trait loci (QTL) analysis is an important tool for dissecting the genetic influences on biological traits. Such analysis is critically important in plant and animal breeding, as well as in understanding the etiology of human diseases. The goal is to determine associations between genetic variability at known locations on chromosomes with variability in the observed traits, also known as phenotypes, to pinpoint the locations of genes controlling the phenotypes. Typically, genotypes are obtained at a large number of locations (referred to as markers) throughout the genome and separate analyses may be done at each location. Complex models may also be fit that permit interactions between genes. A fundamental challenge in QTL analysis is developing methods appropriate for study designs that vary widely in natural and experimental populations.

The focus of this article is experimental populations (Doerge, Zeng, and Weir 1997; Broman and Speed 1999). In such studies, inbred lines are systematically mated to obtain progeny for evaluating the genotype–phenotype associations. Such designs may have increased power relative to natural populations, where natural genetic variability may obscure the associations. The designs are staples in agriculture and animal breeding, where the objective is to manipulate profitable traits. They are also used to investigate human diseases (e.g., diabetes and cancer) in which the homology between animal models (e.g., like rats and mice), and humans may be exploited.

The analysis of experimental populations was first considered by Sax (1923), who proposed t-tests for phenotypic means of different genotype groups at a known marker. This idea was generalized by Lander and Botstein (1989) to interval mapping at loci between known markers, where genotypes are not observed, and simple t-tests may not be appropriate. A likelihood analysis was used, assuming a normally distributed phenotype and a single-gene model. When the assumptions hold, the analysis gives efficient detection and localization of QTLs (Haley and Knott 1992; Jansen 1993; Zeng 1993, 1994; Jansen and Stam 1994; Kao, Zeng, and Teasdale 1999; Sillanpää and Corander 2002). However, for complex traits influenced by multiple and/or interacting QTLs, the single-gene normal models may be inadequate.

An example is the so-called "spike" phenotype (Broman 2003), in which the distribution has a point mass at a single value, the "spike," but is otherwise continuous. Hence the trait has discrete and continuous components, which may have different genetic controls. Such phenotypes arise in cancer studies, in which tumor size has positive probability at zero (absence of tumor). Another example is survival outcomes, in which individuals may never experience the event of interest and may be viewed as "cured" (see Farewell 1977) with event time equaling infinity. Boyartchuk et al. (2001) obtained the death times after *Listeria* infection in a mouse breeding experiment. A large percentage of animals recovered fully and were alive at the end of the study. Their "death" times were set equal to the last follow-up time. Figure 1 provides a histogram of the times; note the "spike" at hour 264.

Broman (2003) studied two approaches to this data. The first approach used standard interval mapping, without decoupling the continuous and discrete components of the trait; the second used standard interval mapping on the continuous component and a binary regression model for the "spike," corresponding to the "cure" probability. This example illustrates the difficulties with nonnormal traits: The analyses depend on the trait being analyzed, and careful consideration of the choice of parametric models is required. This is particularly true with multiple-gene models, where formulating gene interactions depends heavily on the model specification. In this article we use the *Listeria*

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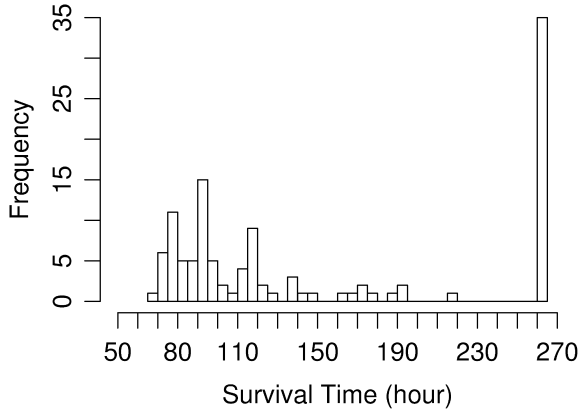

Figure 1. Histogram of the Survival Time of the 116 F2 Female Mice After Infection by Listeria monocytogenes.

dataset as a case study for our proposed methodology, which provides a unified framework for QTL analyses.

1.2 A Unifying Framework

Directly applying traditional QTL mapping strategies using a normal model may lead to low power or false detection of a major locus (Morton and Gray 1984). Specially tailored single-gene models are available, including Poisson (Shepel et al. 1998) and logistic (Mackay and Fry 1996; Yi and Xu 2002) regression. Alternatively, a transformation may be used to normalize the phenotype (Lan et al. 2001). These two approaches are not automatic; there may be no obvious choice of distribution and no natural transformation to normality, particularly with discrete traits. Generalizing to nonnormal traits with multiple QTLs involves customizing the single-QTL models and depends critically on the phenotypic distribution. Nonparametric Wilcoxon rank statistics (Kruglyak and Lander 1995) and Jonckheere–Terpstra tests (Poole and Drinkwater 1996) provide model-free analyses for single-QTL mapping; however, extending these statistics to multiple potentially interacting QTLs is not straightforward.

Zou, Fine, and Yandell (2002) proposed an interesting semiparametric alternative to standard QTL analyses with a single gene. The log ratio of the phenotypic density functions is assumed to satisfy a linear model across genotypes with unspecified baseline density. This exponential tilt model (Anderson 1979) subsumes a range of distributions, including normal, Poisson, binomial, and exponential. In Section 2 we study general phenotypes with multiple genes. The standard models of genetic variability (Falconer and Mackay 1996) for generalized linear models (McCullagh and Nelder 1989), including dominance and additive effects and epistasis between two loci, can be greatly simplified under this formulation. Tests of genetic hypotheses in the generalized linear model reduce to tests of parameters in the tilt model that are common to all link functions. The baseline phenotypic distribution is unspecified, which avoids the need to choose the link function in the generalized linear model or a transformation to normality in standard interval mapping. For the *Listeria* data, the form of the model is the same for the "spike," the continuous component of the trait, and the combined trait.

Zou et al. (2002) estimated a single-gene tilt model for a simple backcross design using a so-called "partial likelihood" from a constrained empirical likelihood (Qin 1999). Later, Zou and Fine (2002) demonstrated that the partial likelihood was closely connected to the conditional distribution of the genotype given the phenotype. In Section 3.3 we further show that the conditional likelihood is robust to selective genotyping bias, when individuals lacking genotypes are omitted. These results are similar to those for case-control studies with selective sampling of diseased and nondiseased individuals (Breslow and Day 1980). In the QTL setup, the genotype is unknown between markers, and the selective sampling is much more subtle. The issue is practically important, because biased sampling of genotypes is a common QTL design strategy for increasing power (Lander and Botstein 1989). Such sampling may also result from an unobserved selection process, which can lead to reduced power or bias in estimates of QTL location and effect sizes. In the *Listeria* analysis in Section 6, the conditional likelihood may give different conclusions than partial likelihood and other standard QTL analyses.

Working in a single-QTL backcross design, Zou et al. (2002) and Zou and Fine (2002) established that the estimators of QTL effects from the partial and conditional likelihoods are consistent and asymptotically normal, and that the partial likelihood is more efficient than the conditional likelihood. Under the null hypothesis of no QTL, the log partial likelihood ratio tests follow chi-squared distributions with 1 degree of freedom, asymptotically. In Section 3 we extend these inferences to multiple-gene models and more complicated breeding designs.

1.3 Genome-Wide Testing

For genome-wide analyses using backcross data, Kruglyak and Lander (1995) showed that interval mapping tests can be approximated by an Ornstein–Uhlenbeck process, with genome-wide thresholds based on its extreme value properties. With multiple-QTL models, the limiting process may not be analytically tractable. An alternative is permutation testing (Churchill and Doerge 1994), which avoids strong assumptions on the marker map and gives exact p values. Unfortunately, it is only suitable for testing single gene models. For multiple QTL, Doerge and Churchill (1996) proposed the conditional empirical threshold (CET) and the residual empirical threshold (RET), both based on large-sample theory. CET is restricted to genome regions unlinked to major QTLs. RET assumes a linear model and performs repermutation after removing previously detected QTL effects. If the model is misspecified or there are gene interactions, then the method is not applicable.

Even when CET and RET are applicable, their computational burden has hindered usage. For a genome of 2000cM, single-QTL testing with 1,000 permutations fits 1 million models in a 2-cM scan. In Section 4 we propose a new resampling method to approximate the genome-wide large sample distribution of our statistics. The method only requires fitting the model once at each locus. It is easily extended to focused genetic hypotheses, like multiple QTL, additivity, and dominance, which are not completely addressed by permutation methods.

The practicability of the exponential tilt model analyses and the new resampling technique are demonstrated in extensive simulations reported in Section 5. Numerical comparisons with available analyses demonstrate improved robustness to model misspecification and improvements in computational efficiency.

In Section 6 we present an analysis of the *Listeria* data using our unified QTL framework and other existing methods. Both single- and double-QTL genome screens are presented, with substantive differences in the results from the different analyses. A discussion of the key issues in analyzing complex genetic architectures concludes in Section 7.

2. MODELING MULTIPLE QUANTITATIVE TRAIT LOCI WITH EXPONENTIAL TILT MODEL

We consider experiments that have two completely inbred parental lines, labeled P1 and P2. Individuals from P1 are homozygous with genotype AA at all loci in the genome, and those from P2 have genotype aa at all loci. These lines are mated, and their F1 progeny are heterozygous Aa at all loci. F1 individuals may be crossed with P1 or P2 to generate a backcross BC1 or BC2. In the sequel, BC always refers to BC2. An F2 population is generated by selfing or mating two F1 individuals (Doerge et al. 1997).

Because of recombination, the genotype at a putative locus between two flanking markers may be unknown. The phenotypic distribution at that locus is a mixture over the possible genotypes. The mixing proportions denote the genotype probabilities conditioned on flanking markers and are determined by a genetic mapping function. The component densities correspond to phenotypic distributions modeled conditionally on genotype at the putative locus. Lander and Botstein (1989) assumed normal distributions with common variance. Here we extend the semiparametric exponential tilt model of Zou et al. (2002) to model phenotype distributions in BC or F2 populations with one or more QTL. We begin with one putative gene to motivate the model specification for two QTL given later, which is more complex.

Let genotypes be represented through q, the number of a alleles. An F2 population has AA, Aa, and aa genotypes, which correspond to $q = 0, 1, 2$. We denote the component densities by $f_q(z)$ and assume that $f_q(z) = f_0(z)\exp(\beta_{0q} + \beta_{1q}z)$, where $\exp(\beta_{0q}) = \{\int f_0(z)\exp(\beta_{1q}z)\}^{-1}$ is the normalizing constant for $f_q(z)$. Including β_{0q} as a separate parameter follows the work of Anderson (1979) and Qin (1999). Without additional parameter constraints, the tilt model is overparameterized. We let $\beta_{00} = \beta_{10} = 0$, yielding an identifiable model.

The tilt model covers the most commonly used continuous and discrete distributions, including normal with common variance, exponential, Poisson, and binomial (Anderson 1979). In standard QTL analysis, densities associated with AA, Aa and aa are $N(\mu + a, \sigma^2)$, $N(\mu + d, \sigma^2)$, and $N(\mu - a, \sigma^2)$ (Falconer and Mackay 1996), where a is the additive effect, d is the dominance effect, μ is the intercept, and σ^2 is the variance, assumed common across genotypes. The genetic effects, a and d, may be formulated for nonnormal distributions using the generalized linear model (GLM) (McCullagh and Nelder 1989). Table 1 relates the tilt parameters (β_{0q}, β_{1q}) to the QTL effects in the most commonly used GLMs.

Table 1 shows that tests about a and d can be based on the exponential tilt model without specifying the link function in the GLM. The same contrasts on the tilt model parameters are used for normal with common variance, exponential, Poisson, and binomial. To be specific, regardless of f_0, inference on either the global null of no QTL effect or on genetic subhypotheses can be made by testing the following contrasts:

1. H_0: no QTL influence $(a = d = 0) \Leftrightarrow H_0 : \beta_{11} = \beta_{12} = \beta_{01} = \beta_{02} = 0$
2. H_0: no additive effect $(a = 0) \Leftrightarrow H_0 : \beta_{12} = \beta_{02} = 0$
3. H_0: full dominance $(a = d) \Leftrightarrow H_0 : \beta_{11} = \beta_{01} = 0$
4. H_0: no dominance effect $(d = 0) \Leftrightarrow H_0 : \beta_{12} + \beta_{10} - 2\beta_{11} = 0$.

This "unifying" formulation is not possible using existing methods, which require correct specification of the distribution or link function for the GLM (Hackett and Weller 1995; Visscher, Haley, and Knott 1996; Xu and Atchley 1995; Rebai 1997).

We now generalize the tilt model to two QTL, allowing for epistasis. Let $\mathbf{q} = (q_1, q_2)$ denote the genotypes of the two QTL, with q_i the number of P2 alleles at the ith QTL; for example, genotype AABB has $\mathbf{q} = (q_1, q_2) = (0, 0)$ and AaBb has $\mathbf{q} = (1, 1)$. Although our setup is quite general, the form of the model is identical to that for BC and single-QTL F2: $f_q(z) = f_0(z)\exp(\beta_{0q} + \beta_{1q}z)$. Here we set $(\beta_{0q}, \beta_{1q}) = (0, 0)$ for model identifiability. The corresponding GLM formulation includes additive effects (a_1, a_2), dominance effects (d_1, d_2), and additive–additive (a_{12}), dominance–dominance

Table 1. Exponential Tilt Model Representation for Single-QTL, F2 Cross

	Genotype	*GLM representation*	β_{0q}	β_{1q}
Normal	AA	$N(\mu + a, \sigma^2)$	0	0
	Aa	$N(\mu + d, \sigma^2)$	$\frac{(a-d)(2\mu+a+d)}{2\sigma^2}$	$(d-a)/\sigma^2$
	aa	$N(\mu - a, \sigma^2)$	$\frac{2a\mu}{\sigma^2}$	$(-2a)/\sigma^2$
Exponential	AA	$E(\alpha, \mu + a)$	0	0
	Aa	$E(\alpha, \mu + d)$	$\log\frac{\mu+d}{\mu+a} - \alpha(a-d)$	$a-d$
	aa	$E(\alpha, \mu - a)$	$\log\frac{\mu-a}{\mu+a} - \alpha(2a)$	$2a$
Poisson	AA	$P(\exp(\mu + a))$	0	0
	Aa	$P(\exp(\mu + d))$	$\exp(\mu+a) - \exp(\mu+d)$	$d-a$
	aa	$P(\exp(\mu - a))$	$\exp(\mu+a) - \exp(\mu-a)$	$-2a$
Binomial	AA	$B(\frac{\exp(\mu+a)}{1+\exp(\mu+a)}, n)$	0	0
	Aa	$B(\frac{\exp(\mu+d)}{1+\exp(\mu+d)}, n)$	$n\log\frac{1+\exp(\mu+a)}{1+\exp(\mu+d)}$	$d-a$
	aa	$B(\frac{\exp(\mu-a)}{1+\exp(\mu-a)}, n)$	$n\log\frac{1+\exp(\mu+a)}{1+\exp(\mu-a)}$	$-2a$

NOTE: In the GLM representation, the mean conditionally on the genotype, q, denoted μ_q, is related to (μ, a, d) through a link function h, which equals identity for normal, N, inverse for exponential, E, log for Poisson, P, and logit for binomial, B. The model is $h(\mu_q) = \mu + a + d$. Note that the exponential density includes a shift parameter α and a rate parameter.

Table 2. Equivalent Null Hypotheses for the GLM and Tilt Model for Two-QTL Analysis in F2

GLM H_0	*Tilt model H_0*
No QTL	all $\beta = 0$
$a_1 = 0$	$\beta_{100} + \beta_{102} - \beta_{120} - \beta_{122} = 0$
$a_2 = 0$	$\beta_{100} + \beta_{120} - \beta_{102} - \beta_{122} = 0$
$d_1 = 0$	$2\beta_{110} - \beta_{100} - \beta_{120} + 2\beta_{112} - \beta_{102} - \beta_{122} + 4\beta_{111} - 2\beta_{101} - 2\beta_{121} = 0$
$d_2 = 0$	$2\beta_{101} - \beta_{100} - \beta_{102} + 2\beta_{121} - \beta_{120} - \beta_{122} + 4\beta_{111} - 2\beta_{110} - 2\beta_{112} = 0$
$a_{12} = 0$	$\beta_{100} + \beta_{122} - \beta_{102} - \beta_{120} = 0$
$d_{12} = 0$	$\beta_{100} + \beta_{102} + \beta_{120} + \beta_{122} - 2\beta_{112} - 2\beta_{110} - 2\beta_{121} - 2\beta_{101} + 4\beta_{111} = 0$
$ad_{12} = 0$	$(\beta_{120} + \beta_{122} - 2\beta_{121}) - (\beta_{100} + \beta_{102} - 2\beta_{101}) = 0$
$da_{12} = 0$	$(\beta_{102} + \beta_{122} - 2\beta_{112}) - (\beta_{100} + \beta_{120} - 2\beta_{110}) = 0$

NOTE: The equivalence holds for normal, exponential, Poisson, and binomial GLM. Conditionally on genotype at two genes, $h(\mu_q) = \mu + a_1 + a_2 + d_1 + d_2 + a_{12} + d_{12} + ad_{12} + da_{12}$. Subscripts i and j on β_{1ij} denote genotype $\mathbf{q} = (i, j)$, or $q_1 = i$ and $q_2 = j$ at loci 1 and 2, respectively.

(d_{12}), and additive–dominance interactions (ad_{12}, da_{12}) (Falconer and Mackay 1996). The contrasts of tilt model parameters needed to test genetic hypotheses specified using the GLM parameters are given in Table 2. Similar to Table 1, inferences can be obtained through the same contrasts of the β's regardless of the link function in the GLM and f_0.

3. INFERENCE USING PARTIAL AND CONDITIONAL LIKELIHOOD

We now discuss inferences for the genetic hypotheses presented in Section 2. In Section 3.1 we establish that the full empirical likelihood for the tilt model has an irregularity arising because the densities f_q are constrained to integrate to 1. In Section 3.2 we discuss alternative analyses based on partial and conditional likelihood that do not enforce these constraints and provide valid tests of the hypotheses. In Section 3.3 we address the robustness of the analyses to selective sampling of either genotypes or of phenotypes.

3.1 Empirical Likelihood Irregularity: Intercross/Multiple QTLs

Inference on the tilt model parameters uses a general mixture model framework. Let T_i be the unobserved mixture indicator for the ith observation, z_i. The density of z_i, conditional on $T_i = q, f_q$, is assumed to satisfy the tilt model. In practice, we observe only $M_i = 1, \ldots, K$, indicating the mixture from which z_i arises. The corresponding mixing probabilities for z_i conditionally on $M_i = k$ are $\tau_{qk} = \Pr(T_i = q | M_i = k)$, which are assumed known. Under the tilt model for z_i conditionally on T_i, the density for z_i conditionally on $M_i = k$ is $f(z_i | M_i = k) = f_0(z_i)\omega(z_i, k, \boldsymbol{\beta})$, where $\omega(z_i, k, \boldsymbol{\beta}) = \sum_q \tau_{qk} \exp(\beta_{0q} + \beta_{1q} z_i)$. This is also a tilt model, involving the original tilt model parameter $\boldsymbol{\beta}$ and the known mixing probabilities τ_{qk}.

In the QTL application, z_i is the value of the phenotype, T_i is the unobserved genotype at the locus of interest, and M_i is the observed genotype at the markers flanking the loci of interest. The distribution of phenotype conditionally on observed flanking marker genotypes must mix over the distribution of the unobserved genotype at the loci of interest, situated in the interval(s) between the flanking markers. The mixing probabilities τ_{qk} are determined by the breeding design, the known marker map, and the mapping function (e.g., Haldane 1919).

With complete data, we observe $(z_i, M_i), i = 1, \ldots, n$. The likelihood for z_i given M_i is $L(\boldsymbol{\beta}, f_0) = \prod_{i=1}^n f_0(z_i) \prod_{i=1}^n \omega(z_i, M_i, \boldsymbol{\beta})$. Unconstrained maximization of this likelihood does not yield valid inferences for $\boldsymbol{\beta}$. Similar to the approach of Qin (1999), we first maximize $L(\boldsymbol{\beta}, f_0)$ over f_0 for fixed $\boldsymbol{\beta}$ under the constraint that $f_0 \in C_\beta = \{f_0 | f_0(z_i) \geq 0, \sum_{i=1}^n f_q(z_i) = \sum_{i=1}^n p_i \exp(\beta_{0q} + \beta_{1q} z_i) = 1\}$, where $p_i = f_0(z_i)$, which ensures that f_q are all proper density functions. This yields a profile likelihood in β that may be maximized to obtain estimates for $\boldsymbol{\beta}$. Qin (1999) used standard empirical likelihood theory to establish the validity of the resulting inferences.

Adapting the approach of Zou et al. (2002) for single-gene backcross models with $q = 2$ and $k = 4$, we find that the profile likelihood has an irregularity with either intercross design or multiple QTL under the hypotheses in Section 2. The problem is that the profile likelihood may not exist for all $\boldsymbol{\beta}$'s in a neighborhood around the null values of $\boldsymbol{\beta}$, as required for the usual empirical likelihood results. The difficulty arises because there is always a $\boldsymbol{\beta}$ in the neighborhood such that no f_0 satisfies the constraints in C_β. To illustrate, consider the case where $q = 2$, as in BC. If the null $f_1 = f_2$ holds with $\beta_{02} = \beta_{12} = 0$, then there is no f_0 in C_β with $\beta_{02} \neq 0$ and $\beta_{12} = 0$. The issue is that if $\beta_{12} = 0$, then f_2 sums to 1 only if $\beta_{02} = 0$. Thus the profile likelihood does not exist on the line $\beta_{02} \neq 0, \beta_{12} = 0$, which is contained in all neighborhoods of $(0, 0)$.

The irregularity is stated in general terms in the following theorem.

Theorem 1 (Extension of thm. 1 of Zou et al. 2002). Let $\boldsymbol{\beta}_T$ denote the true value of $\boldsymbol{\beta}$. Then the following results hold:

a. The set C_β is not empty $\Leftrightarrow \boldsymbol{\beta} \in J_n(y)$, with $J_n(y) := \{\boldsymbol{\beta} | \min_i(\beta_{0q} + \beta_{1q} z_i) \leq 0 \leq \max_i(\beta_{0q} + \beta_{1q} z_i)$ and $\min_i(\beta_{0q} - \beta_{0q^*} + (\beta_{1q} - \beta_{1q^*}) z_i) \leq 0 \leq \max_i(\beta_{0q} - \beta_{0q^*} + (\beta_{1q} - \beta_{1q^*}) z_i)$, and $q \neq q^*, q, q^* = 0, 1, 2\}$.

b. $\beta_T \in J_n(y)$.

c. If $\beta_{1qT} = \beta_{1q^*T}$, or $\beta_{1qT} = 0$, $q \neq q^*$, then there exists no neighborhood $N(\boldsymbol{\beta}_T)$ of $\boldsymbol{\beta}_T$, s.t. $\forall \beta \in N(\boldsymbol{\beta}_T), \boldsymbol{\beta} \in J_n(y)$ as $n \to \infty$.

d. If the condition in (c) is not true, then such a neighborhood $N(\boldsymbol{\beta}_T)$ exists.

The irregularity occurs under all genetic hypotheses in Tables 1 and 2, not only the global null as in the work of Zou et al. (2002). This means that the techniques of Qin (1999) cannot be used to address such biological questions in QTL applications involving intercross or multiple QTLs.

3.2 Extensions of Partial and Conditional Likelihood

It can be shown that the profile empirical log-likelihood for general tilt models is $l[\boldsymbol{\beta}, \tilde{\alpha}(\boldsymbol{\beta})] = l_1[\boldsymbol{\beta}, \tilde{\alpha}(\boldsymbol{\beta})] + l_2(\boldsymbol{\beta}) - n\log n$. The first part involves $\tilde{\alpha}$, a nuisance parameter needed to enforce the density constraints on f_q. The so-called "partial likelihood," $l_2(\boldsymbol{\beta}) = -\sum_{i=1}^n \log\{r(z_i, \boldsymbol{\beta})\} + \sum_{i=1}^n \log\{\omega(z_i, M_i, \boldsymbol{\beta})\}$, where $r(z, \boldsymbol{\beta}) = \sum_q \xi_q \exp(\beta_{0q} + \beta_{1q} z_i)$ and $\xi_q = \sum_k \frac{n_k}{n} \tau_{qk}$, is free of these constraints. Because of difficulties associated with the constraints, Zou et al. (2002) and Zou and Fine (2002) investigated inferences about $\boldsymbol{\beta}$ from the partial likelihood. Because l_2 does not involve f_0, such inferences do not enforce the constraints, thereby avoiding the irregularity.

We now generalize to intercross and multiple QTLs. If (z_i, M_i) are independent and identically distributed, $i = 1, \ldots, n$, then the log-conditional likelihood for M_i given z_i is

$$l_c(\boldsymbol{\beta}) = -\sum_{i=1}^n \log\{R_c(z_i, \boldsymbol{\beta})\} + \sum_{i=1}^n \log\{\omega(z_i, M_i, \boldsymbol{\beta})\} + \sum_{k=1}^K n_k \log(\rho_k),$$

where $\rho_k = \Pr(M_i = k)$, $n_k = \sum_{i=1}^n I(M_i = k)$, $R_c(z_i, \boldsymbol{\beta}) = \sum_{k=1}^K \omega(z_i, M_i = k, \boldsymbol{\beta})\rho_k$, and $I(\cdot)$ is the indicator function. This likelihood uses the conditional distribution of flanking marker genotype given phenotype, unlike the usual QTL analysis, in which the distribution of phenotype given flanking marker genotype is used. Observe that l_c does not depend on f_0 and that $l_c(\boldsymbol{\beta}, \hat{\boldsymbol{\rho}}) = l_2(\boldsymbol{\beta}) + c$, where $\hat{\boldsymbol{\rho}} = (\hat{\rho}_1, \ldots, \hat{\rho}_K) = (n_1/n, \ldots, n_K/n)$ is the maximum likelihood estimator for ρ from the full likelihood for (z_i, M_i). Thus $l_2(\beta)$ is a pseudolikelihood in the sense used by Gong and Samaniego (1981), in that the nuisance parameter ρ is estimated separately from $\boldsymbol{\beta}$ and plugged into the conditional likelihood.

Following Gong and Samaniego (1981, thms. 2.1 and 2.2), the maximum pseudolikelihood estimator $\hat{\boldsymbol{\beta}}_p = \arg\max_{\boldsymbol{\beta}} l_2(\boldsymbol{\beta})$ is consistent and asymptotically normal, because $\hat{\boldsymbol{\rho}} - \boldsymbol{\rho} = O_p(1/\sqrt{n})$. According to Liang and Self (1996, props. 1 and 2), pseudolikelihood ratio statistics follow weighted sums of χ_1^2 distributions in large samples, where the weights depend on the true parameter values. In a backcross, the distribution reduces to χ_1^2 (Zou et al. 2002), but the weights are unknown with F2 and multiple QTL. We summarize our theoretical results for partial likelihood (with $'$ denoting first derivative, and $''$ denoting second derivative).

Theorem 2 (Extension to thm. 2, part 1 of Zou et al. 2002). Assume that all functions are locally bounded and integrable. Then the following results hold:

a. $\hat{\boldsymbol{\beta}}_p$ is consistent in probability for $\boldsymbol{\beta}_T$. Furthermore, $\sqrt{n}(\hat{\boldsymbol{\beta}}_p - \boldsymbol{\beta}_T) \to \mathrm{N}(\mathbf{0}, \boldsymbol{\Sigma})$, where $\boldsymbol{\Sigma} = \mathbf{S}_p^{-1}\mathbf{V}_p\mathbf{S}_p^{-1}$, $\mathbf{S}_p = E\{n^{-1} \times l_2''(\boldsymbol{\beta}_T)\}$, and $\mathbf{V}_p = n^{-1}\operatorname{var}\{l_2'(\boldsymbol{\beta}_T)\}$. The matrix $\mathbf{S}_p$ and $\mathbf{V}_p$ can be consistently estimated by $\hat{\mathbf{S}}_p = n^{-1}\{(l_2''(\hat{\boldsymbol{\beta}}_p)\}^{-1}$, $\hat{\mathbf{V}}_p = n^{-1}\sum_{i=1}^n \hat{Q}_{pi}^{\otimes 2}$ evaluated at $\boldsymbol{\beta}_p = \hat{\boldsymbol{\beta}}_p$, where $\hat{Q}_{pi}$ is defined in the Appendix. $\boldsymbol{\Sigma}$ is estimated by $\hat{\boldsymbol{\Sigma}} = \hat{\mathbf{S}}_p^{-1}\hat{\mathbf{V}}_p\hat{\mathbf{S}}_p^{-1}$.

b. (1) Under H_0, no QTL is present, $2LRT \to \chi_{p/2}^2$, and p is the number of unknown parameters.

(2) Under "subhypotheses," $2LRT \to$ a mixture of χ^2's. For example, in single-QTL model for F2 population, testing no additive effect, $H_0: \beta_{02} = \beta_{12} = 0$, $2LRT \to c_1(\boldsymbol{\beta}_T)\chi_1^2 + c_2(\boldsymbol{\beta}_T)\chi_2^2$, where $c_1(\boldsymbol{\beta}_T)$ and $c_2(\boldsymbol{\beta}_T)$ are related to the eigenvalues of certain matrices defined by Liang and Self (1996).

Conditional likelihood also may be used directly. The flanking marker probabilities $\boldsymbol{\rho}$ are determined by the breeding design, the map function, and the marker map, which are known in the standard QTL setup (Lander and Botstein 1989). This means that $\boldsymbol{\beta}$ may be estimated without estimating $\boldsymbol{\rho}$. Let the conditional maximum likelihood estimator $\hat{\boldsymbol{\beta}}_c = \arg\max_{\boldsymbol{\beta}} l_c(\boldsymbol{\beta})$, with $\boldsymbol{\rho}$ known. Similar to the partial likelihood, the constraints on f_q are not enforced. It follows from standard conditional likelihood theory (Andersen 1970) that $\hat{\boldsymbol{\beta}}_c$ is consistent and asymptotically normal and may be used to conduct Wald and likelihood ratio tests in F2 and multiple-QTL models. The key results are presented in the following theorem.

Theorem 3. Assume that all functions are locally bounded and integrable. Then the following results hold:

a. $\hat{\boldsymbol{\beta}}_c$ is asymptotically unbiased and consistent in probability for $\boldsymbol{\beta}_T$. Further, $\sqrt{n}(\hat{\boldsymbol{\beta}}_c - \boldsymbol{\beta}_T) \to \mathrm{N}(\mathbf{0}, -\mathbf{S}_c^{-1})$, where $\mathbf{S}_c = E\{\frac{1}{n} l_c''(\boldsymbol{\beta}_T)\}$, which can be estimated by $\hat{\mathbf{S}}_c = \frac{1}{n} l_c''(\hat{\boldsymbol{\beta}}_c)$.

b. Under $H_0: \boldsymbol{\beta} = \mathbf{h}(\boldsymbol{\theta})$, $2LRT \to \chi_r^2$, where $\boldsymbol{\beta}$ is a k vector, $\boldsymbol{\theta}$ is a $(k - r)$ vector of unknown parameters under H_0, and $\mathbf{h}$ is a continuous differentiable function.

3.3 Selection Bias in QTL Experiments

Sampling bias may occur in QTL analyses, either by design or by chance. Selective genotyping (Lander and Botstein 1989) is a strategy to improve QTL detection power. Typically, progeny are genotyped only if their phenotypes are "extreme;" selection also may occur based on correlated phenotypes. Natural selection based on the fitness of the phenotypes may occur unnoticed. In selective phenotyping designs (Jin et al. 2004), subjects are phenotyped based on their genotypes. This reduces phenotyping cost while maintaining a desirable detection efficiency. For selective genotyping and selecting phenotyping, both the phenotype distribution and the genotype frequencies in the sample may severely deviate from those of the population. We now show that different methods may be robust to different sampling biases.

If all progeny are sampled, then inference uses n observations, (z_i, M_i). To address selection bias, we introduce a sampling variable, s_i, that equals 1 if the ith progeny is selected and 0 otherwise. Similar but simpler sampling issues arise in case-control studies (Breslow and Day 1980), where M_i is a binary variable (=1, 2) defining disease status and z_i is a risk factor. In the simplest retrospective design, an equal number of individuals with $M_i = 1$ and $M_i = 2$ are sampled, irrespective of z_i. Here $T_i = M_i$, which greatly simplifies the analysis relative to QTL data, where T_i is unobservable.

Following Qin (1998), assuming that conditionally on M_i, z_i satisfies a tilt model with parameters β_{02} and β_{12} ($\beta_{01} = \beta_{11} = 0$, as in BC), then, conditionally on $s_i = 1$ and M_i, z_i satisfies a tilt model with the same β_{12} but a different β_{02}. Using only those (z_i, M_i) with $s_i = 1$, the partial likelihood analysis in Section 2 gives valid inferences about β_{12}. However, the

conditional likelihood may not do so if the disease prevalence $\rho_1 = 1 - \rho_2 = \Pr(M_i = 1)$ is used instead of the sampling fraction $\Pr(M_i = 1|s_i = 1)$. Interestingly, with case-control data, l_2 is equivalent to a prospective logistic regression analysis for $\Pr(M_i = 1|z_i) = \{1 + \exp(\beta_{02} + \beta_{12} z_i\}^{-1}$ (Qin 1998). The robustness of the prospective likelihood to case-control sampling is well known (Prentice and Pyke 1979), but is more complex in our QTL mixture model framework.

We now consider the properties of complete-case analyses using data with $s_i = 1$ under QTL sampling schemes. Naively applying interval mapping (IM) (Lander and Botstein 1989) yields estimators maximizing $\log \prod_{i=1}^{n} \prod_{k=1}^{K} \Pr(z_i|M_i = k, s_i = 1)^{I(M_i=k,s_i=1)}$. The conditional likelihood from sampled individuals is $\prod_{i=1}^{n} \prod_{k=1}^{K} \Pr(M_i = k|z_i, s_i = 1)^{I(M_i=k,s_i=1)}$, where $\Pr(M_i = k|z_i, s_i = 1) = \Pr(z_i|M_i = k, s_i = 1) \Pr(M_i = k|s_i = 1)\{\Pr(z_i|s_i = 1)\}^{-1}$. With selection, $\hat{\rho}_k = n_k/n$ is unbiased for $\Pr(M_i = k|s_i = 1)$, which may not equal $\rho_k = \Pr(M_i = k)$, the flanking marker genotype probabilities defined by the breeding design and map function. Moreover, the distribution of $z_i|M_i = k, s_i = 1$ may not be the same as that of $z_i|M_i = k$.

With selective genotyping, the sampling probabilities depend only on the phenotype z_i, that is, $\Pr(s_i = 1|z_i, M_i = k) = \Pr(s_i = 1|z_i)$. It is well known (Lander and Botstein 1989) that ignoring phenotype-based sampling leads to bias in estimates of QTL effects in the model for $z_i|M_i$. That is, $\Pr(z_i|M_i = k, s_i = 1) \neq \Pr(z_i|M_i = k)$. Observe, however, that $\Pr(M_i = k|z_i, s_i = 1) = \Pr(s_i = 1|z_i, M_i = k) \Pr(M_i = k|z_i) \Pr(z_i)\{\Pr(z_i, s_i = 1)\}^{-1} = \Pr(M_i = k|z_i)$. Thus conditional likelihood estimation using known ρ gives valid results for $\Pr(z_i|M_i = k)$. Substituting $\hat{\boldsymbol{\rho}}$ for $\boldsymbol{\rho}$ may bias the partial likelihood estimator. This occurs because n_k/n may be inconsistent for ρ_k and the model for $\Pr(M_i = k|z_i)$ may be misspecified in large samples, except with random sampling, where $\Pr(s_i = 1|z_i) = \Pr(s_i = 1) = p$ for all z_i.

With selective phenotyping (Jin et al. 2004), sampling depends only on the marker genotypes, $\Pr(s_i = 1|z_i, M_i = k) = \Pr(s_i = 1|M_i = k)$. It follows that $\Pr(z_i|M_i = k, s_i = 1) = \Pr(s_i = 1|z_i, M_i = k) \Pr(z_i|M_i = k) \Pr(M_i = k)\{\Pr(M_i = k, s_i = 1)\}^{-1} = \Pr(z_i|M_i = k)$. Thus inference based on a model for $\Pr(z_i|M_i = k, s_i = 1)$ is directly interpretable in the model for $\Pr(z_i|M_i = k)$, and hence IM is robust against selective phenotyping. Interestingly, because $\hat{\rho}$ estimates $\Pr(M_i = k|s_i = 1)$ consistently and the distribution of $z_i|M_i = k, s_i = 1$ is equivalent to that of $z_i|M_i = k$, inferences from the partial likelihood about the distribution of $z_i|M_i$ are also valid. However, conditional likelihood will generally yield biased results, because ρ_k may not equal $\Pr(M_i = k|s_i = 1)$ unless $\Pr(s_i = 1|M_i = k) = p, k = 1, \ldots, K$.

In some QTL studies, there are data for which both z_i and M_i are observed ($s_i = 1$) and data for which either z_i (selective genotyping) or M_i (selective phenotyping) is observed, but not both ($s_i = 0$). Valid estimators can be obtained from full likelihood for IM and conditional likelihood for tilt models using missing-data methods (Little and Rubin 1987). Difficulties arise when the sampling scheme is hidden or the investigator has only data with $s_i = 1$. In such instances, only the complete-case analysis is possible, as in the *Listeria* data analysis in Section 6. The naive likelihood approach using (z_i, M_i) with $s_i = 1$ may be biased. Our results show that using either IM or partial likelihood is valid when the sampling depends on genotype, whereas conditional likelihood is not. When sampling is phenotype-dependent, both IM and partial likelihood can be biased, whereas conditional likelihood is appropriate.

4. A NEW RESAMPLING METHOD FOR GENOMEWIDE TESTING

A complete QTL analysis requires testing at all loci in the genome. The simultaneous type I error probability is greatly inflated relative to the pointwise level as a result of the large number of tests across the genome. The importance of using appropriate genomewide thresholds is well recognized (Kruglyak and Lander 1995; Churchill and Doerge 1994; Doerge and Churchill 1996). Methods based on extreme-value properties of Gaussian processes have proven practicable with single-QTL models. Permutation testing is more flexible but does not readily extend to complex genetic models with nonnormal phenotypes and may be computationally intensive because of repeated likelihood maximizations. Motivated by these limitations, we develop a computational technique that may be used with partial and conditional likelihood, extending recent work for likelihood analyses (Zou, Fine, Hu, and Lin 2004).

We first describe the resampling for conditional likelihood ratio tests of $H_0 : \boldsymbol{\beta} = \mathbf{0}$, corresponding to no QTL effects. Let $\boldsymbol{\lambda} = (t_1, t_2, \ldots, t_l)$ index the locations of l QTL, where t_i denotes the putative location of the ith QTL. To conduct a likelihood ratio test that controls the type I error rate simultaneously across all λ's, we need to evaluate the distribution of $2LRT(\boldsymbol{\lambda})$ as a process in $\boldsymbol{\lambda}$ under H_0 to determine the threshold. Our approach is to generate numerically from the asymptotic distribution of $2LRT(\boldsymbol{\lambda})$.

We can show that if H_0 holds at all λ, then $2LRT(\boldsymbol{\lambda})$ is asymptotically equivalent to the quadratic form $-n\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda}) \times \mathbf{S}_c(\boldsymbol{\lambda})\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$, where $\mathbf{S}_c(\boldsymbol{\lambda}) = n^{-1} l_c''(\boldsymbol{\beta}_T(\boldsymbol{\lambda}))$. We also can show that as $n \to \infty$, $n^{1/2}\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$ converges to a mean-0 Gaussian process with covariance function $\boldsymbol{\Sigma}(\boldsymbol{\lambda}_1, \boldsymbol{\lambda}_2) = \operatorname{cov}[n^{1/2}\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda}_1), n^{1/2}\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda}_2)]$ under H_0, where $\boldsymbol{\lambda}_1 = (t_{11}, t_{12}, \ldots, t_{1l})$ and $\boldsymbol{\lambda}_2 = (t_{21}, t_{22}, \ldots, t_{2l})$; details are given in the Appendix. This implies that $2LRT(\boldsymbol{\lambda})$ follows a chi-squared process in $\boldsymbol{\lambda}$. At a single $\boldsymbol{\lambda}$, the usual chi-squared result is obtained.

The covariance function $\boldsymbol{\Sigma}$ is quite complicated, and the distribution of the supremum test statistic is analytically intractable for complex designs and multiple-QTL hypotheses. The covariance depends on the genetic map and, with finite unequally spaced markers, does not have the simple form of Lander and Botstein (1989), who derived thresholds analytically for single-QTL backcrosses. We approximate the distribution of the process $\sqrt{n}\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$ by $\hat{\mathbf{W}}_c(\boldsymbol{\lambda}) = n^{-1/2} \sum_i \hat{\mathbf{I}}_{ci}(\boldsymbol{\lambda}) G_i$, where G_i is a random sample from the standard normal, N(0, 1), and $\hat{\mathbf{I}}_{ci}$ is as defined in the Appendix. To approximate the log-likelihood ratio process, $LRT(\boldsymbol{\lambda})$, we then use $\widehat{LP}(\boldsymbol{\lambda}) = -\hat{\mathbf{W}}_c(\boldsymbol{\lambda})\hat{\mathbf{S}}_c(\boldsymbol{\lambda})\hat{\mathbf{W}}_c(\boldsymbol{\lambda})^{\mathrm{T}}$, where $\hat{\mathbf{S}}_c(\boldsymbol{\lambda})$ is given in the Appendix. In large samples, we may approximate the distribution of $LRT(\boldsymbol{\lambda})$ using $\widehat{LP}(\boldsymbol{\lambda})$ and compute the genomewise threshold for an α level supremum test using the $(1 - \alpha)$ quantile of the recorded $\sup_{\lambda}(\widehat{LP}_j(\boldsymbol{\lambda})), j = 1, 2, \ldots, N$, from independent N(0, 1) samples, $(G_{i1}, \ldots, G_{in}), i = 1, \ldots, N$.

Note that we fit the tilt model only once across the entire genome to estimate $\hat{\mathbf{I}}_{ci}$ and $\hat{\mathbf{S}}_c$, which is then fixed when repeatedly generating G_i. This eliminates the repeated likelihood maximization required by the permutation approach.

In general, genetic hypotheses may be formulated as $H_0: \boldsymbol{\beta} = \mathbf{h}(\boldsymbol{\theta})$, where $\boldsymbol{\beta}$ is a k vector, $\boldsymbol{\theta}$ is a $(k-r)$ vector of unknown parameters under the null hypothesis, and $\mathbf{h} = \{h_1, \ldots, h_k\}^T$ is continuously differentiable. For example, testing no epistasis for a two-QTL model in BC gives the null $H_0: \beta_{111} = \beta_{101} + \beta_{110}$, with $k = 6$, $r = 1$, $\boldsymbol{\theta} = (\beta_{001}, \beta_{101}, \beta_{010}, \beta_{110}, \beta_{011})$, $h_i(\theta) = \theta_i, i = 1, \ldots, 5$, and $h_6(\boldsymbol{\theta}) = \theta_2 + \theta_4$. Assume that $\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$ is the unrestricted conditional maximum likelihood estimator and $\tilde{\boldsymbol{\theta}}_c(\boldsymbol{\lambda})$ is the conditional maximum likelihood estimator under H_0 at $\boldsymbol{\lambda}$. The null distribution of $LRT(\boldsymbol{\lambda})$ based on $\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$ and $\hat{\boldsymbol{\theta}}_c(\boldsymbol{\lambda})$ can be approximated by $\widehat{LP}(\boldsymbol{\lambda}) = \tilde{\mathbf{W}}_c(\boldsymbol{\lambda})\tilde{\mathbf{S}}_c(\boldsymbol{\lambda})\tilde{\mathbf{W}}_c(\boldsymbol{\lambda})^{\mathrm{T}} - \hat{\mathbf{W}}_c(\boldsymbol{\lambda})\hat{\mathbf{S}}_c(\boldsymbol{\lambda})\hat{\mathbf{W}}_c(\boldsymbol{\lambda})^{\mathrm{T}}$, where $\tilde{\mathbf{W}}_c(\boldsymbol{\lambda}) = n^{-1/2}\sum_i \tilde{\mathbf{I}}_{ci}(\boldsymbol{\lambda})G_i$. The algorithm is as follows:

1. Compute $\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$, $\hat{\boldsymbol{\theta}}_c(\boldsymbol{\lambda})$, and $LRT(\boldsymbol{\lambda})$.
2. Compute $\hat{\mathbf{I}}_{ci}, \hat{\mathbf{S}}_c, \tilde{\mathbf{I}}_{ci}$, and $\tilde{\mathbf{S}}_c$.
3. Generate N iid samples of standard normal variates, $(G_{j1}, \ldots, G_{jn}), j = 1, \ldots, N$.
4. For $j = 1, \ldots, N$, compute $\hat{\mathbf{W}}_c^j(\boldsymbol{\lambda}) = n^{-1/2}\sum_i \hat{\mathbf{I}}_{ci}(\boldsymbol{\lambda})G_{ji}$, $\tilde{\mathbf{W}}_c^j(\boldsymbol{\lambda}) = n^{-1/2}\sum_i \tilde{\mathbf{I}}_{ci}(\boldsymbol{\lambda})G_{ji}$, and $\widehat{LP}_j(\boldsymbol{\lambda}) = \tilde{\mathbf{W}}_c^j(\boldsymbol{\lambda}) \times \tilde{\mathbf{S}}_c(\boldsymbol{\lambda})\tilde{\mathbf{W}}_c^j(\boldsymbol{\lambda})^{\mathrm{T}} - \hat{\mathbf{W}}_c^j(\boldsymbol{\lambda})\hat{\mathbf{S}}_c(\boldsymbol{\lambda})\hat{\mathbf{W}}_c^j(\boldsymbol{\lambda})^{\mathrm{T}}$.
5. Reject H_0 if $\sup_\lambda LRT(\boldsymbol{\lambda})$ is larger than the $(1-\alpha)$ percentile of $\{\sup_\lambda \widehat{LP}_j(\boldsymbol{\lambda}), j = 1, \ldots, N\}$.

The resampling approach also applies to partial likelihood ratio tests. Let $\hat{\boldsymbol{\beta}}_p(\boldsymbol{\lambda})$ and $\tilde{\boldsymbol{\theta}}_p(\boldsymbol{\lambda})$ be the unrestricted and restricted partial likelihood estimators. Under $H_0: \boldsymbol{\beta} = \mathbf{h}(\boldsymbol{\theta})$, the genomewide distribution of the test statistics can be approximated by $\tilde{\mathbf{W}}_p(\boldsymbol{\lambda})\tilde{\mathbf{S}}_p(\boldsymbol{\lambda})\tilde{\mathbf{W}}_p(\boldsymbol{\lambda})^{\mathrm{T}} - \hat{\mathbf{W}}_p(\boldsymbol{\lambda})\hat{\mathbf{S}}_p(\boldsymbol{\lambda})\hat{\mathbf{W}}_p(\boldsymbol{\lambda})^{\mathrm{T}}$, where $\hat{\mathbf{W}}_p(\boldsymbol{\lambda}) = n^{-1/2}\sum_i \hat{\mathbf{I}}_{pi}(\boldsymbol{\lambda})G_i$, $\tilde{\mathbf{W}}_p(\boldsymbol{\lambda}) = n^{-1/2}\sum_i \tilde{\mathbf{I}}_{pi}(\boldsymbol{\lambda})G_i$, and $\hat{\mathbf{S}}_p(\boldsymbol{\lambda})$, $\tilde{\mathbf{S}}_p(\boldsymbol{\lambda})$, $\hat{\mathbf{I}}_{pi}(\boldsymbol{\lambda})$, and $\tilde{\mathbf{I}}_{pi}(\boldsymbol{\lambda})$ are as given in the Appendix. Although the pointwise null distribution for the partial likelihood ratio test is very complicated, the generality of the resampling methods makes it possible to perform a genomewise QTL scan using l_2. The foregoing recipe for the conditional likelihood ratio tests can be adapted by replacing c with p.

5. NUMERICAL STUDIES

The first set of simulations (results not shown) evaluates the partial (PL) and conditional likelihood (CL) estimators in an F2 cross. The QTL is located at 30 cM on a hypothetical chromosome. Markers are completely genotyped at 20 cM and 40 cM. Datasets with $n = 250$ were simulated 500 times, with $f_0(z)$ following a normal, exponential, or Poisson distribution. Both the PL and CL estimators appear to be consistent. The variances obtained from CL are slightly larger than those from PL, which agrees with previous findings (Zou and Fine 2002) for BC. The empirical variances and model-based variance estimates generally agree.

The next set of simulations illustrates the tilt model with two QTLs and potential epistasis. The QTLs were constructed 40 cM apart on a chromosome with completely genotyped flanking markers. We tested three hypotheses: (1) no QTL; (2) one QTL versus two QTL; and (3) additivity (i.e., no epistasis), using CL and IM likelihood ratio tests and Wald tests from PL (PLW) and CL (CLW). The CLW test rejects $H_0: \mathbf{C}\boldsymbol{\beta} = \mathbf{0}$ if $\mathbf{W}_{cn} > \chi^2_{r,\alpha}$, where $\mathbf{W}_{cn} = (\mathbf{C}\hat{\boldsymbol{\beta}}_c)(-\mathbf{C}\hat{\mathbf{S}}_c^{-1}\mathbf{C}^{\mathrm{T}})^{-1}(\mathbf{C}\hat{\boldsymbol{\beta}}_c)^{\mathrm{T}}$, $-\hat{\mathbf{S}}_c$ estimates the conditional Fisher information matrix and r is the rank of $\mathbf{C}$. The PLW is similar, except that $\hat{\boldsymbol{\beta}}_c$ is replaced by $\hat{\boldsymbol{\beta}}_p$ and $-\hat{\mathbf{S}}_c^{-1}$ with $\hat{\boldsymbol{\Sigma}}$ from Theorem 2, which estimates $\mathrm{var}(\hat{\boldsymbol{\beta}}_p)$. Unless stated otherwise, we use the *scanone/two* functions from the R/qtl package (Broman, Wu, Sen, and Churchill 2003) for interval mapping. The test for the second hypothesis is not implemented in the current version of R/qtl, so we omit these results for IM.

We ran 500 simulations for each model with $n = 100$, 200 (Table 3). The performance of all tests improves as n increases. IM has a high rejection rate, 13–19%, compared to roughly 5% for the other methods in case 9, where the phenotype has a Poisson distribution with two additive QTL. This behavior is more prominent with increasing n and when one QTL has a large effect (not shown). In case 10, with a small interaction, IM detects epistasis at a rate of 61–90%, roughly five times the rates for all other methods, suggesting epistasis can be confounded with misspecified phenotype models in IM. Tilt model tests reject at nominal levels for all null hypotheses. Compared with parametric interval mapping, the semiparametric methods demonstrate comparable power for detecting any QTL and a second QTL. The CL ratio test is more powerful than the Wald tests, with PLW being somewhat less powerful than CLW.

We now evaluate the performance of our proposed resampling method. To demonstrate the computational advantage, we conducted a small experiment with a 100-cM chromosome. Ten backcross populations were simulated with $n = 100$. At each locus, the tilt model was fitted with a genome scan based on either the permutation method or our resampling method. The total computing time was 205 seconds for 10 permutations, 2,169 seconds for 100 permutations and 7,185 seconds for 300 permutations, whereas the corresponding resampling took 24, 34, and 54 seconds. Note that Doerge and Churchill (1996) recommended 1,000 permutations for a significance level of .05 to achieve <13.78% Monte Carlo error. At this level of precision, the new resampling method would achieve more than a 200-fold decrease in computing time.

Next we assessed the size and power of genome screens on a 100-cM region in a BC with $n = 100$ or 200 based on 100 simulated datasets (Table 4). At each locus, we fit $f_1(x) = f_0(x)\exp(\beta_{01} + \beta_{11}x)$, and tested H_0: no QTL, with CL and PL. Thresholds for 5% level tests were obtained by resampling. The tests are compared with IM using permutation thresholds. With 100 iterations, all tests have inflated rejection rates under H_0. This distortion is not apparent with 300 iterations. Power clearly increases with sample size. PL is slightly more powerful than CL, as expected because partial likelihood has been shown to be more efficient than conditional likelihood (Zou and Fine 2002). IM demonstrates somewhat better power in detecting QTL than do tilt model tests.

6. *LISTERIA* CASE STUDY

6.1 Data Description

Boyartchuk et al. (2001) studied survival (in hours) of 116 age-matched female mice after infection with *Listeria monocytogenes*. These mice are from an intercross between the

Table 3. Simulation Results for the Two-QTL Model in BC Cross

Case	aabb	Aabb	aaBb	AaBb	QTL1 effect	QTL2 effect	Epistasis
1	N(2, 1)	N(2, 1)	N(2, 1)	N(2, 1)	0	0	0
2	E(3)	E(3)	E(3)	E(3)	0	0	0
3	P(3)	P(3)	P(3)	P(3)	0	0	0
4	N(2, 1)	N(0, 1)	N(2, 1)	N(0, 1)	−2	0	0
5	N(1.7, 1)	N(.7, 1)	N(1, 1)	N(0, 1)	−1	−.7	0
6	N(−.3, 1)	N(.7, 1)	N(−1, 1)	N(0, 1)	1	−.7	0
7	N(1, 1)	N(1, 1)	N(1, 1)	N(2, 1)	1	1	1
8	P(2)	P(1)	P(2)	P(1)	−.7	0	0
9	P(3)	P(1.5)	P(2)	P(1)	−.7	−.4	0
10	P(4)	P(1.5)	P(2)	P(1)	−.7	−.4	−.3

	IM		Partial likelihood			Conditional likelihood		
Case	test1	test3	test1	test2	test3	test1	test2	test3
$n = 100$								
1	.056	.066	.048	.044	.050	(.036) 060	(.050) 068	(.060) 068
2	.048	.048	.030	.030	.034	(.020) 052	(.030) 056	(.038) 060
3	.050	.068	.024	.028	.052	(.034) 052	(.036) 064	(.064) 070
4	1.000	.062	1.000	.032	.042	(1.000) 1.000	(.040) 060	(.062) 064
5	1.000	.066	1.000	.676	.040	(1.000) 1.000	(.734) 780	(.050) 058
6	.972	.054	.900	.680	.024	(.948) 974	(.736) 788	(.042) 058
7	.980	.608	.978	.694	.532	(.974) 980	(.758) 804	(.578) 606
8	.970	.072	.906	.026	.032	(.942) 966	(.028) 042	(.044) 050
9	1.000	.132	.998	.400	.030	(.998) 1.000	(.468) 574	(.042) 050
10	1.000	.614	1.000	.822	.106	(1.000) 1.000	(.902) 944	(.146) 164
$n = 200$								
1	.064	.042	.046	.052	.042	(.052) 064	(.052) 050	(.040) 040
2	.058	.046	.044	.028	.042	(.042) 060	(.042) 052	(.044) 050
3	.062	.058	.048	.040	.042	(.054) 068	(.044) 060	(.054) 058
4	1.000	.056	1.000	.030	.044	(1.000) 1.000	(.038) 044	(.054) 054
5	1.000	.056	1.000	.964	.060	(1.000) 1.000	(.962) 976	(.070) 070
6	1.000	.050	1.000	.974	.034	(1.000) 1.000	(.974) 988	(.046) 048
7	1.000	.876	1.000	.978	.842	(1.000) 1.000	(.980) 992	(.860) 874
8	1.000	.064	.998	.038	.040	(.996) 1.000	(.043) 050	(.046) 046
9	1.000	.192	1.000	.840	.038	(1.000) 1.000	(.851) 886	(.044) 048
10	1.000	.902	1.000	.996	.190	(1.000) 1.000	(1.000) 1.000	(.210) 226

NOTE: N, E, and P denote normal, exponential, and Poisson distributions *test1*: absence of QTL; *test2*: one QTL; and *test3*: epistasis. Rejection rate is reported for (CLW)CL and PLW.

Table 4. Rejection Rate for Testing Presence of QTL in a Chromosome Scan of a Single-QTL Model in BC Cross

$f_0(z)$	$f_1(z)$	n	No. of permute	No. of resample	IM	Partial	Conditional
N(2, 1)	N(2, 1)	100	100	100	.10	.11	.10
			200	200	.05	.02	.03
			300	300	.04	.06	.06
		200	100	100	.07	.08	.11
			200	200	.05	.03	.02
			300	300	.06	.03	.04
P(3)	P(3)	100	100	100	.05	.06	.10
			200	200	.03	.06	.07
			300	300	.02	.05	.03
		200	100	100	.02	.05	.06
			200	200	.04	.08	.08
			300	300	.07	.07	.07
E(3)	E(3)	100	100	100	.05	.06	.06
			200	200	.02	.06	.04
			300	300	.05	.06	.06
		200	100	100	.05	.05	.08
			200	200	.04	.04	.07
			300	300	.04	.04	.04
N(.6)	N(0, 1)	100	300	300	.67	.56	.53
		200	300	300	.93	.86	.86
P(exp(.6))	P(1)	100	300	300	.76	.69	.65
		200	300	300	.98	.99	.99
B(.5, 1)	B(.646, 1)	100	300	300	.15	.10	.14
		200	300	300	.25	.24	.18

NOTE: N, E, P and B denote normal, exponential, Poisson, and binomial distributions. Threshold was obtained through a genome scan using permutation test for IM method, and our proposed resampling method for partial and conditional likelihood based tests.

BALB/cByJ and C57BL/6ByJ strains, with 133 genetic markers spanning 20 chromosomes. The animals that died from infection had a mean survival of 153.8 hours. Roughly 30% survived past the 264-hour time point and were considered recovered (see Fig. 1). The phenotype can be decomposed into a binary trait, indicating whether the subject survived, and a continuous trait for time to death for those dying within 264 hours (Broman 2003). In addition to testing for QTL on the combined trait, it is of interest to determine whether different genes influence the survival probability and the distribution of death time for those mice that do not survive. Dissecting this complex genetic architecture is the objective of this case study.

6.2 Single QTL Analysis

We first fit single-QTL models to each trait. Previous studies (Boyartchuk et al. 2001; Broman 2003) identified potential modifiers on chromosomes 5 and 13 and a suggestive, but inconclusive, result on chromosome 1. For this reason, our analysis focuses on these three regions. Three methods are examined: IM (normal or binary model), CL, and PL. Genomewise thresholds for IM were obtained from 300 permutations (Churchill and Doerge 1994). For CL and PL, a 5% genomewise error rate is controlled using our proposed resampling technique, with 1,000 runs. LODs are reported in Figure 2.

The results from IM generally agree with the previous studies. The locus on chromosome 1 appears to affect only the time to death for nonsurvivors, whereas the chromosome 5 locus affects the survival trait. The locus on chromosome 13 has a strong influence on the binary trait but is marginally significant for time to death. In all cases, PL has a very similar LOD profile to that of IM. The loss of power resulting from a higher threshold for PL is consistent with the simulations discussed in Section 5. It is worthwhile to highlight the differences between CL and PL. These methods match closely on chromosomes 1 and 5 for both the combined and binary traits; however, the CL LOD is substantially higher on chromosome 13 for all traits and noticeably higher on chromosomes 5 and 1 for the continuous trait.

Because PL is a pseudolikelihood based on CL, differences between the methods should be accounted for by differences between ρ and $\hat{\rho}$. In the *Listeria* dataset, $\rho_k = \Pr(M_i = k)$ is determined by the intercross design and recombination with flanking markers using the Haldane map function. In CL, the known values of ρ_k are used, whereas in PL, ρ_k is estimated by n_k/n. We compared ρ_k and n_k/n at flanking markers to the four LOD peaks [(chr 1, 14cM), (chr 1, 81cM), (chr 5, 29cM), and (chr 13, 26cM)] for all 116 mice and for the 81 mice that died. Deviations were evaluated using chi-squared tests on the nonrecombinants (Table 5). Recombinants were not included because they are rare and may not satisfy the usual assumptions ($np \geq 5$) for the test. The tests are highly significant on chromosome 13 for combined and continuous traits and significant for the continuous trait on chromosomes 1 (at 14 cM) and 5. This reveals distortion in the flanking marker probabilities, which was not noticed in previous analyses.

The investigators are not aware of experimentalwise selection, other than sex and age-related matching (K. Broman, personal communication). Therefore, selection bias is probably not due to selective phenotyping, which is typically per

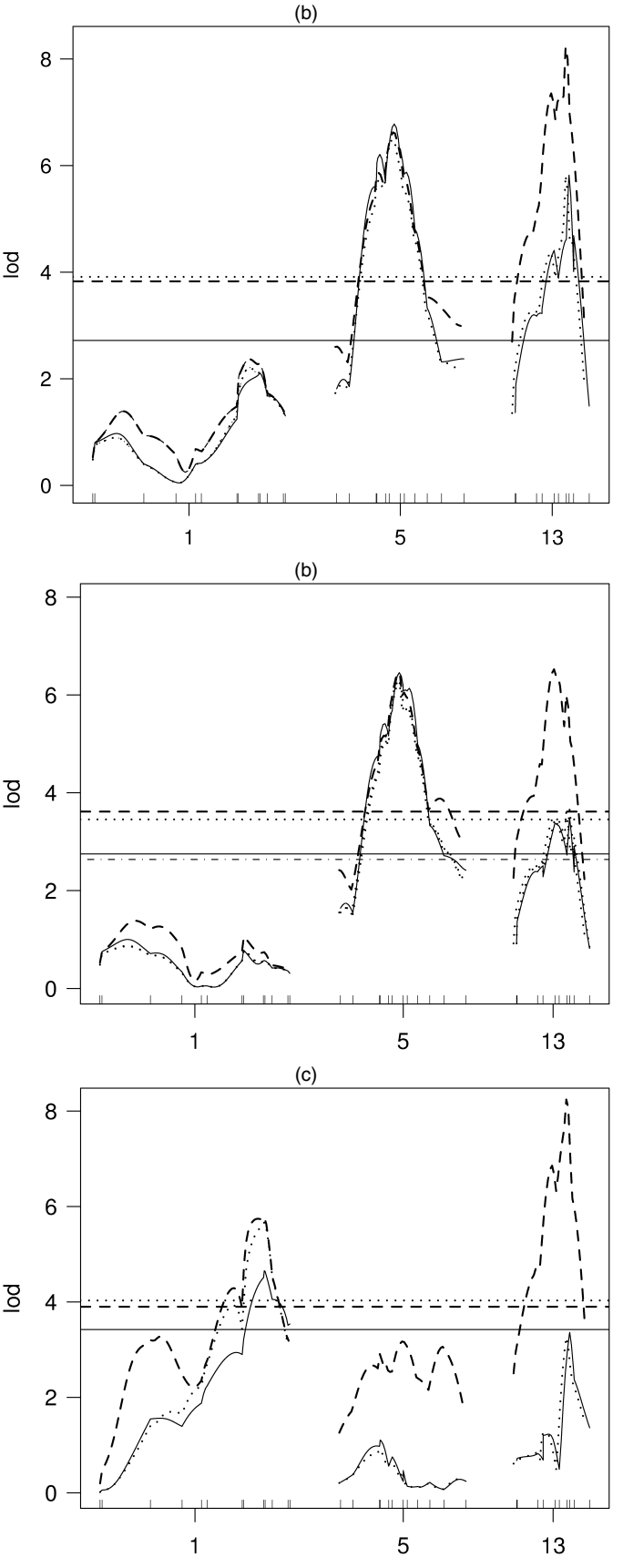

Figure 2. Single-QTL Scan for Chromosomes 1, 5, and 13 Based on IM, CL, or PL, on the Combined (a), Binary (b), and Continuous (c) Traits. (—— IM/perm; – – cond/resamp; ····· part/resamp; –·–· binary/perm.)

Table 5. Chi-Squared Test for $\hat{\rho}$ versus ρ

Data	*chr*	*cM*	ρ	$\hat{\rho}$	*p*
Combined/	1	14	.164, .346, .164	.164, .371, .095	.176
binary	1	81	.246, .492, .246	.276, .457, .250	.698
$n = 116$	5	29	.225, .451, .225	.250, .440, .216	.819
	13	26	.240, .480, .240	.172, .397, .397	$<.001$
Continuous	1	14	.164, .346, .164	.185, .407, .062	.040
$n = 81$	1	81	.246, .492, .246	.234, .457, .284	.697
	5	29	.225, .451, .225	.358, .432, .099	.002
	13	26	.244, .487, .244	.136, .370, .481	$<.001$

NOTE: Only nonrecombinants are tested, corresponding to flanking marker genotypes of (AA/BB, Aa/Bb, aa/bb).

protocol. It seems reasonable that the flanking marker distortions in the whole sample of 116 mice is attributable to hidden selective genotyping, based on natural selection of correlated phenotypes. As discussed in Section 3.3, PL and IM tend to give biased results in this scenario, whereas CL is robust and gives valid parameter estimates for the underlying phenotype model. The continuous trait analyses were restricted to those 81 mice not recovering from their infections. This introduces another layer of selective genotyping, because mice are selected based on whether they died, that is, if their survival time was <264 hours. Hence it is not surprising that quite large deviations are seen from ρ on chromosomes 1 and 5 for the continuous trait for this subsample.

CL appears to be preferable to PL and IM if the interest lies in the underlying models for the combined and binary traits. However, the estimates from IM and PL may be meaningful for the QTL influence on the survival of mice that are not "cured," that is, conditional on $s_i = 1$.

6.3 Two-QTL Model With Epistasis

A multiple-QTL model may improve over single-QTL mapping because of its ability to distinguish linked QTL and to identify epistasis. We fit two-QTL models to the *Listeria* data for the three traits. We fit the tilt model on all pairwise positions on different chromosomes or at least 40cM apart on the same chromosome. Resampling with 300 iterations provides genomewise thresholds at significance levels of .05 and .10. The results are compared with two-dimensional genome scans of IM with 100 permutations using R/qtl in Table 6. Because chromosome 13 is only 35 cM, the two-QTL model is not fitted on this chromosome.

Although multiple QTL have been identified with single-QTL analyses, two-QTL IM failed to reject the null of no QTL with the combined trait. The analysis of the binary data using IM based on normal assumptions was unstable and failed to converge. Unfortunately, two-QTL IM for binary data is not available in R/qtl. Using the 81 mice that died, IM detected two QTL on chromosomes 5 and 13, with a p value between .05 and .10. It is somewhat surprising that the two-QTL IM results are rather different from the single-QTL results, contradicting the wisdom that simultaneously adjusting for multiple QTL leads to increased power.

Using CL and PL, we detected the joint presence of two QTL for most pairs. The strongest QTL effects were located on chromosomes 5 and 13 for both the combined and binary data. Any combination of either of these QTLs and positions on chromosome 1 were significant. For the continuous trait, the strongest QTLs were on chromosomes 1 and 13; these were significant with CL but not with PL. This difference may be explained in part by previously observed genotype distortions.

Table 6. Two-QTL Model Analysis Across the Genome With a 2-cM scan

	LOD	*chr 1:5*	*chr 1:13*	*chr 5:13*	*chr 1:1*	*chr 5:5*	*T.95*	*T.90*
Spike data								
IM	J	20.7(14/30)	21.7(12/56)	13.3(12/6)	11.1(16/28)	14.2(28/26)	28.3	25.2
	E	19.7(14/30)	18.1(12/56)	9.8(12/6)	3.5(16/28)	1.6(28/26)	26.4	24.4
CL	J	10.6(76/12)	13.9(76/26)	15.1(36/30)	10.3(14/84)	9.9(12/54)	10.4	9.6
	E	3.2(76/12)	3.0(74/18)	2.3(36/30)	4.8(14/84)	.4(18/58)	6.6	5.7
PL	J	10.7(82/26)	11.5(76/26)	11.9(24/18)	10.5(22/78)	7.5(18/60)	9.8	8.8
	E	2.7(84/10)	3.1(76/26)	2.9(6/24)	4.6(16/84)	.3(18/60)	6.1	5.6
Binary data								
CL	J	12.4(36/28)	10.3(36/18)	14.6(34/22)	8.3(30/82)	10.4(0/46)	8.8	8.3
	E	4.4(92/36)	3.3(34/10)	2.5(58/4)	2.6(20/80)	.7(0/44)	4.4	4.1
PL	J	10.1(44/26)	6.6(74/26)	11.7(26/26)	8.2(24/82)	8.8(4/60)	8.7	8.2
	E	3.7(92/50)	2.7(48/0)	2.3(60/4)	3.9(24/82)	.9(4/60)	4.2	3.8
NonSurvival Data								
IM	J	8.8(64/78)	8.3(82/14)	10.9(78/16)	4.9(4/14)	5.2(12/26)	12.0	10.7
	E	4.4(64/78)	2.4(82/14)	4.1(78/16)	3.8(4/14)	.8(12/26)	10.0	9.3
CL	J	11.1(82/16)	14.8(26/80)	12.3(30/22)	10.6(30/72)	8.0(0/50)	10.4	9.6
	E	4.0(0/30)	2.0(0/32)	1.5(40/22)	1.9(30/72)	1.8(20/60)	6.1	5.4
PL	J	8.9(82/18)	9.5(26/82)	5.6(4/24)	8.9(26/72)	4.2(0/58)	12.5	11.1
	E	3.7(2/36)	2.0(0/40)	1.8(4/24)	2.3(30/70)	1.4(8/60)	8.4	7.2

NOTE: J, testing no QTL; E, testing epistasis. The position estimates for QTL (pos 1/pos 2) are reported corresponding to the maximum LOD, where pos 1 and 2 are on chromosome *i* and *j* from the header (*i:j*). T.95 denotes the genome-wise threshold at significance level of .05, and T.90 is the threshold at .10. These are obtained from 100 permutation for IM or 300 resampling for CL and PL.

Two QTLs were inferred on chromosome 1, near positions 14–30 and 70–84; the joint models were significant at level .05 based on both PL and CL for the combined trait and moderately significant at level .10 for the binary trait. Epistasis was not detected using IM, which assumes that the maximum LOD for testing epistasis occurs at the same location with maximum LOD testing for no QTL, which may not be correct. The PL and CL analyses suggest a moderate gene interaction ($p = .05$–$.10$) between the region 80–92 cM on chromosome 1 and region 30–50 cM on chromosome 5 for the binary trait.

7. DISCUSSION

The semiparametric tilt model allows us to make unified inferences about genetic effects in QTL experiments without specifying f_0, thereby accommodating arbitrary phenotypes. If knowledge of f_0 is available, then fully parametric models can be fit, and the resulting likelihood inferences will generally be more efficient than those based on the tilt model, assuming that the model is specified correctly. This is the price that must be paid for not specifying the baseline density function.

The flexibility of the tilt model is useful in the *Listeria* case study, where the presence of a "spike" corresponding to "cure" makes the phenotype definition unclear. The specification of parametric models critically depends on the definition (Broman 2003). Whereas the focus in this article was on QTL analysis for experimental populations, similar issues occur with "spike" data in human populations. Epstein, Lin, and Boehnke (2003) proposed a variance component Tobit model for familial data in which the time at which the "spike" occurs serves as a censoring time and the underlying event time is modeled using a normal distribution. It would be worthwhile to investigate similar parametric models for censored phenotype data in experimental populations. As noted in Section 6, one may wish to model the conditional distribution given no "cure" and the probability of "cure" separately (as in Farewell 1977), which is not possible using the Tobit model. Furthermore, if one is interested in analyzing the combined trait, then the "cure" time should not be treated as a censoring time, because the point mass at that time point is of scientific interest. It is well known that using proper distributions, like the normal, is inappropriate with a "cure," because the distribution of the combined trait is a mixture of a proper distribution and a point mass at the "cure" time, and hence is improper.

Bias may occur in QTL analyses with nonrandom sampling when naive complete case analyses are used. Selective sampling can affect QTL analyses very differently, depending on the model and method of estimation. With phenotype-dependent sampling, CL provides unbiased estimates, whereas IM and PL may not be valid. Alternatively, a sample may be selected based on the genotypes or on some other traits that happen to be mapped to the same or closely linked genes. Here CL may be biased, whereas IM/PL analyses are appropriate. In practice, the sampling scheme may be hidden, and comparing IM, PL, and CL may be useful for diagnosing such biases. If data on individuals with $s_i = 0$ are available, then a formal likelihood analysis based on either the distribution of z_i given M_i or the distribution of M_i given z_i will give valid inferences, assuming that s_i satisfies missing at random (Little and Rubin 1987).

Extensive empirical studies have suggested that interval mapping with the normality assumption is rather robust against nonnormal distributions in single-QTL analyses. In this article we have shown that this is not always the case, especially with multiple-QTL analysis and epistasis. IM may falsely detect epistasis and overestimate small gene–gene interactions when the baseline phenotype follows a nonnormal distribution. Interestingly, in the two-QTL analysis of the *Listeria* data, the failure to detect any QTL in joint two-QTL models may be due in part to violation of normality by the "spike" phenotype. This result clearly contrasts with the findings of the two-QTL analyses based on the tilt model, which were in agreement with single-QTL analyses.

The proposed resampling method offers increased efficiency and versatility in obtaining p values and thresholds for QTL studies. The computational gains may be quite large, particularly with multiple-QTL models, where repeated maximization may be prohibitive.

QTL analysis often involves multiple correlated traits. These data present special problems, especially when genetic interactions between the traits are of interest. We are currently investigating extensions of the exponential tilt model to multiple-trait QTL analyses, which assume that the same set of QTL affects all traits (pleiotropy), but with different effects on different traits. Analyses based on a joint model that accounts for correlation among the multiple traits may have increased power for QTL detection and yield more efficient parameter estimators. The joint model also gives formal tests for phenotypic interactions and other biologically interesting concepts. The formulation extends readily to other experimental crosses, including designs involving two inbred parents (Liu and Zeng 2000).

APPENDIX: DETAILS OF RESAMPLING TECHNIQUE

Under the null $H_0 : \boldsymbol{\beta} = \mathbf{0}$, a first-order linear approximation as $n \to \infty$ is $\sqrt{n}\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda}) = -n^{-1/2}\sum_{i=1}^{n} \mathbf{S}_c^{-1}(\boldsymbol{\lambda})Q_{ci}(\boldsymbol{\lambda}) + o_p(1)$, where $Q_{ci}(\boldsymbol{\lambda}) = \omega^{-1}(z_i, \boldsymbol{\beta})\omega'(z_i, \boldsymbol{\beta}) - R_c^{-1}(z_i, \boldsymbol{\beta})R_c'(z_i, \boldsymbol{\beta})$. The covariance function $\boldsymbol{\Sigma}(\boldsymbol{\lambda}_1, \boldsymbol{\lambda}_2) = E\{\mathbf{I}_{ci}(\boldsymbol{\lambda}_1)\mathbf{I}_{ci}^{\mathrm{T}}(\boldsymbol{\lambda}_2)\}$, where $\mathbf{I}_{ci}(\boldsymbol{\lambda}) = -\mathbf{S}_c(\boldsymbol{\lambda})^{-1}Q_{ci}(\boldsymbol{\lambda})$. The covariance may be estimated by $n^{-1} \times \sum_i^n \hat{\mathbf{I}}_{ci}(\boldsymbol{\lambda}_1)\hat{\mathbf{I}}_{ci}^{\mathrm{T}}(\boldsymbol{\lambda}_2)$, where $\hat{\mathbf{I}}_{ci}(\boldsymbol{\lambda}) = -\hat{\mathbf{S}}_c(\boldsymbol{\lambda})^{-1}\hat{Q}_{ci}(\boldsymbol{\lambda})$ and $\hat{\mathbf{S}}_c(\boldsymbol{\lambda})$ and $\hat{Q}_{ci}(\boldsymbol{\lambda})$ are $\mathbf{S}_c$ and Q_{ci} with $\boldsymbol{\beta} = \hat{\boldsymbol{\beta}}_c$.

Conditioned on the observed data (but not on G_i), for any fixed n, $\hat{\mathbf{W}}_c(\boldsymbol{\lambda}_1), \ldots, \hat{\mathbf{W}}_c(\boldsymbol{\lambda}_m)$ is multivariate Gaussian, where $\boldsymbol{\lambda}_j = (t_{j1}, t_{j2}, \ldots, t_{jl})$, $j = 1, \ldots, m < \infty$, and $\mathrm{cov}\{\hat{\mathbf{W}}_c(\boldsymbol{\lambda}_i), \hat{\mathbf{W}}_c(\boldsymbol{\lambda}_j)\} = n^{-1} \times \sum_i \hat{\mathbf{I}}_{ci}(\boldsymbol{\lambda}_i)\hat{\mathbf{I}}_{ci}^{\mathrm{T}}(\boldsymbol{\lambda}_j)$. As $n \to \infty$, the covariance converges uniformly almost surely to $\boldsymbol{\Sigma}(\boldsymbol{\lambda}_i, \boldsymbol{\lambda}_j)$. Thus $n^{1/2}\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$ and $\hat{\mathbf{W}}_c(\boldsymbol{\lambda})$ have the same limiting process, and generating from $\hat{\mathbf{W}}_c(\boldsymbol{\lambda})$ provides samples from the distribution of $n^{1/2}\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$. Because $LRT(\boldsymbol{\lambda})$ is a smooth function of $n^{1/2}\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda})$ and $\hat{\mathbf{S}}_c(\boldsymbol{\lambda})$, $-\hat{\mathbf{W}}_c(\boldsymbol{\lambda})\hat{\mathbf{S}}_c(\boldsymbol{\lambda})\hat{\mathbf{W}}_c(\boldsymbol{\lambda})^{\mathrm{T}}$ and $LRT(\boldsymbol{\lambda})$ follow the same distribution as $n \to \infty$.

Under the general null $H_0 : \boldsymbol{\beta} = \mathbf{h}(\boldsymbol{\theta})$, the distribution of $\sqrt{n}\{\hat{\boldsymbol{\beta}}_c(\boldsymbol{\lambda}) - \boldsymbol{\beta}(\boldsymbol{\lambda})\}$ can be approximated by that of $\hat{\mathbf{W}}_c(\boldsymbol{\lambda})$, and the distribution of $\sqrt{n}\{\tilde{\boldsymbol{\theta}}_c(\boldsymbol{\lambda}) - \boldsymbol{\theta}(\boldsymbol{\lambda})\}$ can be approximated by that of $\tilde{\mathbf{W}}_c(\boldsymbol{\lambda})$, where $\tilde{\mathbf{I}}_{ci}(\boldsymbol{\lambda}) = -\tilde{\mathbf{S}}_c(\boldsymbol{\lambda})^{-1}\tilde{Q}_{ci}(\boldsymbol{\lambda})$, $\tilde{Q}_{ci}(\boldsymbol{\lambda}) = Q_i(\boldsymbol{\lambda})[\mathbf{h}'(\tilde{\boldsymbol{\theta}}_c)]$, and $\tilde{\mathbf{S}}_c(\boldsymbol{\lambda}) = [\mathbf{h}'(\tilde{\boldsymbol{\theta}}_c)]^{\mathrm{T}}\mathbf{S}_c(\boldsymbol{\lambda})[\mathbf{h}'(\tilde{\boldsymbol{\theta}}_c)]$. It follows that $LRT(\boldsymbol{\lambda})$ and $\widehat{LP}(\boldsymbol{\lambda})$ have the same limiting distribution as processes in $\boldsymbol{\lambda}$.

For the partial ratio likelihood tests, $\hat{\mathbf{I}}_{pi}(\boldsymbol{\lambda}) = -\hat{\mathbf{S}}_p(\boldsymbol{\lambda})^{-1}\hat{Q}_{pi}(\boldsymbol{\lambda})$, $\tilde{\mathbf{I}}_{pi}(\boldsymbol{\lambda}) = \{[\mathbf{h}'(\tilde{\boldsymbol{\theta}})]^{\mathrm{T}}\hat{\mathbf{S}}_p(\boldsymbol{\lambda})\mathbf{h}'(\tilde{\boldsymbol{\theta}})\}^{-1}\hat{Q}_{pi}(\boldsymbol{\lambda})[\mathbf{h}'(\tilde{\boldsymbol{\theta}})]$,

$$\hat{Q}_{pi}(\boldsymbol{\lambda}) = \frac{\omega'(z_i, \boldsymbol{\beta}_p(\boldsymbol{\lambda}), M_i)}{\omega(z_i, \hat{\boldsymbol{\beta}}_p(\boldsymbol{\lambda}), M_i)} - \frac{r'(z_i, \boldsymbol{\beta}_p(\boldsymbol{\lambda}))}{r(z_i, \boldsymbol{\beta}_p(\boldsymbol{\lambda}))} - \sum_{k=1}^{K} I(M_i = k)\left(\frac{1}{n_k}\sum_{j=1}^{n}\left[\frac{\omega'(z_j, \boldsymbol{\beta}_p(\boldsymbol{\lambda}), M_j = k)}{\omega(z_j, \boldsymbol{\beta}_p(\boldsymbol{\lambda}), M_j = k)}\right]\right),$$

and $\tilde{\mathbf{S}}_p(\boldsymbol{\lambda})$ and $\hat{\mathbf{S}}_p(\boldsymbol{\lambda})$ are $\tilde{\mathbf{S}}_c(\boldsymbol{\lambda})$ and $\hat{\mathbf{S}}_c(\boldsymbol{\lambda})$, with $\hat{\boldsymbol{\beta}}_p(\boldsymbol{\lambda})$ and $\tilde{\boldsymbol{\theta}}_p(\boldsymbol{\lambda})$ in place of $\boldsymbol{\beta}(\boldsymbol{\lambda})$ and $\theta(\boldsymbol{\lambda})$.

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