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INVESTIGATIONS

vqtl: An R package for Mean-Variance QTL Mapping

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ABSTRACT We present vqtl, an R package that implements mean-variance QTL mapping. This QTL mapping approach tests for genetic loci that influence the mean of the phenotype, termed mean QTL, the variance of the phenotype, termed variance QTL, or some combination of the two, termed mean-variance QTL. It is unique in its ability to correct for variance heterogeneity arising from nuisance factors, such as sex, batch, or housing. This package provides a functions for geneticists to conduct genome scans, run permutations to assess the statistical significance of their findings, and make informative plots to communicate their results. Because this package is interoperable with the popular R/qtl package and uses many of the same data structures and input patterns, it will be easy for geneticists to analyze future experiments with R/vqtl as well as re-analyze past experiments, possibly discovering new QTL.

KEYWORDS

QTL mapping, vQTL, mvQTL, variance heterogeneity, DGLM, R

INTRODUCTION

QTL mapping studies in experimental crosses have provided important insights on nearly every trait of interest in human health and disease. Advances in model organism genotyping (Williams *et al.* 1990) and phenotyping (Yang *et al.* 2014) as well as in statistical methods (Lander and Botstein 1989; Martínez and Curnow 1992) and software tools (Broman *et al.* 2003; Mulligan *et al.* 2017) have supported these discoveries.

Traditional analyses assumed that the mapping population had homogenous variance and it was analyzed in search of "mean QTL", regions of the genome where allelic variation drives heterogeneity of phenotype mean. But, recent work has challenged that homogenous variance assumption by seeking to identify an additional type of QTL; those that influence the extent of residual variation, termed "variance QTL" (vQTL) (Paré *et al.* 2010; Rönnegård and Valdar 2011, 2012; Cao *et al.* 2014).

QTL analysis seeking to identify vQTL has recently entered the mainstream of genetic mapping efforts (Yang *et al.* 2012; Hulse and Cai 2013; Ayroles *et al.* 2015; Wei *et al.* 2016; Wang and Payseur 2017; Wei *et al.* 2017), but there remains great heterogeneity among the statistical methods used to conduct these analyses. We developed a standardized method for QTL mapping that can detect mQTL, vQTL, and a generalization of the two that we term "mvQTL". This approach, which we term "mean-variance QTL mapping", can be applied to intercross and backcross designs. In two companion articles, we characterize this method and competitors in the setting where a background factor drives variance heterogeneity [Corty and Valdar 2018+ [BVH]] and use it to discover new QTL from two existing data resources [Corty *et al* 2018+]

Here, we provide a practical guide to using the R package vqtl, which implements mean-variance QTL mapping. First, we simulate an F2 intercross using the popular R/qtl package and four phenotypes — a phenotype determined entirely by random noise, and one with each of the three kinds of QTL. We conduct a genome scan on each of the four phenotypes, using the standard interval mapping approach (Lander and Botstein 1989), and the mean-variance approach to QTL mapping, which includes a test for mQTL, a test for vQTL, and a test for mvQTL. We plot the association statistics of all four tests in LOD score units and discuss some drawbacks to this traditional plotting unit. We conduct permutation scans and use the results of the permutation scans to put the four tests on a level, and more easily interpretable, playing field. And finally, we plot the results to communicate the effects that led to the detection of a QTL, and use the bootstrap to estimate its confidence interval.

SIMULATE AN F2 INTERCROSS AND FOUR TRAITS

We used the popular R/qtl package to simulate an example experimental cross. This cross consisted of 200 male and 200 female F2 offspring, with 3 chromosomes of length 100 cM, each tagged by 11 equally-spaced markers and estimated genotype probabilities at 2cM intervals with R/qtl's hidden Markov model.

We simulated four phenotypes:

1. phenotype1 consists only of random noise and will serve as an example of negative results for all tests.

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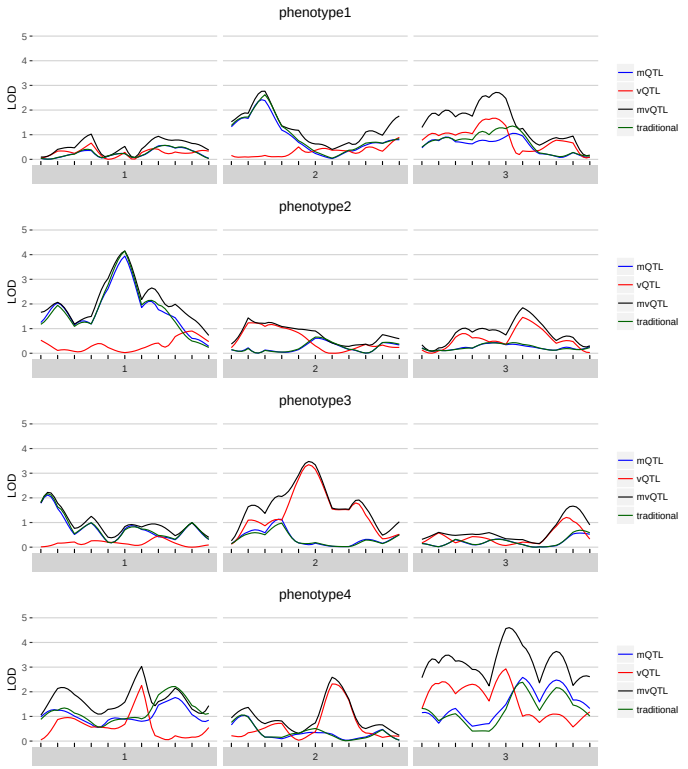

Figure 1 For each of the four simulated phenotypes, the genome scan shows the LOD score of each test – mean, variance, and joint – in blue, red, and black, respectively. The traditional test is in green and globally similar to the mean test.

2. `phenotype2` has an mQTL at the center of chromosome one.

3. `phenotype3` has a vQTL at the center of chromosome two.

4. `phenotype4` has an mvQTL at the center of chromosome three.

We additionally consider `phenotype1x` through `phenotype4x`, which have the same type of genetic effects as `phenotype1` through `phenotype4`, and, as an additional wrinkle, females have greater residual variance than males. All the same analyses and plots that are shown for `phenotype1` through `phenotype4` are shown for `phenotype1x` through `phenotype4x` in the appendix.

SCAN THE GENOME

The central function for genetic mapping in package `qtl` is `scanone` (Broman *et al.* 2003). Analogously, the central function for in package `vqtl` is `scanonevar`. It takes three required inputs:

1. `cross` is an object that contains the genetic and phenotypic information from an exerimental cross, as defined in package `qtl`.

2. `mean.formula` is a two-sided formula, specifying the phenotype to be mapped, the covariates to be corrected for, and the QTL terms to be fitted, with keywords `mean.QTL.add` and `mean.QTL.dom`

3. `var.formula` is a one-sided formula, specifying the variance covariates to be corrected for as well as the QTL terms to be fitted, using keywords `var.QTL.add` and `var.QTL.dom`.

For example, to scan a phenotype named `p1`, we run:

```
scanonevar(cross = test_cross,
    mean.formula = p1 ~ sex + mean.QTL.add + mean.QTL.dom,
    var.formula = ~ sex + var.QTL.add + var.QTL.dom)
```

Unlike `scanone`, which only tests for mQTL, `scanonevar` computes a test statistic for mQTL, vQTL, and mvQTL at each locus. For each type of QTL, the test statistic is the LOD score, the base 10 logarithm of the ratio of the likelihood of an alternative model to that of a null model. For all three types of QTL, the alternative model is the same:

$$\text{mean} \propto \text{covariate effects} + \text{locus effects}$$
$$\log(\text{variance}) \propto \text{covariate effects} + \text{locus effects}$$

The mQTL test compares that alternative against a null model that omits the locus effects on phenotype mean, whereas the vQTL test compares it with a null model that omits the locus effects on phenotype variance, and the mvQTL test omits both locus effects.

The results of `scanonevar` on each of the four phenotypes can be plotted directly, with the LOD score as the measure of association (Figure 1). Calling `summary` on the output of `scanonevar` produces a summary of how the scan was conducted and what the results were. With this example dataset, it takes five seconds to run one genome scan on a Intel Core i5.

The LOD Score – Problems and an Alternative

The LOD score is the traditional association statistic in QTL mapping, but its interpretation can be difficult becuase it cannot be compared across tests with different degrees of freedom. For example, associations from a sex chromosome are not be comparable with those from an autosome when, as is common, fewer parameters are used to model a locus on a sex chromosome. Another example, directly relevant here, is that the mvQTL test has more parameters than the mQTL test and the vQTL test and thus the LOD score is not comparable across those test classes.

These difficulties in interpreting the LOD score are evident in Figure 1. It seems that there are no important signals in the genome scan of `phenotype1` and it is visually clear that the most interesting signals for `phenotype2`, `phenotype3`, and `phenotype4` are on chromosomes one, two, and three, respectively. But important questions remain: (1) If we didn't have the genome scans from `phenotype2` – `phenotype4` available for comparison, would we be confident there are no statistically significant signals related to `phenotype1`? (2) How can we compare the results of the mvQTL test to the results of the other tests? (3) How could we compare the results of tests on autosomes to tests on sex chromosomes (if they were present)? (4) How often do we expect to observe results of this magnitude or greater when there is no true association, due simply to sampling variation and the multiplicity of tests conducted?

To put all genetic loci and all three tests on a level playing field, we consider two types of p-values: (1) Asymptotic p-values are calculated by `scanonevar` using the χ^2 distribution with the appropriate degrees of freedom for each locus. Though these p-values overcome the questions 1 through 3 of working with LOD scores described above, they leave question 4 unresolved. (2) Empirical, family-wide error rate (FWER)-corrected p-values resolve all the above questions with interpreting LOD scores so they are our preferred association statistics in most cases.

ASSESS THE SIGNIFICANCE OF RESULTS

To calculate the empirical, FWER-controlling, p-value of each test at each locus we advocate use of a permutation procedure [Corty

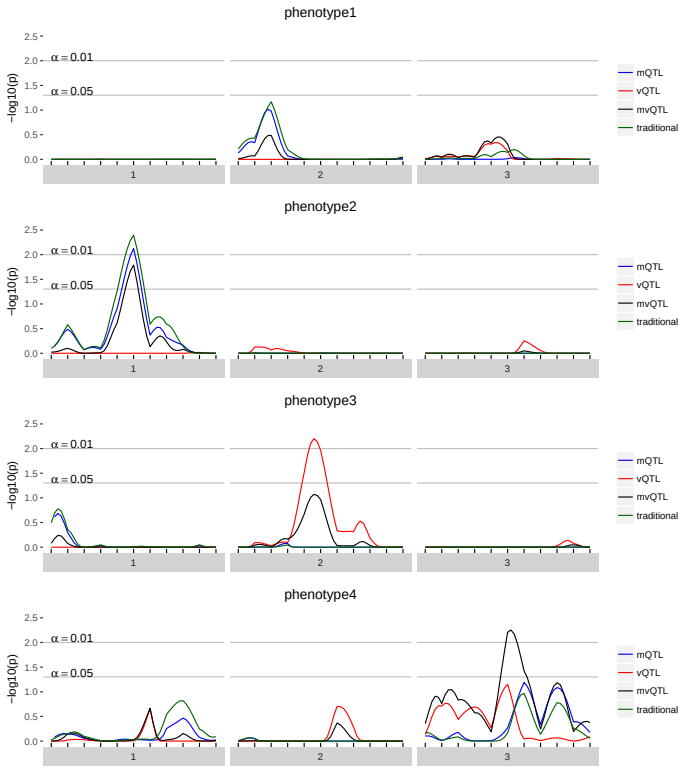

Figure 2 For each of the four simulated phenotypes, the genome scan shows the -log10 of the FWER-corrected p-value of each test – mean, variance, and joint – in blue, red, and black, respectively. Thus, a value of 2 implies that the quantity of evidence against the null is such that we expect to see this much or more evidence once per hundred genome scans when there is no true effect.

and Valdar 2018+ [BVH]]. Like previous work on permutation-based thresholds for genetic mapping (Churchill and Doerge 1994; Carlborg and Andersson 2002), this procedure sidesteps estimation of the effective number of tests.

In brief, this approach involves conducting many genomes scans on pseudo-null data generated through permutation to maintain as much of the character of the data as possible, while breaking the tested phenotype-genotype association. Specifically, the design matrix of the QTL is permuted in the mean portion of the mQTL alternative model, the variance portion of the vQTL alternative model, and in both portions of the mvQTL alternative model.

For each test (mQTL, vQTL, and mvQTL), the highest observed test statistic is extracted from each permutation scan and the collection of statistics that results is used to fit a generalized extreme value (GEV) density (Stephenson 2002). The observed LOD scores from the genome scan are then transformed by the cumulative distribution function of the extreme value density to estimate the FWER-controlling p-values. This approach is implemented in the function, `scanonevar.perm`, which requires two inputs:

1. `sov` is the `scanonevar` object, the statistical significance of which will be assessed through permutation.

2. `n.perms` is the number of permutations to conduct.

The object returned by `scanonevar.perm` is a `scanonevar` object with two additional piece of information, an empirical p-value for each test at each locus and the per-permutation maxima that were used to calculate those p-values. These FWER-corrected p-values are straightforwardly interpretable — $p = 0.05$ for a specific test at a specific locus implies that in 5% of similar experiments where there is no true genotype-phenotype association, we would expect to observe a locus with this much or more evidence of association in this test.

Accurate estimation of the FWER-controlled p-values requires many permutation scans. We recommend 1000 (Churchill and Doerge 1994; Carlborg and Andersson 2002). These permutation scans can be run on multiple processors by specifying the optional `n.cores` argument, which defaults to the total number of cores on the computer minus 1. On an Intel Core i5, running 100 permutations on this dataset takes about five minutes. When many phenotypes are studied, or if faster runtimes are needed, these permutation scans can be broken into groups with different values for `random.seed`, run on separate computers, and combined with the `c` function. This function combines the permutations from all the inputted scans, re-estimates the extreme value density, re-evaluates the observed LOD scores in the context of new extreme value density, and returns a new `scanonevar` object with more precisely estimated empirical p-values.

COMMUNICATE SIGNIFICANT FINDINGS

Having identified some QTL, we want to visualize the estimated genetic and covariate effects at the QTL. Because the `vqtl` package models both mean and variance effects, existing plotting utilities aren't able to display the entirety of the modeling results. To understand and communicate the results of a `vqtl` scan at one particular locus, we developed the `mean_var_plot`. This plot shows information about the phenotype mean on the horizontal axis and information about the phenotype variance on the vertical axis. There are both "model-free" and "model-based" versions of this plotting utility. For brevity, we show only the more useful "model-based" version.

In each `mean_var_plot` in Figure 3, the location of the dot shows the estimated mean and standard deviation of each genotype group, with the mean indicated by the horizontal position and the standard deviation indicated by the vertical position. The horizontal lines extending to the left and right from each dot show the standard error of the mean estimate, and the vertical lines extending up and down from each dot show the standard error of the standard deviation estimate. There are two types of grouping factors considered by the function `mean_var_plot_model_based`: (1) `focal.groups` are groups that are modeled and the prediction for each group is plotted. For example, a genetic marker is the `focal.group` in each plot in Figure 3; `D1M1` in the top left, `D1M6` in the top right, etc. (2) `nuisance.groups` are groups that are modeled, but then averaged over before plotting. When there are many grouping factors thought to play a role in determining the mean and variance of an individual's phenotype, such as sex, treatment, and batch, we recommend putting just one or two in `focal.groups` and the others in `nuisance.groups` for clarity, cycling through which are displayed to gain a thorough understanding of the factors that determine the mean and variance of the phenotype.

Additional plotting utilities, `phenotype_plot`, `effects_plot` and `mean_var_plot_model_free` are described in the online documentation, available on CRAN.

ESTABLISH A CONFIDENCE INTERVAL FOR THE QTL

Finally, it is important to assess the genetic precision of a discovered QTL for bioinformatic follow-up. The function

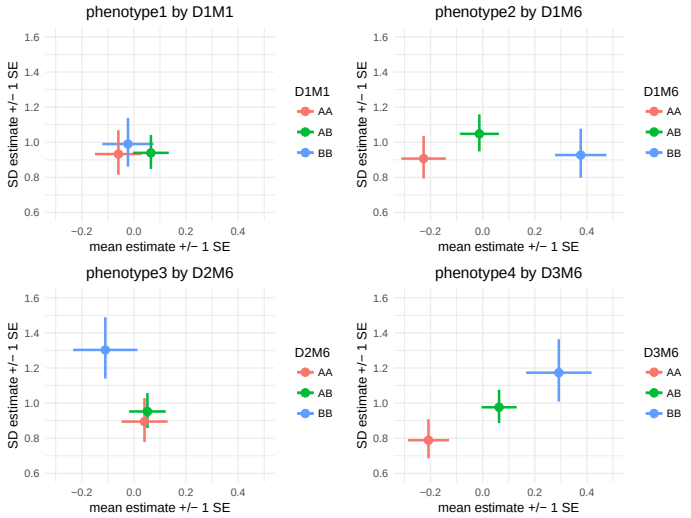

Figure 3 `mean_var_plots` show the estimated genotype effects at a locus, with mean effects on the horizontal axis and variance effects on the vertical axis. Horizontal lines indicate standard errors for mean effects and vertical lines indicate standard errors for variance effects. For `phenotype1`, the pattern of overlapping estimates and standard errors is consistent with the fact that there are no genetic effects, and the *p*-value was not statistically significant at any locus. For `phenotype2`, the pattern of horizontal, but not vertical, separation visually illustrates the identified mQTL. For `phenotype3`, the pattern of vertical, but not horizontal, separation visually illustrates the identified vQTL. For `phenotype4`, the pattern of two dimensional separation without either total horizontal or vertical separation illustrates an mvQTL with neither mean nor variance effect strong enough to define an mQTL or vQTL.

`scanonevar.boot` implements the non-parametric bootstrap Visscher *et al.* (1996). This function takes, as arguments, a `scanonevar` object, the name of the chromosome containing the QTL, and `num.resamples`, the number of bootstrap resamplings desired. As with `scanonevar.perm`, the `n.cores` argument can be used to spread the bootstraps over many computational cores and defaults to the number of cores available minus two, and bootstraps can be run on separate computers and combined with `c` to increase the precision of the estimate of the confidence interval.

We recommend 1000 resamples to establish 80% and 90% confidence intervals. With the datasets simulated here, it takes 20 minutes to run 1000 bootstrap resamples on an Intel core i5.

CONCLUSION

We have demonstrated typical usage of the `R` package `vqtl` for mean-variance QTL mapping in an F2 intercross. This package is appropriate for crosses and phenotypes where genetic factors or covariates or are known or suspected to influence phenotype variance. In the case of genetic factors, they can be mapped, as illustrated in Corty *et al* 2018+. In the case of covariates, they can be accommodated, which can increase power and control false positive rate, as described in Corty and Valdar 2018+ [BVH].

RESOURCES

The scripts used to simulate genotypes and phenotypes, conduct the genome scans, and plot the results are available as a public, static Zenodo repository at DOI:10.5281/zenodo.1173799. The package `vqtl` and its documentation are freely available on `CRAN` at https://CRAN.R-project.org/package=vqtl.

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APPENDIX

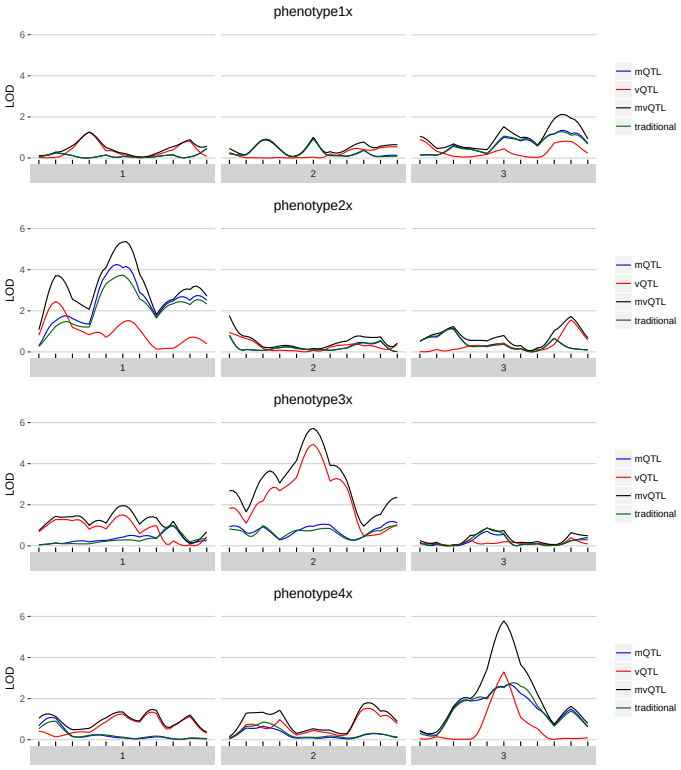

Figure A1 For each of the four simulated phenotypes with background variance heterogeneity, the genome scan shows the LOD score of each test – mean, variance, and joint – in blue, red, and black, respectively. The traditional test is in green and globally similar to the mean test.

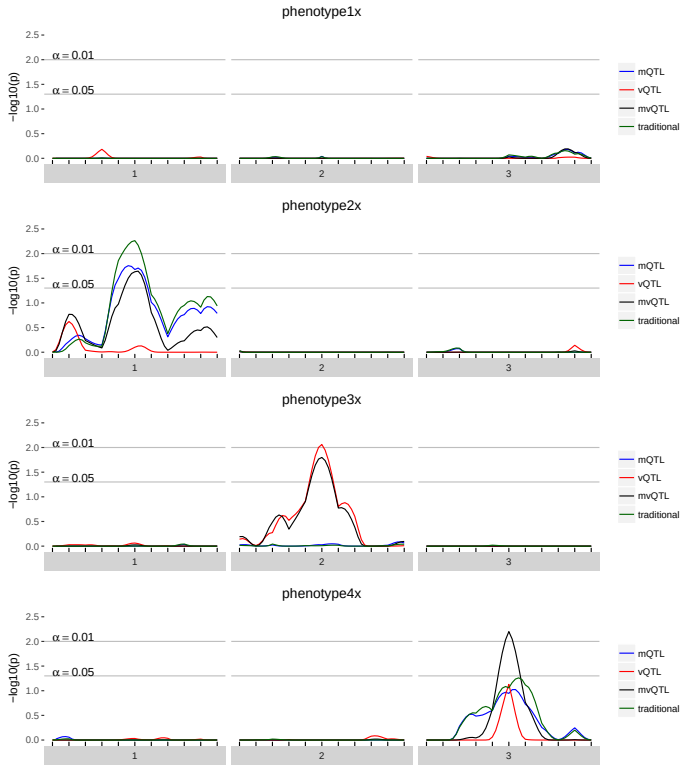

Figure A2 For each of the four simulated phenotypes with background variance heterogeneity, the genome scan shows the -log10 of the FWER-corrected *p*-value of each test – mean, variance, and joint – in blue, red, and black, respectively. Thus, a value of 3 implies that the quantity of evidence against the null is such that we expect to see this much or more evidence once per thousand genome scans when there is no true effect.

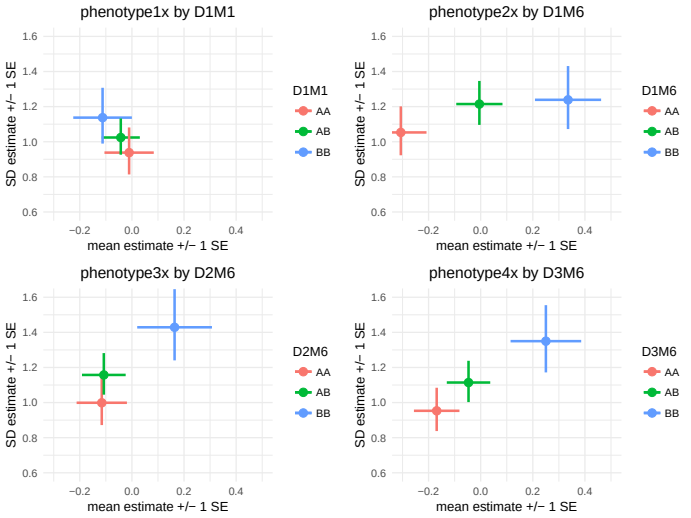

Figure A3 `mean_var_plots` show the estimated genotype effects at a locus, with mean effects on the horizontal axis and variance effects on the vertical axis. Horizontal lines indicate standard errors for mean effects and vertical lines indicate standard errors for variance effects. For `phenotype1x`, the pattern of overlapping estimates and standard errors is consistent with the fact that there are no genetic effects, and the *p*-value was not statistically significant at any locus. For `phenotype2x`, the pattern of horizontal, but not vertical, separation visually illustrates the identified mQTL on a background of variance heterogeneity. For `phenotype3x`, the pattern of vertical, but not horizontal, separation visually illustrates the identified vQTL on a background of variance heterogeneity. For `phenotype4x`, the pattern of two dimensional separation without either total horizontal or vertical separation illustrates an mvQTL with neither mean nor variance effect strong enough to define an mQTL or vQTL on a background of variance heterogeneity.