

QTL Mapping using Diversity Outbred Mice

Daniel M. Gatti

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1 Introduction

Quantitative Trait Locus (QTL) mapping in DO mice is performed in several steps. First, we use the founder haplotype contributions to perform linkage mapping. In the mapping model, we adjust for kinship between DO mice using the R package QTLRel. Then, we perform permutations to determine and empirical significance threshold. Next, we select chromosomes with QTL peaks above the significance threshold, examine the founder allele effects and determine support intervals. Finally, we impute the founder SNPs onto the DO genomes to perform association mapping in the QTL intervals.

2 Mapping Models

2.1 Linkage Mapping

Linkage mapping involves the use of founder haplotype probabilities. We perform point mapping at each marker on the array. We fit an additive model that regresses the phenotype on the eight founder haplotype contributions and incorporates an adjustment for the kinship between samples.

$$y = X\alpha + H\beta + Zu + \varepsilon \tag{1}$$

where:

- n is the number of samples
- y is an $n \times 1$ vector of phenotype values for each sample
- X is an $n \times p$ matrix of p fixed covariates (sex, diet, etc.)
- α is a $p \times 1$ vector of fixed effects
- H is an $n \times 8$ matrix of founder haplotype contributions (each row sums to 1)
- β is an 8×1 vector of founder haplotype effects
- Z is an $n \times n$ matrix of error covariances between samples
- u is an $n \times 1$ vector of ???
- ε is an $n \times 1$ vector of residual errors

2.2 Association Mapping

Between each pair of markers, we assign the genotype state with the highest probability to each DO sample. We then query the Sanger Mouse Genomes SNP file to obtain all of the founder SNPs in the interval.

For each Sanger SNP, we impute the Sanger SNPs onto DO genomes as follows:

$$a_j = \sum_{i=1}^8 s_i h_{ij} \quad (2)$$

where:

- a is the allele call (coded as 0, 1 or 2) for sample j
- s is the Sanger founder allele call (coded as 0 or 1)
- h is the founder haplotype contribution of founder i for sample j

$$y = X\alpha + A\beta + Zu + \varepsilon \quad (3)$$

where:

- n is the number of samples
- y is an $n \times 1$ vector of phenotype values for each sample
- X is an $n \times p$ matrix of p fixed covariates (sex, diet, etc.)
- α is a $p \times 1$ vector of fixed effects
- A is an $n \times 3$ matrix of imputed allele calls
- β is an 3×1 vector of allele effects
- Z is an $n \times n$ matrix of error covariances between samples
- u is an $n \times 1$ vector of ???
- ε is an $n \times 1$ vector of residual errors

3 QTL Mapping

We will use example data from Svenson et.al, *Genetics*, 2012. Briefly, 149 mice (75 F, 74 M) were placed on either a chow ($n = 100$) or a high fat diet ($n = 49$). A variety of clinical phenotypes were measured at two time points, roughly 14 weeks apart. In this example, we will map the hemoglobin distribution width (HDW) at the second time point. We will load this data from the Bioconductor data package `MUGAExampleData`.

```
> library(DOQTL)
> library(MUGAExampleData)
> data(pheno)
> data(model.probs)
```

QTL mapping requires phenotype and genotype data. Here, we have a `data.frame` of phenotypes called `pheno` and a 3D array of founder haplotype contributions (num.samples x 8 founders x num.markers) called `model.probs`. The sample IDs must be in `rownames(pheno)` and `dimnames(model.probs)[[1]]` and they must match each other. We will map the hemoglobin distribution width at time point 2 (HDW2).

First, we need to create a kinship matrix using the founder contributions.

```
> K = kinship.probs(model.probs)
```

Second, we need to create a matrix of additive covariates to run in the model. In this case, we will use sex, diet and CHOL1. Note that the sample IDs must be in `rownames(covar)`.

```
> covar = data.frame(sex = as.numeric(pheno$Sex == "M"), diet = as.numeric(pheno$Diet == "hf"))
> rownames(covar) = rownames(pheno)
```

Third, we need to get the marker locations on the array.

```
> load(url("ftp://ftp.jax.org/MUGA/muga_snps.Rdata"))
```

Fourth, we map the phenotype using `scanone`.

```
> qtl = scanone(pheno = pheno, pheno.col = "HDW2", probs = model.probs, K = K,
+             addcovar = covar, snps = muga_snps)
```

```
[1] "HDW2"
```

Fifth, we run permutations to determine significance thresholds. We recommend running at least 1,000 permutations. In this demo, we run 100 permutations to save time.

```
> perms = scanone.perm(pheno = pheno, pheno.col = "HDW2", probs = model.probs,
+                    K = K, addcovar = covar, snps = muga_snps, nperm = 100)
> thr = quantile(perms, probs = 0.95)
```

We then plot the LOD curve for the QTL.

```
> plot(qtl, sig.thr = thr, main = "HDW2")
```

The largest peak appears on Chr 9. The linkage mapping model (Eqn. 1) produces an estimate of the effect of each founder allele at each marker. We can plot these effects (model coefficients) on Chr 9 to see which founders contribute to a high HDW.

```
> coefplot(qtl, chr = 9)
```

Note that the DO mice with alleles from three strains, 129S1/SvImJ, NZO/HILtJ and WSB/EiJ, have lower changes in cholesterol than the other five strains. Remember these strains because they will appear again below. We then determine the width of the QTL support interval using `bayesint`. Note that this

function only provides reasonable support intervals if there is a single QTL on the chromosome.

```
> interval = bayesint(qtl, chr = 9)
> interval
```

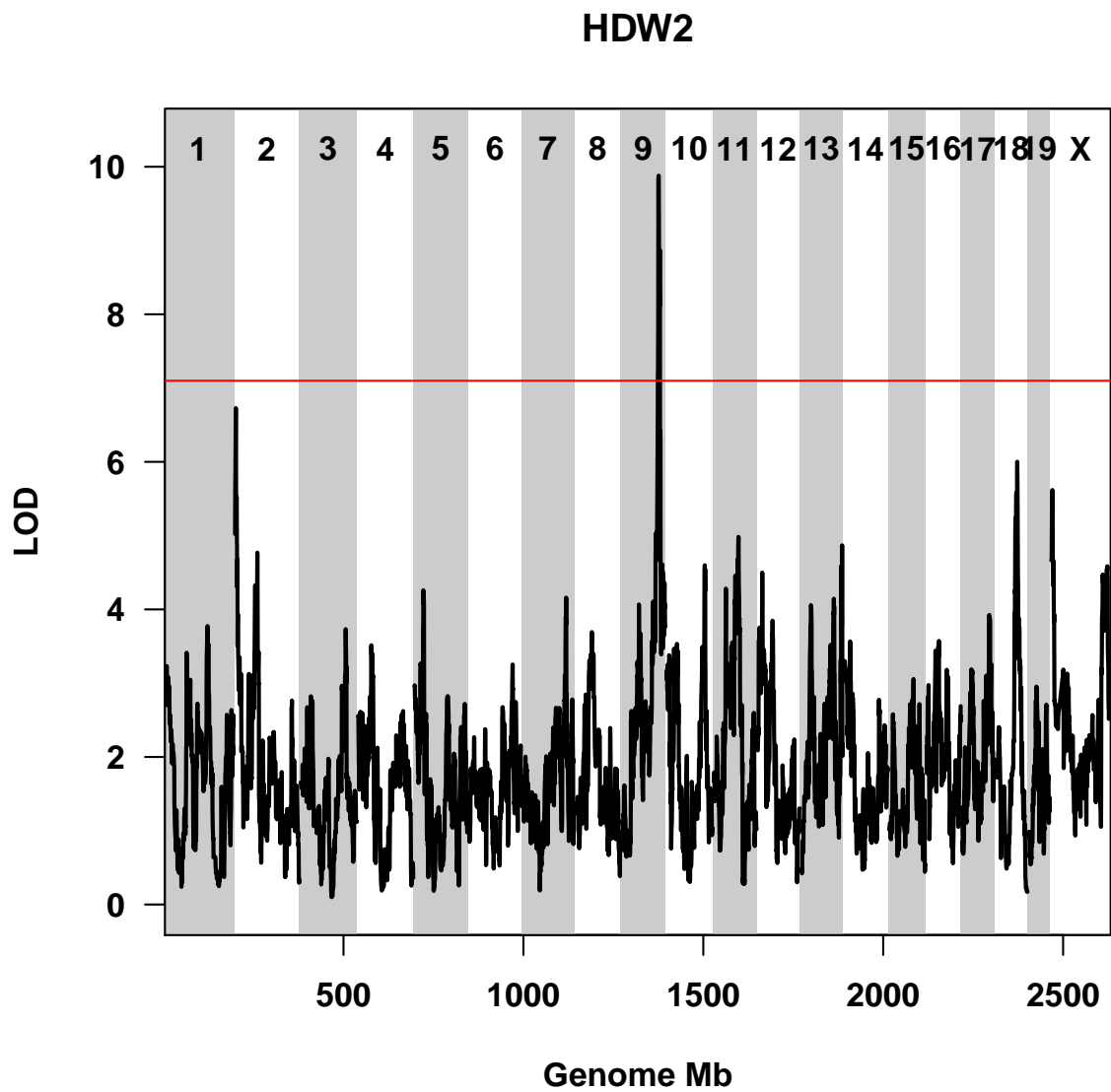


Figure 1: QTL plot of HDW2. The LOD of the mode in Eqn. 1 is plotted along the mouse genome. The red line is the $p < 0.05$ significance threshold.

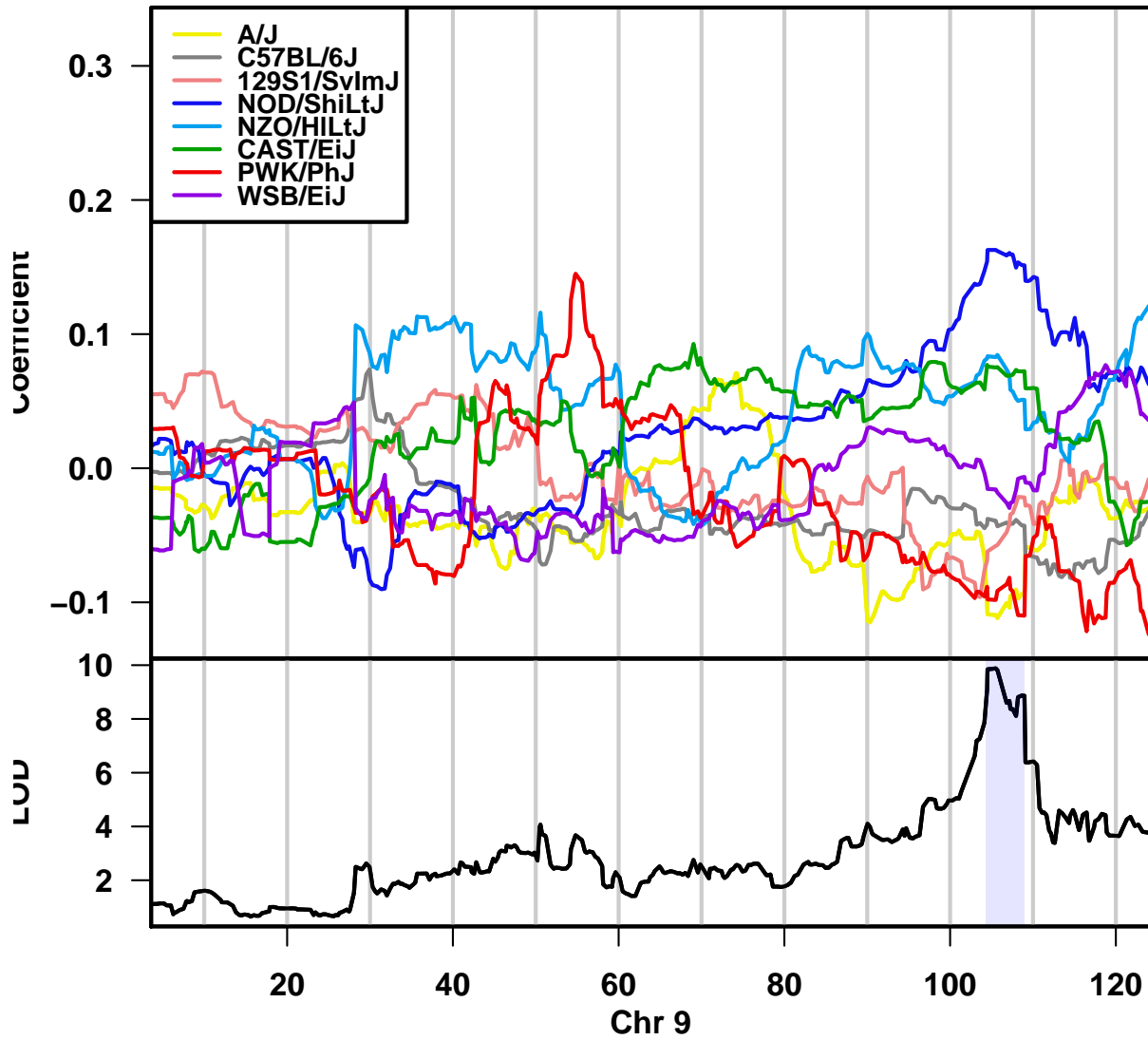


Figure 2: Coefficient plot of HDW2 on Chr 9. The top panel shows the 8 estimated founder allele effects along Chr 9. The NOD/ShiLtJ allele contributes to high values and the A/J and PWK/PhJ alleles contribute to low values. The bottom panel shows the LOD score.

	SNP_ID	Chr	Mb_NCBI38	cM	perc.var	lrs	lod
1	<NA>	9	104.3100	56.6120	0.251632	39.16096	8.503694
4105	UNC091160886	9	105.5128	56.7432	0.286097	45.49603	9.879338
3	<NA>	9	108.9811	59.6310	0.256933	40.11901	8.711732

The QTL support interval is 4.7 Mb wide. Finally, we narrow the candidate gene list by imputing the founder SNPs onto the DO genomes. This idea is essentially association mapping in an outbred population.

```
> ma = assoc.map(pheno = pheno, pheno.col = "HDW2", probs = model.probs, K = K,
+               addcovar = covar, snps = muga_snps, chr = interval[1,2],
+               start = interval[1,3], end = interval[3,3])

[1] "Mapping with 135 samples."
[1] "Retrieving SNPs..."
[1] "Retrieved 66750 SNPs."
[1] "Finding unique SNP patterns..."
[1] "Calculating LOD"

> tmp = assoc.plot(ma, thr = 4)
> unique(tmp$sdps)

[1] 00010000 00010100 00111100 00011000
79 Levels: 00000001 00000010 00000011 00000100 00000101 00000110 ... 10111111
```

We can get the genes in the QTL interval using the `get.mgi.features()` function.

```
> mgi = get.mgi.features(chr = interval[1,2], start = interval[1,3],
+                       end = interval[3,3], type = "gene", source = "MGI")
> nrow(mgi)

[1] 168

> head(mgi)

      seqid source type      start      stop score strand phase      ID
1         9   MGI gene 104288240 104337728      .      -      . MGI:MGI:1928480
135        9   MGI gene 104426113 104426187      .      +      . MGI:MGI:4358922
142        9   MGI gene 104481368 104481510      .      -      . MGI:MGI:5455681
146        9   MGI gene 104547286 105034544      .      +      . MGI:MGI:1921270
305        9   MGI gene 104994916 104995019      .      +      . MGI:MGI:5453168
379        9   MGI gene 105053239 105077476      .      +      . MGI:MGI:2137204

      Name Parent
1      Acpp      NA
135  Mir2136      NA
142  Gm25904      NA
146   Cpne4      NA
305  Gm23391      NA
379  Mrp13      NA

                                     Dbxref
1  VEGA:OTTMUSG00000024988,NCBI_Gene:56318,ENSEMBL:ENSMUSG00000032561
```

```

[1] "Mapping with 135 samples."
[1] "Retrieving SNPs..."
[1] "Retrieved 66750 SNPs."
[1] "Finding unique SNP patterns..."
[1] "Calculating LOD"

```

```

[1] 00010000 00010100 00111100 00011000
79 Levels: 00000001 00000010 00000011 00000100 00000101 00000110 ... 10111111

```

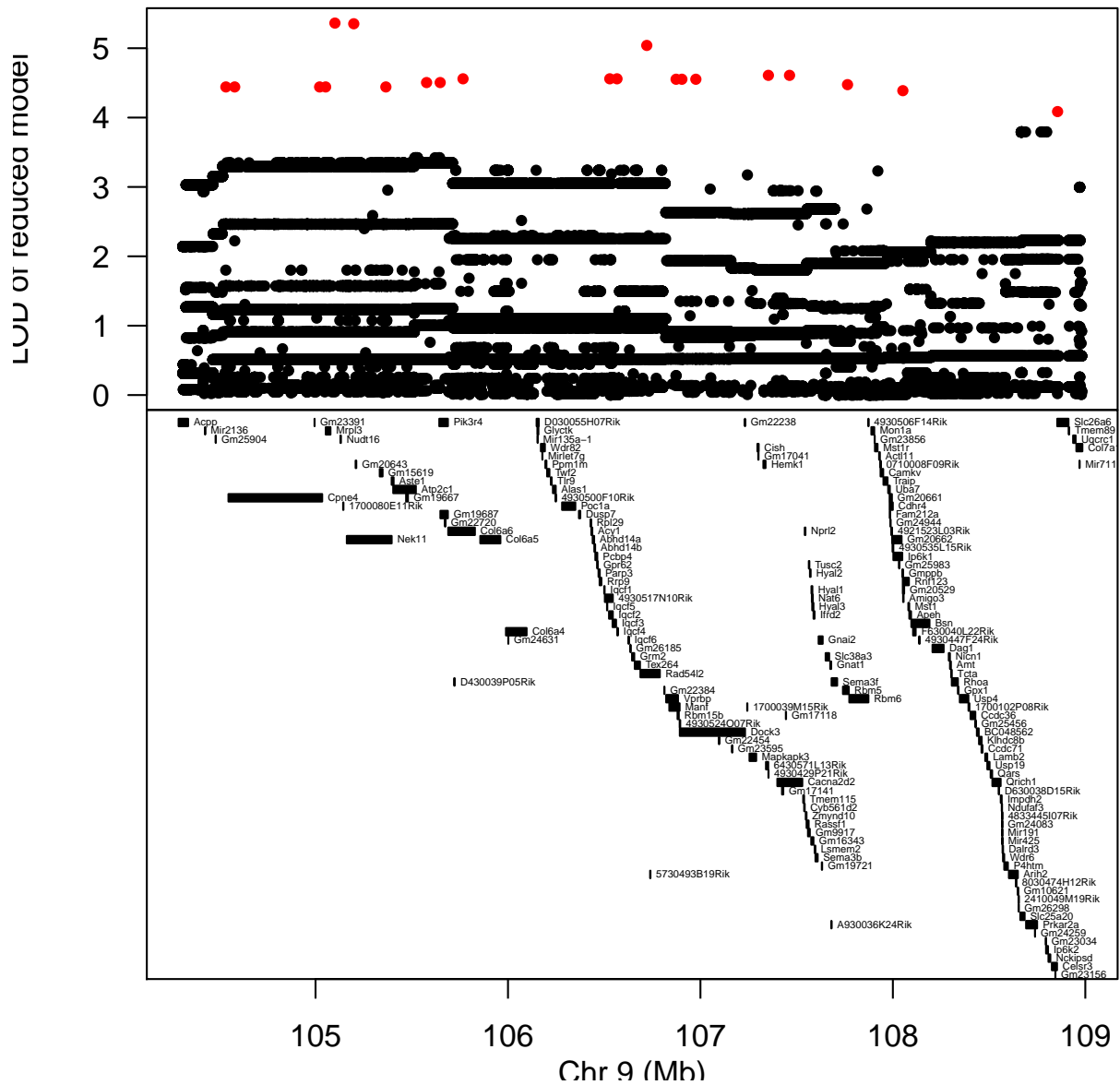


Figure 3: Association mapping plot of HDW2 in the Chr 9 support interval. The top panel shows the LOD score from association mapping (Eqn. 3) in the QTL support interval. The bottom panel shows the genes and non-coding RNAs from the Mouse Genome Informatics database.

```

135          NCBI_Gene:100316725,ENSEMBL:ENSMUSG00000089406
142          ENSEMBL:ENSMUSG00000089116
146 VEGA:OTTMUSG00000023466,NCBI_Gene:74020,ENSEMBL:ENSMUSG00000032564
305          ENSEMBL:ENSMUSG00000088204
379 VEGA:OTTMUSG00000023521,NCBI_Gene:94062,ENSEMBL:ENSMUSG00000032563
          mgiName          bioType
1      acid phosphatase%2c prostate protein coding gene\r
135          microRNA 2136          miRNA gene\r
142          predicted gene%2c 25904          snoRNA gene\r
146          copine IV protein coding gene\r
305          predicted gene%2c 23391          miRNA gene\r
379 mitochondrial ribosomal protein L3 protein coding gene\r

```

There are 169 genes in the QTL support interval. Several SNPs have LOD scores above 4. This is a somewhat arbitrary cutoff and an appropriate threshold will be supplied in future version of DOQTL. In this case, there may be more than one variant that influences the phenotype.