

R/qtl2: software for mapping quantitative trait loci with high-dimensional data and multi-parent populations

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Abstract

R/qrtl2 is an interactive software environment for mapping quantitative trait loci (QTL) in experimental populations. The R/qrtl2 software is a complete rewrite of the widely-used R/qrtl software package. The new software expands the scope of R/qrtl to include multi-parent populations derived from more than two founder strains, such as the Collaborative Cross and Diversity Outbred mice, heterogeneous stocks, and MAGIC plant populations. R/qrtl2 is designed to handle modern high-density genotyping data and high-dimensional molecular phenotypes including gene expression and proteomics. R/qrtl2 includes the ability to perform genome scans using a linear mixed model to account for population structure, and also includes features to impute SNPs based on founder strain genomes and to carry out association mapping. The R/qrtl2 software provides all of the basic features needed for QTL mapping, including graphical displays and summary reports, and it can be extended through the creation of add-on packages. R/qrtl2 comes with a test framework and is free and open source software written in the R and C++ programming languages.

Introduction

There has been a resurgence of interest in the mapping of quantitative trait loci (QTL) in experimental organisms, spurred in part by the use of gene expression phenotypes (eQTL mapping; see Albert and Kruglyak 2015) to more rapidly identify the underlying genes, and by the development of multi-parent populations, including heterogeneous stocks (Mott *et al.* 2000; Mott and Flint 2002), MAGIC lines (Cavanagh *et al.* 2008; Kover *et al.* 2009), the Collaborative Cross (Churchill *et al.* 2004), and Diversity Outbred mice (Churchill *et al.* 2012; Svenson *et al.* 2012).

There are numerous software packages for QTL mapping in experimental populations, including Mapmaker/QTL (Lincoln and Lander 1990), QTL Cartographer (Basten *et al.* 2002), R/qtl (Broman *et al.* 2003; Broman and Sen 2009), and MapQTL (Van Ooijen 2009). There are a smaller number of packages for QTL analysis in multi-parent populations, including DOQTL (Gatti *et al.* 2014), HAPPY (Mott *et al.* 2000), and mpMap (Huang and George 2011).

The original R/qtl (hereafter, R/qtl1) is widely used, and has a number of advantages compared to proprietary alternatives. R/qtl1 includes a quite comprehensive set of QTL mapping methods, including multiple-QTL exploration and model selection (Arends *et al.* 2010; Broman and Speed 2002; Manichaikul *et al.* 2009), as well as extensive visualization and data diagnostics tools (Broman and Sen 2009). Further, users and developers both benefit by it being an add-on package for the general statistical software, R (R Core Team 2018). A number of other R packages have been written to work in concert with R/qtl1, including ASMap (Taylor and Butler 2017), ctl (Arends *et al.* 2016), dlmap (Huang *et al.* 2012), qtlcharts (Broman 2015), vqtl (Corty and Valdar 2018), and wgaim (Taylor and Verbyla 2011).

R/qtl1 has a number of limitations (see Broman 2014), the most critical of which is that the

central data structure generally limits its use to biparental crosses. Also, R/qt11 was designed at a time when a dataset with more than 100 genetic markers was considered large.

Rather than extend R/qt11 for multi-parent populations, we decided to start fresh. R/qt12 is a completely redesigned R package for QTL analysis that can handle a variety of multi-parent populations and is suited for high-dimensional genotype and phenotype data. To handle population structure, QTL analysis may be performed with a linear mixed model that includes a residual polygenic effect. The R/qt12 software is available from its web site (<https://kbroman.org/qt12>) as well as GitHub (<https://github.com/rqt1/qt12>).

Features

QTL analysis in multi-parent populations can be split into two parts: calculation of genotype probabilities using multipoint SNP genotypes, and the genome scan to evaluate the association between genotype and phenotype, using those probabilities. As with R/qt11, we use a hidden Markov model (HMM; see Broman and Sen 2009, App. D) for the calculation of genotype probabilities. As the source of genotype information, R/qt12 considers SNP genotypes, generally derived from microarrays. At present, we focus solely on marker genotypes rather than array intensities or allele counts/dosages from genotyping-by-sequencing (GBS) assays.

R/qt12 includes implementations of the usual sorts of two-way crosses (backcross, intercross, doubled haploids, two-way recombinant inbred lines by selfing or sibling mating, and two-way advanced intercross populations), and several different types of multi-parent populations (4- and 8-way recombinant inbred lines by sibling mating; 4-, 8-, and 16-way recombinant inbred lines by selfing; 3-way advanced intercross populations, Diversity Outbred mice, heterogeneous stocks, 19-way MAGIC lines like the Kover *et al.* (2009) Arabidopsis lines, and 6-way doubled haploids following a design of maize MAGIC lines being developed at the University of Wisconsin–Madison.

A key component of the HMM is the transition matrix (or “step” probabilities), which are specific to the cross design. Transitions represent locations where the ancestry of chromosomal segments change from one founder strain haplotype to another. The transition probabilities for multi-way recombinant inbred lines are taken from Broman (2005). The transition probabilities for heterogeneous stocks and Diversity Outbred mice are taken from Broman (2012b), which uses the results of Broman (2012a).

At present, R/qrtl2 assumes dense marker information and a low level of uncertainty in the haplotype reconstructions, so that we may rely on Haley-Knott regression (Haley and Knott 1992) for genome scans to establish genotype-phenotype association. This may be performed either with a simple linear model (as in Haley and Knott 1992), or with a linear mixed model (Yu *et al.* 2006; Kang *et al.* 2008; Lippert *et al.* 2011) that includes a residual polygenic effect to account for population structure. The latter may also be performed using kinship matrices derived using the “leave-one-chromosome-out” (LOCO) method (see Yang *et al.* 2014).

To establish statistical significance of evidence for QTL, accounting for a genome scan, R/qrtl2 facilitates the use of permutation tests (Churchill and Doerge 1994). For multi-parent populations with analysis via a linear mixed model, we permute the rows of the haplotype reconstructions as considered in Cheng and Palmer (2013).

R/qrtl2 includes a variety of data diagnostic tools, which can be particularly helpful for data on multi-parent populations where the SNP genotypes are incompletely informative (i.e., SNP genotypes do not fully define the corresponding founder haplotype). These include SNP genotyping error LOD scores (Lincoln and Lander 1992) and estimated crossover counts.

R/qrtl2 reproduces the functionality of DOQTL (Gatti *et al.* 2014) but targets a broader set of multi-parent populations, in addition to Diversity Outbred mice. (DOQTL will ultimately be deprecated and replaced with R/qrtl2.) Fig. 1 contains a reproduction, using R/qrtl2, of Fig. 5 from Gatti *et al.* (2014). This is a QTL analysis of constitutive neutrophil counts in 742 Diversity Outbred mice (from generations 3–5) that were genotyped with the first generation Mouse Universal Genotyping Array (MUGA) (Morgan *et al.* 2016), which contained 7,851 markers, of which we are using 6,413.

The regression model that R/qrtl2 applies in a genome scan is determined by the the HMM

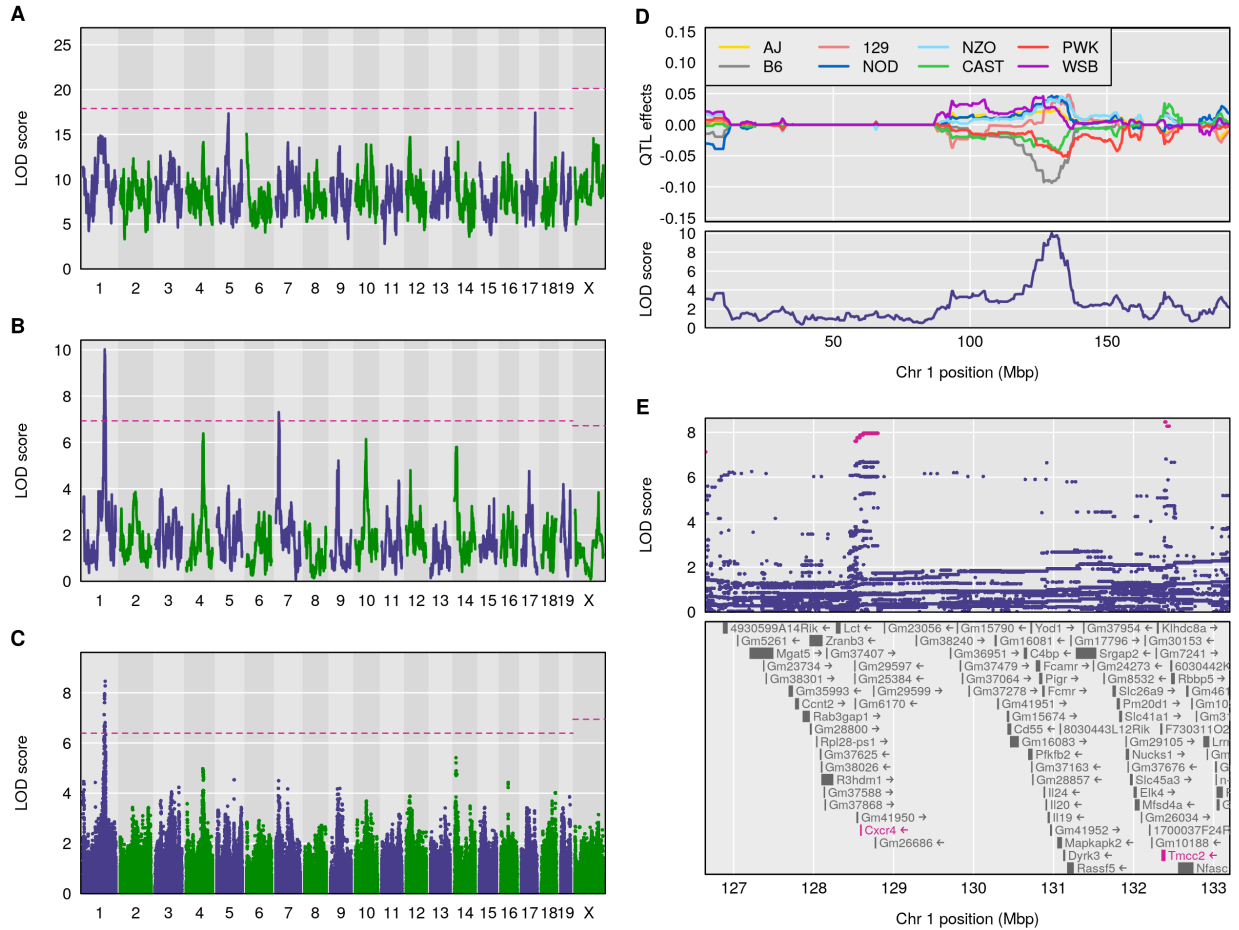


Figure 1: Reconstruction of Fig. 5 from Gatti *et al.* (2014), on the mapping of constitutive neutrophil counts in 742 Diversity Outbred mice. (A) LOD scores from a genome scan using the full model (comparing all 36 genotypes for the autosomes and 44 genotypes for the X chromosome); the dashed horizontal line indicates the 5% genome-wide significance threshold, based on a permutation test. (B) LOD scores from a genome scan with an additive allele model (compare the 8 founder haplotypes). (C) LOD scores from a SNP association scan, using all SNPs that were genotyped in the eight founder lines. (D) Best linear unbiased predictors (BLUPs) of the eight haplotype effects in the additive model, along with the LOD curve on chromosome 1. (E) SNP association results in the region of the chromosome 1 QTL, along with genes in the region; SNPs with LOD scores within 1.5 of the maximum are highlighted in pink. All figures are produced with R/qt12.

output in the genotype probabilities data structure. For an 8-parent MPP such as the DO mice, there are 36 possible diplotypes (44 on the X chromosome) and the genome scan will be based on a regression model with 35 degrees of freedom. With so many degrees of freedom, the model typically lacks power to detect QTL. An alternative representation collapses the 36 states to 8 founder "dosages" and uses a regression model with 7 degrees of freedom, assuming that the founder effects are additive at any given locus. R/qtl2 has the ability to incorporate SNP (and other variant) data from founder strains and to impute biallelic genotypes for every SNP. The genome scan on imputed SNPs is equivalent to an association mapping scan and can employ a additive (one degree of freedom) or general (two degrees of freedom) regression model.

Fig. 1A contains the LOD curves from a genome scan using a full model comparing all 36 possible genotypes with log neutrophil count as the phenotype and with sex and log white blood cell count as covariates. The horizontal dashed line indicates the 5% genome-wide significance level, derived from a permutation test, with separate thresholds for the autosomes and the X chromosome, using the technique of Broman *et al.* (2006). Fig. 1B contains the LOD curves from a genome scan using an additive allele model (corresponding to a test with 7 degrees of freedom), and Fig. 1C contains a SNP association scan, using a test with 2 degrees of freedom. All of these analyses use a linear mixed model with kinship matrices derived using the "leave-one-chromosome-out" (LOCO) method.

Fig. 1D shows the estimated QTL effects, assuming a single QTL with additive allele effects on chr 1, and sliding the position of the QTL across the chromosome. This is analogous to the estimated effects in Fig. 5D of Gatti *et al.* (2014), but here we present Best Linear Unbiased Predictors (BLUPs), taking the QTL effects to be random effects. This results in estimated effects that have been shrunk towards 0, which helps to clean up the figure and focus attention on the

region of interest.

Fig. 1E shows individual SNP association results, for the 6 Mbp region on chr 1 that contains the QTL. As with the DOQTL software, we use all available SNPs for which genotype data are available in the 8 founder lines, and impute the SNP genotypes in the Diversity Outbred mice, using the individuals' genotype probabilities along with the founder strains' SNP genotypes.

Fig. 1 shows a number of differences from the results reported in Gatti *et al.* (2014), including that we see nearly-significant loci on chr 5 and 17 in the full model (Fig. 1A), and we see a second significant QTL on chr 7 with the additive allele model (Fig. 1B). Also, in Fig. 1E, we see associated SNPs not just at ~ 128.6 Mbp near the *Cxcr4* gene (as in Gatti *et al.* 2014), but also a group of associated SNPs at ~ 132.4 Mbp, near *Tmcc2*. The differences between these results and those of Gatti *et al.* (2014) are largely due to differences in genotype probability calculations (data not shown).

Data and software availability

The data for Fig. 1 are available at the Mouse Phenotype Database (<https://phenome.jax.org/projects/Gatti2>). In addition, R/qtl2 input files for these data are available at GitHub (<https://github.com/rqtl/qtl2data>).

The R/qtl2 software is available from its web site (<https://kbroman.org/qtl2>) as well as GitHub (<https://github.com/rqtl/qtl2>). The software is licensed under the GNU General Public License version 3.0.

The code to create Fig. 1 is available at GitHub at https://github.com/kbroman/Paper_Rqtl2.

Implementation

R/qrtl2 is developed as a free and open source add-on package for the general statistical software, R (R Core Team 2018). Much of the code is written in R, but computationally intensive aspects are written in C++. (Computationally intensive aspects of R/qrtl1 are in C.) We use Rcpp (Eddelbuettel and François 2011; Eddelbuettel 2013) for the interface between R and C++, to simplify code and reduce the need for copying data in memory.

Linear algebra calculations, such as matrix decomposition and linear regression, are a central part of QTL analysis. We use RcppEigen (Bates and Eddelbuettel 2013) and the Eigen C++ library (Guennebaud *et al.* 2010) for these calculations. For the fit of linear mixed models, to account for population structure with a residual polygenic effect, we closely followed code from PyLMM (Furlotte 2015). In particular, we use the basic technique described in Kang *et al.* (2008), of taking the eigen decomposition of the kinship matrix.

We use roxygen2 (Wickham *et al.* 2017) to develop the documentation. R packages make it easy to provide detailed documentation to users, but the R documentation format is tedious and separate from the function definitions and so requires the maintenance of two versions of function documentation in separate places. Roxygen2 is a documentation tool that uses specially-formatted comments near the original code to generate the detailed R documentation files.

In contrast to R/qrtl1, which includes almost no formal software tests, R/qrtl2 includes extensive unit tests to ensure correctness. We use the R package testthat (Wickham 2011) for this purpose. The use of unit tests helps us to catch bugs earlier, and revealed several bugs in R/qrtl1.

Discussion

R/qrtl2 is a reimplementaion of the R package R/qrtl1, for QTL mapping in multi-parent populations, with improved handling of high-dimensional data. A variety of multi-parent populations have been implemented, including heterogeneous stocks, the Collaborative Cross, Diversity Outbred mice, and certain types of MAGIC lines. R/qrtl2 includes the ability to perform genome scans via a linear mixed model, accounting for population structure via a residual polygenic effect.

While the basic functionality of R/qrtl2 is complete, there are a number of areas for further development. In particular, we would like to further expand the set of crosses that may be considered, including partially-inbred recombinant inbred lines (so that we may deal with residual heterozygosity, which presently is ignored). We have currently been focusing on exact calculations for specific designs, but the mathematics involved can be tedious. We would like to have a more general approach for genotype reconstruction in multi-parent populations, along the lines of RABBIT (Zheng *et al.* 2015) or STITCH (Davies *et al.* 2016). Plant researchers have been particularly creative in developing unusual sets of MAGIC populations, and by our current approach, each design requires the development of design-specific code, which is difficult to sustain.

Another important area of development is the handling of genotyping-by-sequencing (GBS) data. We are currently focusing solely on called genotypes. With low-coverage GBS data, it is difficult to get quality genotype calls at individual SNPs, and there will be considerable advantage to using the pairs of allele counts and combining information across SNPs. Extending the current HMM implementation in R/qrtl2 to handle pairs of allele counts for GBS data appears

straightforward.

At present, QTL analysis in R/qtl2 is solely by genome scans with single-QTL models. Consideration of multiple-QTL models will be particularly important for exploring the possibility of multiple causal SNPs in a QTL region, along the lines of the CAVIAR software (Hormozdiari *et al.* 2014) or the method of Deng and Pan (2018).

We have currently focused solely on Haley-Knott regression (Haley and Knott 1992) for QTL analysis. This has a big advantage in terms of computational speed, but it does not fully account for the uncertainty in genotype reconstructions. But the QTL analysis literature has a long history of methods for dealing with this genotype uncertainty, including interval mapping (Lander and Botstein 1989) and multiple imputation (Sen and Churchill 2001). While this has not been a high priority in the development of R/qtl2, ultimately we will include implementations of these sorts of approaches, to better handle regions with reduced genotype information.

R/qtl2 is an important update to the popular R/qtl software, expanding the scope to include multi-parent populations, providing improved handling of high-dimensional data, and enabling genome scans with a linear mixed model to account for population structure. R/qtl1 served as an important hub upon which other developers could build; we hope that R/qtl2 can serve a similar role for the genetic analysis of multi-parent populations.

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