Linear models for GWAS

I: Introduction and linear regression

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Microsoft Research

Current topics in computational biology
UCLA
October 15^{th}, 2012

Course structure

October 15^{th}

- Introduction
  - Terminology
  - Study design
  - Data preparation
  - Challenges and pitfalls
  - Course overview
- Linear regression
  - Parameter estimation
  - Statistical testing

October 17^{th}

- Linear mixed models
  - Population structure correction
  - Parameter estimation
  - Variance component modeling
  - Phenotype prediction
- Further challenges and outlook

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Outline

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Introduction
- Why QTL mapping
- Terminology & background
- Methodological challenges

Linear Regression

Hypothesis Testing

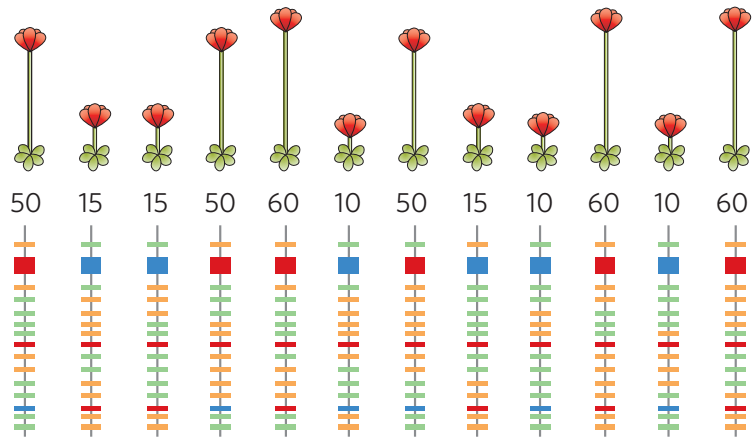
Multiple Hypothesis Testing

Model Checking

Genotype to phenotype mapping

Given:

- Genotype for multiple individuals
  - Single nucleotide polymorphisms (SNPs), microsatelite markers
- Quantitative traits (phenotypes) for the same individuals
  - disease, height, gene-expression, . . .



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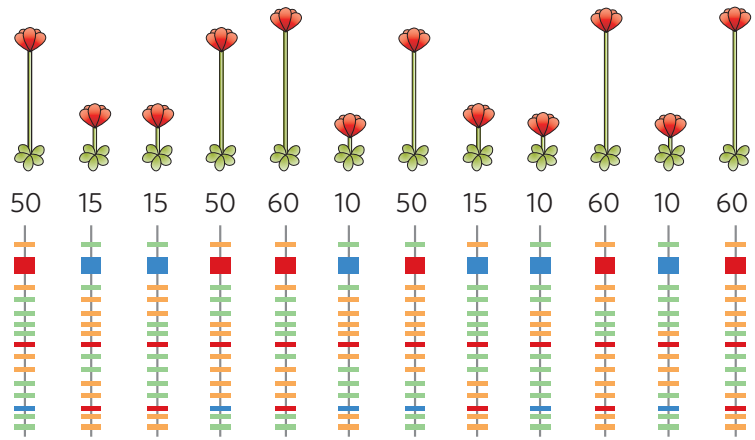

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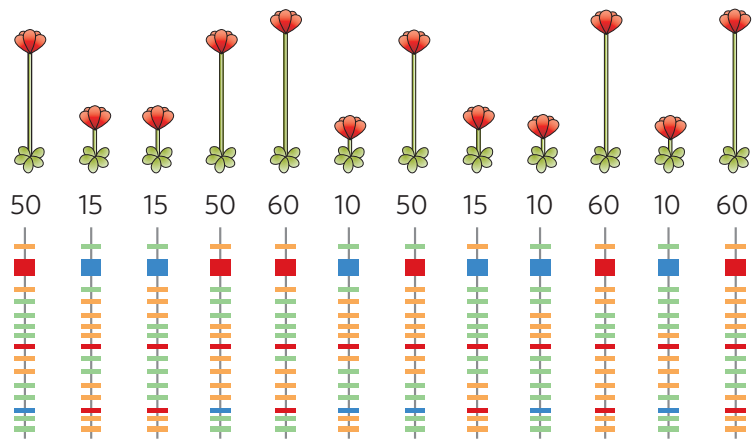

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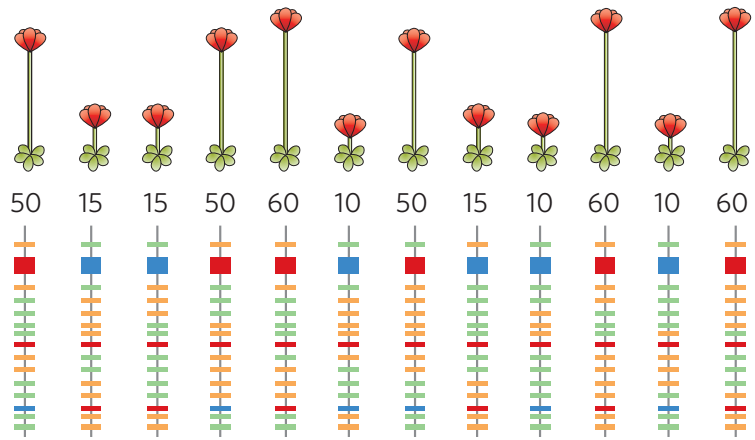
Use of GWAs in plant systems

- Basic biology
  - Understand the makeup of molecular pathways
  - Dissect the genetic component of natural variation.
  - Genotype-environment interactions
- Breeding
  - Mine for markers causal for phenotype to assist in breeding decisions.
  - Maximization of yield, pathogene resistance, etc.



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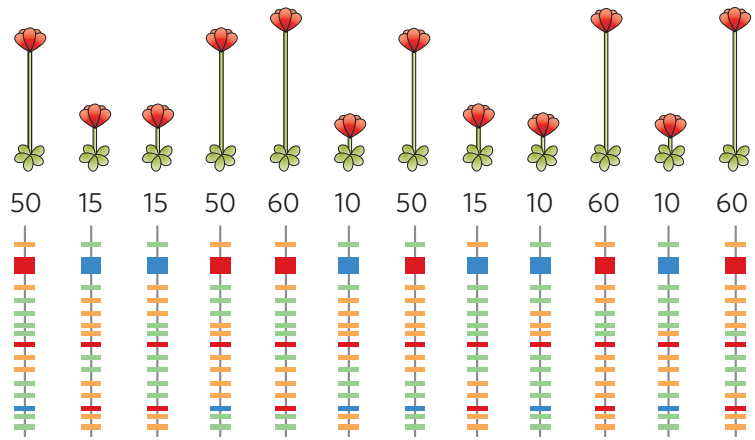

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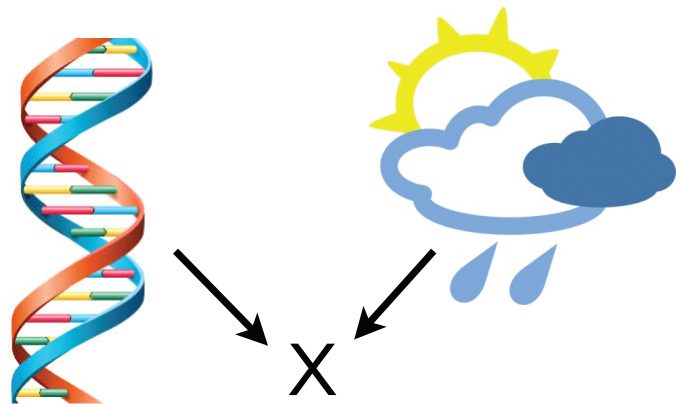

Personalized medicine & health

- Adapting treatment to the patients genetic make-up.
  - Targeting patients who can benefit.
  - Appropriate dosage of a drug by using genetic variants to understand drug metabolism (e.g., anti-depressants, beta blockers, opioid analgesics).
  - Disease subcategorization
- Risk prediction
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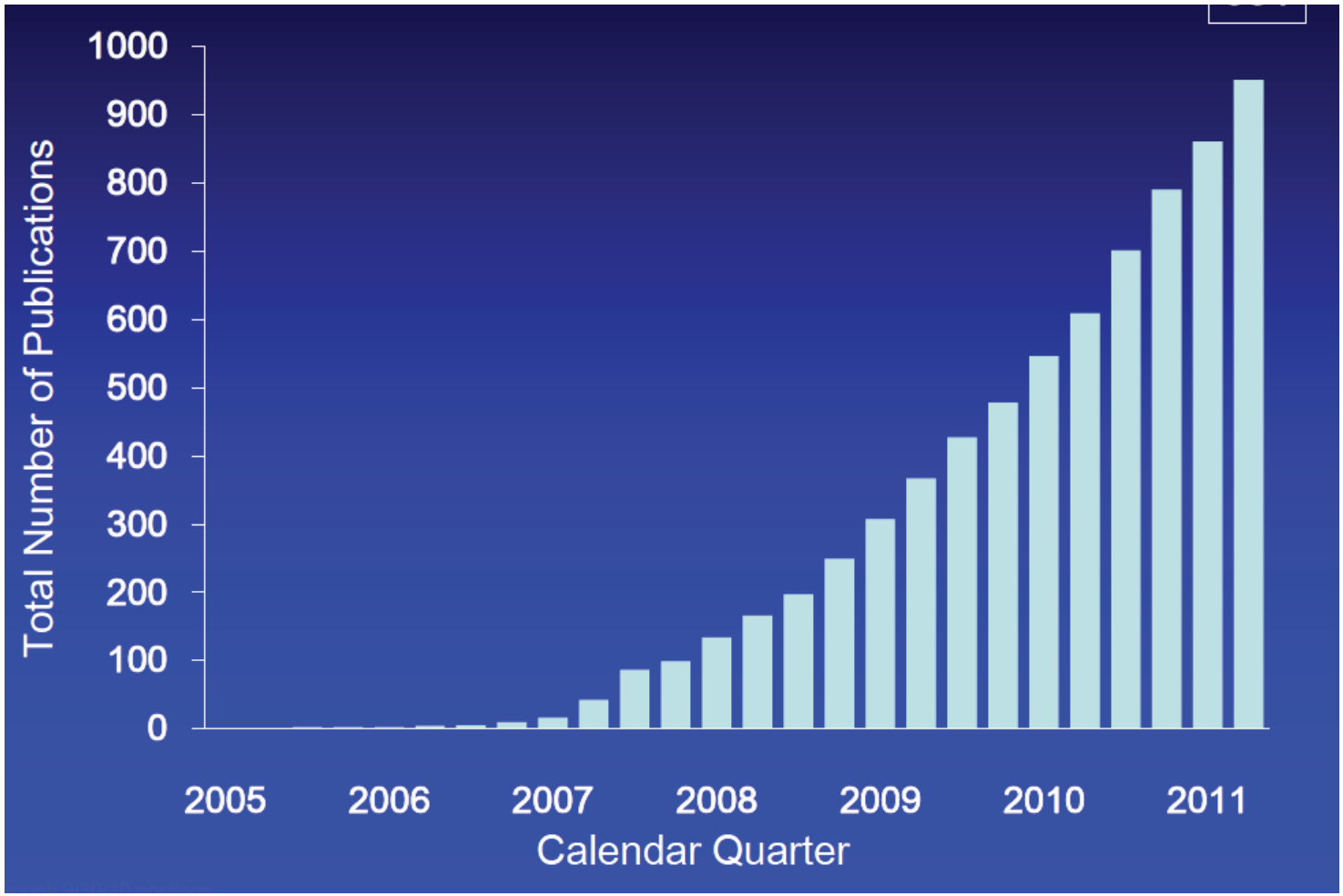

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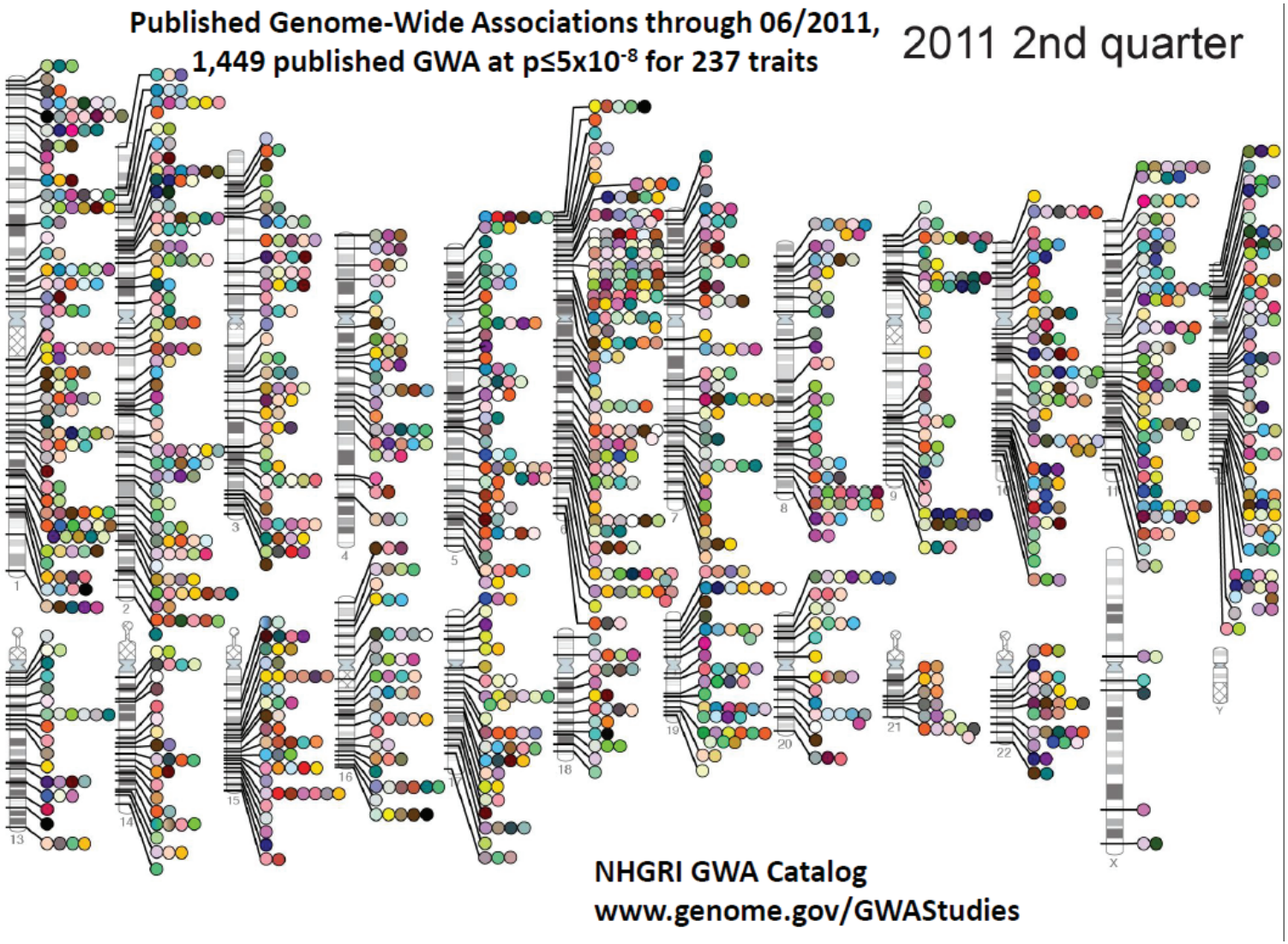

Some definitions

- Genotype denotes the genetic state of an individual.
  - Denoted by $\mathbf{x}^n$ for individual n.
- Phenotype denotes the state of a trait of an individual.
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- A locus is a position or limited region in the genome.
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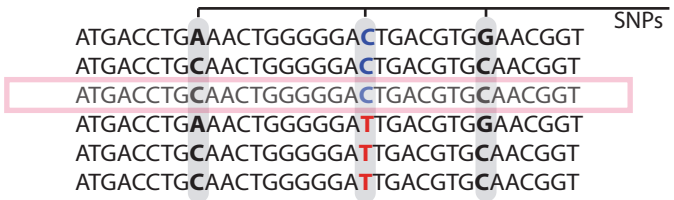
- An allele is the genetic state of a locus.



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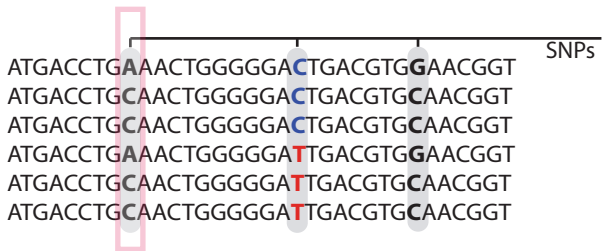

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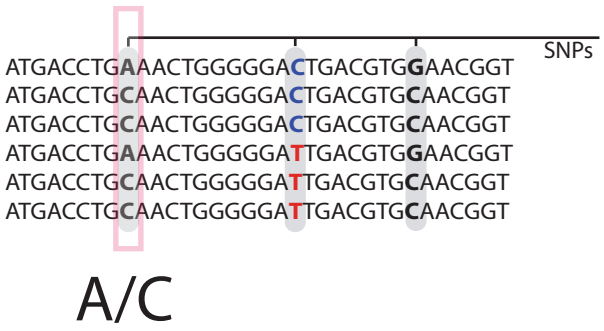

More definitions

- An organism/cell is haploid if it only has one chromosome set or identical chromosome sets.
  - e.g. *A. thaliana*, sperm cells or inbred lab strains
- An organism/cell is diploid if it has two separately inherited homologous chromosomes.
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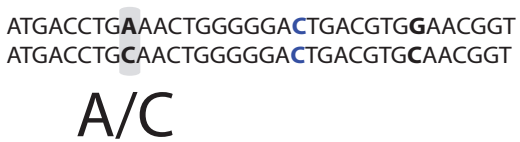
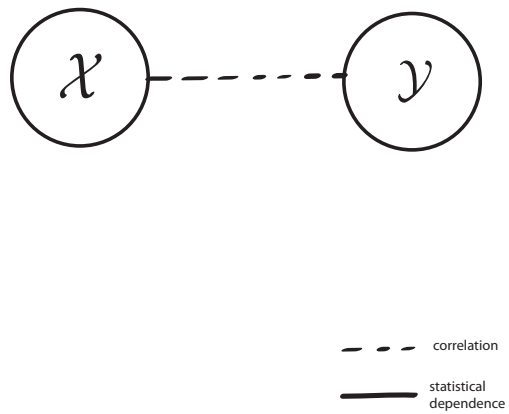


Statistical association

Association is any relationship between two measured quantities that renders them statistically dependent.

- Direct association
- Indirect association
  - Can be beneficial
    - e.g.: Linkage
  - Can be harmful
    - e.g.: Population structure



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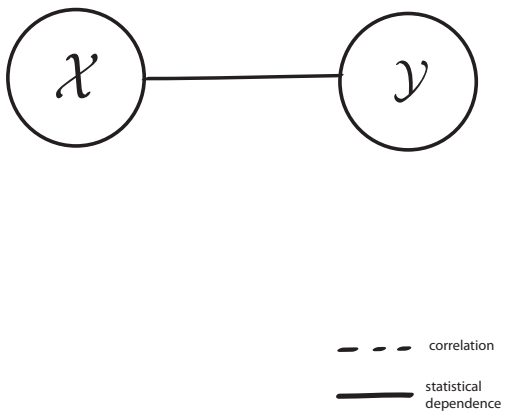

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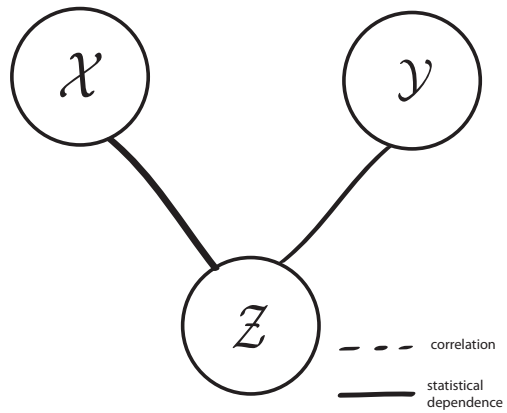

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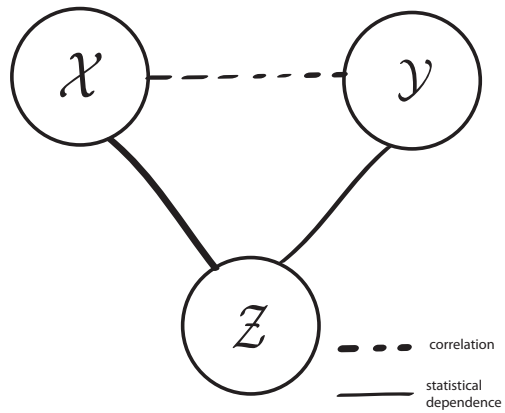

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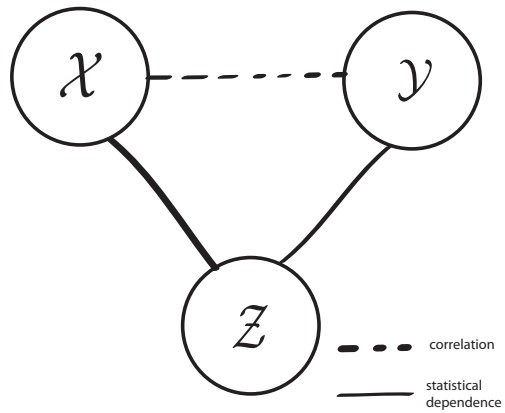

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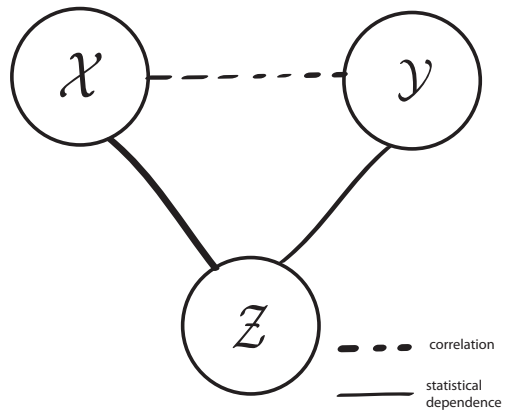

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Result

Example GWAS on *A. thaliana*

- Phenotype: Flowering time at 10 degrees
- Test every SNP in the genome for association with floweringtime
- Position vs. Log10(P-value) (Manhattan plot)

[Atwell et al., 2010]

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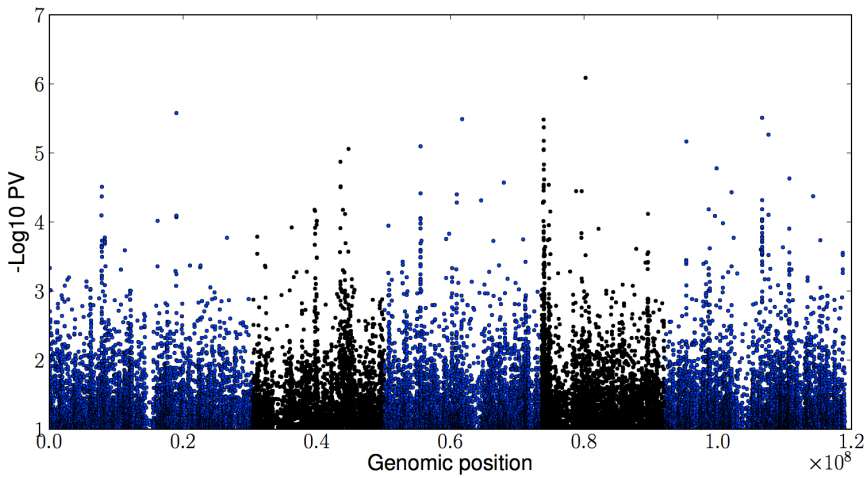

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Genetic designs

- Natural population
  - Global sampling of plants, human or animals.
  - Samples may exhibit varying degrees of relatedness.
  - Typically diploid.
- Inbred F2 crosses
  - Mapping of the differences of founder strains
  - Plant- and animal systems
  - No relatedness
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- Multi-parent crosses
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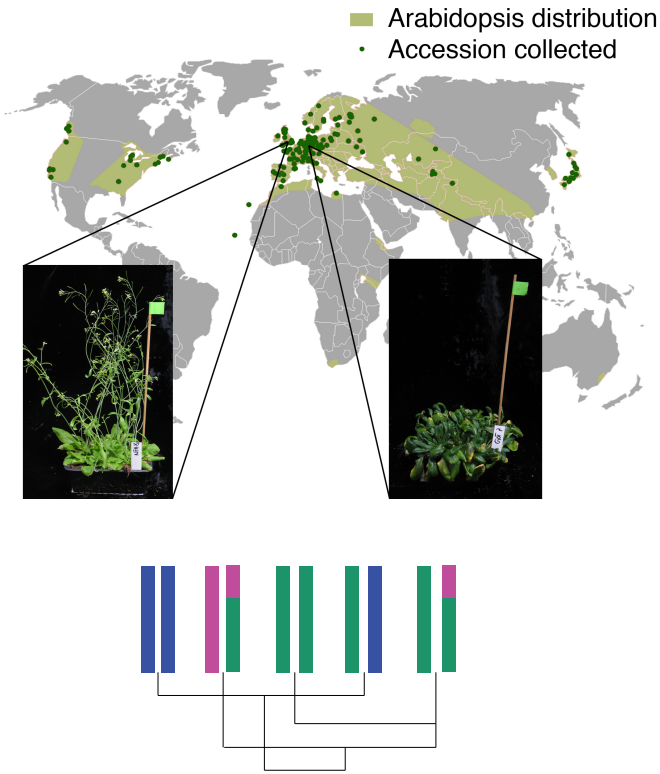

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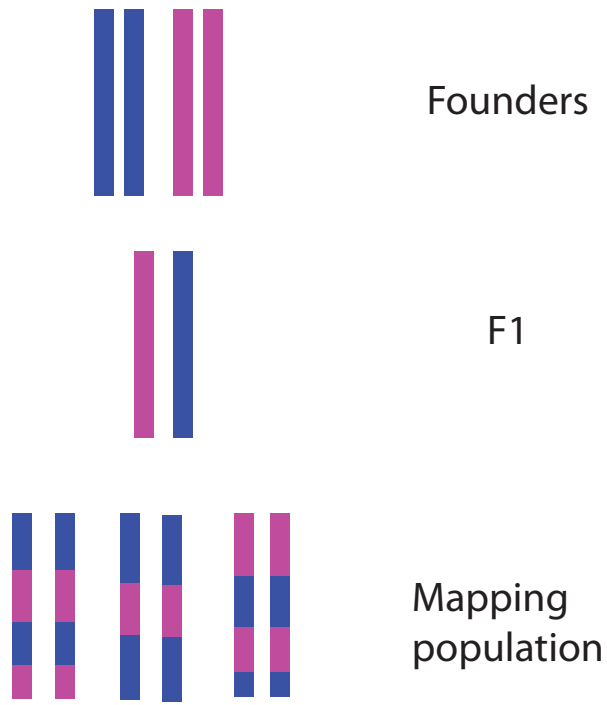

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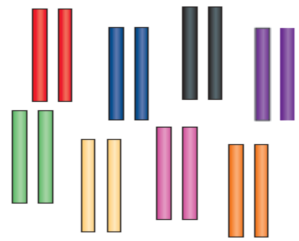
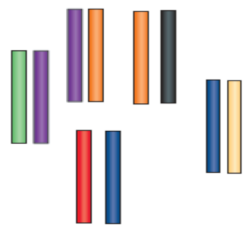
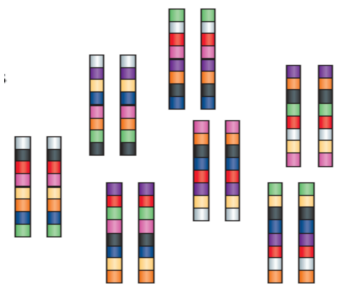


n founders



n (n-1)
F1

n (n-1)
out crosses



selfing

Genetic designs

Genotype encoding

A simple encoding scheme, ignoring dominance:

- A locus is heterozygous if it differs between paternal and maternal haplotypes.
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A/C

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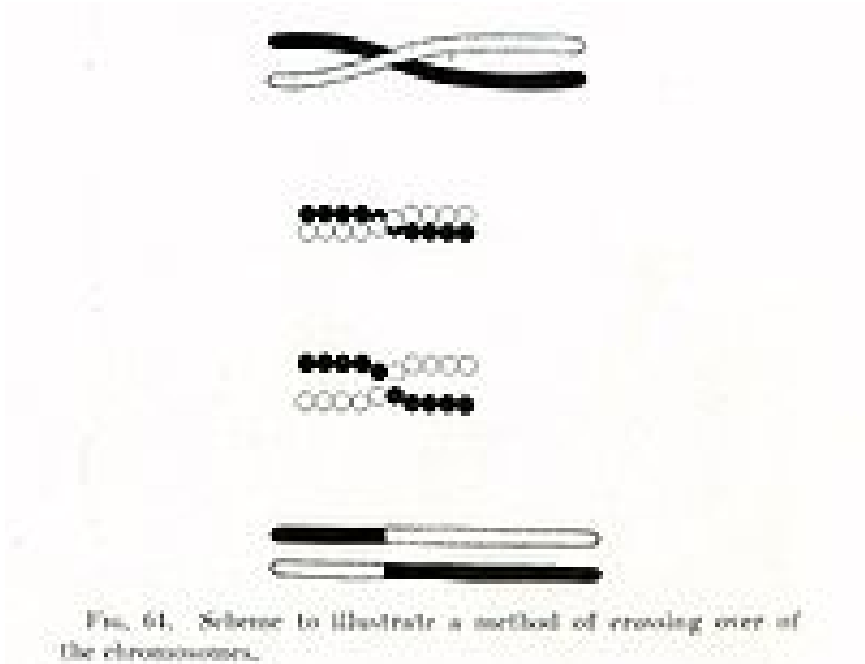
A/A

Linkage Disequilibrium

Physical linkage

- Recombination causes linkage between loci.
- Linkage is not uniform along the chromosome.
- Recombination hotspots on the chromosome lead to conserved haplotype blocks in strong linkage.
- Linkage can be used to chose *tag*-SNPs to cover all linked regions.
  - Tradeoff between resolution and genotyping cost.



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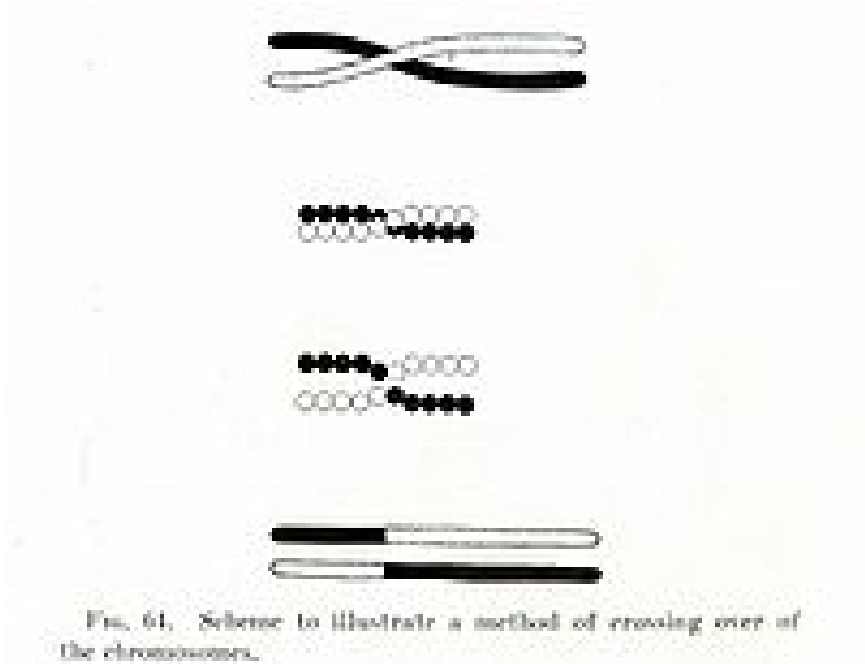

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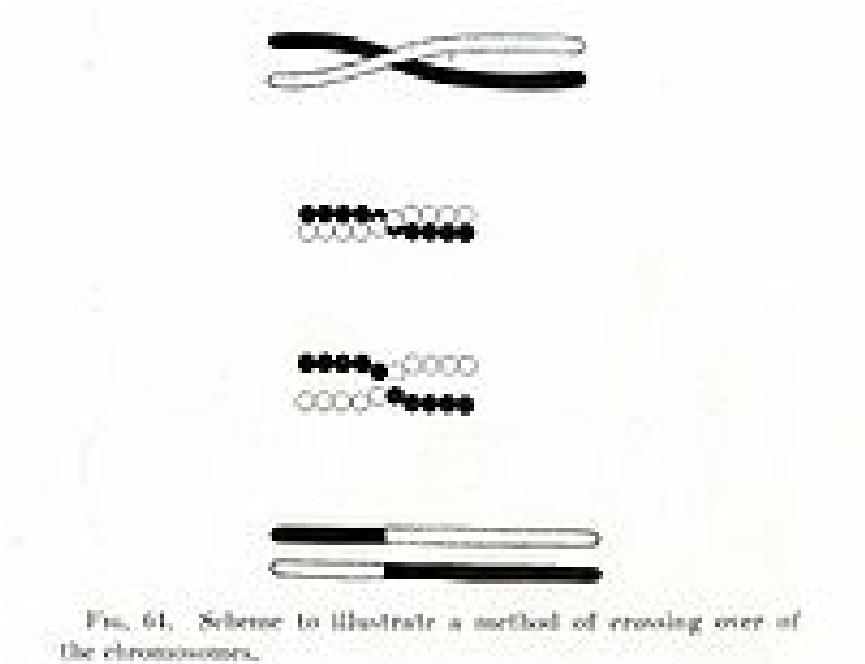

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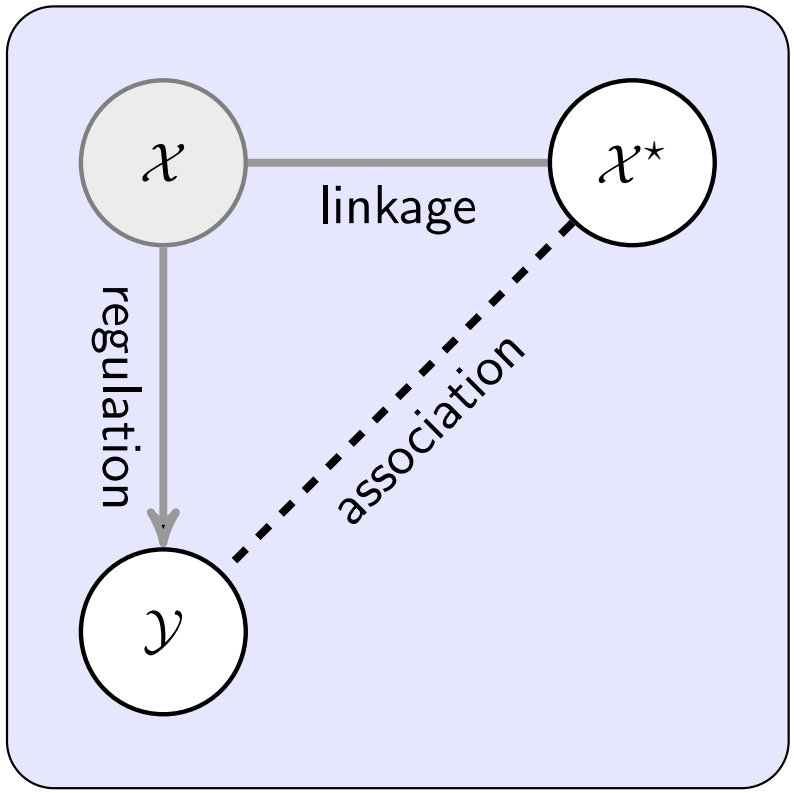

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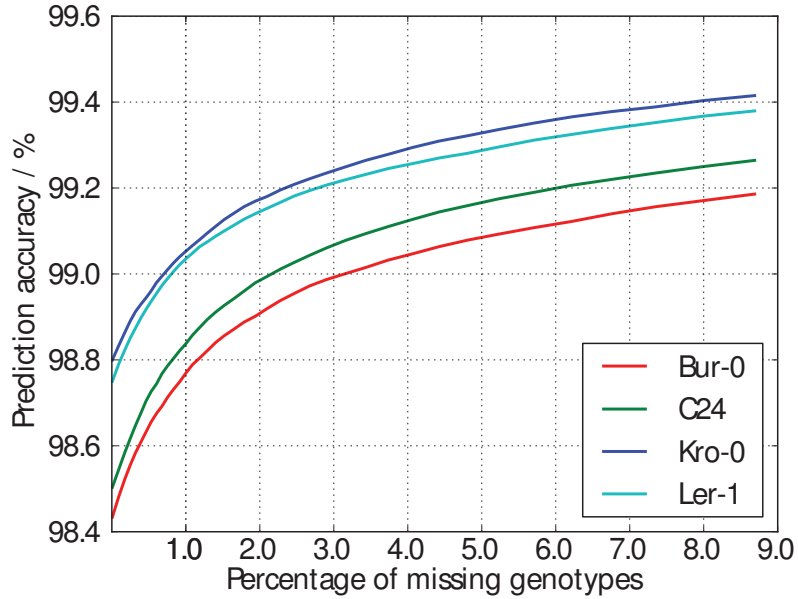

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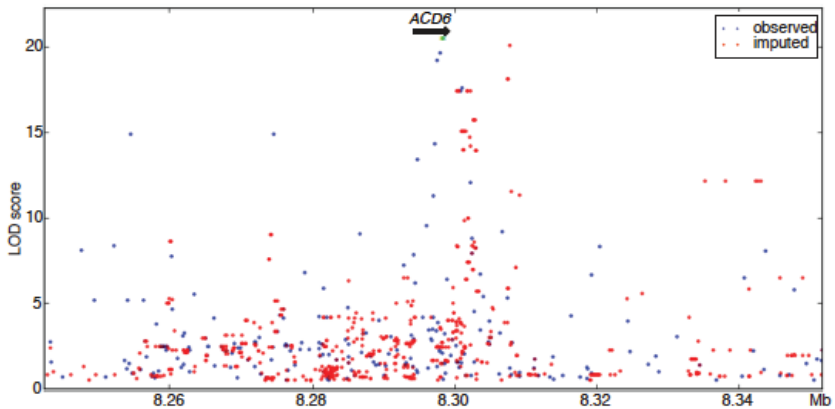

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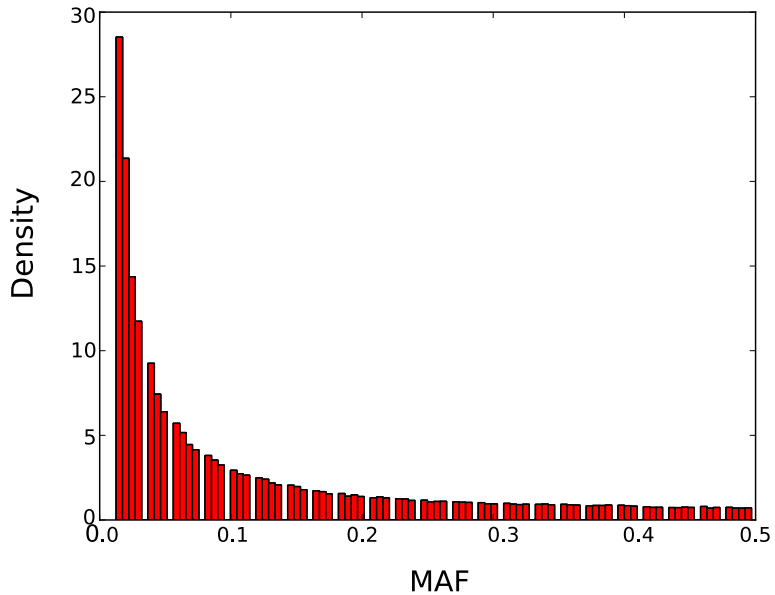

Minor allele frequency from 160 *A. thaliana* lines; 2.3 million genome-wide SNPs from NGS sequencing

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- Most parametric models are based on Gaussianity assumptions
- Phenotype residuals are often non-Gaussian
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  - Use of prior knowledge
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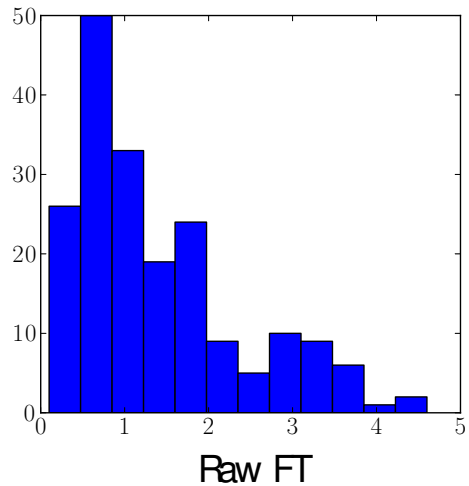

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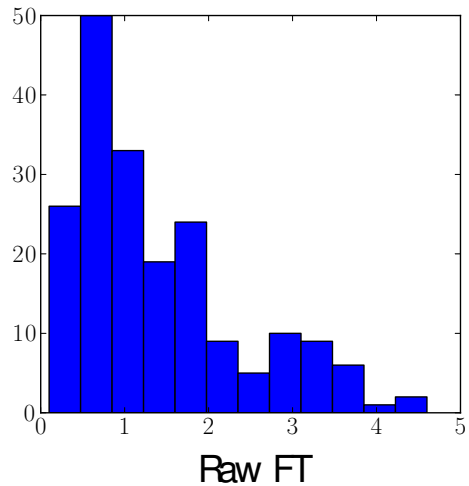

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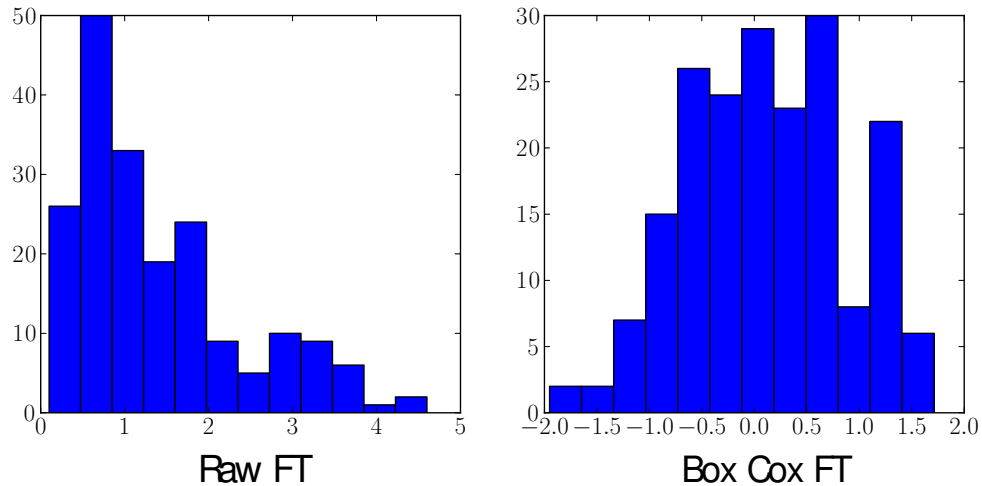

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Linkage Disequilibrium

Gametic Phase Disequilibrium

- Association between two loci.
- Deviation from random co-inheritance between loci.
- LD can be caused by recombination, population structure, epistasis
- Measures of LD between two loci x_1 and x_2 are D and r^2.
  - $D = f_{AB} - f_A f_B$
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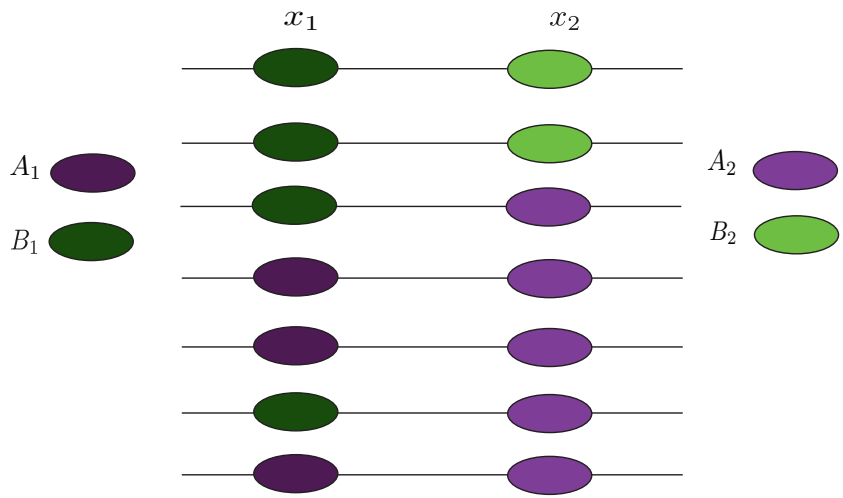

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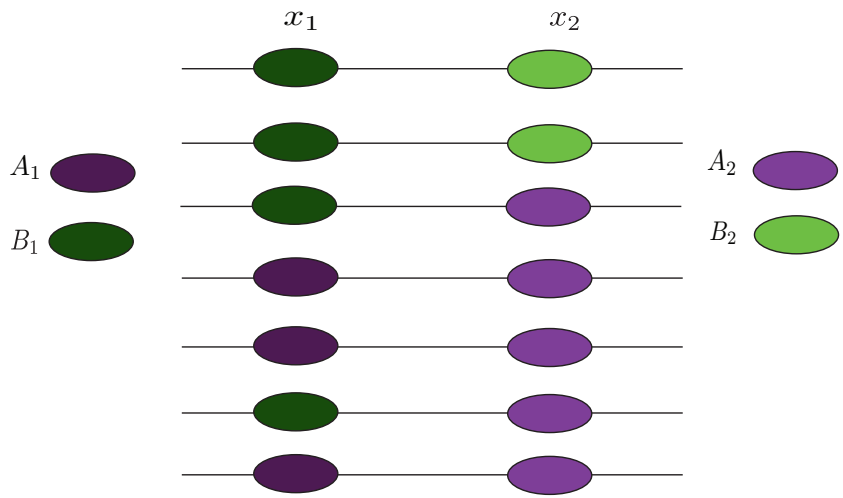

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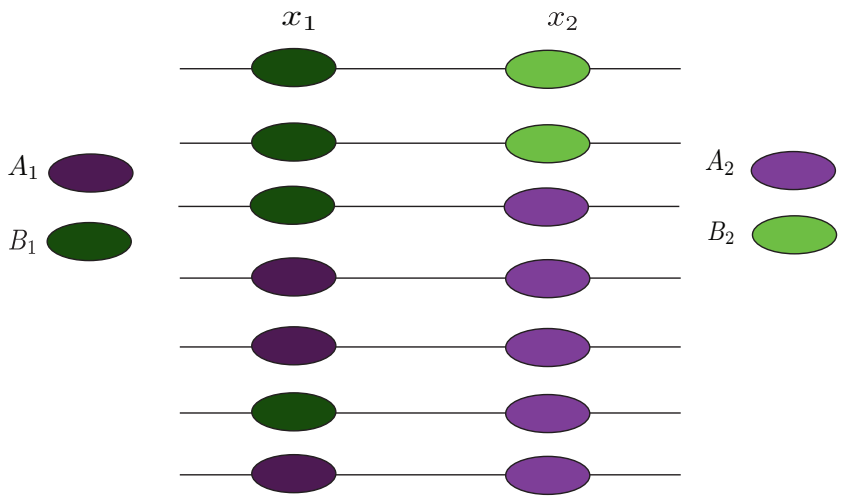

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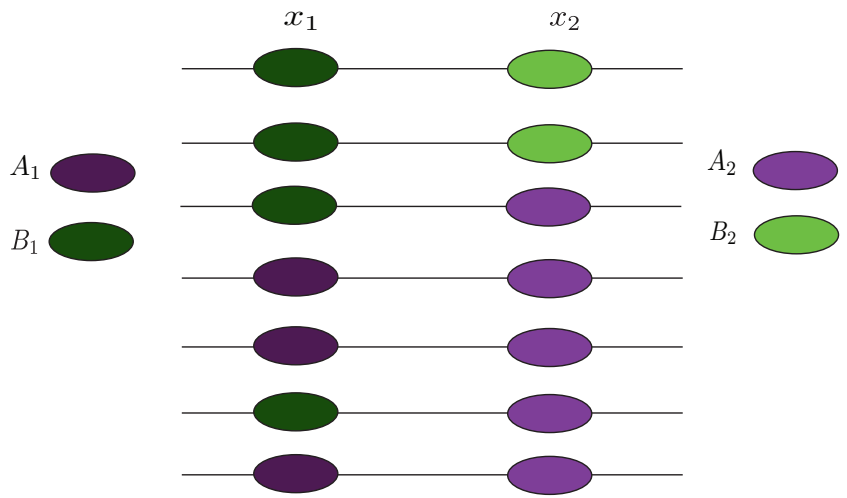

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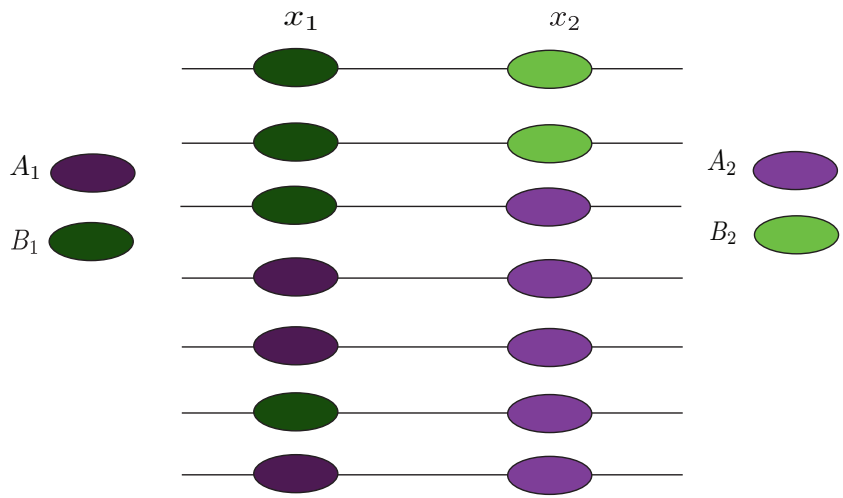

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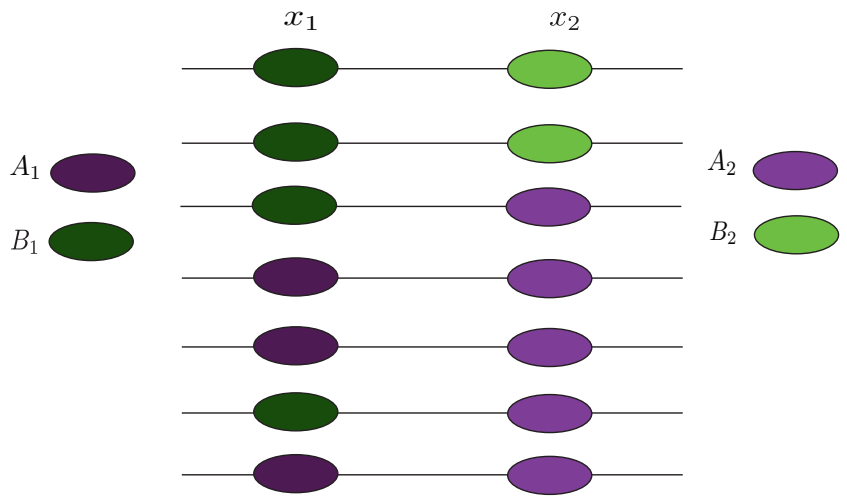

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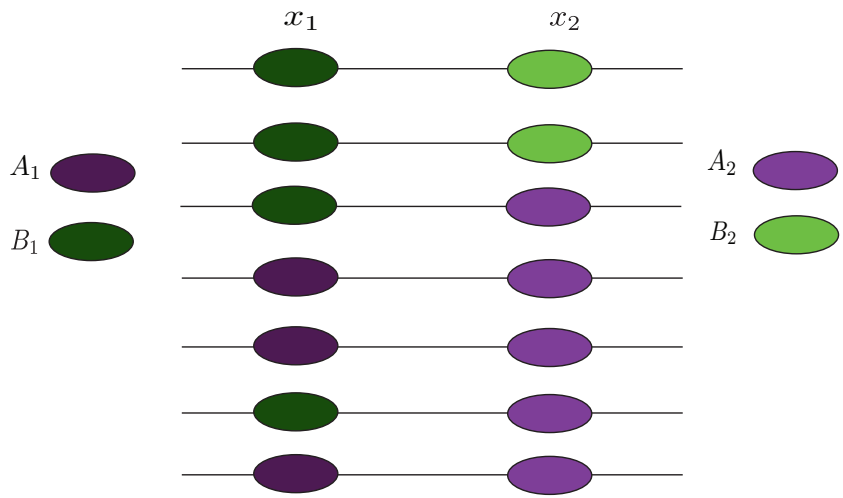

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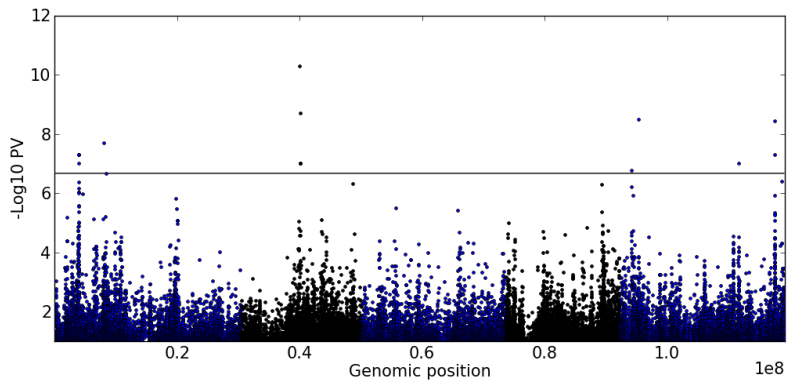
Multiple hypothesis testing

- In GWAS, the number of statistical tests commonly is on the order of 10^6.
- At significane level of 0.01 we would expect 10,000 false positives
- Thus, individual P-values < 0.01 are not significant anymore.
- Correction for multiple hypothesis testing is critical!



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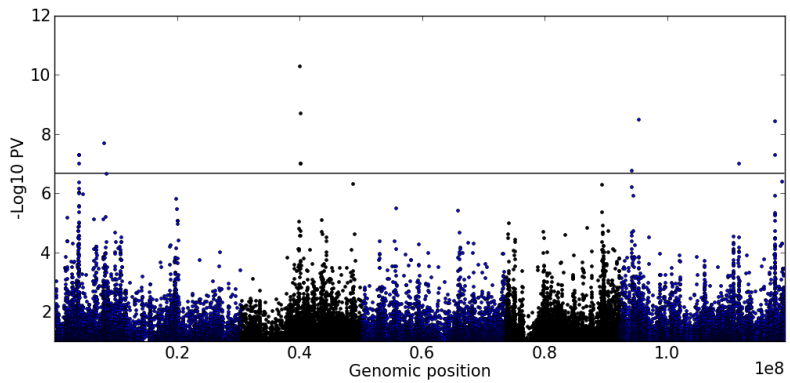

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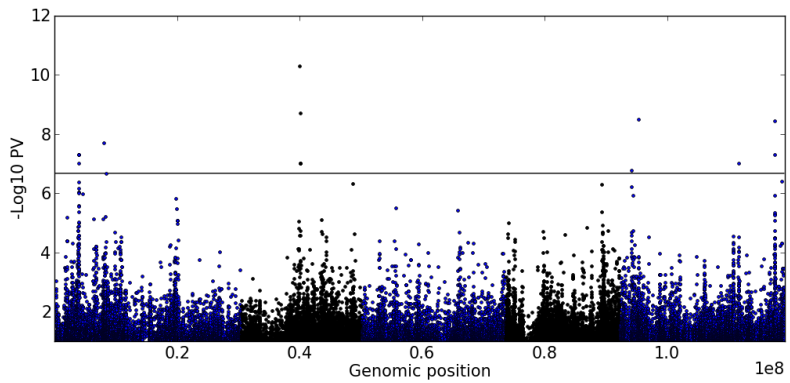

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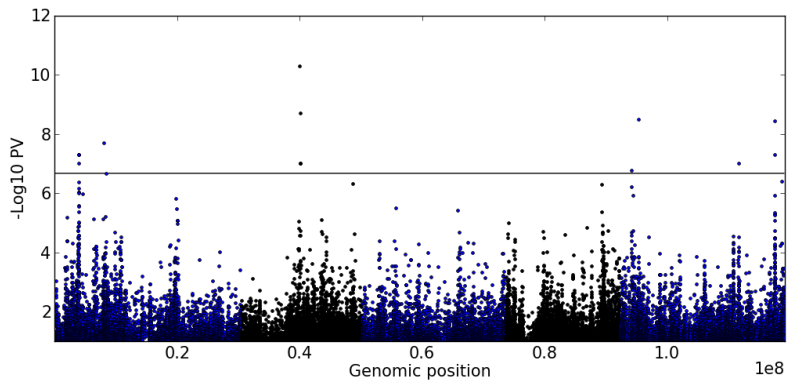

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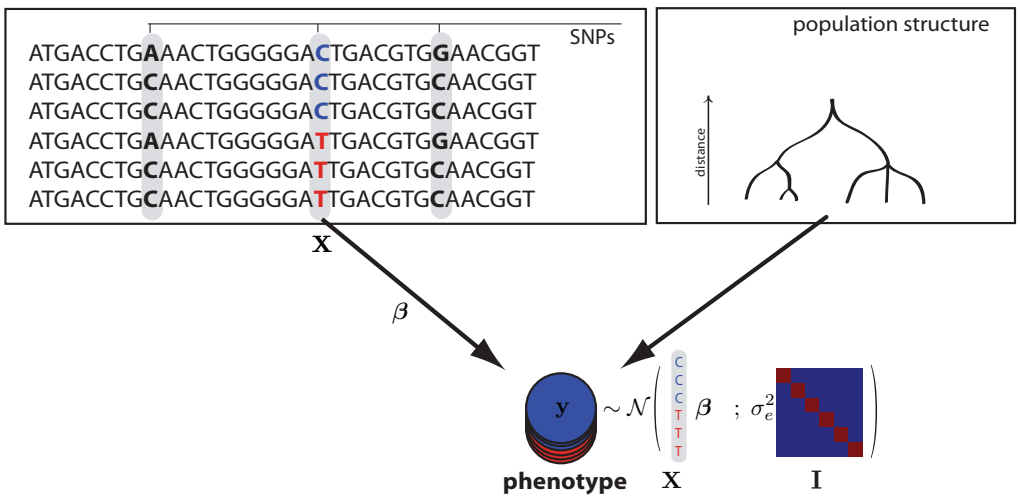

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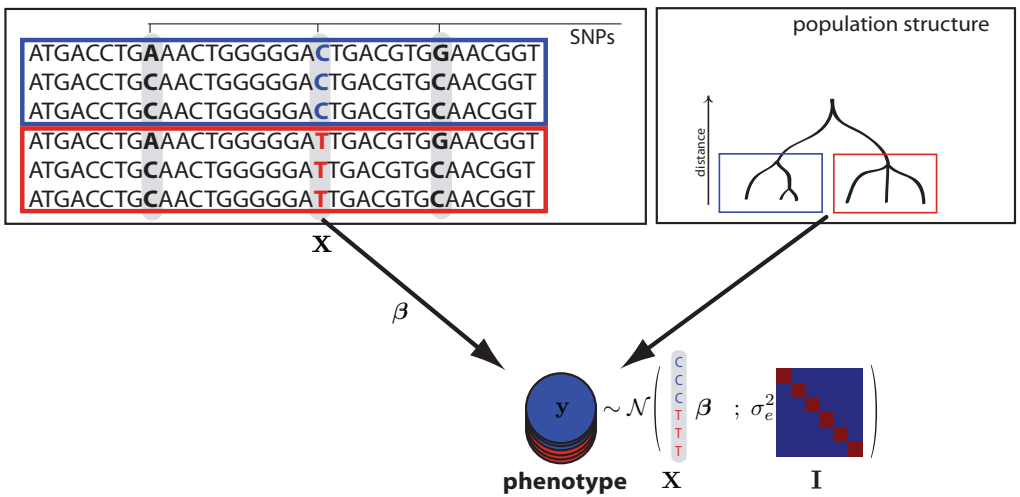

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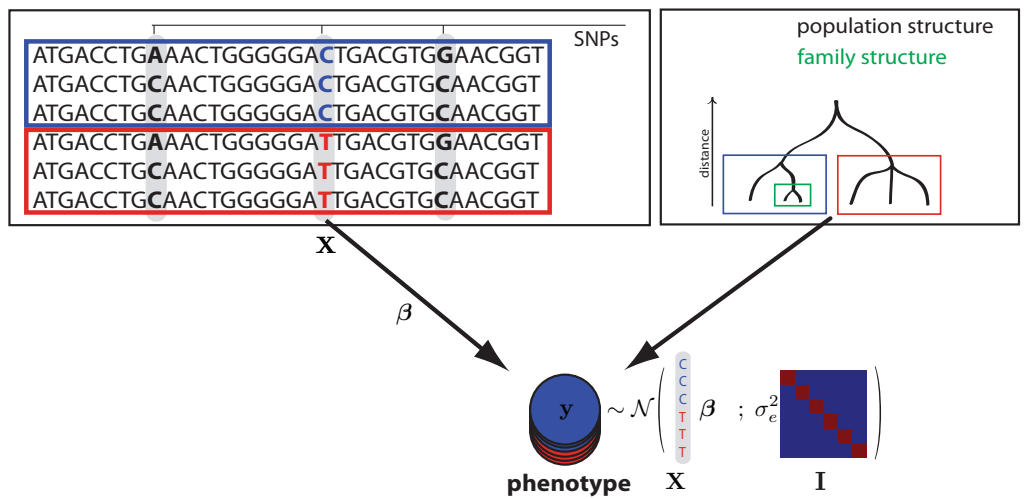

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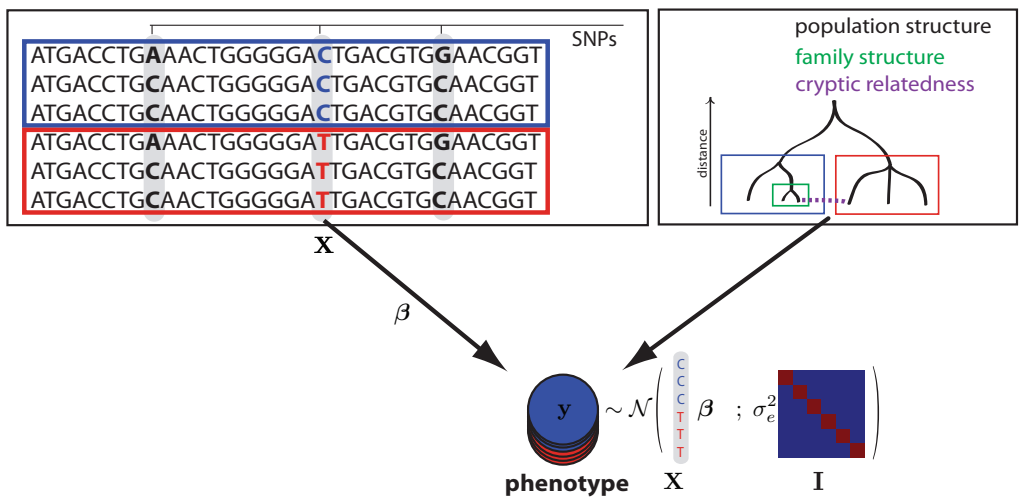

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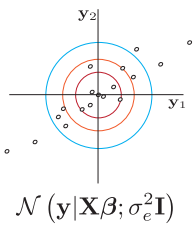

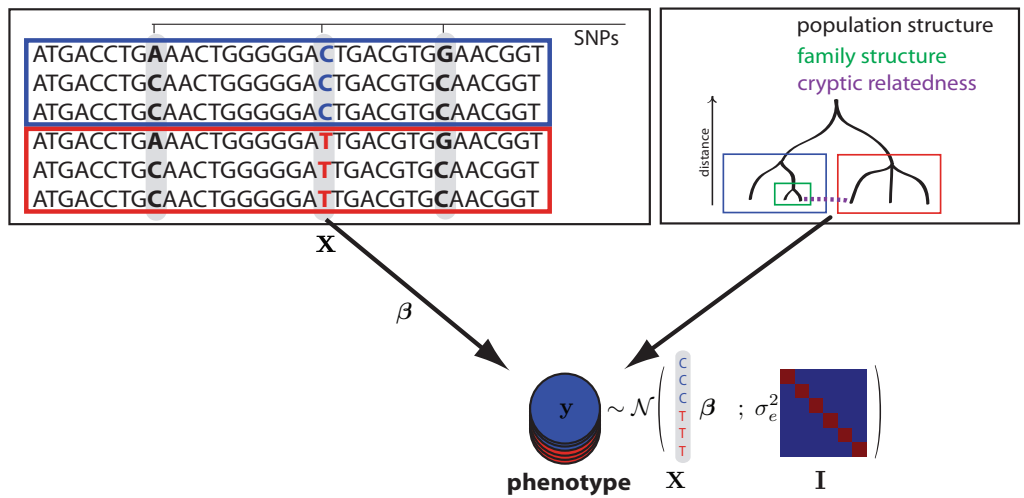

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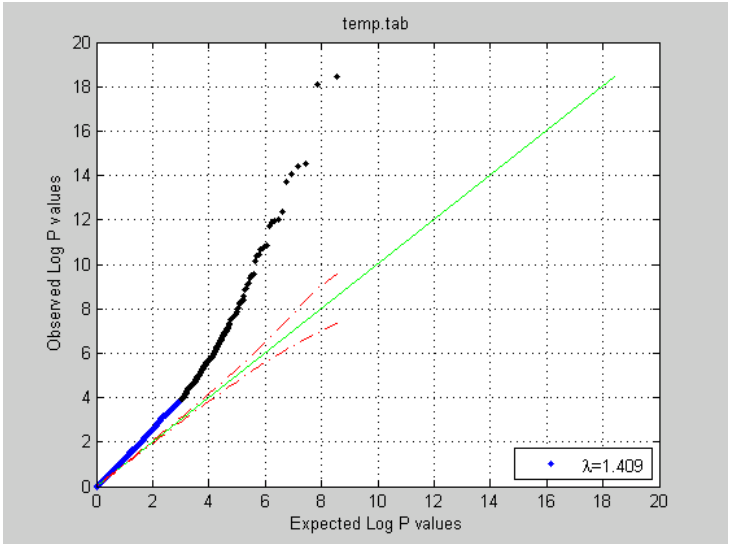

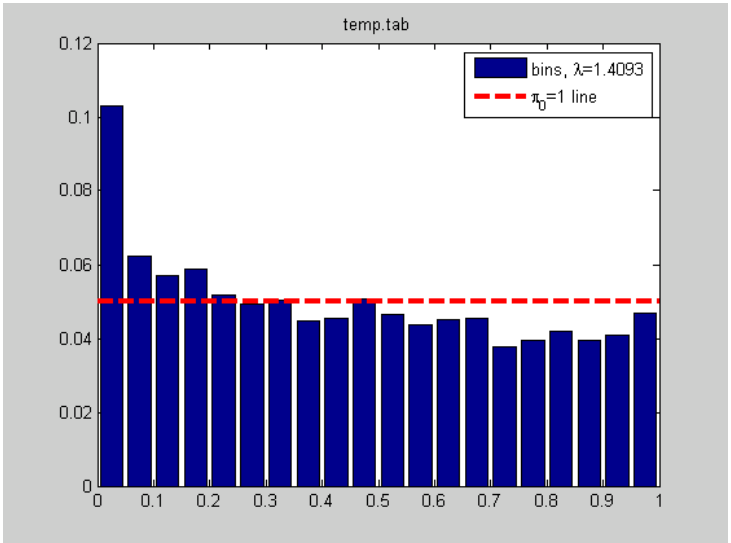

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- Small number of samples, large number of hypotheses
- Rare variants
- Small effect sizes
- Complex phenotypes have multiple regulators
- Increase power by
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Outline

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Introduction
- Why QTL mapping
- Terminology & background
- Methodological challenges

Linear Regression

Hypothesis Testing

Multiple Hypothesis Testing

Model Checking

Regression

Noise model and likelihood

- Given a dataset $\mathcal{D} = \{\mathbf{x}^n, y^n\}_{n=1}^N$, where $\mathbf{x}^n = \{x_1^n, \ldots, x_S^n\}$ is S dimensional, fit parameters $\boldsymbol{\theta}$ of a regressor f with added Gaussian noise:

$$y^n = f(\mathbf{x}^n; \boldsymbol{\theta}) + \epsilon^n \quad \text{where} \quad p(\epsilon \,|\, \sigma^2) = \mathcal{N}\left(\epsilon \,\middle|\, 0, \sigma^2\right).$$

- Equivalent likelihood formulation:

$$p(\mathbf{y} \,|\, \mathbf{X}) = \prod_{n=1}^{N} \mathcal{N}\left(y^n \,\middle|\, f(\mathbf{x}^n), \sigma^2\right)$$

Regression

Choosing a regressor

- Choose f to be linear:

$$p(\mathbf{y} \,|\, \mathbf{X}) = \prod_{n=1}^{N} \mathcal{N}\left(y^n \,\middle|\, \mathbf{x}^n \cdot \boldsymbol{\theta} + c, \sigma^2\right)$$

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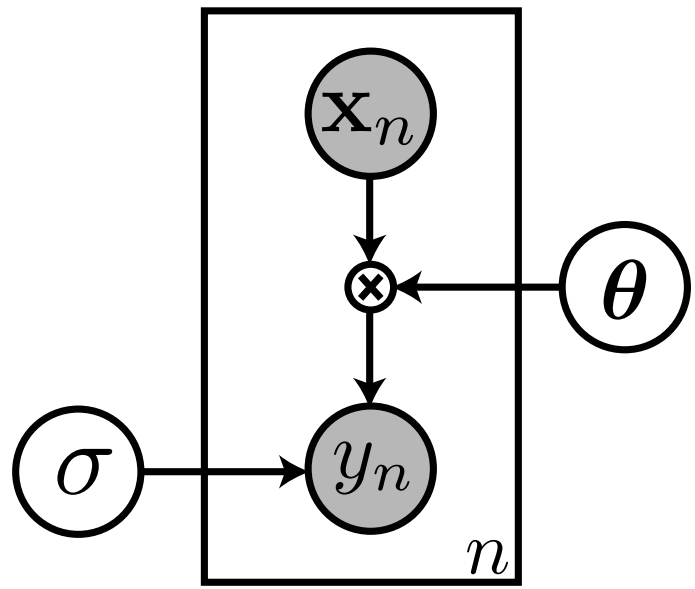

Equivalent graphical model

Linear Regression

Maximum likelihood

- Taking the logarithm, we obtain

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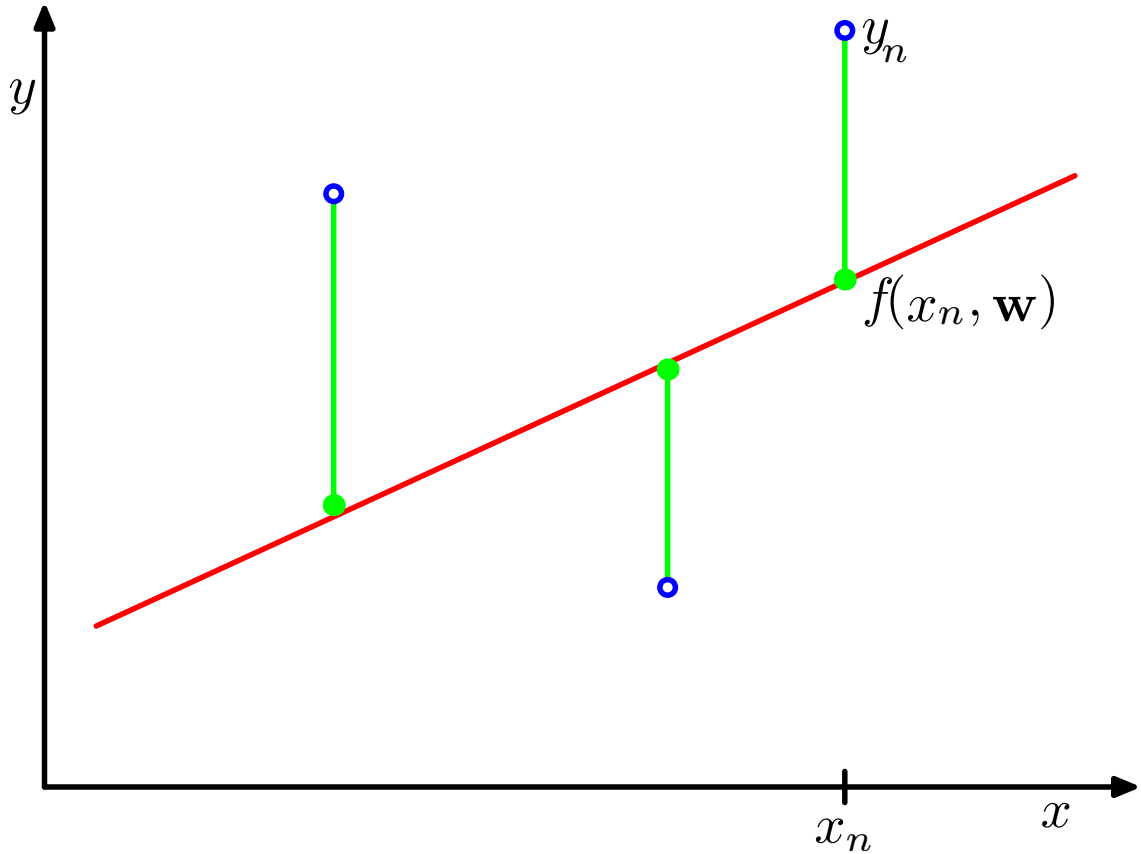
$$= -\frac{N}{2} \ln 2\pi\sigma^2 - \frac{1}{2\sigma^2} \underbrace{\sum_{n=1}^{N} (y^n - \mathbf{x}^n \cdot \boldsymbol{\theta})^2}_{\text{Sum of squares}}$$

- The likelihood is maximized when the squared error is minimized.
- Least squares and maximum likelihood are equivalent.

Linear Regression and Least Squares



(C.M. Bishop, Pattern Recognition and Machine Learning)

$$E(\boldsymbol{\theta}) = \frac{1}{2}\sum_{n=1}^{N}(y^n - \mathbf{x}^n \cdot \boldsymbol{\theta})^2$$

Linear Regression and Least Squares

- Derivative w.r.t. a single weight entry θ_i

$$\frac{d}{\mathrm{d}\theta_i} \ln p(\mathbf{y} \mid \boldsymbol{\theta}, \sigma^2) = \frac{d}{\mathrm{d}\theta_i} \left[-\frac{1}{2\sigma^2} \sum_{n=1}^{N} (y^n - \mathbf{x}^n \cdot \boldsymbol{\theta})^2 \right]$$

$$= \frac{1}{\sigma^2} \sum_{n=1}^{N} (y^n - \mathbf{x}^n \cdot \boldsymbol{\theta}) x_i$$

- Set gradient w.r.t. θ to zero

$$\nabla_{\boldsymbol{\theta}} \ln p(\mathbf{y} \mid \boldsymbol{\theta}, \sigma^2) = \frac{1}{\sigma^2} \sum_{n=1}^{N} (y^n - \mathbf{x}^n \cdot \boldsymbol{\theta}) \mathbf{x}^{n\mathrm{T}} = 0$$

$$\Longrightarrow \boldsymbol{\theta}_{\mathsf{ML}} = \underbrace{(\mathbf{X}^{\mathrm{T}}\mathbf{X})^{-1}\mathbf{X}^{\mathrm{T}}}_{\text{Pseudo inverse}} \mathbf{y}$$

- Here, the matrix $\mathbf{X}$ is defined as $\mathbf{X} = \begin{bmatrix} x_1^1 & \dots & x_S^1 \\ \dots & \dots & \dots \\ x_1^N & \dots & x_S^N \end{bmatrix}$

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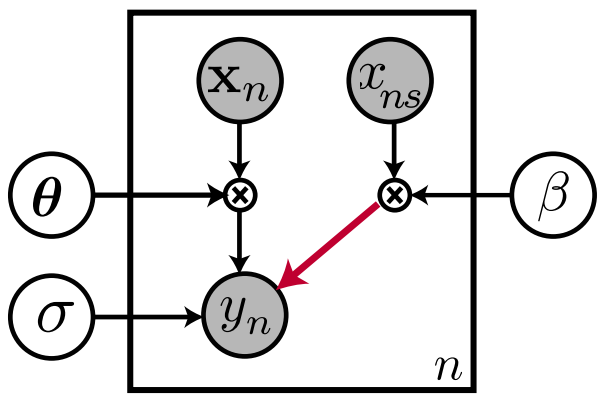
Testing in Linear Regression

Likelihood Ratio Test

$$p(\mathbf{y} \mid \mathbf{X}) = \prod_{n=1}^{N} \mathcal{N}\left(y^n \mid \mathbf{x}^n \cdot \boldsymbol{\theta} + x_s^n \beta, \sigma^2\right)$$

- x_s^n: SNP to be tested
- $\mathbf{x}^n$: regression covariates (including bias term)
  - Race
  - Known background SNPs
  - Environment



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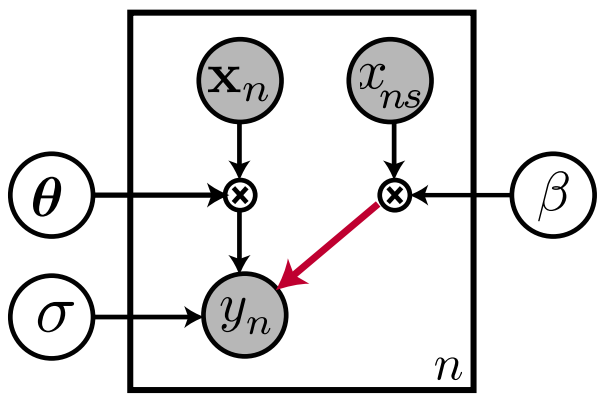

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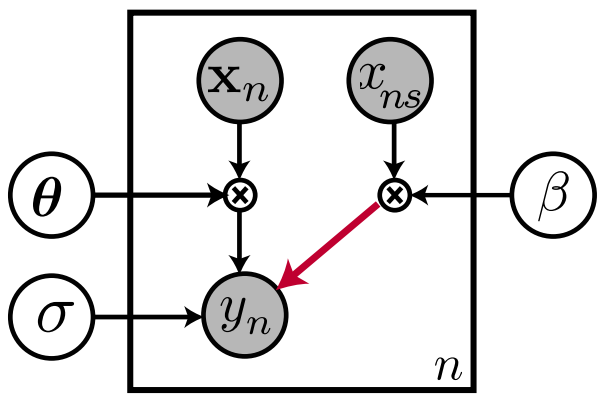

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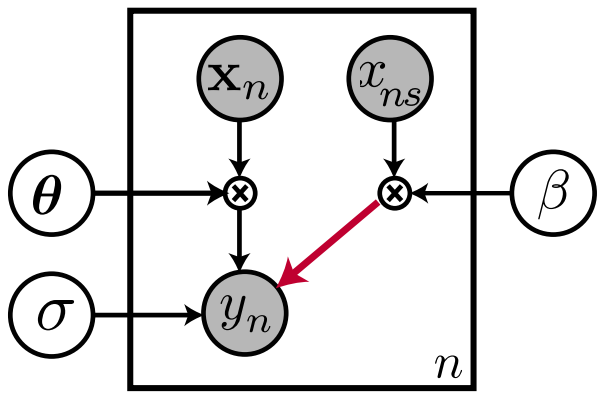

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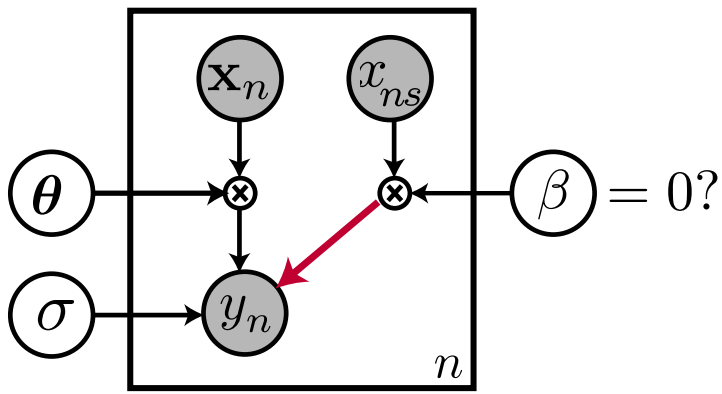

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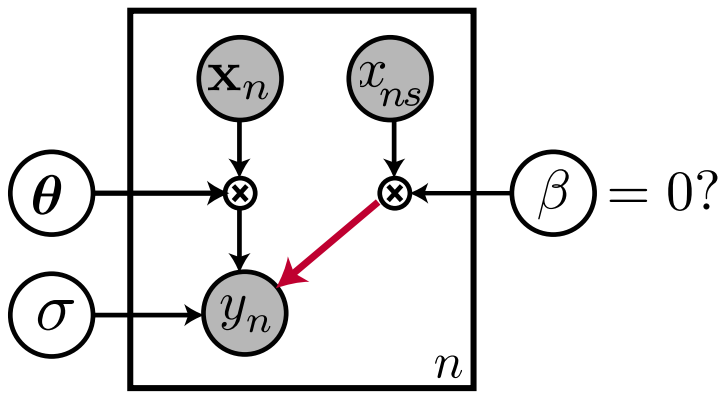

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- Why QTL mapping
- Terminology & background
- Methodological challenges

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Hypothesis Testing

Multiple Hypothesis Testing

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Hypothesis Testing

Example:

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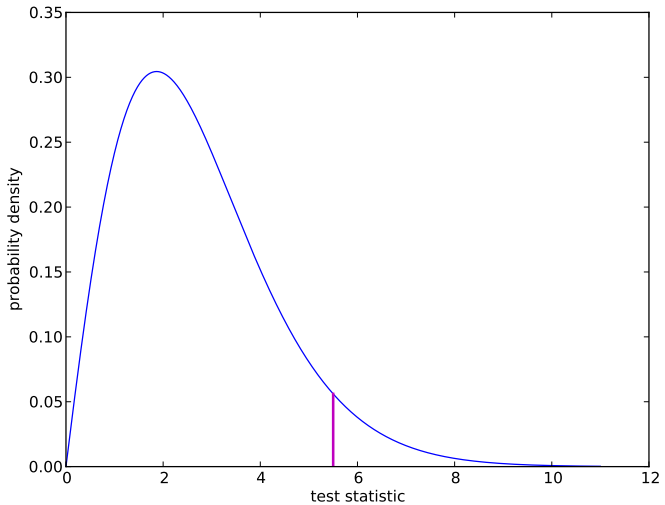

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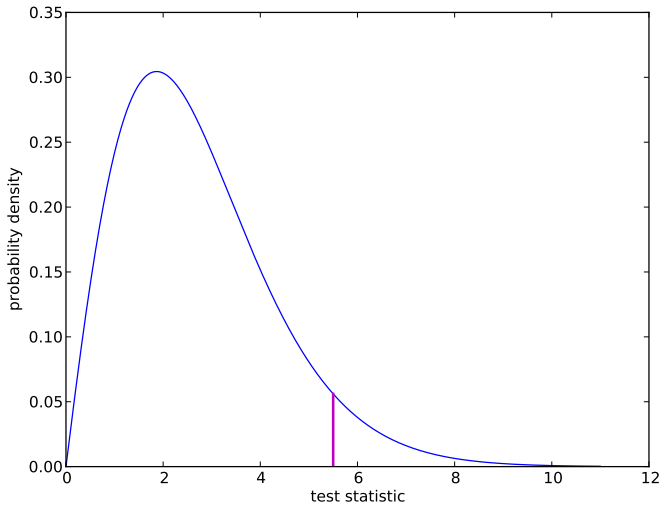

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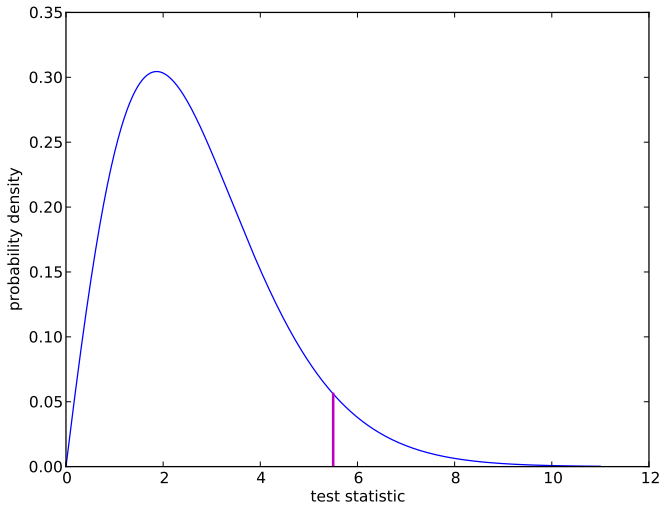

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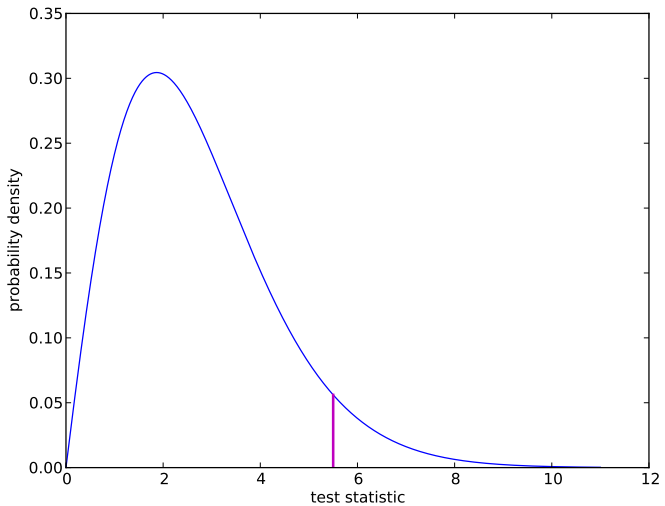

P-value

definition

- Probability of observing a test statistic at least as extreme (e.g. likelihood ratio statistic), given that $\mathcal{H}_0$ is true.
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- Need to know the null distribution of test statistics. (usually unknown)
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Repeat M times:

- Permute phenotype $\mathbf{y}$ and covariates $\mathbf{x}$ jointly over individuals.
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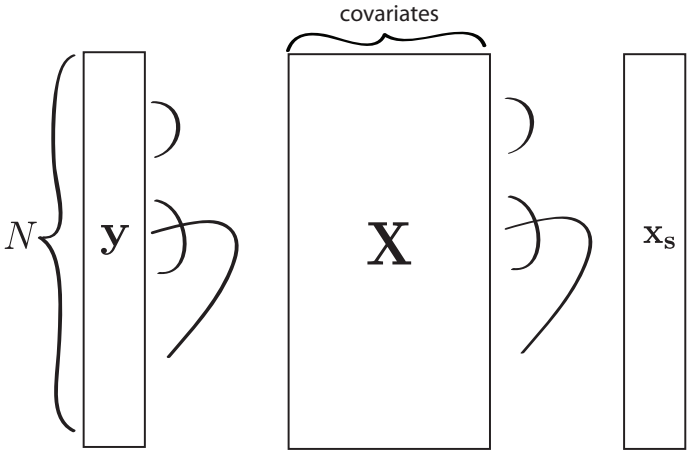

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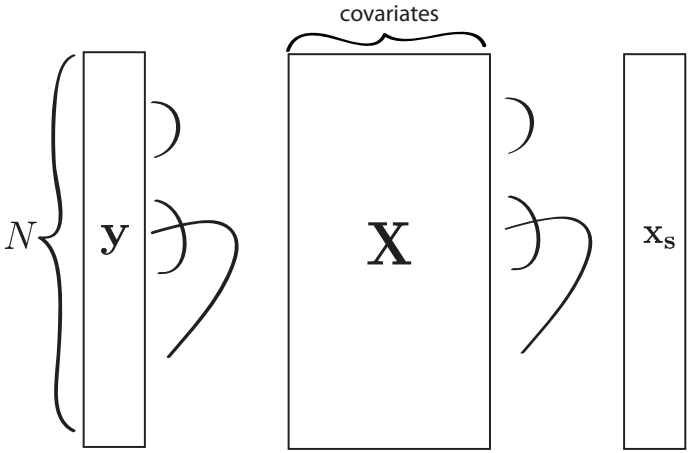

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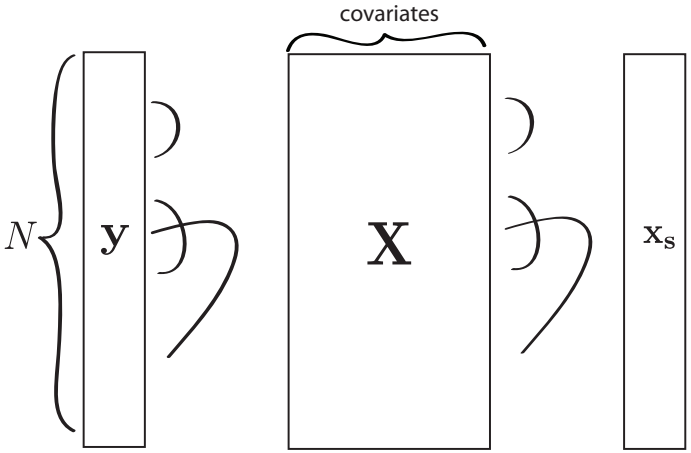

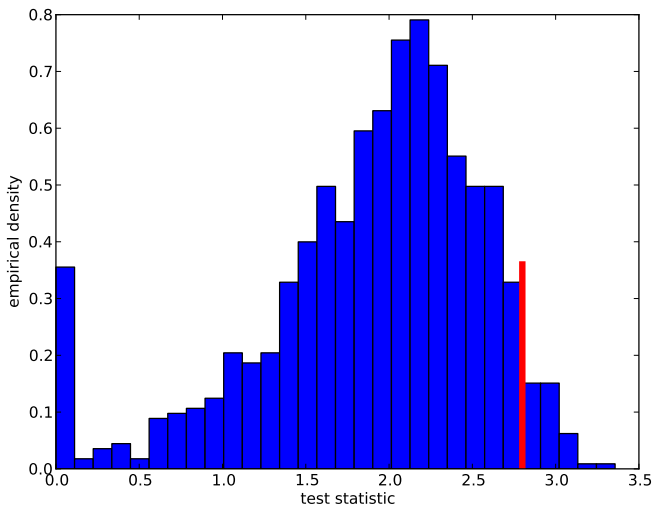

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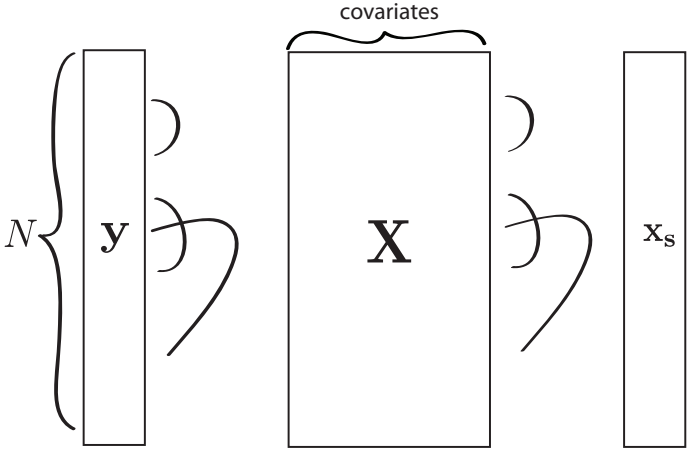
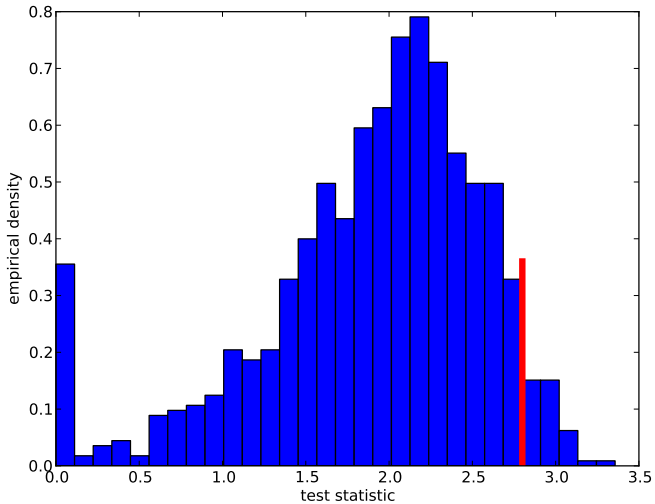
The P-value is the quantile of real test statistic in artificial null distribution.





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Likelihood Ratio Test revisited

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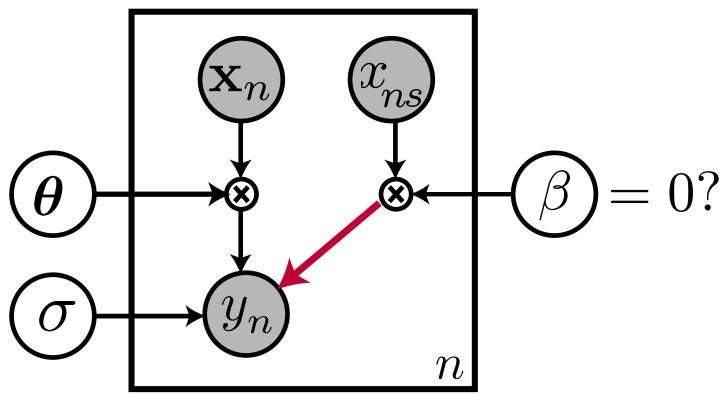

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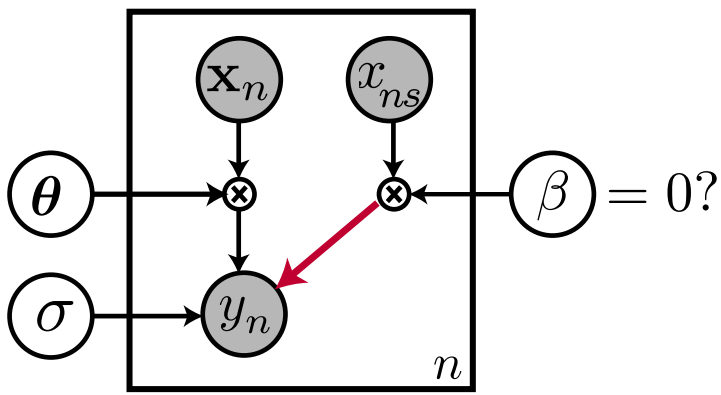

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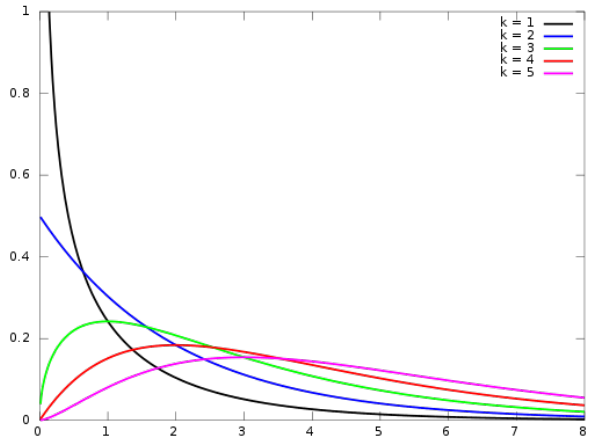

(source: Wikipedia)

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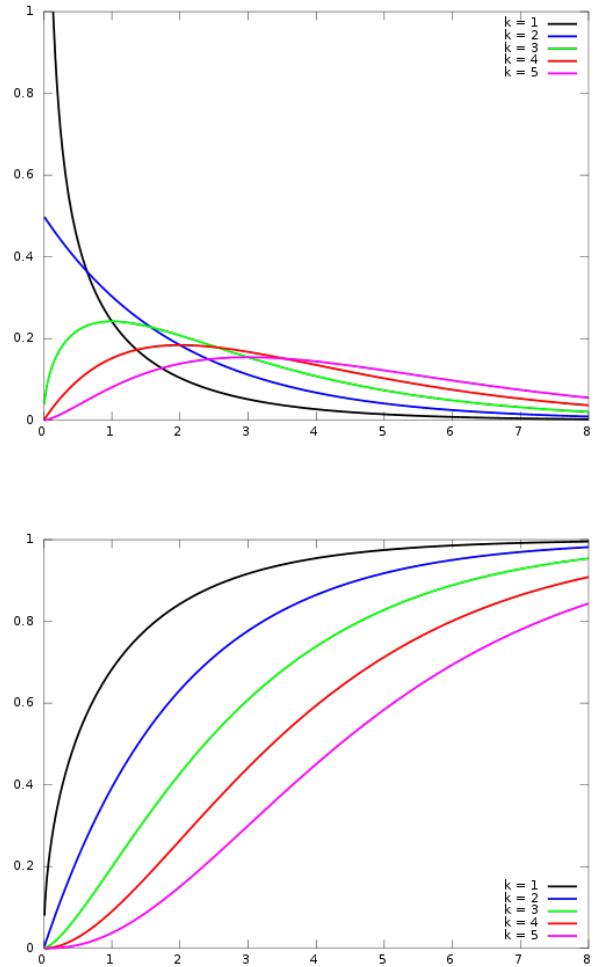

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- Significance level α equals probability of type-1 error.
- In GWAS we perform $S = 10^6$ tests
- At $\alpha = 0.01$ we would expect 10000 type-1 errors!
- Probability of at least 1 type-1 error is $1 - (1 - \alpha)^S \to 1$.
- Individual P-values < 0.01 are not significant anymore.

	$\mathcal{H}_0$ holds	$\mathcal{H}_0$ doesn't hold
$\mathcal{H}_0$ accepted	true negatives	false negatives type-2 error
$\mathcal{H}_0$ rejected	false positives type-1 error	true positives

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Need to correct for multiple hypothesis testing!

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Multiple Hypothesis Testing

Family-Wise Error Rate (FWER)

- Probability of at least one type-1 error.
- Correct by bounding the FWER.
- Bonferroni correction: $P_B = P \cdot S$
- Equivalently $P < \frac{\alpha}{S}$ significant.
- Bounds the FWER $1 - (1 - \alpha/S)^S$ by α

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Outline

Introduction
- Why QTL mapping
- Terminology & background
- Methodological challenges

Linear Regression

Hypothesis Testing

Multiple Hypothesis Testing

Model Checking

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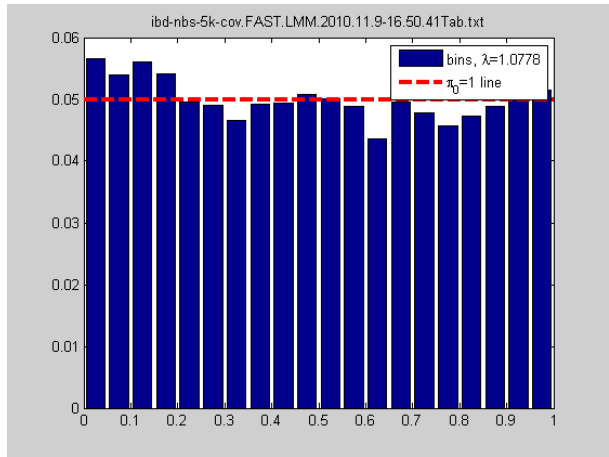
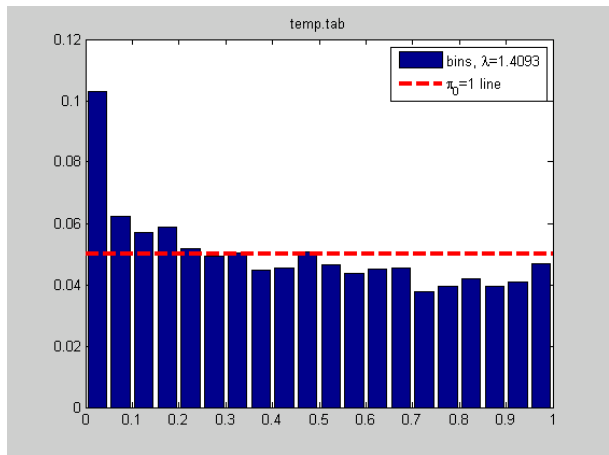
- Do my estimated P-values match the true null distribution?
  - By definition uniformly distributed under null distribution.
- Do the empirical results match my assumptions on the null model?
- In GWAS we perform a large number of tests. (usually in the order of 10^6)
- Use the strong prior knowledge that in GWAS almost all of the test SNPs have no effect on the phenotype.
- Empirical test statistics should follow the null distribution





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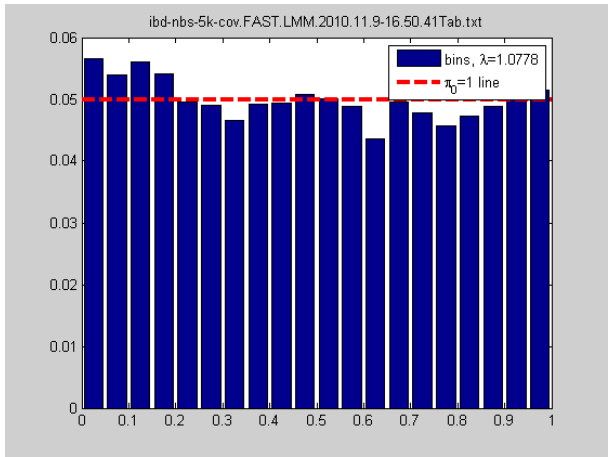

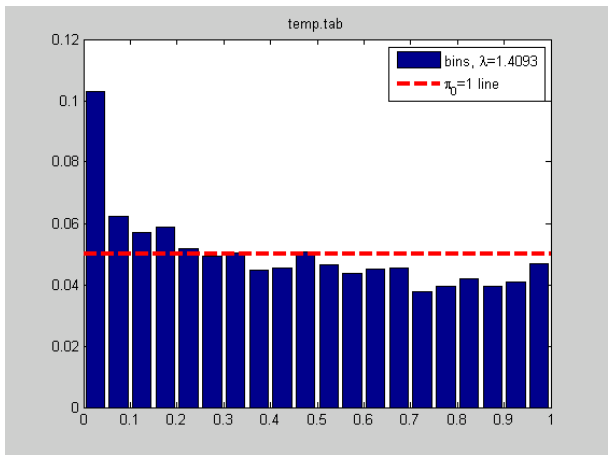

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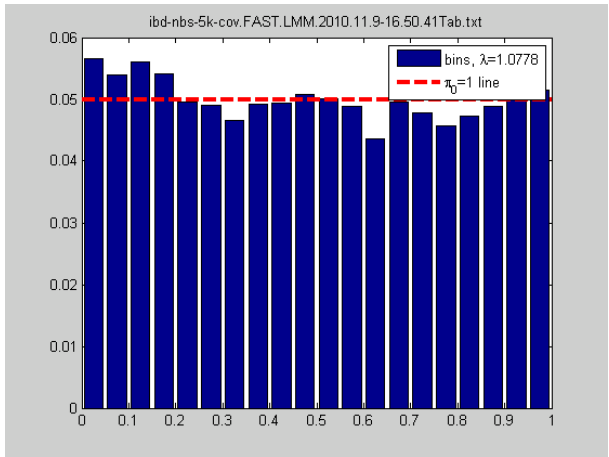

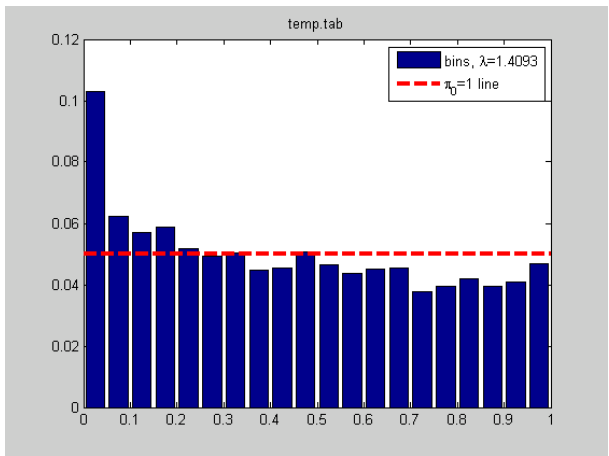

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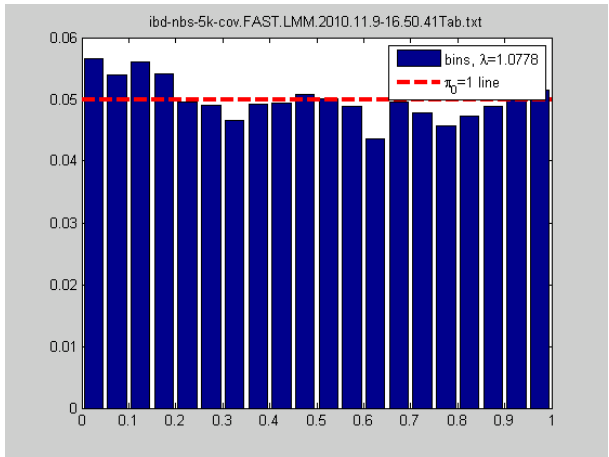

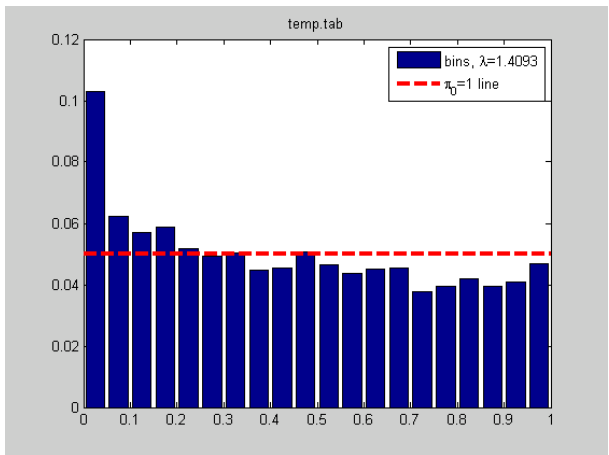

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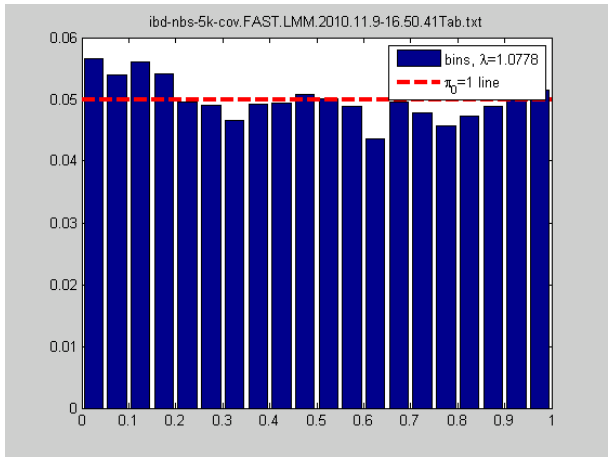

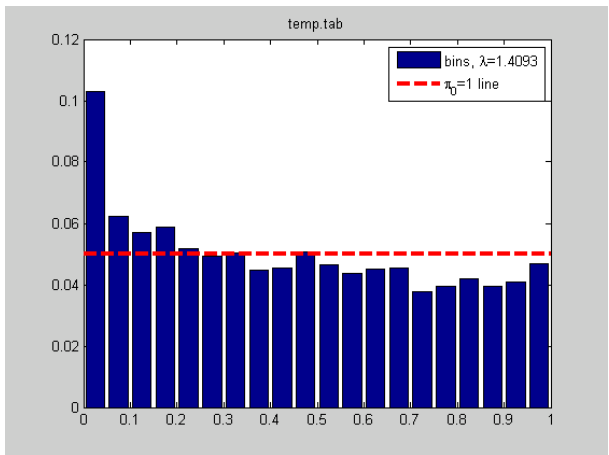

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QQ-plot

Compare quantiles of the empirical test statistic distribution to assumed null distribution.

- Sort test statistics
- Plot test statisitcs against (y-axis) quantiles of the theoretical null-distribution (x-axis)
  - for example: 2LR vs. χ^2_1
- If the plot is close to the diagonal, the distributions match up
- Deviation from the diagonal indicates inflation or deflation of test statistics.

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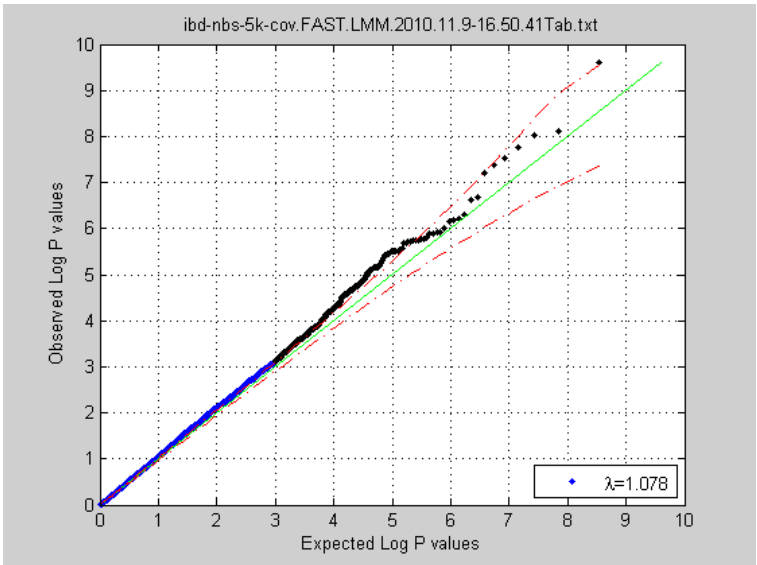

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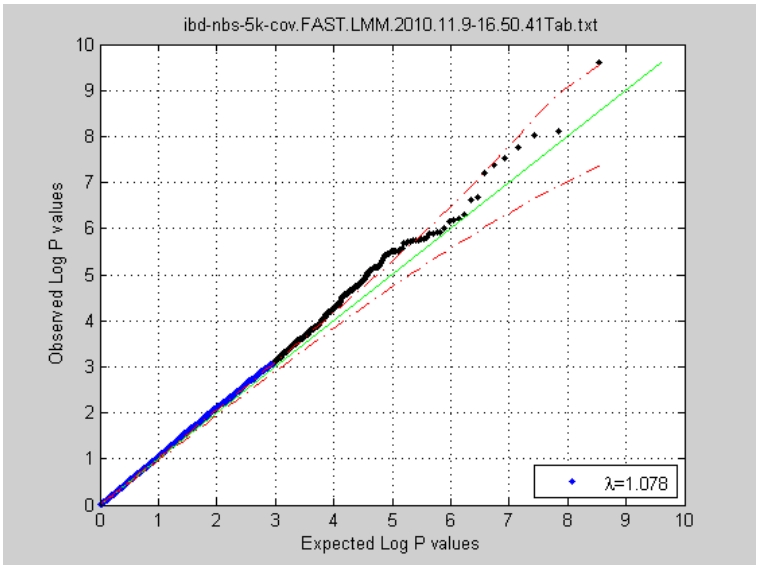

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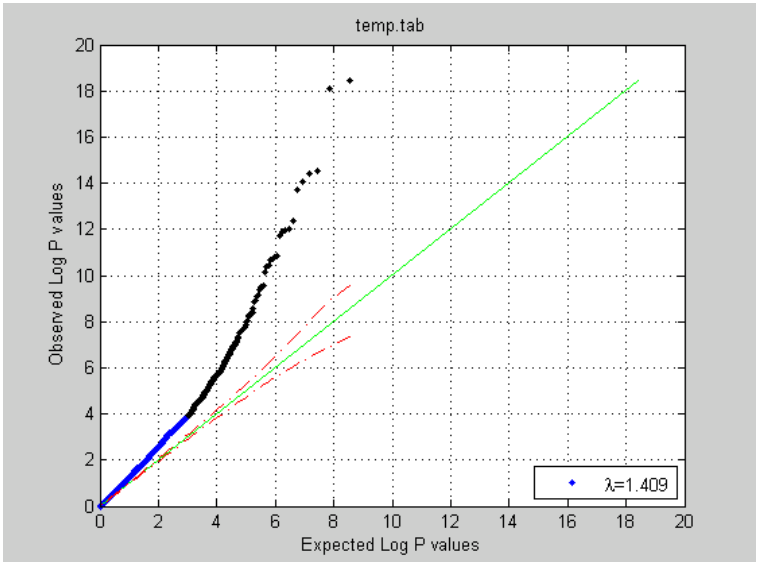

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  - Study design
  - Data preparation
- Challenges and pitfalls
  - Power
  - Multiple hypothesis testing
  - Population structure
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Acknowledgements

- **Joint course material**
  O. Stegle
- **Why QTL mapping**
  D. Weigel, K. Borgwardt

References I

S. Atwell, Y. Huang, B. Vilhjálmsson, G. Willems, M. Horton, Y. Li, D. Meng, A. Platt, A. Tarone, T. Hu, et al. Genome-wide association study of 107 phenotypes in arabidopsis thaliana inbred lines. *Nature*, 465(7298):627–631, 2010.

B. Browning and S. Browning. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *The American Journal of Human Genetics*, 84(2):210–223, 2009.

J. Cao, K. Schneeberger, S. Ossowski, T. Günther, S. Bender, J. Fitz, D. Koenig, C. Lanz, O. Stegle, C. Lippert, X. Wang, F. Ott, J. Müller, C. Alonso-Blanco, K. Borgwardt, K. Schmid, and D. Weigel. Whole-genome sequencing of multiple *arabidopsis thaliana* populations. *Nature Genetics*, 43(10):956–963, 10 2011. doi: 10.1038/ng.911.

J. Spitzer. A primer on box-cox estimation. *The Review of Economics and Statistics*, 64(2): 307–313, 1982.

G. Upton and I. Cook. Oxford dictionary of statistics, 2002.