Overview and introduction

Christoph Lippert[1], **Oliver Stegle**[2]

[1] Microsoft Research, Los Angeles, USA
[2] Max-Planck-Institutes Tübingen, Germany

Basel
09. September 2012

Time table

- 09:10–09:20 Welcome
- 09:20–10:00 Introduction and background
- 10:00–10:30 Linear models I
- 10:30–11:00 **Coffee break**
- 11:00–11:30 Linear models I contd.
- 11:30–11:45 Demonstrations I
- 11:45–12:15 Linear models II
- 12:15–13:30 **Lunch**
- 13:30–14:00 Linear models II contd.
- 14:00–14:30 Advanced mixed models
- 14:30–15:00 Demonstrations II
- 15:00–15:30 **Coffee break**
- 15:30–16:15 High-dimensional traits, gene expression
- 16:15–17:00 Discussion, questions, etc.

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Outline

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Why QTL mapping

Terminology & background

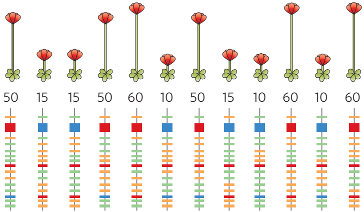
Methodological challenges

Tutorial outline & resources

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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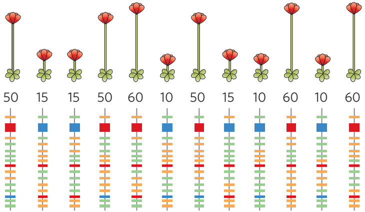

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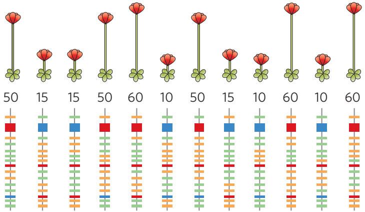

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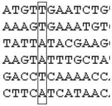

ATGTTGAATCTG
AAAGTGAAATGT
TATTATACGAAG
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Goal:

- Identify causal loci that explain phenotypic differences.

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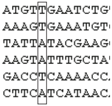

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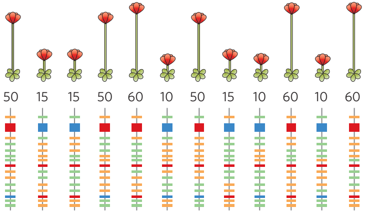
Use of GWAs in plant systems

- 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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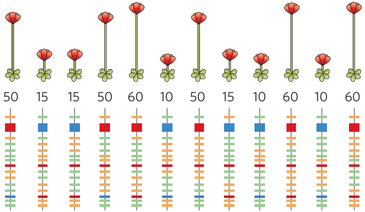

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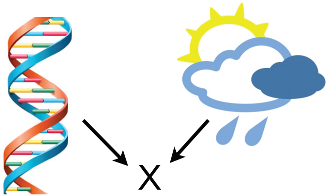

Personalized medicine & health

- 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
  - Known causal variants help to identify individuals with higher risk to develop a particular disease.
  - Improved monitoring of high-risk groups.

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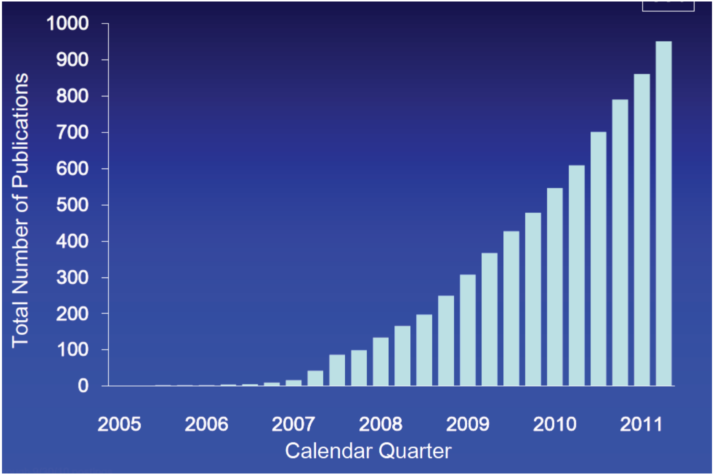

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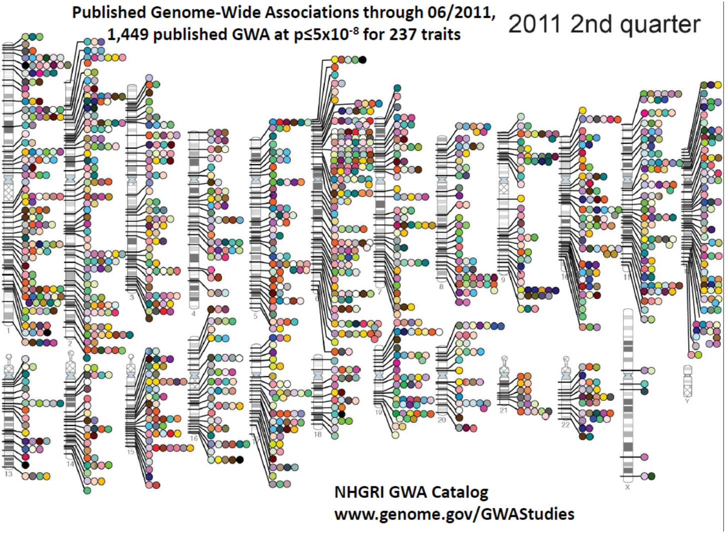

Published Genome-Wide Associations through 06/2011, 1,449 published GWA at p≤5x10^{-8} for 237 traits

2011 2nd quarter

NHGRI GWA Catalog
www.genome.gov/GWAStudies

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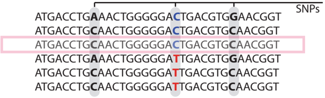
Some definitions

- 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.
  - Denoted by $\mathbf{y}^n$ for individual n.
- A locus is a position or limited region in the genome.
  - Denoted by $\mathbf{x}_s$ for locus (or SNP) s.
- An allele is the genetic state of a locus.


SNPs
ATGACCTGAAACTGGGGGACTGACGTGGAACGGT
ATGACCTGCAACTGGGGGACTGACGTGCAACGGT
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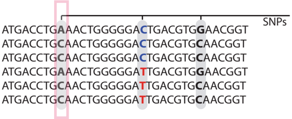

ATGACCTGAAACTGGGGGACTGACGTGGAACGGT
ATGACCTGCAACTGGGGGACTGACGTGCAACGGT
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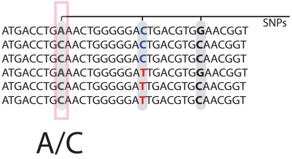
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A/C

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.
  - e.g. *human*
- An organism/cell is polyploid if it has more than two homologous chromosomes.
  - e.g. *sugar cane* is hexaploid.

image source: Wikipedia

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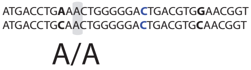
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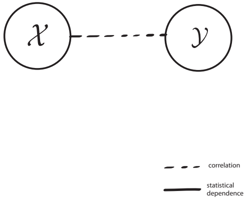
A/C

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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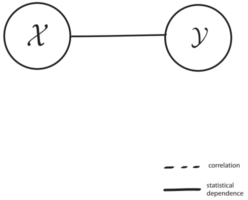

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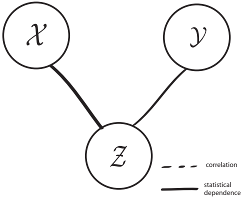

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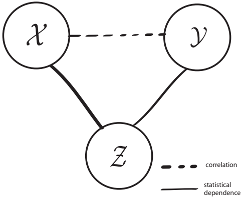

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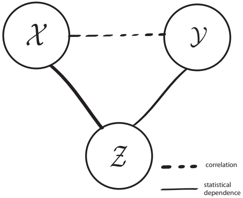

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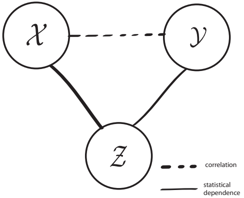

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Result

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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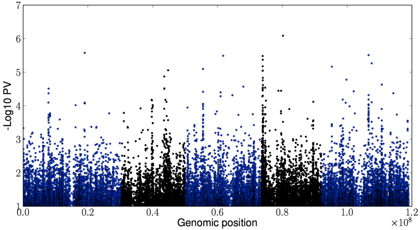

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Genetic designs

- 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
  - Typically haploid.
- Multi-parent crosses
  - Increased genetic diversity
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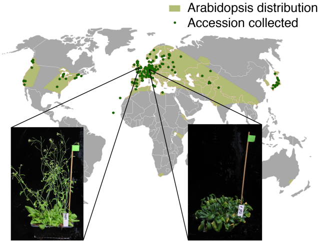

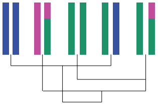

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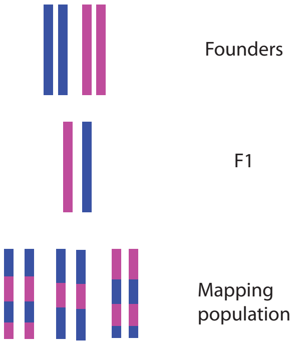

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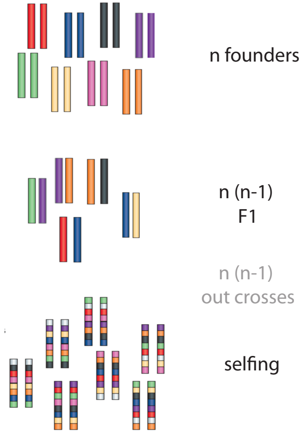

Genetic designs

Genotype encoding

A simple encoding scheme, ignoring dominance:

- A locus is heterozygous if it differs between paternal and maternal haplotypes.
  - heterozygous allele usually encoded as 1
- A locus is homozygous if it matches between paternal and maternal haplotypes.
  - homozygous *major* allele usually encoded as 0
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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.

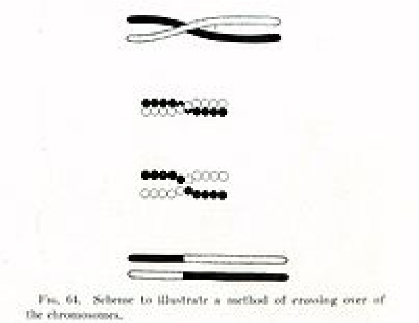
FIG. 64. Scheme to illustrate a method of crossing over of the chromosomes.

image source: Wikipedia

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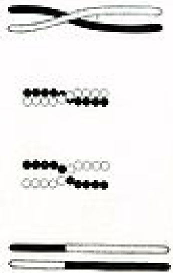

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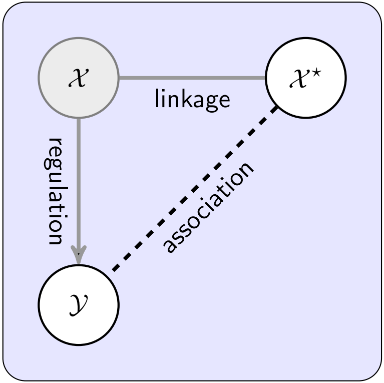

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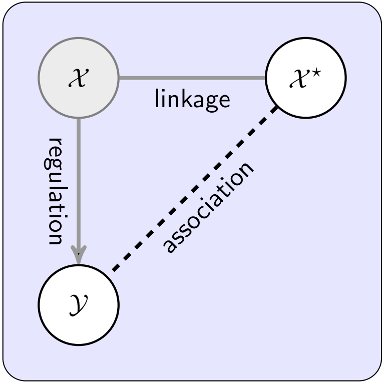

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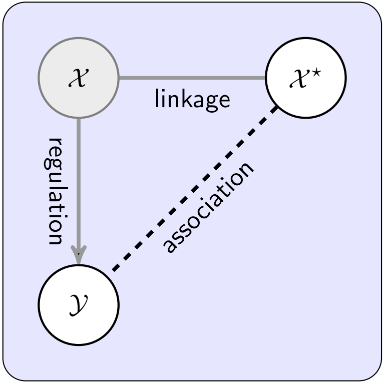
- Recombination hotspots on the chromosome lead to conserved haplotype blocks in strong linkage.
- Linkage can be used to chose *tag*-SNPs to cover all linked regions.
  - Tradeoff between resolution and genotyping cost.



Phenotypes

- Binary
  - case, control
- Continuous
  - Gaussian
  - Non-Gaussian
- Multivariate
- Other

- e.g. disease status
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Genotype

- Imputation of missing values
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  - Beagle, IMPUTE
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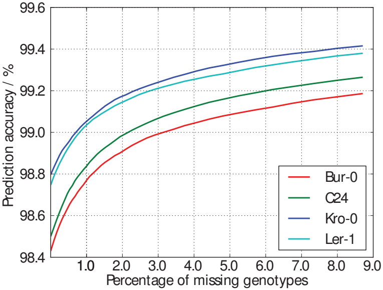

Genotype imputation accuracy from SNP-chip to 80Genomes reference panel [Cao et al., 2011].

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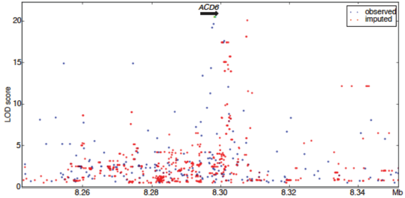

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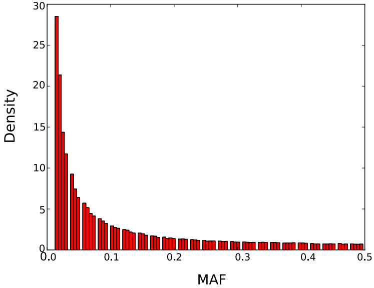

Minor allele frequency from 160 *A. thaliana* lines; 2.3 million genome-wide SNPs from NGS sequencing

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- Most parametric models are based on Gaussianity assumptions
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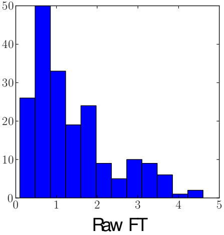

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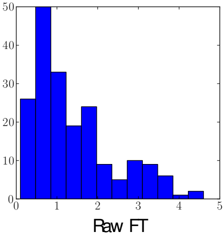

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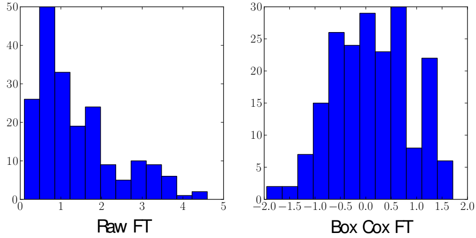

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Linkage Disequilibrium

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.
  - $D = f_{AA} - f_{.A} f_{A.}$
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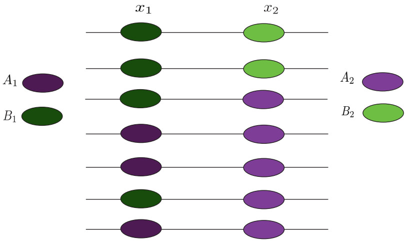

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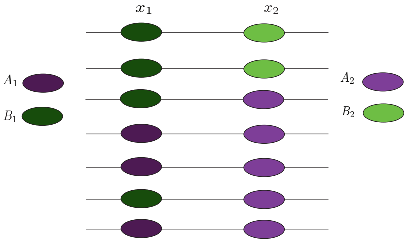

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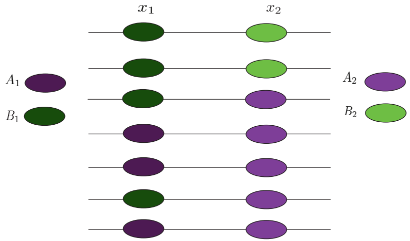

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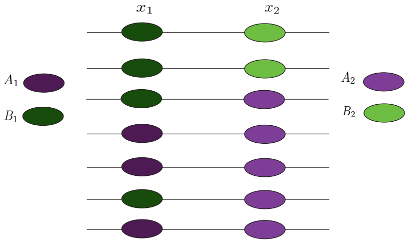

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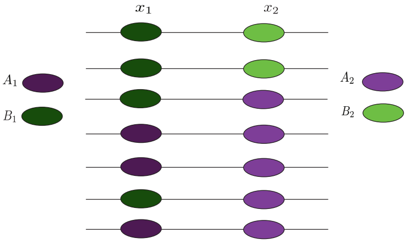

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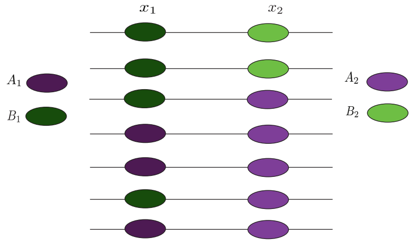

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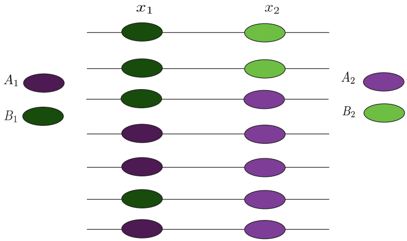

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Outline

Why QTL mapping

Terminology & background

Methodological challenges

Tutorial outline & resources

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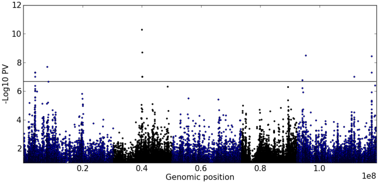
Multiple hypothesis testing

- 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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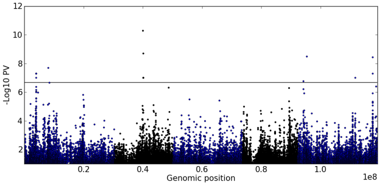

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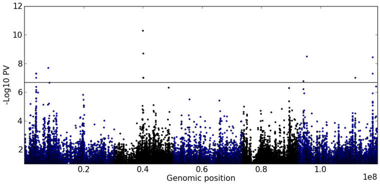

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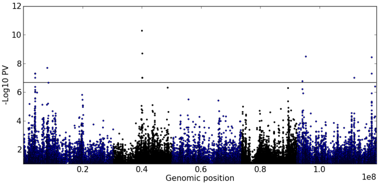

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Population structure

- Confounding structure leads to false positives.
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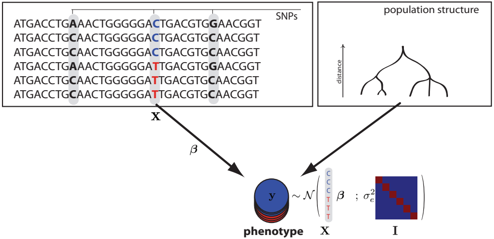
  - Family structure
  - Cryptic relatedness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X
population structure
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phenotype
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C C C T T T
X
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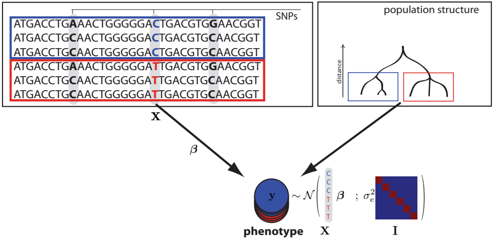
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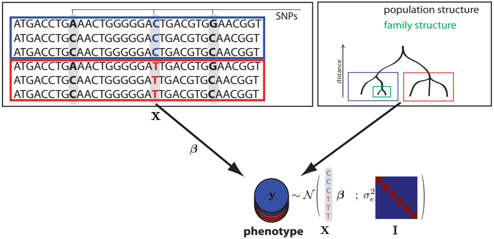
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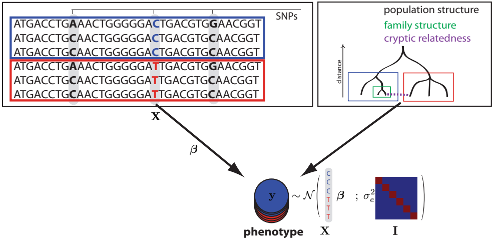
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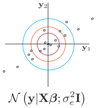

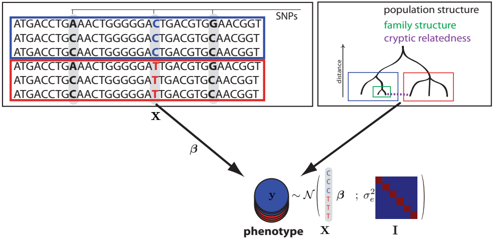
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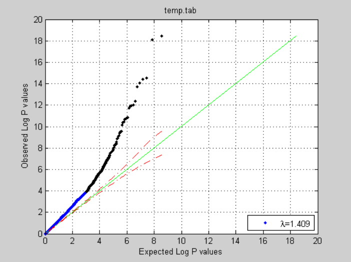

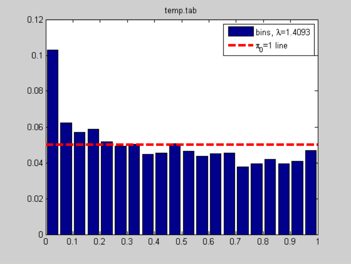

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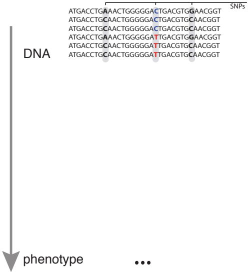
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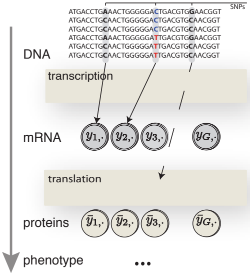
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DNA
transcription
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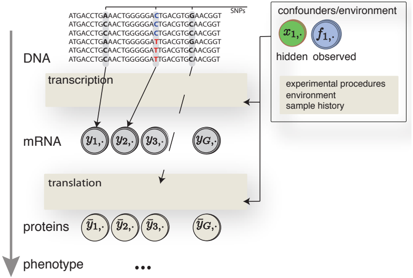

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- Increase power by
  - Conditioning on known effects
  - Testing compound hypotheses (e.g. test all (rare) variants in a window)

Challenges

Statistical power and resolution

- Small number of samples, large number of hypotheses
- Rare variants
- Small effect sizes
- Complex phenotypes have multiple regulators
- Increase power by
  - Conditioning on known effects
  - Testing compound hypotheses (e.g. test all (rare) variants in a window)

Challenges

Non-independent traits

- Measured expression levels for thousands of genes
- Growth related phenotypes
  - e.g. weight, BMI
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Outline

Why QTL mapping

Terminology & background

Methodological challenges

Tutorial outline & resources

Topics covered

- Linear models 1
  - Significance testing, multiple hypothesis correction, correction for population structure
- Linear models 2
  - Composite variance analysis, multi-trait models, phenotype prediction, LASSO
- Advanced topics
  - Improved linear mixed models
  - Association mapping of high-dimensional traits
- Practical demonstations and demos to take away
- Opportunities for open discussion, questions and scientific exchange

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Software used in examples

FaST-LMM

- Large-scale genome-wide association studies
- Command line tool
- Correction for population structure on up to 100k samples
- `http://mscompbio.codeplex.com`

[Lippert et al., 2011]

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- Efficient open source C++ toolbox for advanced GWAS analyses
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Resources

Datasets and resources used in this tutorial

- Wellcome Trust Case Control Consortium [Burton et al., 2007]
  - Data access and details: `http://www.wtccc.org.uk/`.
- *A. thliana* GWAS on 107 phenotypes [Atwell et al., 2010]
  - Data publicly available `https://cynin.gmi.oeaw.ac.at/home/resources/atpolydb/genomic-polymorphism-data-in-arabidopsis-thaliana`
- eQTL datasets from yeast [Smith and Kruglyak, 2008]
  - Data is also included in the examples of PEER [Stegle et al., 2012]
    - Data download: `http://www.nature.com/nprot/journal/v7/n3/extref/nprot.2011.457-S1.zip`

Questions?

Acknowledgements

- **Why QTL mapping**
  Detlef Weigel, Karsten Borgwardt

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