



# Mixed Model Analysis and Permutation for Recombinant Inbred Strain Panels

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Shirng-Wern Tsaih, ScD  
The Jackson Laboratory

# Recombinant inbred (RI) strains

- A great resource to dissect simple and complex traits
- Formed from an initial cross between two different inbred strains followed by an F1 intercross and 20 generations of strict brother-sister mating.
- The genomes of each of these RI strain are a homozygous mosaic of chromosomal regions from the two founders.

# Strength of RI sets

- An immortal mapping population because each RI strain is nearly homozygous
  - Only need to genotype each line once (reproducible genotype)
  - Measure multiple individuals when heritability is low
  - Can be used to study gene-environment interaction
  - 3.6 fold map expansion when compared to a typical F2 population
    - Advantageous for mapping closely linked loci

# Limitation

- Each genotype is represented by a single RI strain
  - Individuals from the same RI strain have the identical genome
- Limited power in the existing mouse RI panels
  - Most existing panels: < 40 strains
  - BXD panel: 80 strains (original set + new set)
  - LXS panel: 77 strains (2003) -> ~ 65 strains (2009)

# LXS RI strains

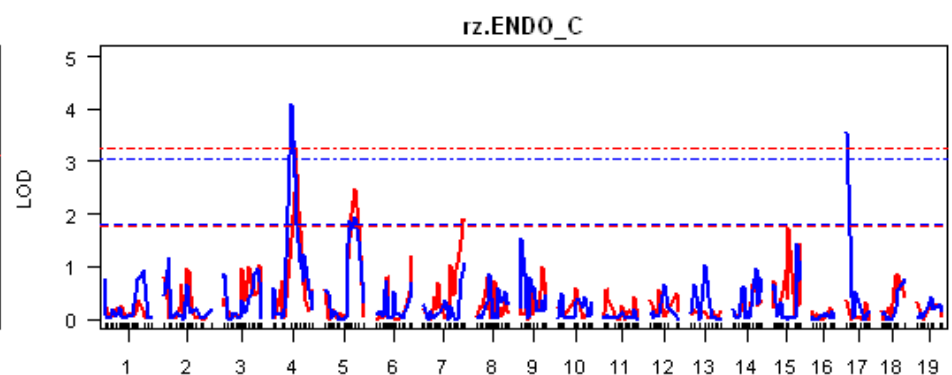
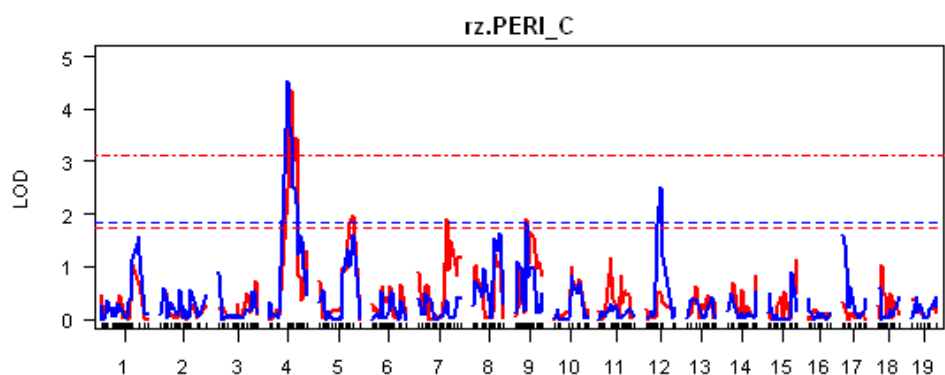
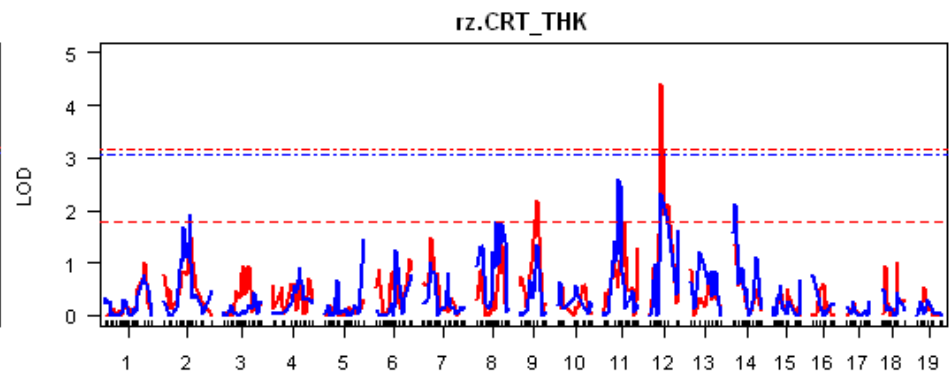
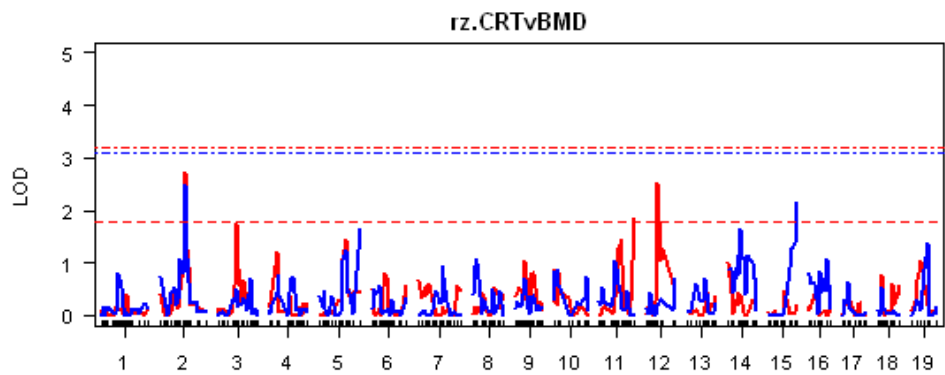
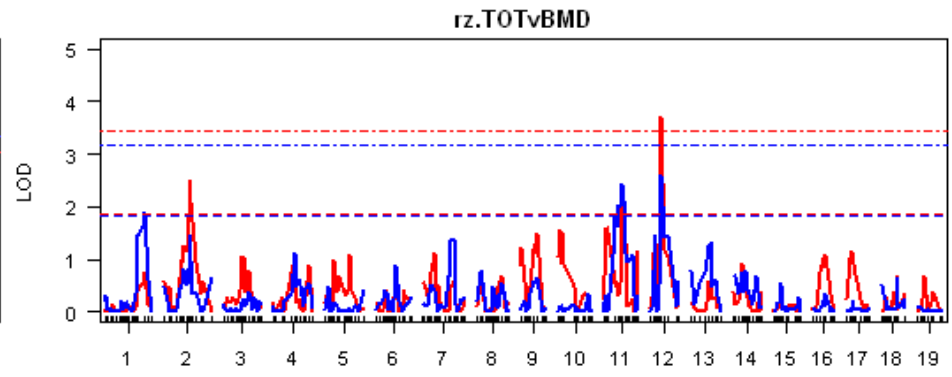
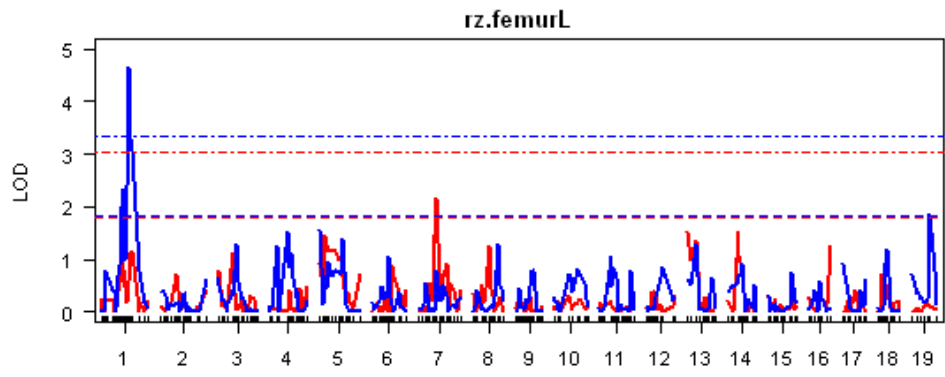
- The Inbred Long Sleep and the Inbred Short Sleep strains have been selected for sensitivity (ILS) and lack of sensitivity (ISS) to alcohol
  - Animal model of the effects of ethanol on the nervous system
  - Dietary restriction and lifespan study

# Motivation

- QTL study of BMD in LXS RI strains
- Sample collection
  - Femurs of female and male mice from the LXS RI panel (Dr. Bennett, Institute for Behavioral Research, Boulder, CO)
  - BMD measurements by pQCT (Beamer Lab, JAX)
    - Femur length, total BMD, endosteal circumference, periosteal circumference, cortical thickness, cortical BMD
    - 53 RI strain

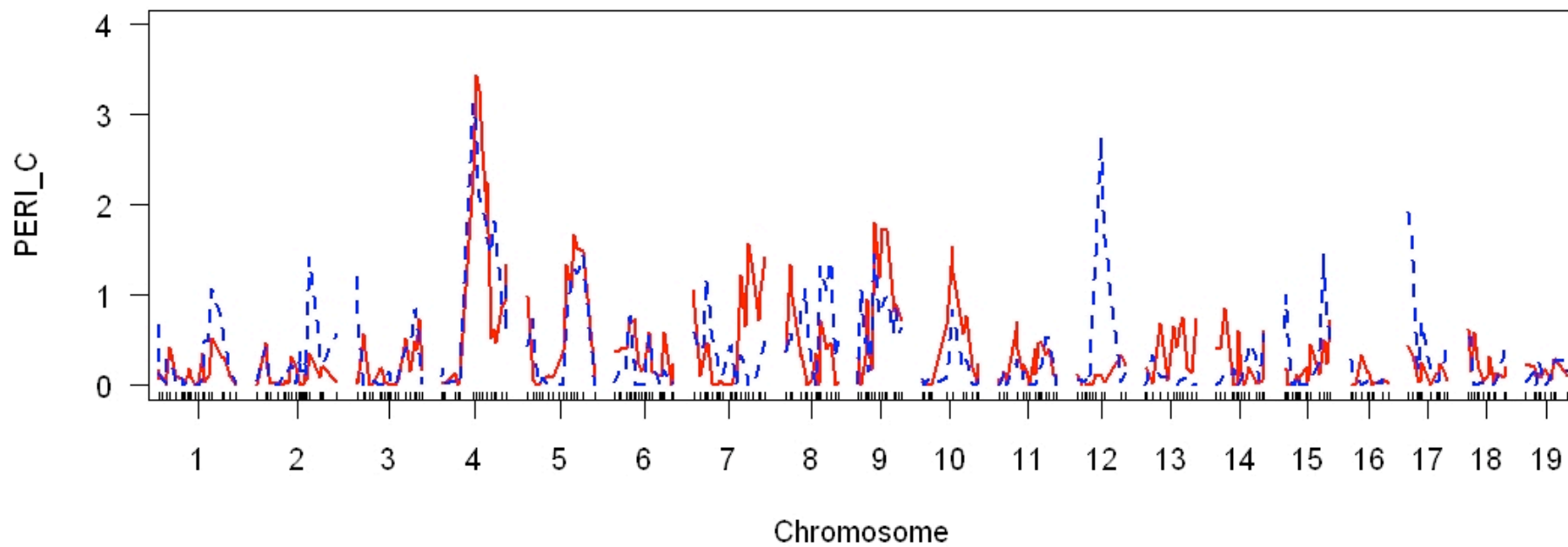
# Motivation

- Marker data
  - Strain distribution pattern (SDP) of 330 SSLP markers has been characterized in 77 LXS RI strains
    - (Dr. Bennett, Institute for Behavioral Research, Boulder, CO)
    - Data cleaning: remove strains without phenotype data
- QTL analysis
  - Strain mean analysis by sex (R/qtl)
    - Sex difference?



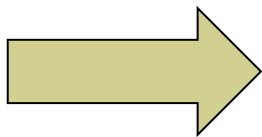


# PERI\_C



## We need

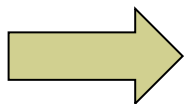
- Combine male and female RI data in one single data set
- Correlation among RI progeny
  - Uncorrelated: animals from different RI strain
  - Correlated: **male and female** animals within the same RI strain
- A new model that takes into account the correlation structure among the RI progeny



Mixed-effect model approach

# New scan models

- Mixed-effect model approach:
  - Add a random effect term *Strain* to account for the correlation structure in RI panel
    - $Y = \text{Sex} + Q + \text{Strain}$
  - To look for sex-dependent QTL, we use the same approach as in F2 and BC1 populations
    - Interactive scan model:  $Y = \text{Sex} * Q + \text{Strain}$



**A permutation method that also takes into account the correlation structure**

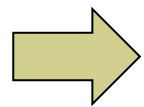
# Free (data) Permutation

- **Idea:**
  - Trait observations are reassigned without replacement among the subjects (Churchill and Doerge (1994) )
- Commonly used in simple experimental designs
  - F2, BC
  - Strain mean model of RI strains with balanced experiment

# Exact permutation

- **Idea**

- Data are permuted as units within levels of the random factor (**Strain**)
- Males and females of a given RI strain are assigned the same permuted genotype



In the permuted data, these animals will have the same relatedness before and after permutation.

Anderson and Braak (2003); Zou et al. (2006)

# Residual permutation of reduced model

- **Idea:**
  - Fit a reduced model of no QTL, take the residuals
  - Randomly shuffle the residuals among individuals (residual permutation sample)

Anderson and Braak (2003)

# Simulation study 1 with real LXS genotypes

- 53 strains
- Common QTL on Chr 4
- Mixed-effect scan
- Permutation tests:
  - Free, Exact and Residuals (alpha = 0.05)

Chr	Free	Exact	Residual
-	2	4	4
Chr 4	94	92	92
Not Chr 4	4	4	4
Threshold	3.1	3.3	3.3

100 simulations

# Simulation study 2 with real LXS genotypes

- 53 RI strains
- Common QTL: Chr 4
- Sex-specific QTL : Chr 7
- Mixed-effect scan model
- Permutation tests:
  - Data vs Residuals (alpha=0.05)

Chr	Data	Residual
-	14	25
Chr 4	76	74
Chr 7	1	1
Chr 4 & 7	9	9
Threshold	4.3	4.8

100 simulations

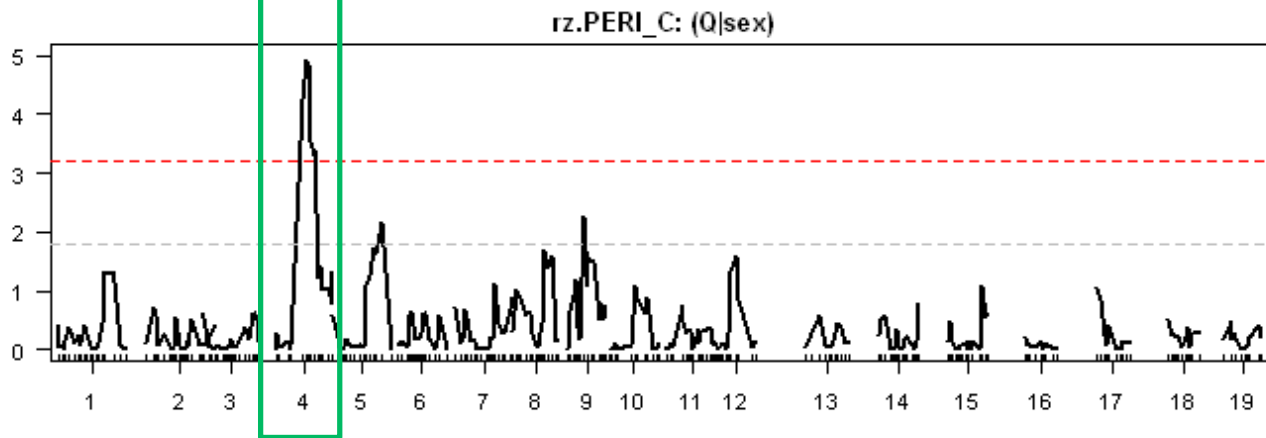


## Simulation study 3 with real LXS genotypes

- 70 RI strains (1 F + 1 M) with 1 common QTL
- Fixed-effect scan + data permutation ( $\alpha=0.05$ )
- Mixed-effect scan + exact permutation ( $\alpha=0.05$ )

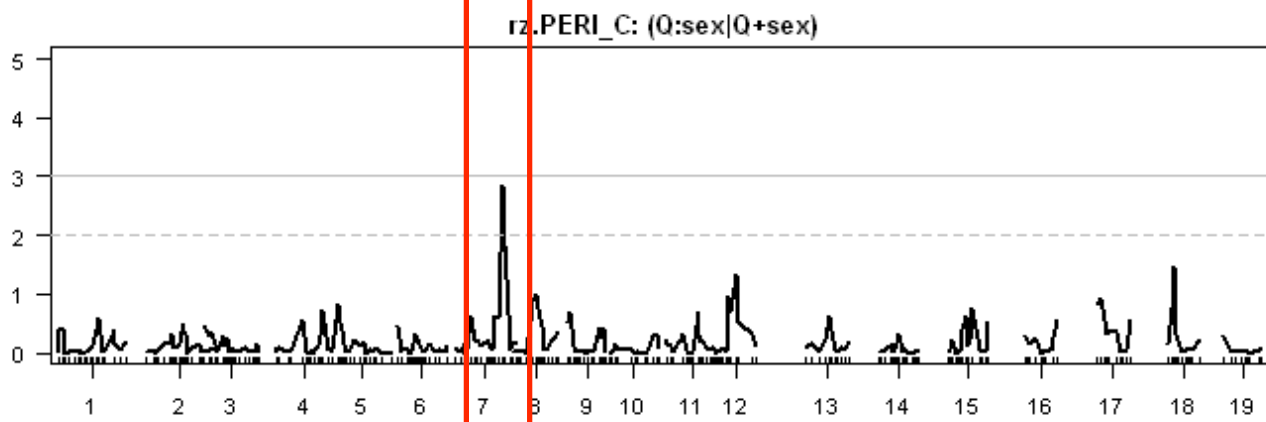
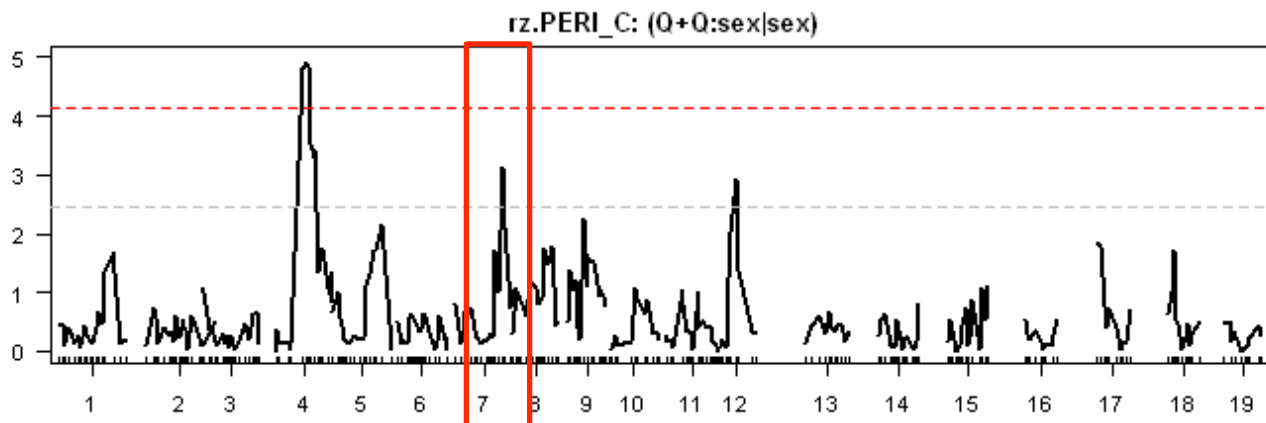
QTL effect	Mixed		Fixed	
	FP	FN	FP	FN
0.1	5%	8%	8%	8%
0.2	10%	5%	14%	4%
0.3	14%	0%	16%	0%

1000 simulations



Common QTL:  
Chr 4 \*\*

Sex-specific QTL:  
Chr 7



Threshold lines for  
the top 2 panels  
are based on 100  
permutation tests

# Acknowledgements

Gary Churchill (Jackson Laboratory)  
Churchill Lab members

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Wes Beamer (Jackson Laboratory)

Beth Bennett (University of Colorado at Boulder)

Ellison Medical Foundation

# The Churchill Group

<http://churchill.jax.org>

Our lab is actively applying a systems approach to study the genetics of health and disease, incorporating new statistical methods for the investigation of complex disease-related traits in the mouse.



*Career opportunities are currently available for:*

## Postdoctoral Researchers

The Jackson Laboratory was voted #2 in a poll of postdocs conducted by *The Scientist* in 2009 and is an EOE/AA employer

## Biostatisticians

## Software Engineers

To apply submit a CV to Imogen Hurley ([Imogen.Hurley@jax.org](mailto:Imogen.Hurley@jax.org))

