

Testing pleiotropy vs. separate QTL in multiparental populations

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ABSTRACT

The high mapping resolution of multiparental populations, combined with technology to measure tens of thousands of phenotypes, presents a need for quantitative methods to enhance understanding of the genetic architecture of complex traits. When multiple traits map to a common genomic region, knowledge of the number of distinct loci provides important insight into the underlying mechanism and can assist planning for subsequent experiments. We extend the method of [Jiang and Zeng \(1995\)](#), for testing pleiotropy with a pair of traits, to the case of more than two alleles. We also incorporate polygenic random effects to account for population structure. We use a parametric bootstrap to determine statistical significance. We apply our methods to a behavioral genetics data set from Diversity Outbred mice, where we find evidence for presence of two distinct loci in a 2.5 cM region. Our methods have been incorporated into the R package `qt12pleio`.

KEYWORDS Quantitative trait locus; pleiotropy; multivariate analysis; linear mixed effects models; systems genetics

Complex trait studies in multiparental populations present new challenges in statistical methods and data analysis. Among these is the development of strategies for multivariate trait analysis. The joint analysis of two or more traits allows one to address additional questions, such as whether two traits share a single pleiotropic locus.

Previous research addressed the question of pleiotropy vs. separate QTL in two-parent crosses. [Jiang and Zeng \(1995\)](#) developed a likelihood ratio test for pleiotropy vs. separate QTL for a pair of traits. Their approach assumed that each trait was affected by a single QTL. Under the null hypothesis, the two traits were affected by a common QTL, and under the alternative hypothesis the two traits were affected by distinct QTL. [Knott and Haley \(2000\)](#) used linear regression to develop a fast approximation to the test of [Jiang and Zeng \(1995\)](#), while [Tian *et al.* \(2016\)](#) used the methods from [Knott and Haley \(2000\)](#) to dissect QTL hotspots in a F_2 population.

Multiparental populations, such as the Diversity Outbred (DO) mouse population ([Churchill *et al.* 2012](#)), enable high-precision mapping of complex traits ([de Koning and McIntyre 2014](#)). The DO mouse population began with progenitors of the Collaborative Cross (CC) mice ([Churchill *et al.* 2004](#)). Each DO mouse is a highly heterozygous genetic mosaic of alleles from the eight CC founder lines. Random matings among non-siblings have maintained the DO population for more than 23 generations ([Chesler *et al.* 2016](#)).

Several limitations of previous pleiotropy vs. separate QTL tests prevent their direct application in multiparental populations. First, multiparental populations can have complex patterns of relatedness among subjects, and failure to account for these patterns of relatedness may lead to spurious results ([Yang *et al.* 2014](#)). Second, previous tests allowed for only two founder lines ([Jiang and Zeng](#)

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42 1995). Finally, Jiang and Zeng (1995) assumed that the null distribution of the test statistic follows a
43 chi-square distribution.

44 We developed a pleiotropy vs. separate QTL test for two traits in multiparental populations. Our
45 test builds on research that Jiang and Zeng (1995), Knott and Haley (2000), Tian *et al.* (2016), and
46 Zhou and Stephens (2014) initiated. Our innovations include the accommodation of k founder alle-
47 les per locus (compared to the traditional two founder alleles per locus) and the incorporation of
48 multivariate polygenic random effects to account for relatedness. Furthermore, we implemented a
49 parametric bootstrap test to assess statistical significance (Efron 1979; Tian *et al.* 2016).

50 Below, we describe our likelihood ratio test for pleiotropy vs. separate QTL. In simulation studies,
51 we find that it is slightly conservative, and that it has power to detect two separate loci when the
52 univariate LOD peaks are strong. We further illustrate our approach with an application to data on
53 a pair of behavior traits in a population of 261 DO mice (Logan *et al.* 2013; Recla *et al.* 2014). We find
54 modest evidence for distinct QTL in a 2.5-cM region on mouse Chromosome 8.

55 **Methods**

56 Our strategy involves first identifying two traits that map to a common genomic region. We then per-
57 form a two-dimensional, two-QTL scan over the genomic region, with each trait affected by one QTL
58 of varying position. We identify the QTL position that maximizes the likelihood under pleiotropy
59 (that is, along the diagonal where the two QTL are at a common location), and the ordered pair of
60 positions that maximizes the likelihood under the model where the two QTL are allowed to be dis-
61 tinct. The logarithm of the ratio of the two likelihoods is our test statistic. We determine statistical
62 significance with a parametric bootstrap.

63 **Data structures**

64 The data consist of three objects. The first is an n by k by m array of allele probabilities for n subjects
65 with k alleles and m marker positions on a single chromosome [derived from the observed SNP
66 genotype data by a hidden Markov model; see Broman *et al.* (2019)]. The second object is an n by 2
67 matrix of phenotype values. Each column is a phenotype and each row is a subject. The third object
68 is an n by c matrix of covariates, where each row is a subject and each column is a covariate.

69 One additional object is the genotype-derived kinship matrix, which is used in the linear mixed
70 model to account for population structure. We are focusing on a defined genomic interval, and
71 we prefer to use a kinship matrix derived by the “leave one chromosome out” (LOCO) method
72 (Yang *et al.* 2014), in which the kinship matrix is derived from the genotypes for all chromosomes
73 except the chromosome under test.

74 **Statistical Models**

75 Focusing on a pair of traits and a particular genomic region of interest, the next step is a two-
76 dimensional, two-QTL scan (Jiang and Zeng 1995). We consider two QTL with each affecting a
77 different trait, and consider all possible pairs of locations for the two QTL. For each pair of posi-
78 tions, we fit the multivariate linear mixed effects model defined in Equation 1. Note that we have
79 assumed an additive genetic model throughout our analyses, but extensions to design matrices that
80 include dominance are straightforward.

$$vec(Y) = Xvec(B) + vec(G) + vec(E) \quad (1)$$

81 where Y is the n by 2 matrix of phenotypes values; X is a $2n$ by $2(k + c)$ matrix that contains the k
82 allele probabilities for the two QTL positions and the c covariates in diagonal blocks; B is a $(k + c)$

83 by 2 matrix of allele effects and covariate effects; G is a n by 2 matrix of random effects; and E is a
 84 n by 2 matrix of random errors. n is the number of mice. The ‘vec’ operator stacks columns from
 85 a matrix into a single vector. For example, a 2 by 2 matrix inputted to ‘vec’ results in a vector with
 86 length 4. Its first two entries are the matrix’s first column, while the third and fourth entries are the
 87 matrix’s second column.

88 We also impose distributional assumptions on G and E :

$$G \sim MN_{n \times 2}(0, K, V_g) \quad (2)$$

89 and

$$E \sim MN_{n \times 2}(0, I, V_e) \quad (3)$$

90 where $MN_{n \times 2}(0, V_r, V_c)$ denotes the matrix-variate (n by 2) normal distribution with mean being the
 91 n by 2 matrix with all zero entries and row covariance V_r and column covariance V_c . We assume
 92 that G and E are independent.

93 **Parameter inference and log likelihood calculation**

94 Inference for parameters in multivariate linear mixed effects models is notoriously difficult and
 95 can be computationally intense (Meyer 1989, 1991). Thus, we estimate V_g and V_e under the null
 96 hypothesis of no QTL, and then take them as fixed and known in our two-dimensional, two-QTL
 97 genome scan. We use restricted maximum likelihood methods to fit the model:

$$vec(Y) = X_0 vec(B) + vec(G) + vec(E) \quad (4)$$

98 where X_0 is a $2n$ by $2(c + 1)$ matrix whose first column of each diagonal block in X_0 has all entries
 99 equal to one (for an intercept); the remaining columns are the covariates.

100 We draw on our R implementation (Boehm 2018) of the GEMMA algorithm for fitting a multivari-
 101 ate linear mixed effects model with expectation-maximization (Zhou and Stephens 2014). We use
 102 restricted maximum likelihood fits for the variance components V_g and V_e in subsequent calcula-
 103 tions of the generalized least squares solution \hat{B} .

$$\hat{B} = (X^T \hat{\Sigma}^{-1} X)^{-1} X^T \hat{\Sigma}^{-1} vec(Y) \quad (5)$$

104 where

$$\hat{\Sigma} = \hat{V}_g \otimes K + \hat{V}_e \otimes I_n \quad (6)$$

105 where \otimes denotes the Kronecker product, K is the kinship matrix, and I_n is a n by n identity matrix.
 106 We then calculate the log likelihood for a normal distribution with mean $X vec(\hat{B})$ and covariance $\hat{\Sigma}$
 107 that depends on our estimates of V_g and V_e (Equation 6).

108 **Pleiotropy vs. separate QTL hypothesis testing framework**

109 Our test applies to two traits considered simultaneously. Below, λ_1 and λ_2 denote putative locus
 110 positions for traits one and two. We quantitatively state the competing hypotheses for our test as:

$$\begin{aligned} H_0 : \lambda_1 &= \lambda_2 \\ H_A : \lambda_1 &\neq \lambda_2 \end{aligned} \quad (7)$$

111 Our likelihood ratio test statistic is:

$$\text{LOD} = \log_{10} \left[\frac{\max_{\lambda_1, \lambda_2} L(B, \Sigma, \lambda_1, \lambda_2)}{\max_{\lambda} L(B, \Sigma, \lambda, \lambda)} \right] \quad (8)$$

112 where L is the likelihood for fixed QTL positions, maximized over all other parameters.

113 **Visualizing profile LOD traces**

114 The output of the above analysis is a two-dimensional \log_{10} likelihood surface. To visualize these
115 results, we followed an innovation of [Zeng *et al.* \(2000\)](#) and [Tian *et al.* \(2016\)](#), and plot three traces:
116 the results along the diagonal (corresponding to the null hypothesis of pleiotropy), and then the
117 profiles derived by fixing one QTL's position and maximizing over the other QTL's position.

118 We define the LOD score for our test:

$$\text{LOD}(\lambda_1, \lambda_2) = ll_{10}(\lambda_1, \lambda_2) - \max ll_{10}(\lambda, \lambda) \quad (9)$$

119 where ll_{10} denotes \log_{10} likelihood.

120 We follow [Zeng *et al.* \(2000\)](#) and [Tian *et al.* \(2016\)](#) in defining profile LOD by the equation

$$\text{profile LOD}_1(\lambda_1) = \max_{\lambda_2} \text{LOD}(\lambda_1, \lambda_2) \quad (10)$$

121 We define profile $\text{LOD}_2(\lambda_2)$ analogously. The profile LOD_1 and profile LOD_2 traces have the same
122 maximum value, which is non-negative and gives the overall LOD test statistic.

123 We construct the pleiotropy trace by calculating the log-likelihoods for the pleiotropic models at
124 every position.

$$\text{LOD}_p(\lambda) = ll_{10}(\lambda, \lambda) - \max ll_{10}(\lambda, \lambda) \quad (11)$$

125 By definition, the maximum value for this pleiotropy trace is zero.

126 **Bootstrap for test statistic calibration**

127 We use a parametric bootstrap to determine statistical significance ([Efron 1979](#)). While [Jiang and Zeng](#)
128 [\(1995\)](#) used quantiles of a chi-squared distribution to determine p-values, this does not account
129 for the two-dimensional search over QTL positions. We follow the approach of [Tian *et al.* \(2016\)](#),
130 and identify the maximum likelihood estimate of the QTL position under the null hypothesis of
131 pleiotropy. We then use the inferred model parameters under that model and with the QTL at that
132 position to simulate bootstrap data sets according to the model in equations 1–3. For each of b boot-
133 strap data sets, we perform a two-dimensional QTL scan (over the genomic region of interest) and
134 derive the test statistic value. We treat these b test statistics as the empirical null distribution, and
135 calculate a p-value as the proportion of the b bootstrap test statistics that equal or exceed the ob-
136 served one, with the original data, $p = \#\{i : \text{LOD}_i^* \geq \text{LOD}\} / b$ where LOD_i^* denotes the LOD score
137 for the i th bootstrap replicate and LOD is the observed test statistic.

138 **Data & Software Availability**

139 Our methods have been implemented in an R package, `qt12pleio`, available at GitHub:

140 <https://github.com/fboehm/qt12pleio>

141 Custom R code for our analyses and simulations are at GitHub:

142 <https://github.com/fboehm/qt2pleio-manuscript>

143 The data from [Recla *et al.* \(2014\)](#) and [Logan *et al.* \(2013\)](#) are available at the Mouse Phenome Database:

Table 1 Type I error rates for all runs in our 2^3 experimental design. We set (marginal) genetic variances (*i.e.*, diagonal elements of V_g) to 1 in all runs. V_e was set to the 2 by 2 identity matrix in all runs. We used allele probabilities at a single genetic marker to simulate traits for all eight sets of parameter inputs. In the column “Allele effects partitioning”, “ABCD:EFGH” means that lines A–D carry one QTL allele while lines E–H carry the other allele. “F:ABCDEGH” means the QTL has a private allele in strain F.

Run	$\Delta(\text{Allele effects})$	Allele effects partitioning	Genetic correlation	Type I error rate
1	6	ABCD:EFGH	0	0.032
2	6	ABCD:EFGH	0.6	0.035
3	6	F:ABCDEGH	0	0.040
4	6	F:ABCDEGH	0.6	0.045
5	12	ABCD:EFGH	0	0.038
6	12	ABCD:EFGH	0.6	0.042
7	12	F:ABCDEGH	0	0.025
8	12	F:ABCDEGH	0.6	0.025

144 <https://phenome.jax.org/projects/Chesler4> and <https://phenome.jax.org/projects/Recla1>.
 145 They are also available in R/qtl2 format at <https://github.com/rqtl/qtl2data>.

146 Simulation studies

147 We performed two types of simulation studies, one for type I error rate assessment and one to
 148 characterize the power to detect separate QTL. To simulate traits, we specified X , B , V_g , K , and V_e
 149 matrices (Equations 1–3). For both we used the allele probabilities from a single genomic region
 150 derived empirically from data for a set of 479 Diversity Outbred mice from Keller *et al.* (2018).

151 Type I error rate analysis

152 To quantify type I error rate (*i.e.*, false positive rate), we simulated 400 pairs of traits for each of eight
 153 sets of parameter inputs (Table 1). We used a 2^3 factorial experimental design with three factors:
 154 allele effects difference, allele effects partitioning, and genetic correlation, *i.e.*, the off-diagonal entry
 155 in the 2 by 2 matrix V_g .

156 We chose two strong allele effects difference values, 6 and 12. These ensured that the univariate
 157 phenotypes mapped with high LOD scores to the region of interest. For the allele partitioning factor,
 158 we used either equally frequent QTL alleles, or a private allele in the CAST strain (F). For the residual
 159 genetic correlation (the off-diagonal entry in V_g), we considered the values 0 and 0.6. The marginal
 160 genetic variances (*i.e.*, the diagonal entries in V_g) for each trait were always set to one.

161 We performed 400 simulation replicates per set of parameter inputs, and each used $b = 400$
 162 bootstrap samples. For each bootstrap sample, we calculated the test statistic (Equation 8). We
 163 then compared the test statistic from the simulated trait against the empirical distribution of its 400
 164 bootstrap test statistics. When the simulated trait’s test statistic exceeded the 0.95 quantile of the
 165 empirical distribution of bootstrap test statistics, we rejected the null hypothesis. We observed that

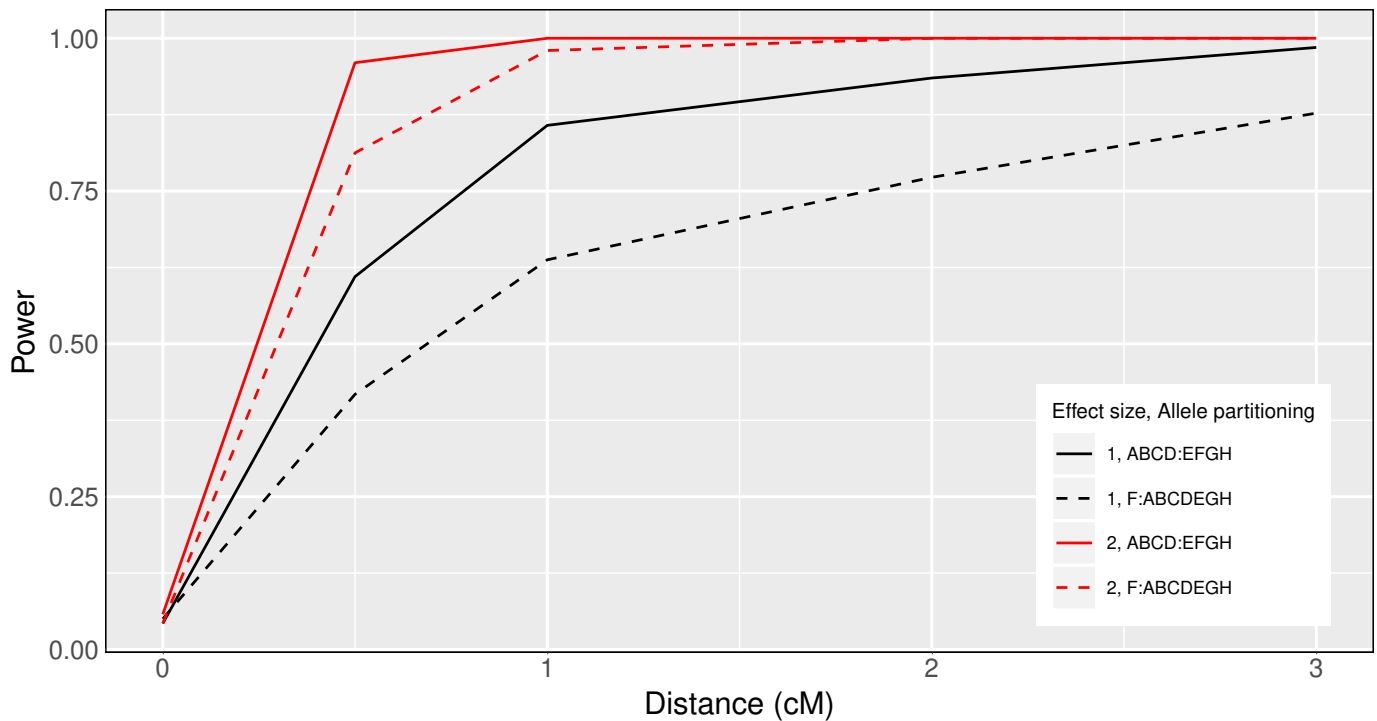


Figure 1 Pleiotropy vs. separate QTL power curves for each of four sets of parameter settings. Factors that differ among the four curves are allele effects difference and allele partitioning. Red denotes high allele effects difference, while black is the low allele effects difference. Solid line denotes the even allele partitioning (ABCD:EFGH), while dashed line denotes the uneven allele partitioning (F:ABCDEGH).

166 the test is slightly conservative over our range of parameter selections (Table 1), with estimated type
 167 I error rates < 0.05 .

168 **Power analysis**

169 We also investigated the power to detect the presence of two distinct QTL. We used a $2 \times 2 \times 5$
 170 experimental design, where our three factors were allele effects difference, allele effects partitioning,
 171 and inter-locus distance. The two levels of allele effects difference were 1 and 2. The two levels of
 172 allele effects partitioning were as in the type I error rate studies, ABCD:EFGH and F:ABCDEGH
 173 (Table S1). The five levels of interlocus distance were 0, 0.5, 1, 2, and 3 cM. V_g and V_e were both set
 174 to the 2 by 2 identity matrix in all power study simulations.

175 We simulated 400 pairs of traits per set of parameter inputs. For each simulation replicate, we
 176 calculated the likelihood ratio test statistic. We then applied our parametric bootstrap to determine
 177 the statistical significance of the results. For each simulation replicate, we used $b = 400$ bootstrap
 178 samples. Because the bootstrap test statistics within a single set of parameter inputs followed ap-
 179 proximately the same distribution, we pooled the $400 * 400 = 160,000$ bootstrap samples per set
 180 of parameter inputs and compared each test statistic to the empirical distribution derived from the
 181 160,000 bootstrap samples. However, for parameter inputs with interlocus distance equal to zero,
 182 we did not pool the 160,000 bootstrap samples; instead, we proceeded by calculating power (*i.e.*,
 183 type I error rate, in this case), as we did in the type I error rate study above.

184 We present our power study results in Figure 1. Power increases as interlocus distance increases.
 185 The top two curves correspond to the case where the QTL effects are largest. For each value for the

186 QTL effect, power is greater when the QTL alleles are equally frequent, and smaller when a QTL
187 allele is private to one strain. One can have high power to detect that the two traits have distinct
188 QTL when they are separated by > 1 cM and when the QTL have large effect.

189 Application

190 To illustrate our methods, we applied our test to data from Logan *et al.* (2013) and Recla *et al.* (2014),
191 on 261 DO mice measured for a set of behavioral phenotypes. Recla *et al.* (2014) identified *Hydin* as
192 the gene that underlies a QTL on Chromosome 8 at 57 cM for the “hot plate latency” phenotype (a
193 measure of pain tolerance). The phenotype “percent time in light” in a light-dark box (a measure
194 of anxiety) was measured on the same set of mice (Logan *et al.* 2013) and also shows a QTL near
195 this location, which led us to ask whether the same locus affects both traits. The two traits show a
196 correlation of -0.15 (Figure S1).

197 QTL analysis with the LOCO method, and using sex as an additive covariate, showed multiple
198 suggestive QTL for each phenotype (Figure S2; Table S2). For our investigation of pleiotropy, we
199 focused on the interval 53–64 cM on Chromosome 8. The univariate QTL results for this region are
200 shown in Figure 2.

201 The estimated QTL allele effects for the two traits are quite different (Figure 3). With the QTL
202 placed at 55 cM, for “percent time in light”, the WSB and PWK alleles are associated with large
203 phenotypes and NOD with low phenotypes. For “hot plate latency”, on the other hand, CAST and
204 NZO show low phenotypes and NOD and PWK are near the center.

205 In applying our test for pleiotropy, we performed a two-dimensional, two-QTL scan for the pair
206 of phenotypes. With these results, we created a profile LOD plot (Figure 4). The profile LOD for
207 “percent time in light” (in brown) peaks near 55 cM, as was seen in the univariate analysis. The
208 profile LOD for “hot plate latency” (in blue) peaks near 57 cM, also similar to the univariate analysis.
209 The pleiotropy trace (in gray) peaks near 55 cM.

210 The likelihood ratio test statistic for the test of pleiotropy was 1.2. Based on a parametric bootstrap
211 with 1,000 bootstrap replicates, the estimated p-value was 0.11, indicating weak evidence for distinct
212 QTL for the two traits.

213 Discussion

214 We developed a test of pleiotropy vs. separate QTL for multiparental populations, extending the
215 work of Jiang and Zeng (1995) for multiple alleles and with a linear mixed model to account for
216 population structure (Kang *et al.* 2010; Yang *et al.* 2014). Our simulation studies indicate that the test
217 has power to detect presence of separate loci, especially when univariate trait associations are strong
218 (Figure 1). Type I error rates indicate that our test is slightly conservative (Table 1).

219 In the application of our method to two behavioral phenotypes in a study of 261 Diversity Out-
220 bred mice (Recla *et al.* 2014; Logan *et al.* 2013), we obtained weak evidence ($p=0.11$) for the presence
221 of two distinct QTL, with one QTL (which contained the *Hydin* gene) affecting only “hot plate la-
222 tency” and a second QTL affecting “percent time in light” (Figure 4).

223 Founder allele effects plots provide further evidence for the presence of two distinct loci. As
224 Macdonald and Long (2007) and King *et al.* (2012) have demonstrated in their analyses of multi-
225 parental *Drosophila* populations, a biallelic pleiotropic QTL would result in allele effects plots that
226 have similar patterns. While we do not know that “percent time in light” and “hot plate latency”
227 arise from biallelic QTL, the dramatic differences that we observe in allele effects patterns further
228 support the argument for two distinct loci.

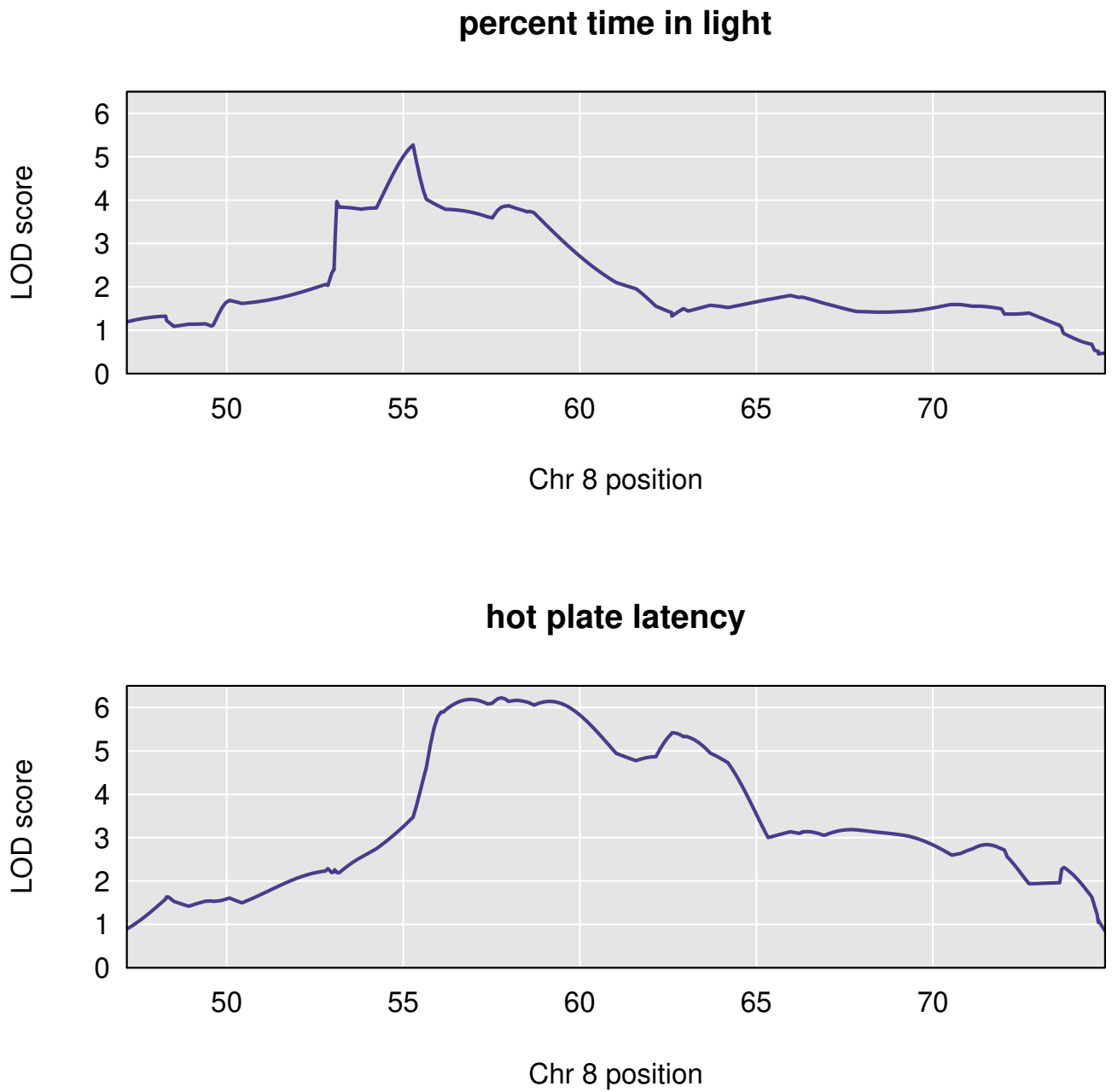


Figure 2 Chromosome 8 univariate LOD scores for percent time in light and hot plate latency reveal broad, overlapping peaks between 53 cM and 64 cM. The peak for percent time in light spans the region from approximately 53 cM to 60 cM, with a maximum near 55 cM. The peak for hot plate latency begins near 56 cM and ends about 64 cM.

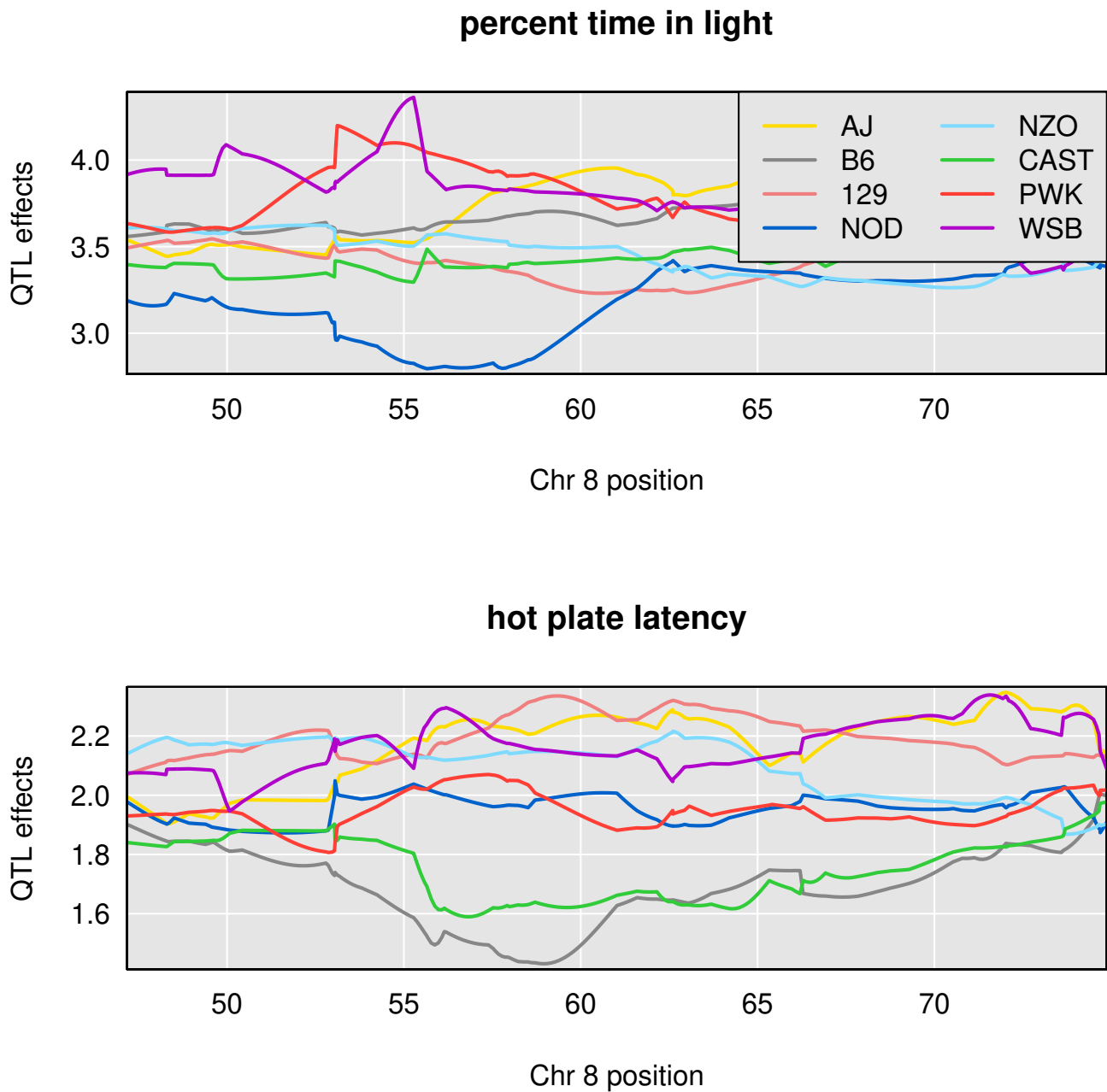


Figure 3 Chromosome 8 univariate LOD scores for percent time in light and hot plate latency reveal broad, overlapping peaks between 53 cM and 64 cM. The peak for percent time in light spans the region from approximately 53 cM to 60 cM, with a maximum near 55 cM. The peak for hot plate latency begins near 56 cM and ends about 64 cM.

percent time in light and hot plate latency

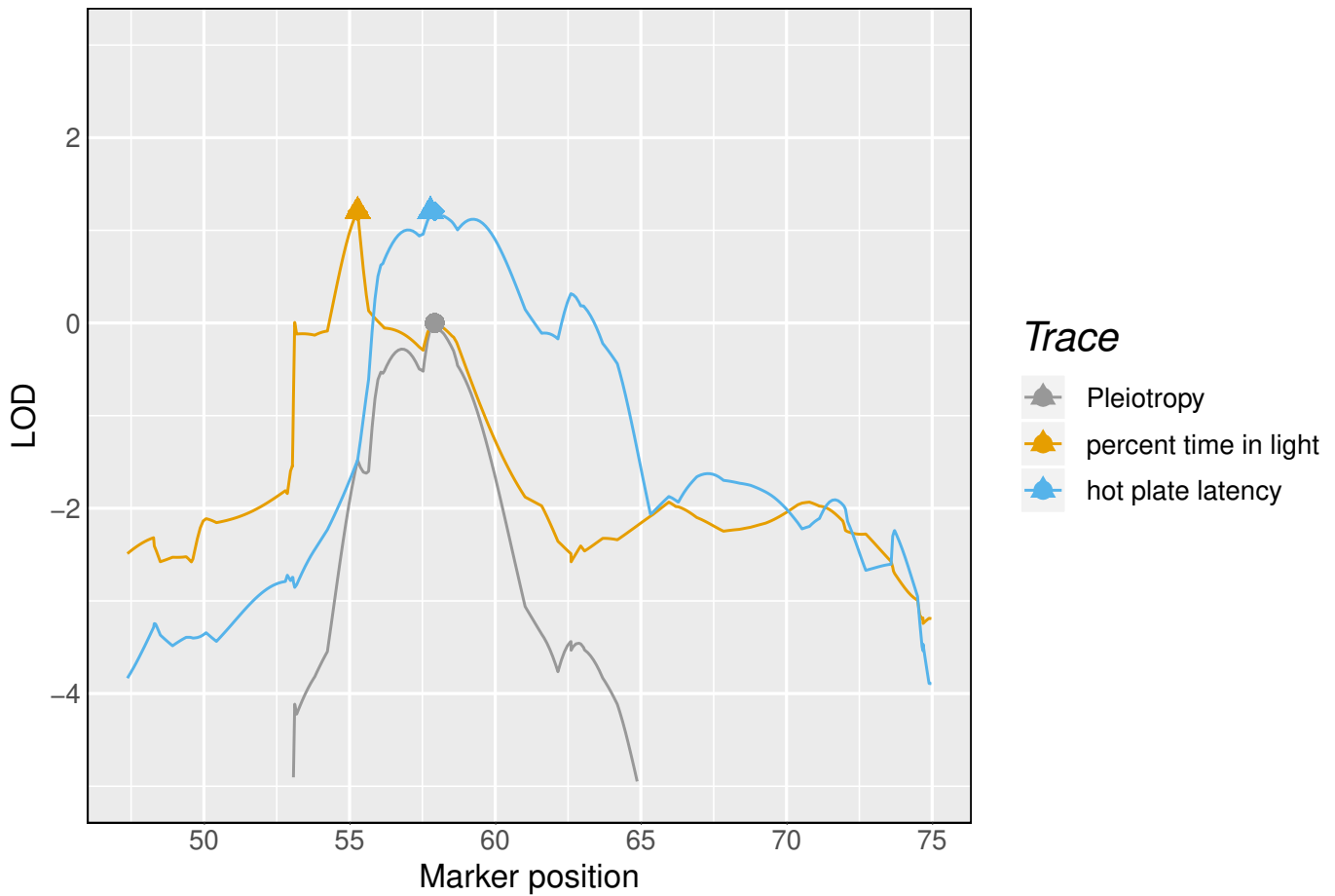


Figure 4 Profile LOD curves for the pleiotropy vs. separate QTL hypothesis test for “percent time in light” and “hot plate latency”. Gray trace denotes pleiotropy LOD values. Triangles denote the univariate LOD maxima, while diamonds denote the profile LOD maxima. For “percent time in light”, the brown triangle obscures the smaller brown diamond. Likelihood ratio test statistic value corresponds to the height of the blue and brown traces at their maxima.

229 We have implemented our methods in an R package `qt12pleio`, but analyses can be computa-
230 tionally intensive and time consuming. `qt12pleio` is written mostly in R, and so we could likely
231 obtain improved computational speed by porting parts of the calculations to a compiled language
232 such as C or C++. To accelerate our multi-dimensional QTL scans, we have integrated C++ code
233 into `qt12pleio`, using the `Rcpp` package (Eddelbuettel *et al.* 2011).

234 Another computational bottleneck is the estimation of the variance components V_g and V_e . To
235 accelerate this procedure, especially for the joint analysis of more than two traits, we will consider
236 other strategies for variance component estimation, including that described by Meyer *et al.* (2018).
237 Meyer *et al.* (2018), in joint analysis of dozens of traits, implement a bootstrap strategy to estimate
238 variance components for lower-dimensional phenotypes before combining bootstrap estimates into
239 valid covariance matrices for the full multivariate phenotype. Such an approach may ease some of
240 the computational burdens that we encountered.

241 We view tests of pleiotropy as complementary to mediation tests and related methods that have
242 become popular for inferring biomolecular causal relationships (Chick *et al.* 2016; Schadt *et al.* 2005;
243 Baron and Kenny 1986). A mediation test proceeds by including a putative mediator as a covariate
244 in the regression analysis of phenotype and QTL genotype; a substantial reduction in the association
245 between genotype and phenotype corresponds to evidence of mediation.

246 Mediation analyses and our pleiotropy test ask distinct, but related, questions. Mediation analysis
247 seeks to establish causal relationships among traits, including molecular traits, or dependent biolog-
248 ical and behavioral processes. Pleiotropy tests examine whether two traits share a single source of
249 genetic variation, which may act in parallel or in a causal network. Pleiotropy is required for causal
250 relations among traits. In many cases, the pleiotropy hypothesis is the only reasonable one.

251 Schadt *et al.* (2005) argued that both pleiotropy tests and causal inference methods may contribute
252 to gene network reconstruction. They developed a model selection strategy, based on the Akaike
253 Information Criterion (Akaike 1974), to determine which causal model is most compatible with the
254 observed data. Schadt *et al.* (2005) extended the methods of Jiang and Zeng (1995) to consider more
255 complicated alternative hypotheses, such as the possibility of two QTL, one of which associates with
256 both traits, and one of which associates with only one trait. As envisioned by Schadt *et al.* (2005), we
257 foresee complementary roles emerging for our pleiotropy test and mediation tests in the dissection
258 of complex trait genetic architecture.

259 Two related approaches for identifying and exploiting pleiotropy deserve mention. First, CAPE
260 (Combined Analysis of Pleiotropy and Epistasis) is a strategy for identifying higher-order relation-
261 ships among traits and marker genotypes (Tyler *et al.* 2013, 2016) and has recently been extended
262 for use with multiparental populations, including DO mice (Tyler *et al.* 2017). CAPE exploits the
263 pleiotropic relationship among traits in order to characterize the underlying network of QTLs, and
264 it can suggest possible pleiotropic effects, but it does not provide an explicit test of pleiotropy. Sec-
265 ond, Schaid *et al.* (2016) described a test for pleiotropy in the context of human genome-wide asso-
266 ciation studies (GWAS). Their approach is fundamentally different from ours, in that rather than
267 ask whether traits are affected by a common locus or distinct loci, they ask whether the traits are all
268 affected by a particular SNP or only some are. The difference in these approaches may be attributed
269 to the difference in mapping resolution between human GWAS and experimental populations.

270 Technological advances in mass spectrometry and RNA sequencing have enabled the acquisi-
271 tion of high-dimensional biomolecular phenotypes (Ozsolak and Milos 2011; Han *et al.* 2012). Mul-
272 tiparental populations in *Arabidopsis*, maize, wheat, oil palm, rice, *Drosophila*, yeast, and other
273 organisms enable high-precision QTL mapping (Yu *et al.* 2008; Tisné *et al.* 2017; Stanley *et al.* 2017;
274 Raghavan *et al.* 2017; Mackay *et al.* 2012; Kover *et al.* 2009; Cubillos *et al.* 2013). The need to analyze
275 high-dimensional phenotypes in multiparental populations compels the scientific community to de-

276 velop tools to study genotype-phenotype relationships and complex trait architecture. Our test, and
277 its future extensions, will contribute to these ongoing efforts.

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Table S1 Eight founder lines and their one-letter abbreviations.

Founder allele	One-letter abbreviation
A/J	A
C57BL/6J	B
129S1/SvImJ	C
NOD/ShiLtJ	D
NZO/H1LTJ	E
Cast/EiJ	F
PWK/PhJ	G
WSB/EiJ	H

Table S2 Both “hot plate latency” and “percent time in light” demonstrate multiple QTL peaks with LOD scores above 5.

phenotype	chr	pos	LOD score
percent time in light	8	55.28	5.27
hot plate latency	8	57.77	6.22
percent time in light	9	36.70	5.42
hot plate latency	9	46.85	5.22
percent time in light	11	63.39	6.46
hot plate latency	12	43.52	5.13
percent time in light	15	15.24	5.67
hot plate latency	19	47.80	5.48

hot plate latency vs. percent time in light

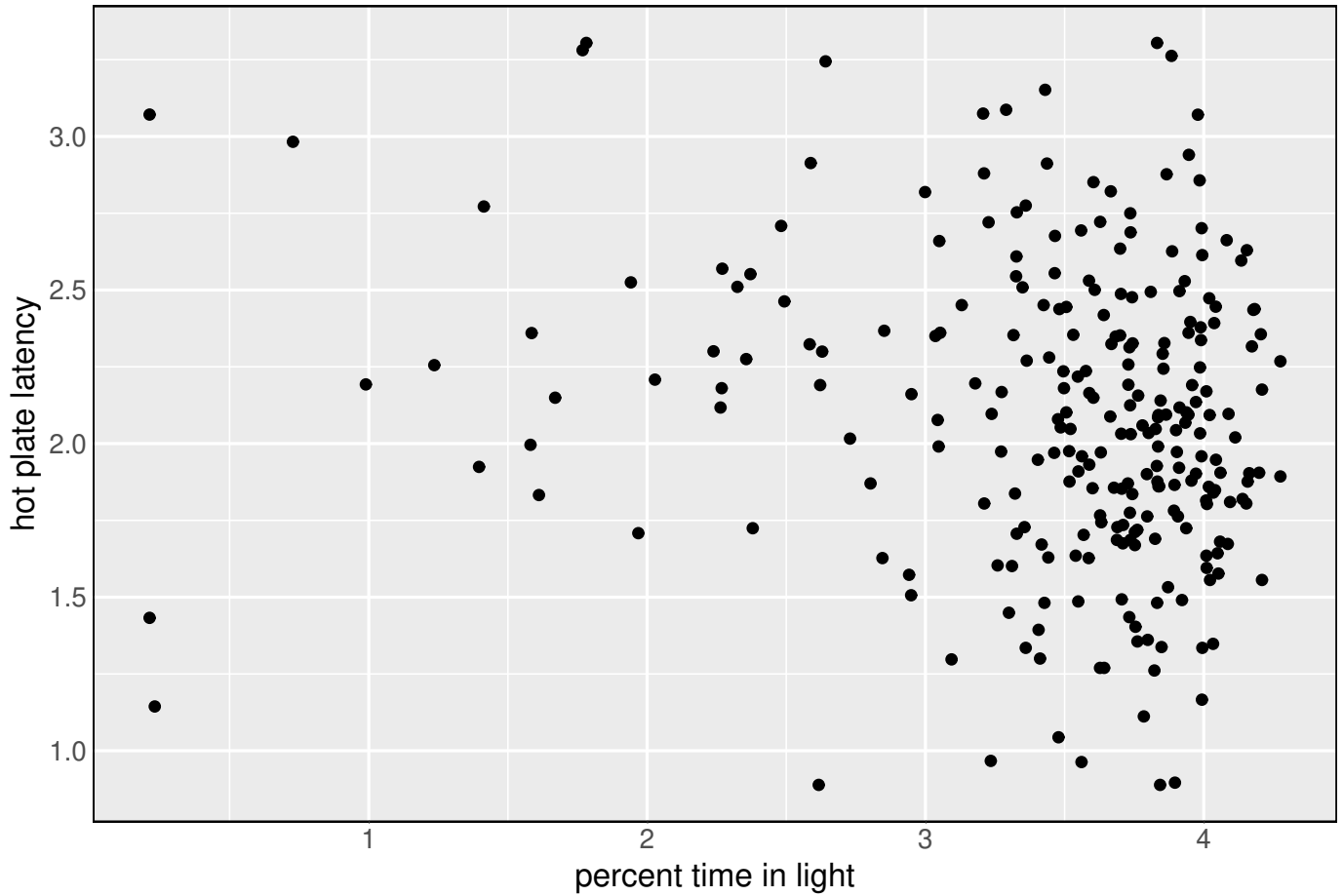


Figure S1 Scatter plot of “hot plate latency” against “percent time in light”, after applying logarithm transformations and winsorizing both traits.

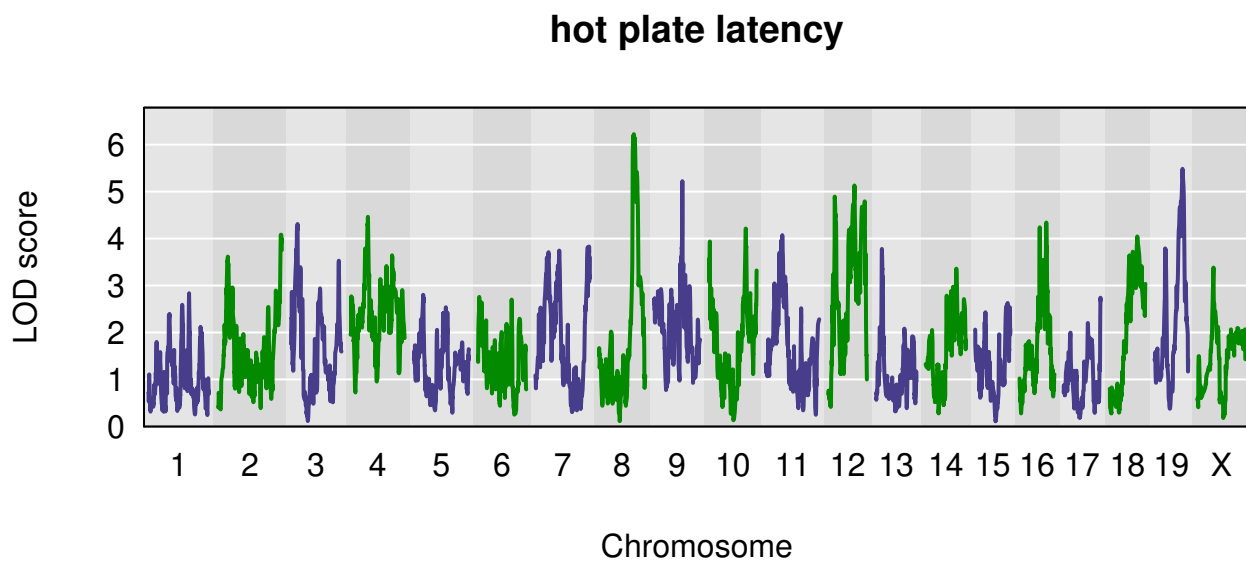
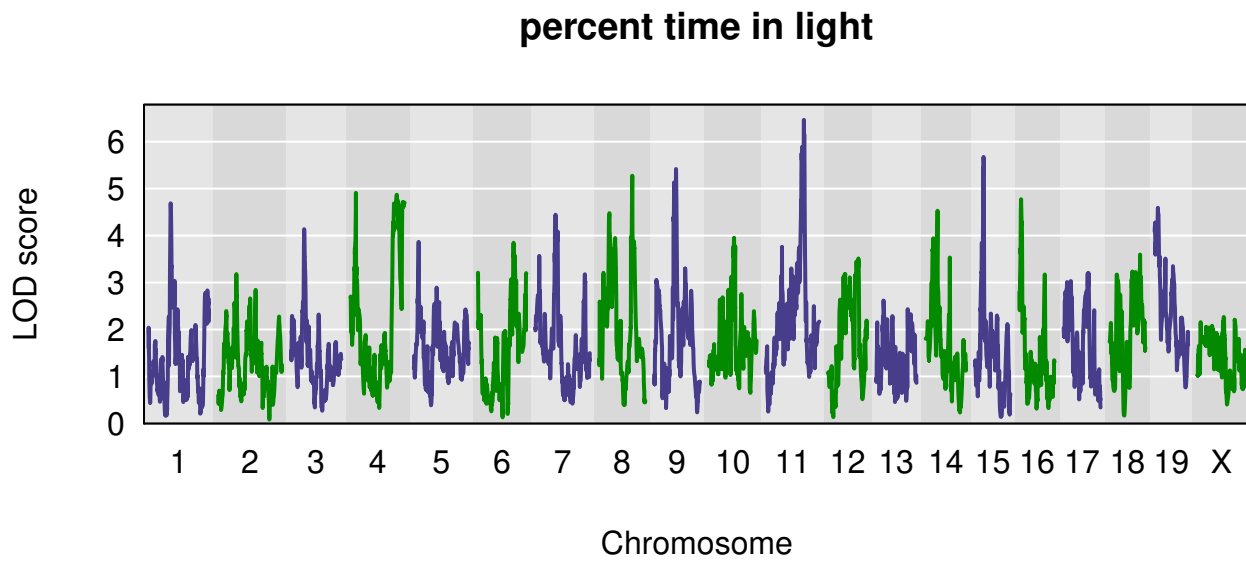


Figure S2 Genome-wide QTL scan for percent time in light reveals multiple QTL, including one on Chromosome 8.