https://goo.gl/bSX3De

trans-National Infrastructure for Plant Genomic Science

Part of the transPLANT European Plant Genomics Infrastructure

Hands-on tutorial to Genome-wide Association Studies (GWAS)

Ümit Seren

Exploring Plant Variation Data Workshop

Jul. 1st-3rd 2015

Outline

- Introduction
  - Motivation
  - Why plants (*A. thaliana*)?
  - Population Structure
- GWAS methods
  - Linear model
  - Non-parametric test
  - Linear Mixed Model
  - Advanced Linear Mixed Models
  - Caveats & Problems
- Hands-on tutorial
  - Introduction to GWA-Portal
  - Step by step guide
- Summary

Suggested literature

- Hastie, Tibshirani, and Friedman. (2009) *The Elements of Statistical Learning: Data Mining, Inference, and Prediction.* A very good book. A pdf can be downloaded here: http://www-stat.stanford.edu/~tibs/ElemStatLearn/.
- Lynch and Walsh. (1998) *Genetics and Analysis of Quantitative Traits*. This book is an outstanding classical reference for quantitative geneticists.
- Nature Genetics. (2008-2013) *Genome-wide association studies*. Series about best practices for doing GWAS in humans. http://www.nature.com/nrg/series/gwas/index.html

Motivation, Why plants (*A. thaliana*) ?, Population Structure

Introduction

Motivation

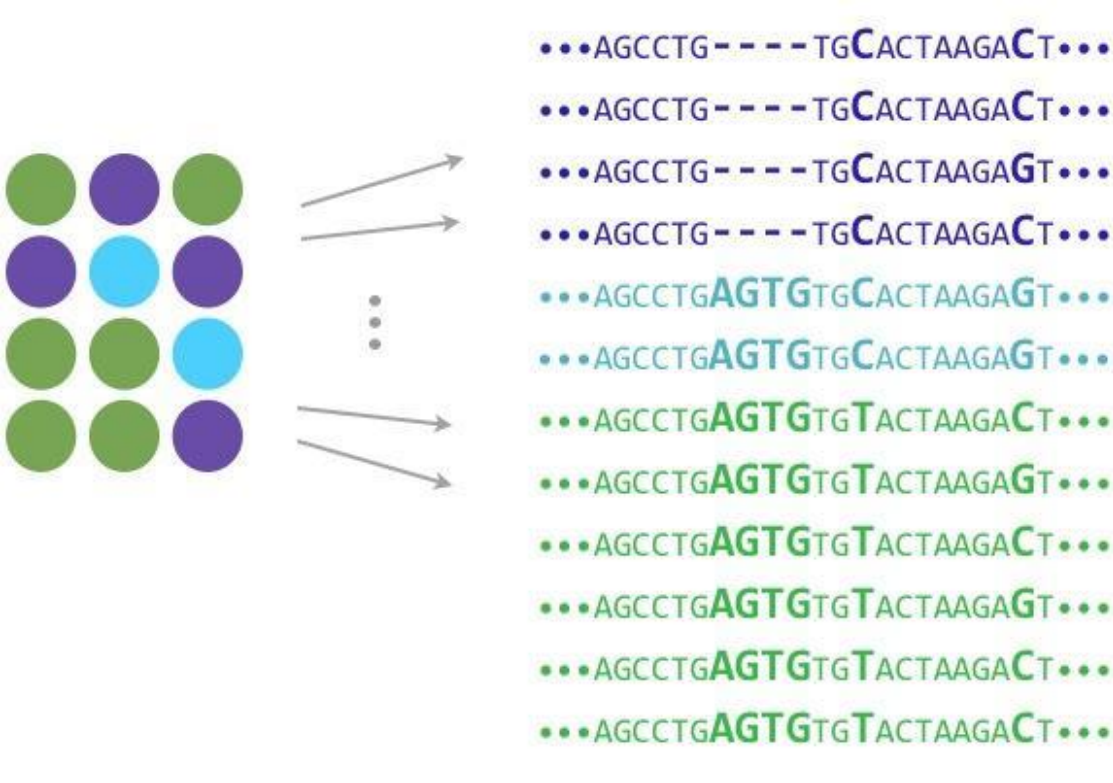
Motivation

- Identifying large amounts of associations efficiently is a problem that arises frequently in modern genomics data.
  - Understand the genetics of important human diseases. Data is typically in the form of case control data with ascertainment bias.
  - Understand the genetics of other important traits, e.g. traits with medical or agricultural relevance.
  - Identifying expression QTLs.
  - Cancer genetics, for identifying problematic mutations.
  - Understand interaction between genotypes and the environment.
- As genomics datasets become more common and sample sizes grow, the need for efficient tests increases.

Motivation

- Studying the genetics of natural variation
- Understanding the genetic architecture of traits of ecological and agricultural importance
- Identifying the genomic regions that control genetic variation
- Test association at many variants instead of some and hypothesis-free instead of hypothesis-driven.

Phenotype ⟷ Genomic marker



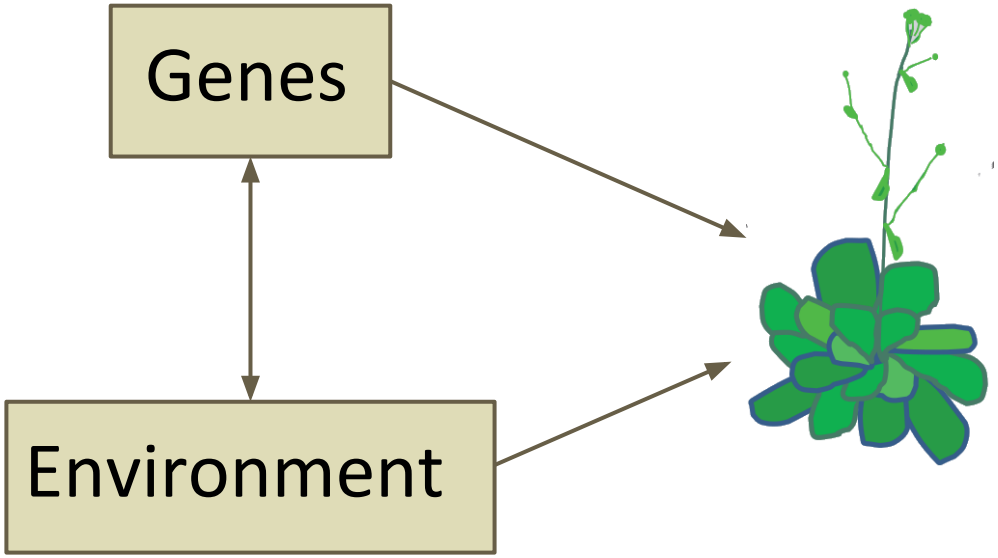
SNPs

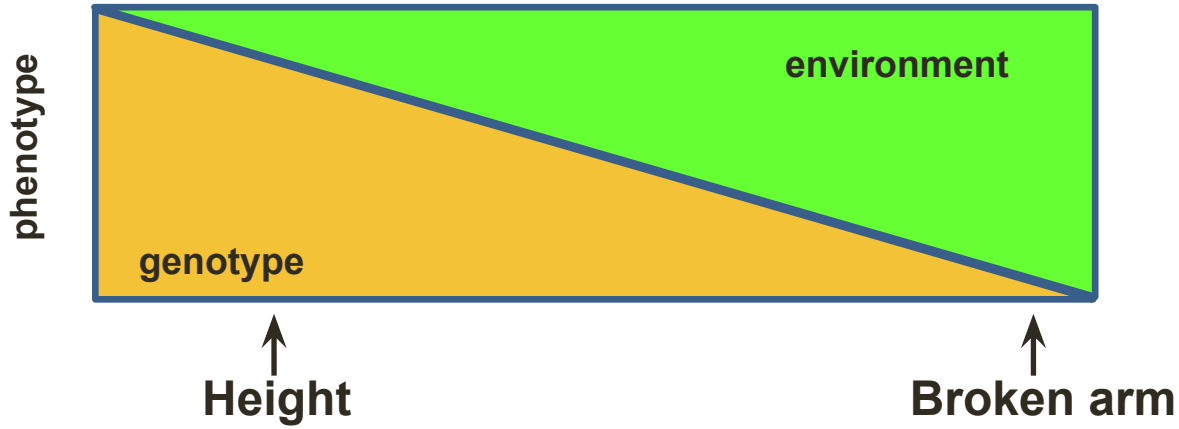
Indels

CNVs

Epigenetic markers

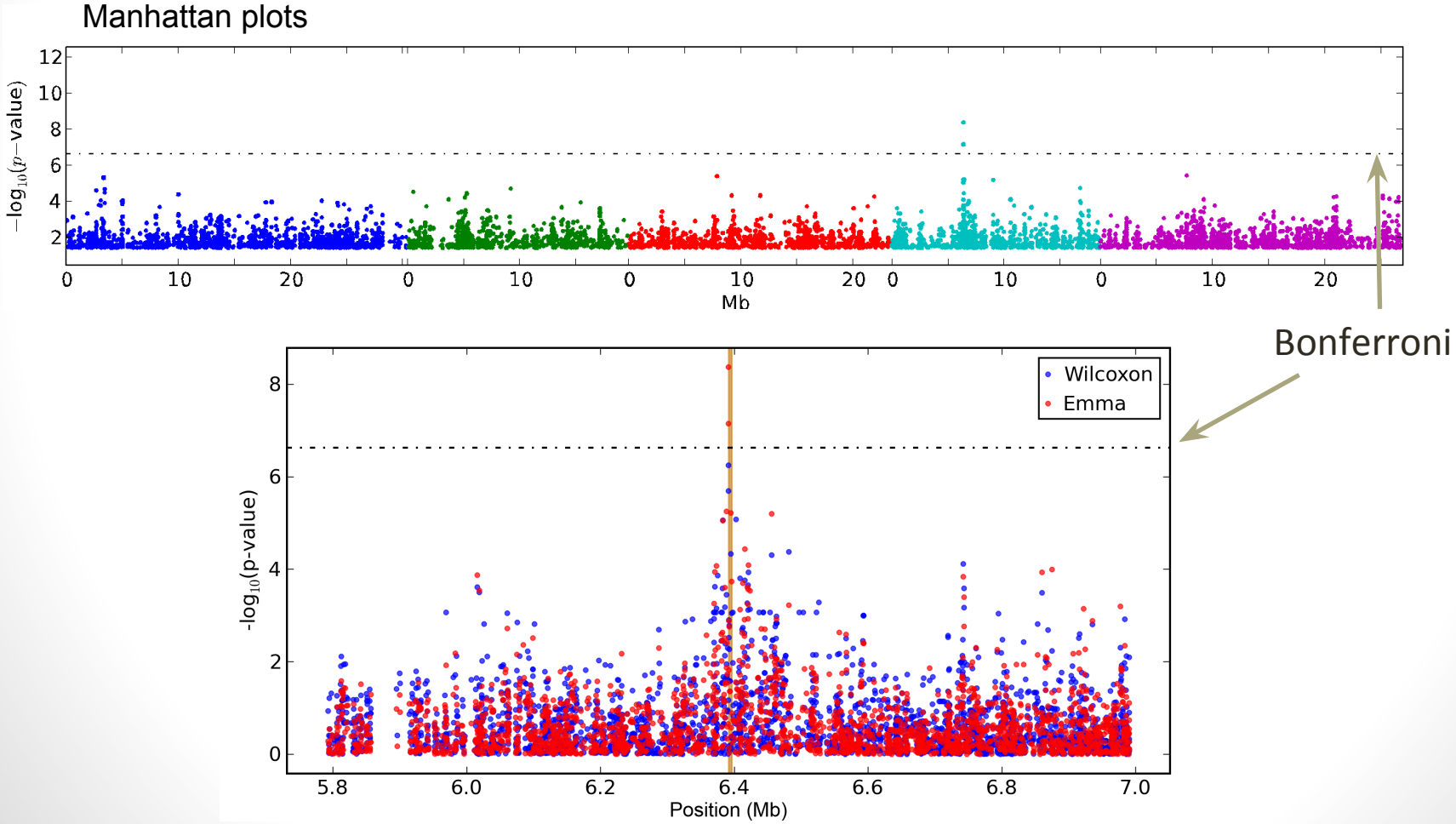
Phenotype = Genotype + Environment + GxE





A simple GWAS example

- Sodium concentration measured in *A. thaliana* leaves.



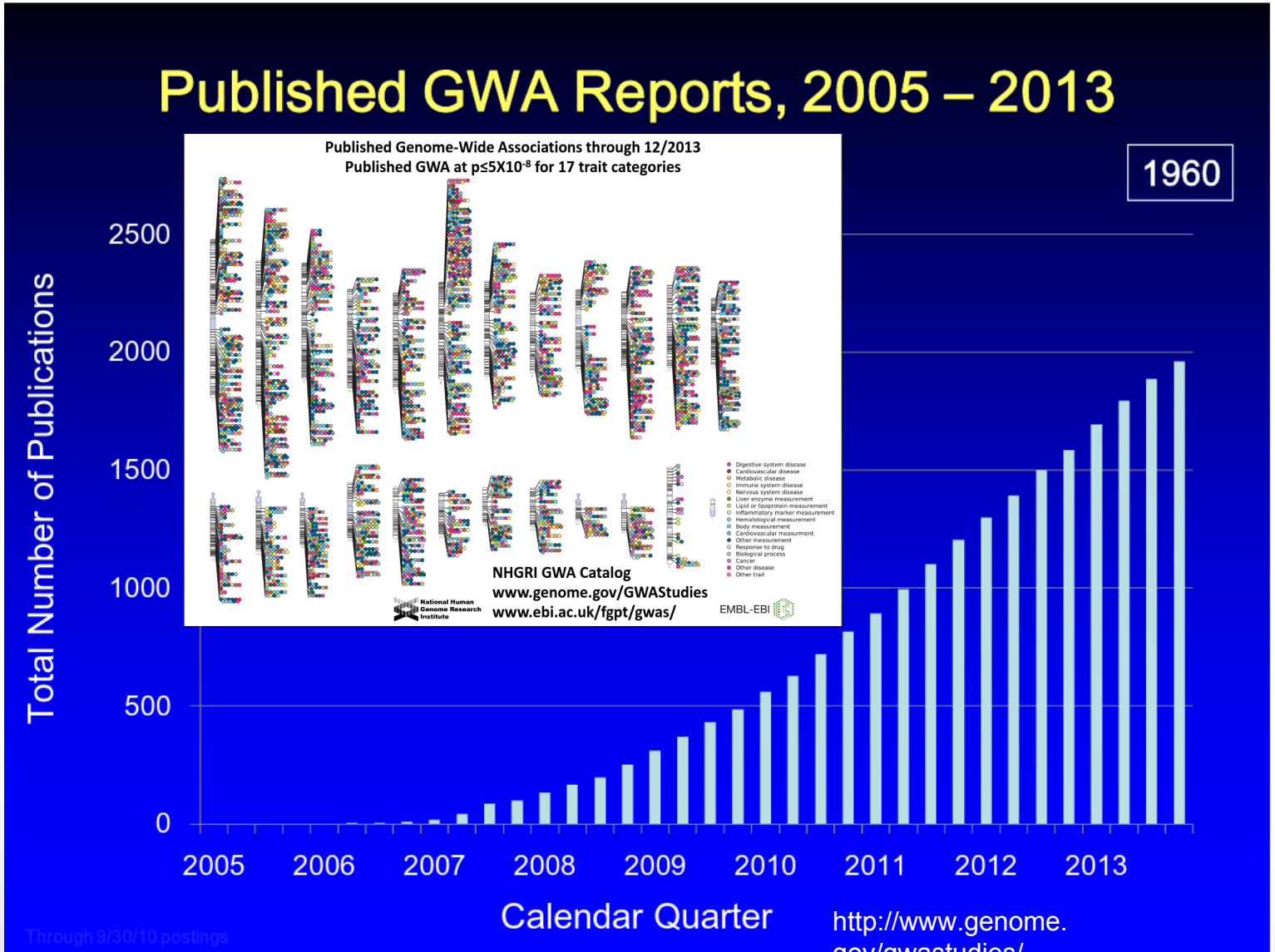
Multiple testing correction

- In GWAS a large number of marker tests are conducted, which leads to a multiple testing problem.
- Using a 5% significance threshold, we would expect 5% of the markers that have true marker effects of 0 to be significant.
- Solutions include:
  - **Bonferroni correction:** By assuming markers are independent we can obtain a conservative bound on the probability of rejecting the null hypothesis for one or more markers.

$$1 - P(T_1 \leq t, \ldots, T_m \leq t | H_0) \leq \alpha$$

    for a given significance threshold α.
  - Other common methods include adjusted Bonferroni correction depending on rank, and permutations.

GWAS - a success story



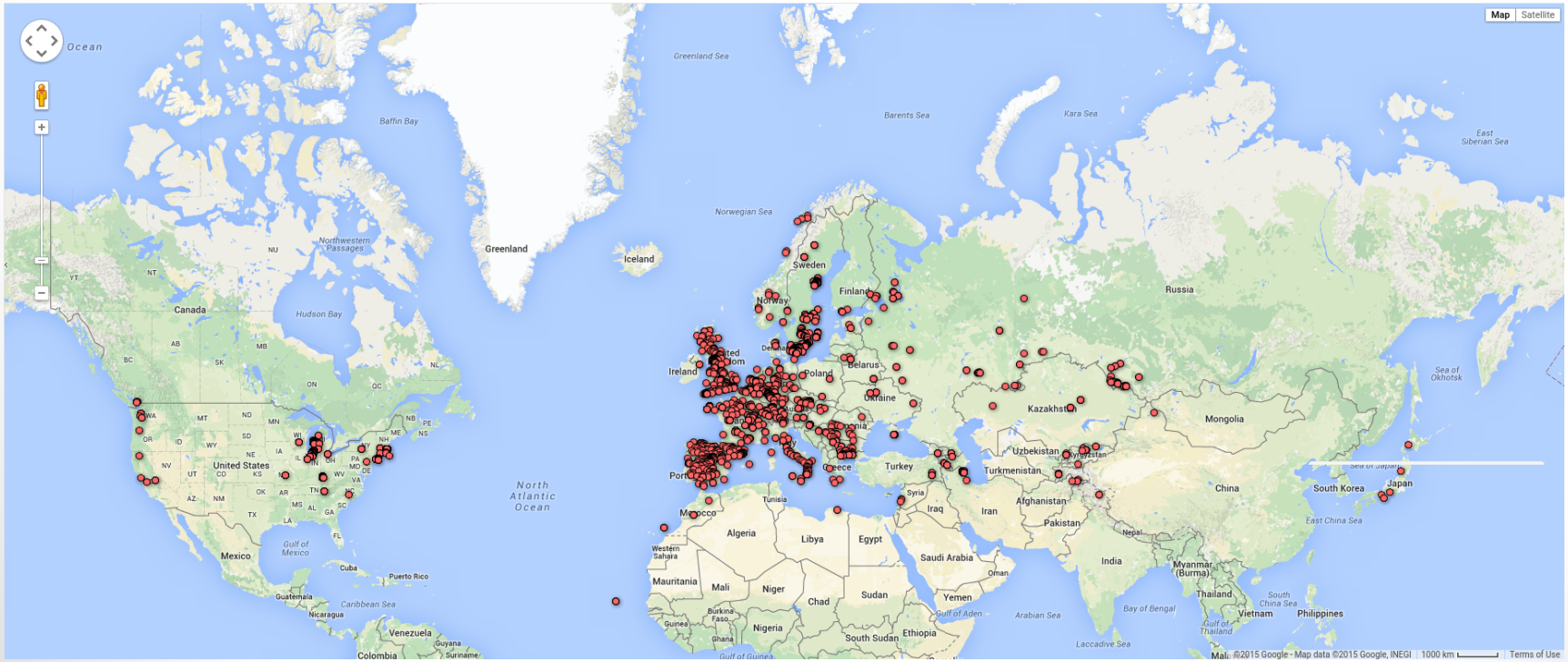
Why plants (*A. thaliana*)?

- Replicates usually available either through clonal propagation or the existence of inbred lines
- Relationship with breeding
- *A.thaliana*: the model plant
  - small size
  - rapid life cycle
  - small genome (~150 Mb, 5 Chr.)
  - inbred (self-fertilization)
  - transgenics (follow up)
  - mutant collections (follow up)

Why plants (*A. thaliana*)?

Availability of lines

- Curated information about 7522 accessions (https://goo.gl/lwGah)



Why plants (*A. thaliana*)?
Availability of genotypes

Genotyping data:

- 250k Affymetrix genotyping array (Horton *et al*., 2012)
  - 250.000 probes → after filtering 214.051 SNPs for 1307 accessions.
  - Expected resolution is pretty good (average SNP density 1 per 550 bp | LD decays on average within 10 kb. Kim *et al.*, 2007)

Full-sequence data:

- Small sets:
  - Long et al., 2013 (181 accessions)
  - Cao *et al.*, 2010 (80 accessions)
  - Schmitz *et al.*, 2013 (195 accessions)
- 1001genomes (http://the1001genomes.org):
  - Joint effort of MPI, GMI, Salk and Monsanto
  - 10 Million SNPs and 500k structural var. for 1135 accessions
  - Imputation → 2029 accessions

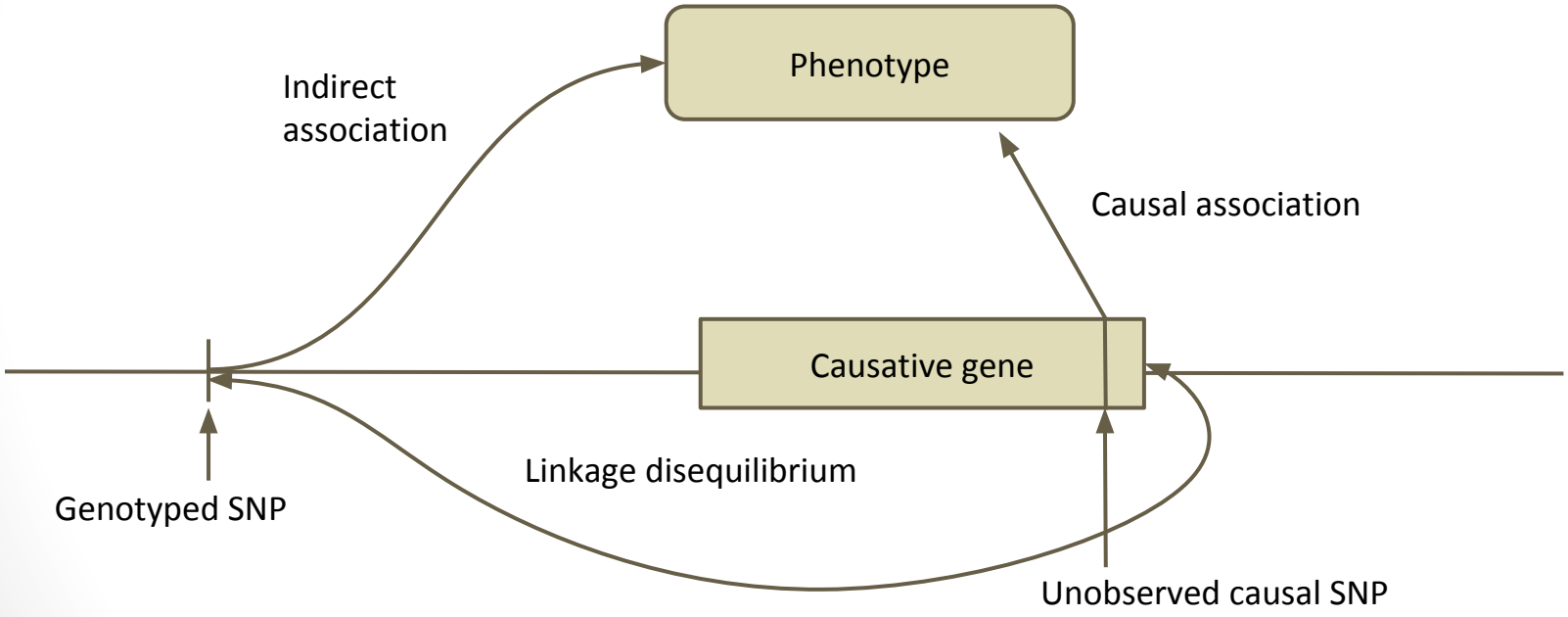
Why plants (*A. thaliana*)?
Availability of phenotypes

- Atwell *et al.*, 2010:
  - 107 phenotypes on up to 197 accessions
  - 4 categories: flowering (23), defence (23), ionomics (18), development (18)
  - https://github.com/Gregor-Mendel-Institute/atpolydb

- Other sources on larger datasets:
  - Baxter *et al.*, 2010: sodium concentration on 342 accessions.
  - Li *et al.*, 2010: flowering time for 473 accessions grown in 4 controlled environments
  - Unpublished data: flowering time, germination, leaf morphology, metabolite levels, gene expression

Linkage disequilibrium

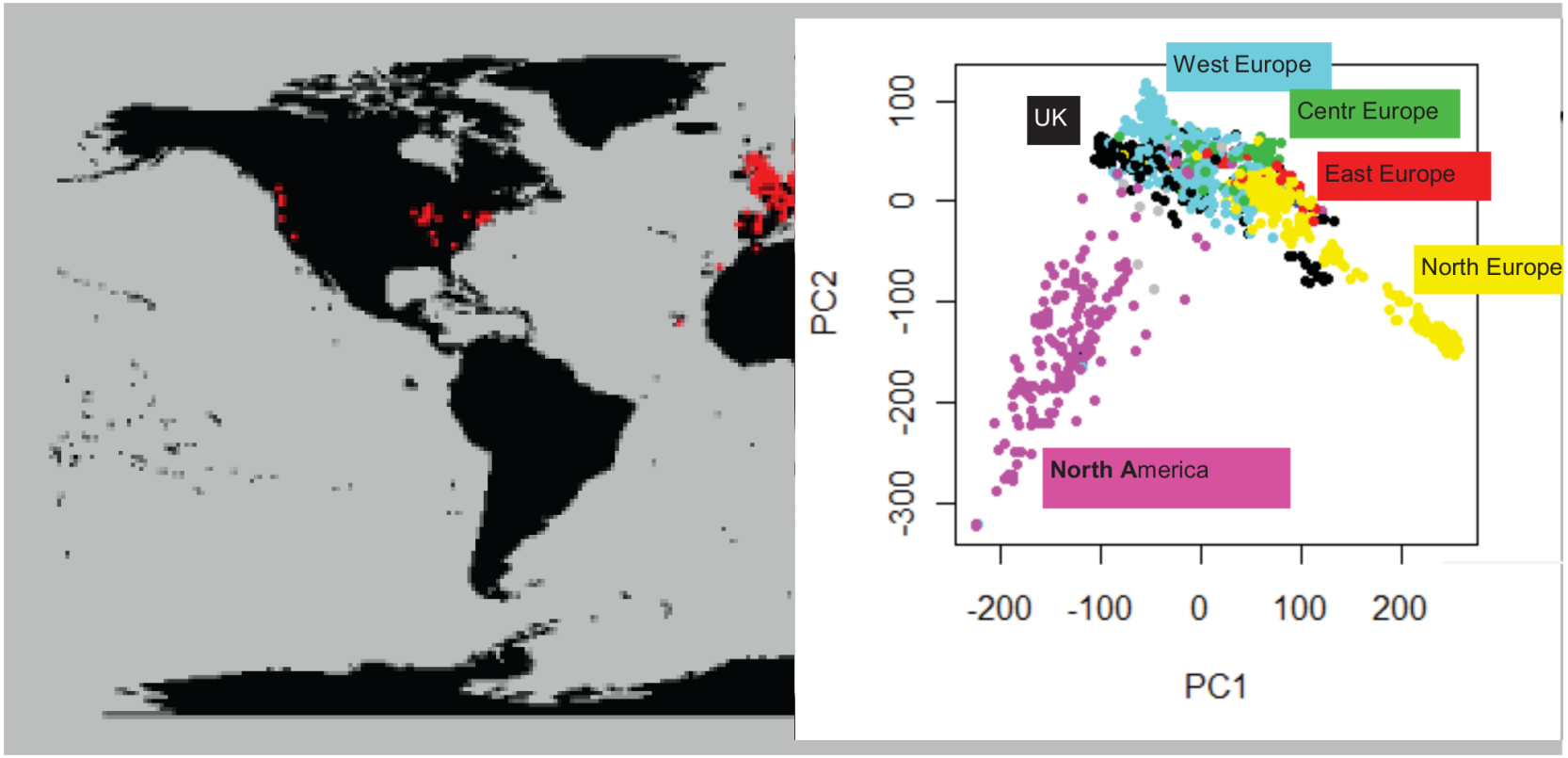
- Neighboring markers will tend to be inherited together, causing linkage disequilibrium (LD) between the two markers



- Since LD causes correlations between markers, in a given population we expect a lot of redundancy in the genotypes.

Population Structure

- Isolation by distance (Platt *et al*, 2010)
- Accessions tend to cluster in sub-populations according to their geographic origin

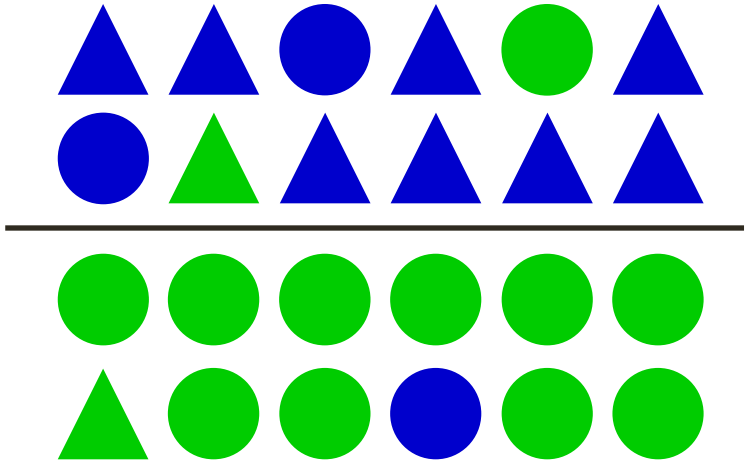


Population Structure

- Confounding due to population structure may arise if it correlates with the trait in question.

Sub-population 1

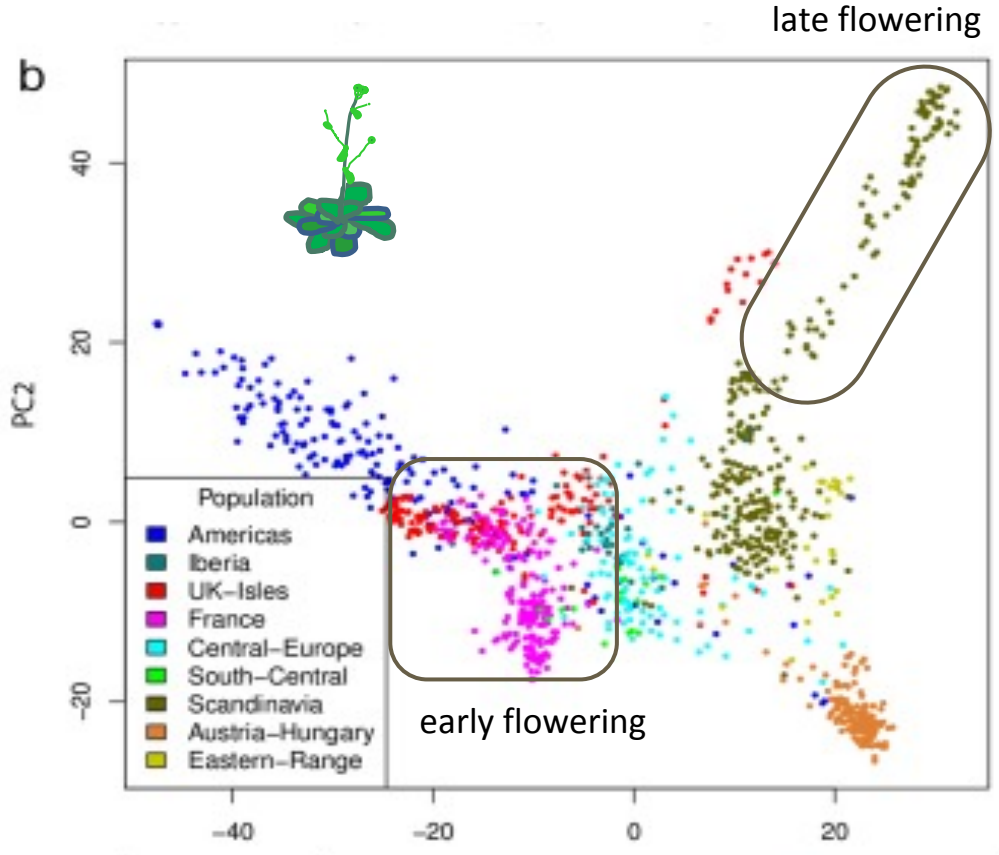
Sub-population 2



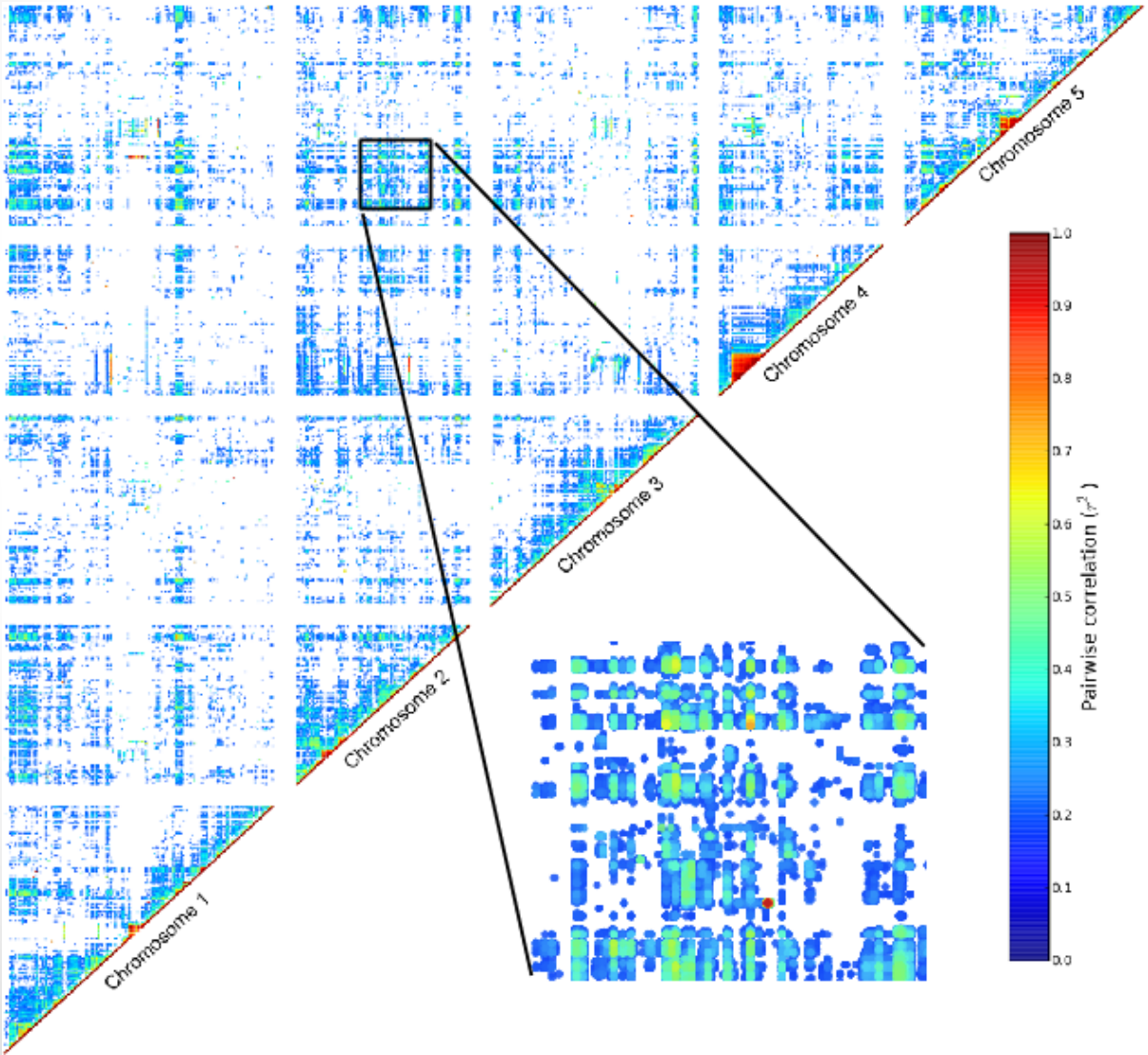
- Any variant which is fixed for different alleles in each sub-population will show an association.

Examples of Population Structure Confounding

- Humans:
  - Genetic marker for skin color might also be associated with malaria resistance because the trait is correlated with the population structure.
- A. thaliana:
  - Flowering time is correlated with latitude
  - Disease resistance is **NOT** correlated with population structure



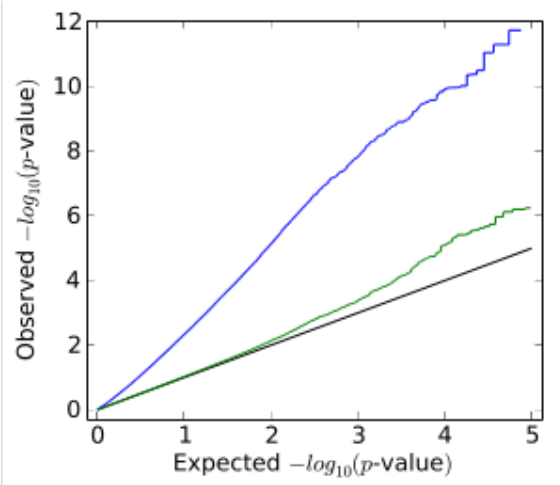
Population Structure is reflected in long range LD.

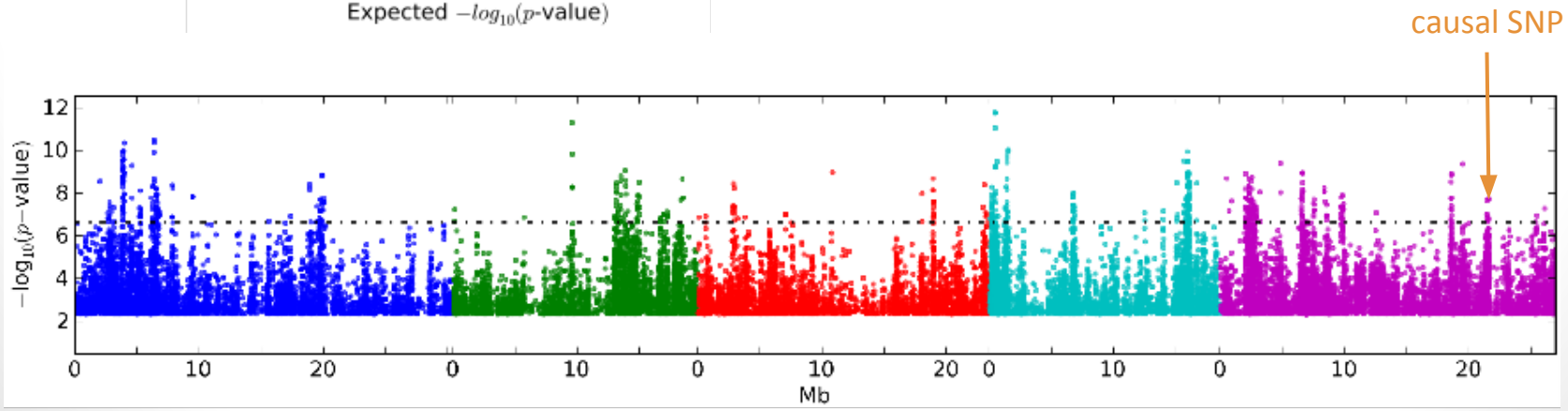


Linkage disequilibrium in *A. thaliana*, 214K SNPs and 1307 accessions.

Implication for Association Studies

- Test statistic is inflated
- High false positive rate





Association mapping in structured populations

- **Genomic contro**l**:** Scale down the test-statistic so that its median becomes the expected median. Heavily used, but does not solve the problem (Devlin & Roeder 1999, Biometrics)!
- **Structured association** (Pritchard et al. 2000, Am.J.Hum.Genet.)
- **PCA approach:** Accounting for structure using the first **n** principle components of the genotype matrix (Price *et al.*, 2006). However when population structure is very complex, e.g. in *A. thaliana*, too many PCs are needed.
- **Mixed Model approach:** Model the genotype effect as a random term in a mixed model, by explicitly describing the covariance structure between the individuals (Yu et al. 2006, Nature Genet.; Kang et al. 2008, Genetics).

Linear Model, Non-parametric test, Linear Mixed Model, Advanced Linear Mixed Models & Caveats & Problems

GWAS Methods

Linear Model (LM)

A linear model generally refers to linear regression models in statistics.

$$y_j = \sum_{j=1}^{P} \beta_j x_{ij} + \epsilon_i \qquad Y = X'\beta + \epsilon$$

- **Y** typically consists of the phenotype values, or case-control status for **N** individuals.
- **X** is the **N**x**P** genotype matrix, consisting of **P** genetic variants (e.g. SNPs).
- β is a vector of P effects for the genetic variants.
- ϵ is still just known as the *noise* or *error* term.

Non-parametric tests (KW)

- Both the t-test and the F-test assume that the underlying distribution is Gaussian, i.e. for a single SNP, the conditional phenotype distribution is Gaussian.
  - This is obviously not true for most traits.
- Alternatively we can employ non-parametric tests.
- For binary markers (SNPs coded as 0-1), we can use the Wilcoxon rank sum test, or a Fisher's exact test.
- For more general markers (more that two alleles) we can employ a Kruskal-Wallis, Wilcoxon rank-sum test, or the Spearman rank correlation.

Linear Mixed Model (LMM)

- Linear model and Non-parametric tests don't account for population structure

$$Y = X\beta + u + \epsilon, \quad u \sim N(0, \sigma_g K), \quad \epsilon \sim N(0, \sigma_e I)$$

- Initially proposed in Association mapping by Yu et al. (2006)
- **Y** typically consists of the phenotype values, or case-control status for N individuals.
- **X** is the **N**x**P** genotype matrix, consisting of **P** genetic variants (e.g. SNPs).
- **u** is the random effect of the mixed model with var(u) = σ g K
- **K** is the **N** x **N** kinship matrix inferred from genotypes
- β is a vector of **P** effects for the genetic variants.
- ϵ is a **N** x **N** matrix of residual effects with var(ε) = σ e I

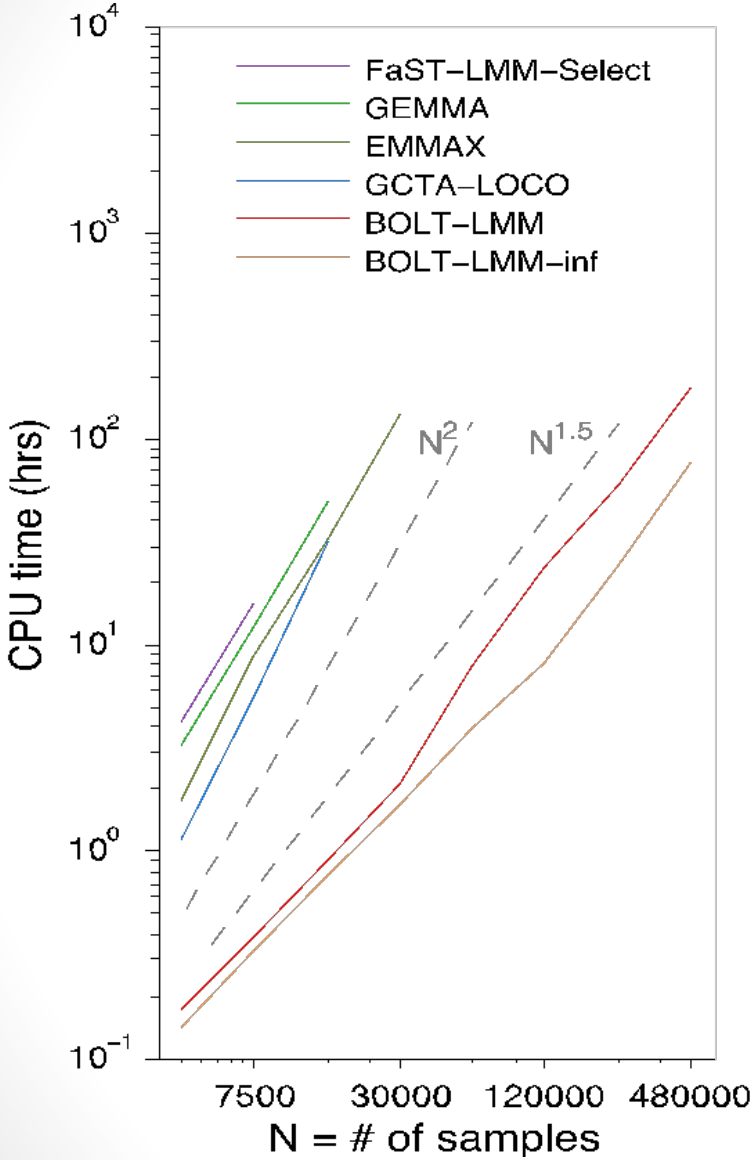
Kinship

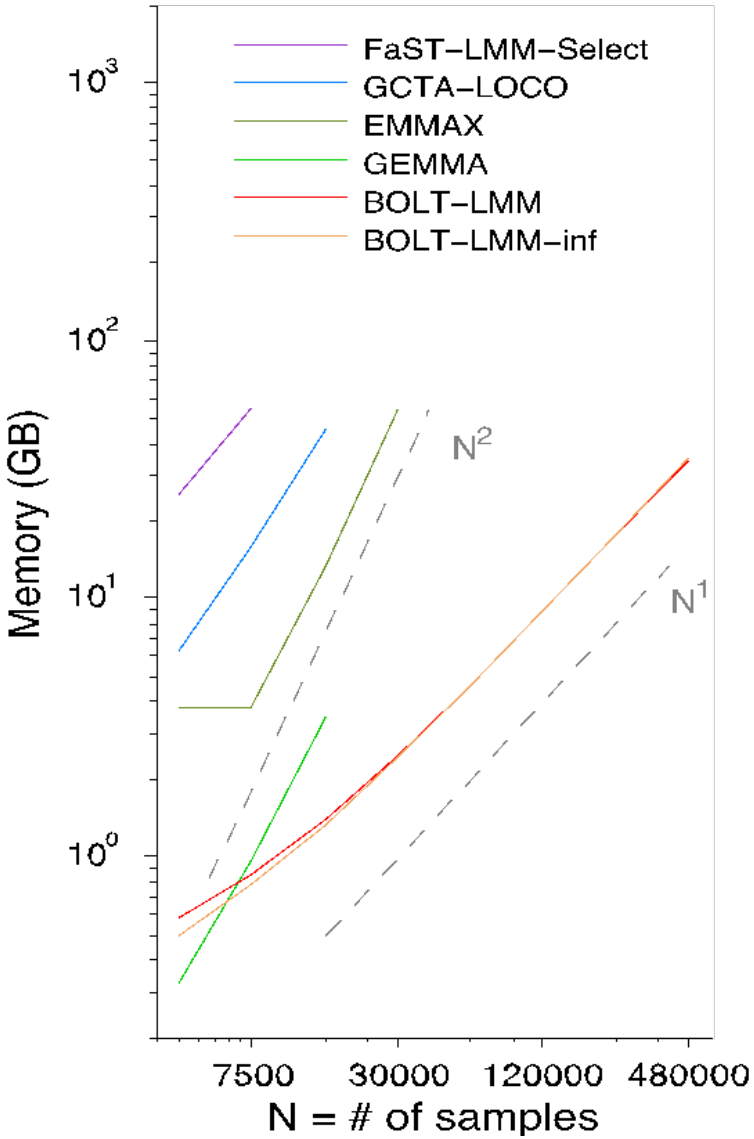
- The kinship measures the degree of relatedness, and is in general different from the covariance matrix.
- It is estimated using either pedigree (family relationships) data or (lately) using genotype data.
  - When estimating it from pedigree data, one normally assumes that the ancestral founders are “unrelated”.
  - They are sensitive to confounding by cryptic relatedness.
- Alternatively the kinship can be estimated from genotype data.
  - Genotype data may be incomplete.
  - Weights or scaling of genotypes can impact the kinship.
- A. thaliana using an IBS matrix works pretty well (Zhao *et al.*, 2007, Atwell *et al.*, 2010)

Linear Mixed Model (LMM)

- Original implementation: EMMA (Kang *et al.*, 2008)
  - Problem: $O(PN^3) \rightarrow$ 1 GWAS in 1 day (500k individuals)
- Approximate methods $O(PN^2)$:
  - GRAMMAR (Aulchenko *et al.*, 2007) http://www.genabel.org/packages/GenABEL
  - P3D (Zhang *et al.*, 2010) http://www.maizegenetics.net/#!tassel/c17q9
  - EMMAX (Kang *et al.*, 2010) http://genetics.cs.ucla.edu/emmax/
- Exact methods:
  - FaST LMM (Lippert *et al.*, 2011) http://mscompbio.codeplex.com/
  - GEMMA (Zhou *et al.*, 2012) http://www.xzlab.org/software.html
- This is too slow for large samples (>20000 individuals), i.e. exactly the sample sizes where one might expect to see most gains.
  - BOLT-LMM (Loh *et al.*, 2015), **O(PN)** https://data.broadinstitute.org/alkesgroup/BOLT-LMM/?

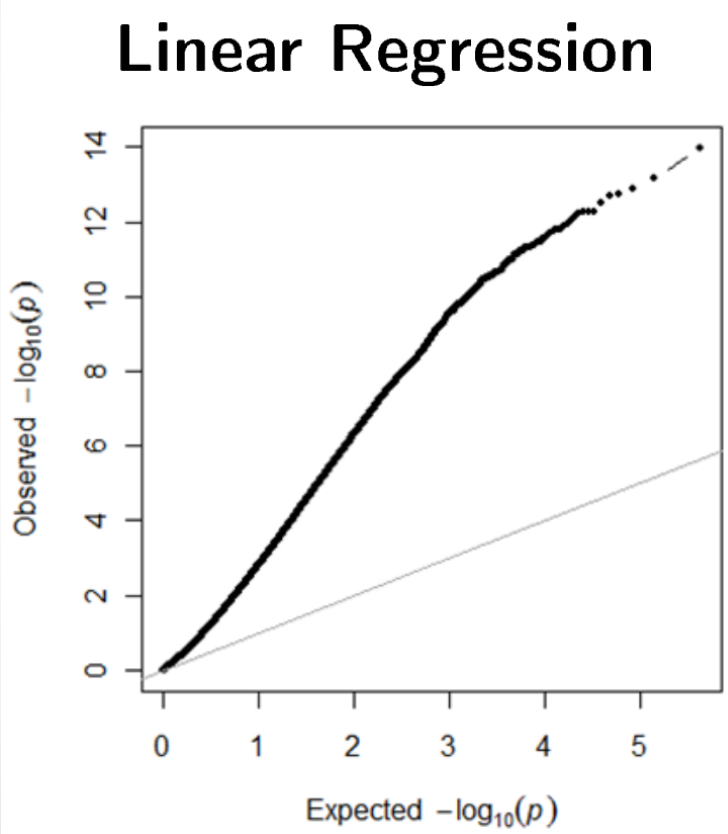
BOLT-LMM

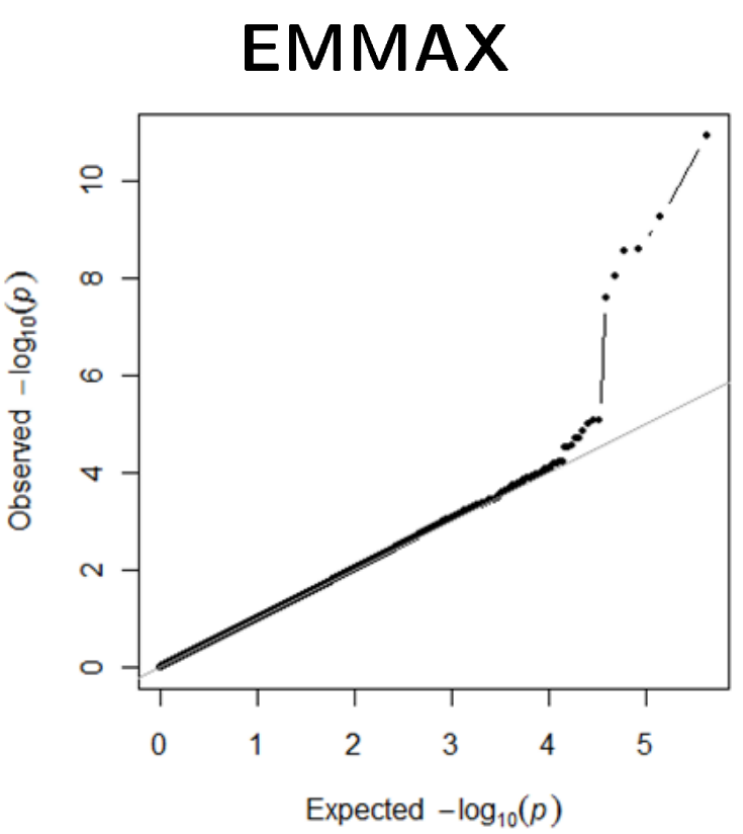




Po-Ru Loh *et al.* (Nat Genet 2015)

LMM reduces test statistic inflation

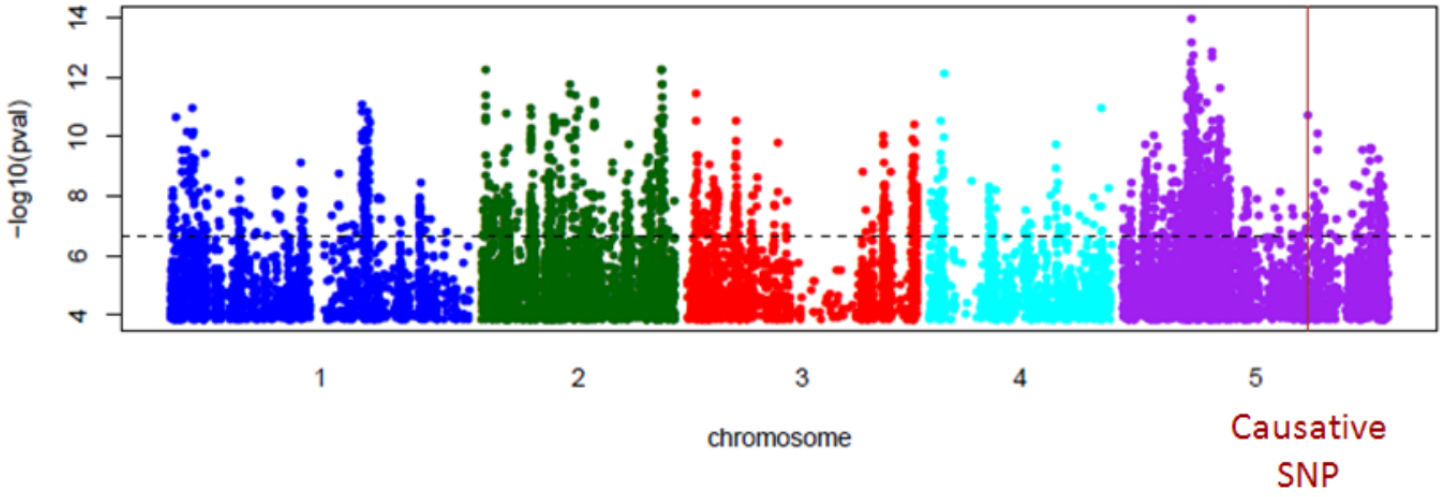




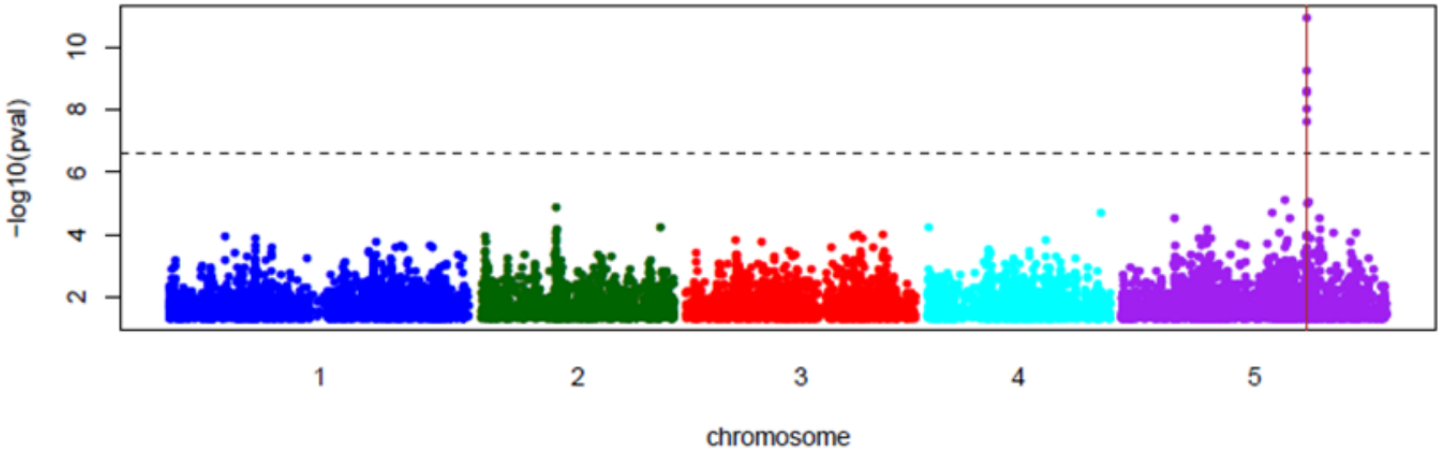
LMM reduces false positive rate

GWAS for a simulated phenotype

Linear Regression



EMMAX



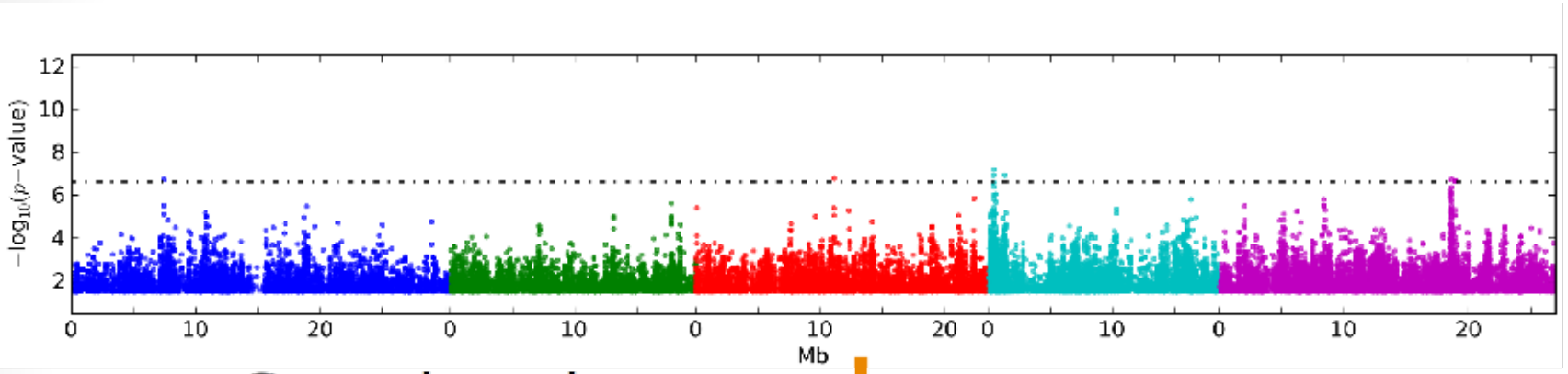
Advanced Mixed Models

The mixed-model performs pretty well, but GWAS power remain limited and need to be improved:

- **M**ulti **L**ocus **M**ixed **M**odel (MLMM, Segura *et al.*, 2012):
  - Single SNP tests are wrong model for polygenic traits
  - Increase in power compared to single locus models
  - Detection of new associations in published datasets
  - Identification of particular cases of (synthetic associations) and/or allelic heterogeneity
- **M**ulti **T**rait **M**ixed **M**odel (MTMM, Korte *et al.*, 2012):
  - Traits are often correlated due to **pleiotropy** (shared genetics) or **linkage** between causative polymorphisms.
  - Combining correlated traits in a single model should thus increase detection power
  - When multiple phenotypes consists in a single trait measure in multiple environments, **plasticity** can be studies through the assessment of GxE interaction

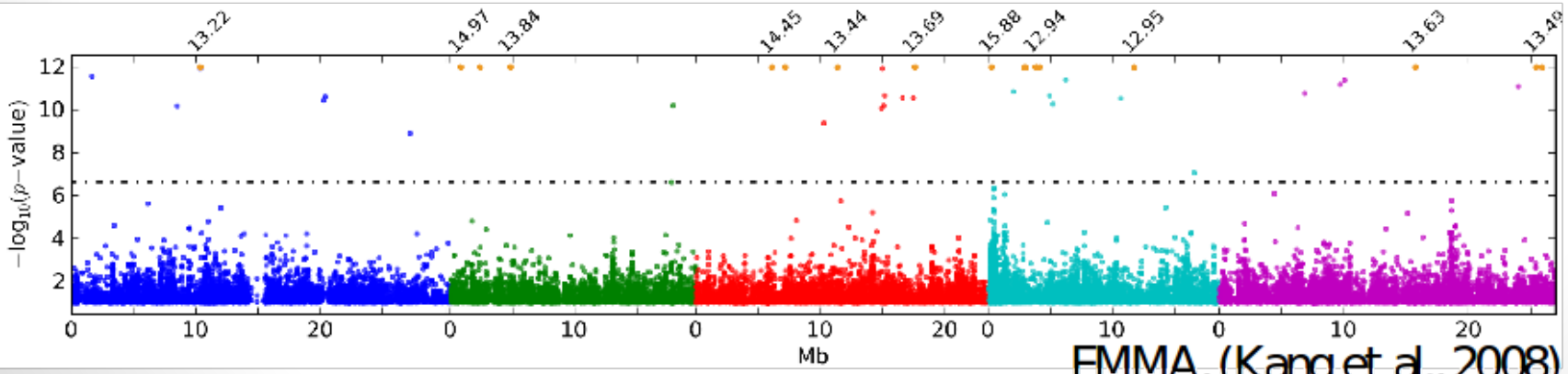
Caveats & Problems

Accounting for population structure does not alway work:



Wilcoxon rank sum test

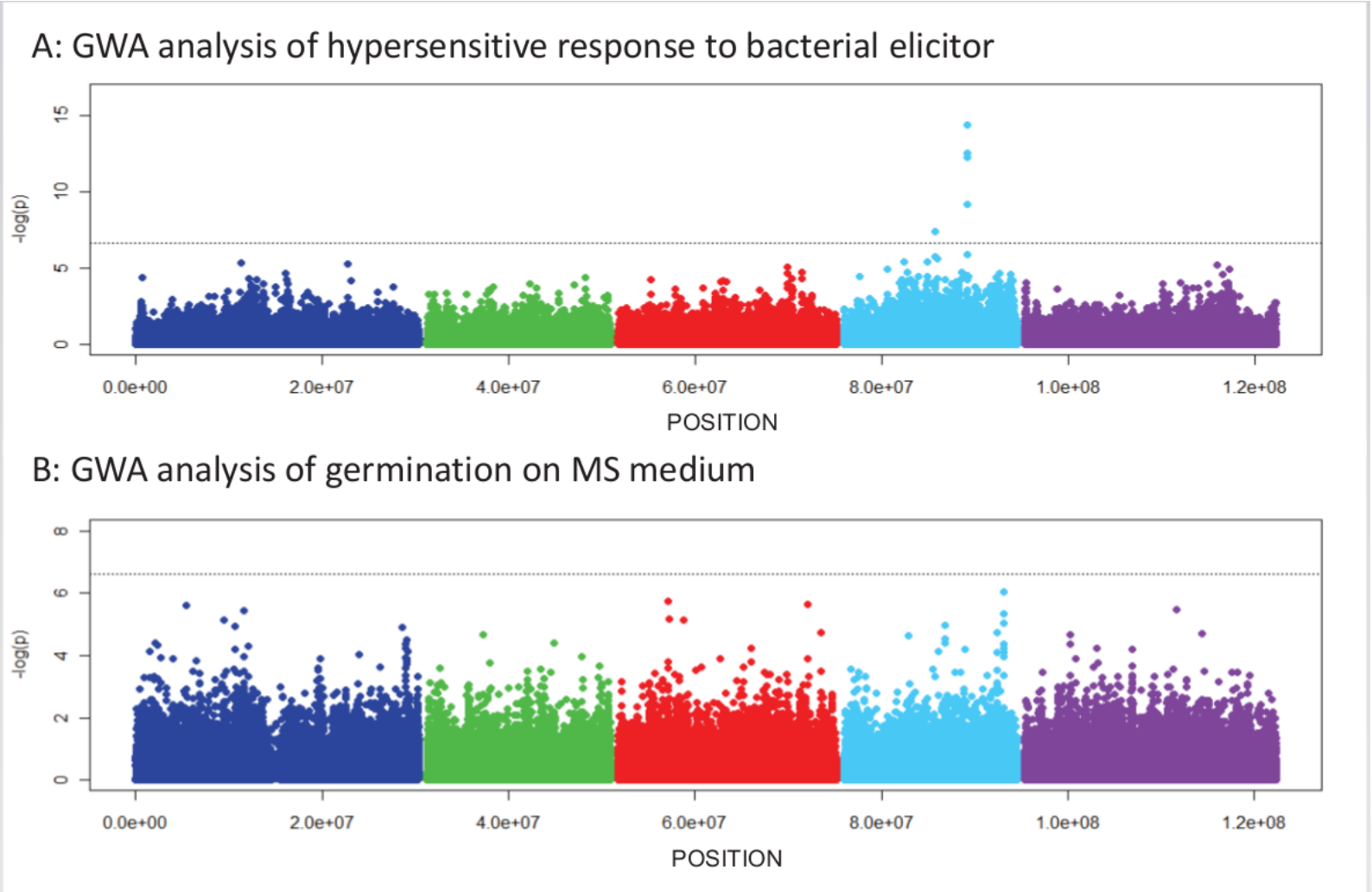
Sometimes it doesn't



EMMA, (Kang et al., 2008)

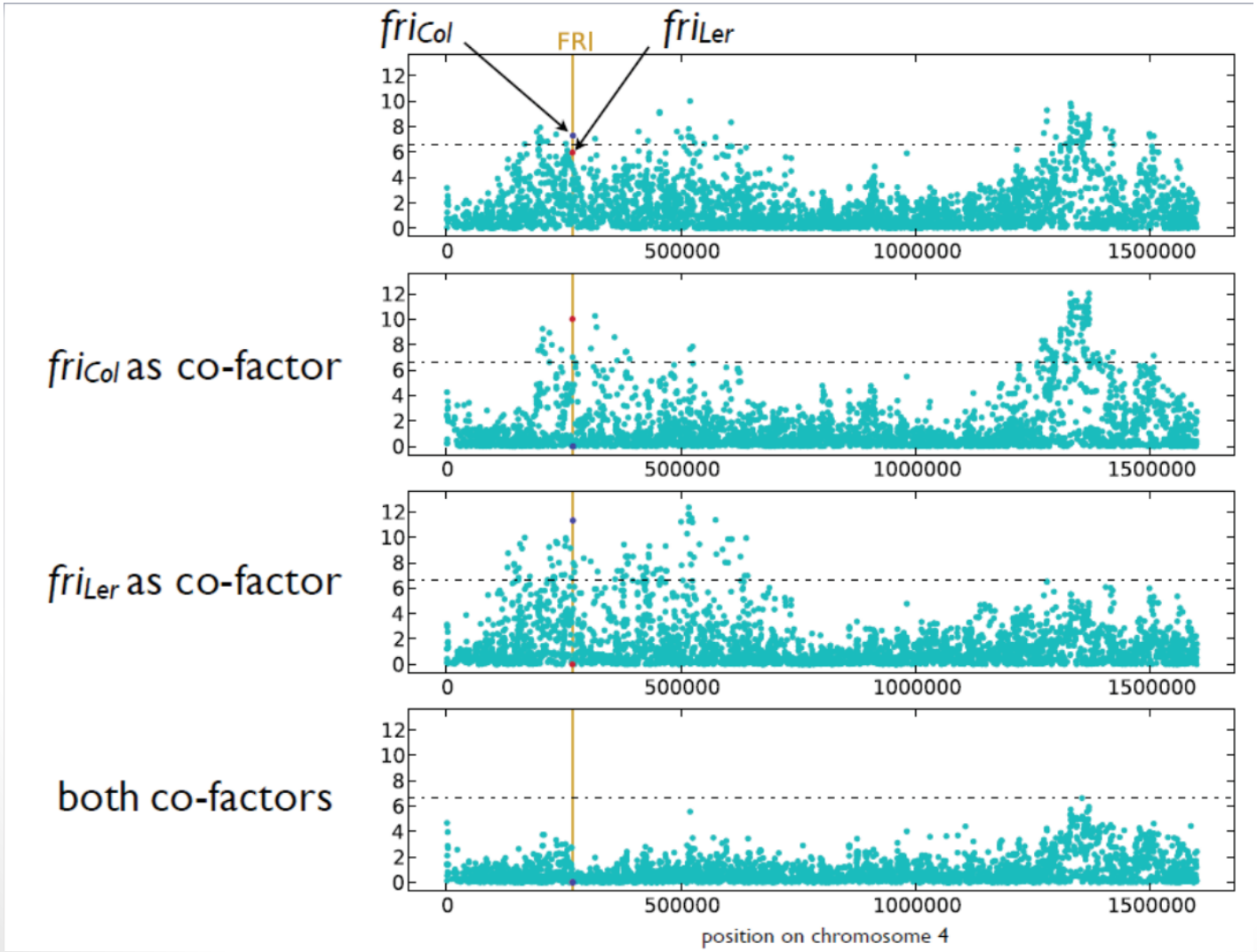
Caveats & Problems

Difficult to decide which peaks are significant (Solution: permutation)



Caveats & Problems

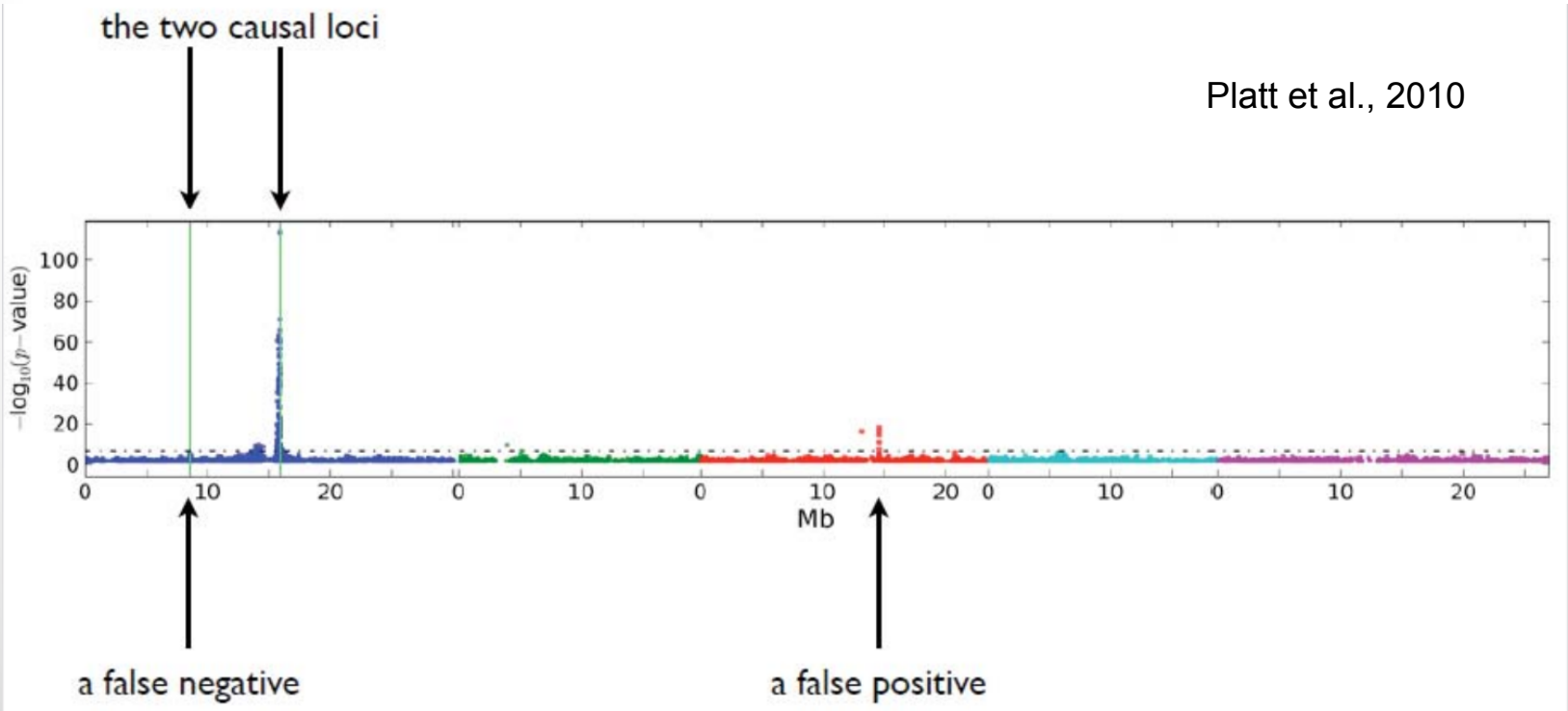
Peaks are complex and make it difficult to pinpoint causative site



Caveats & Problems

Condition under which GWAS will be positively misleading:

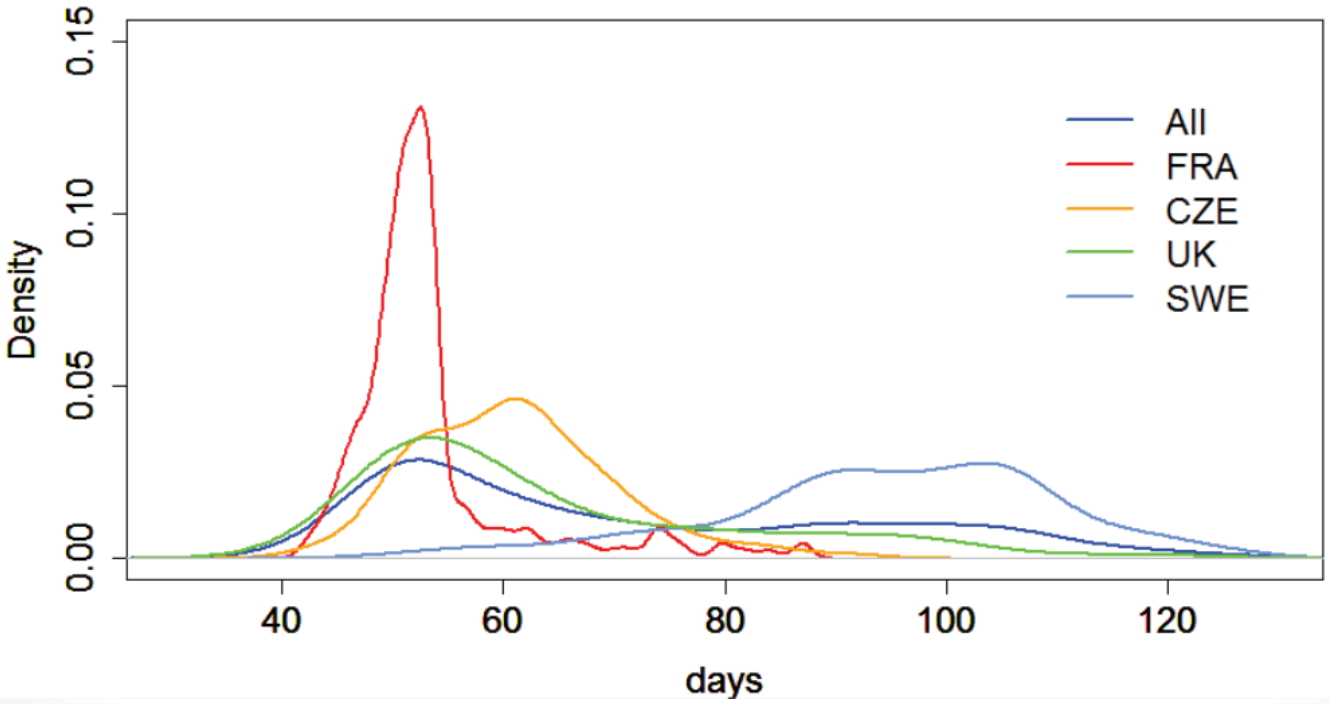
- Correlation between causal factors and unlinked non-causal markers
- More than one causal factor
- Epistasis



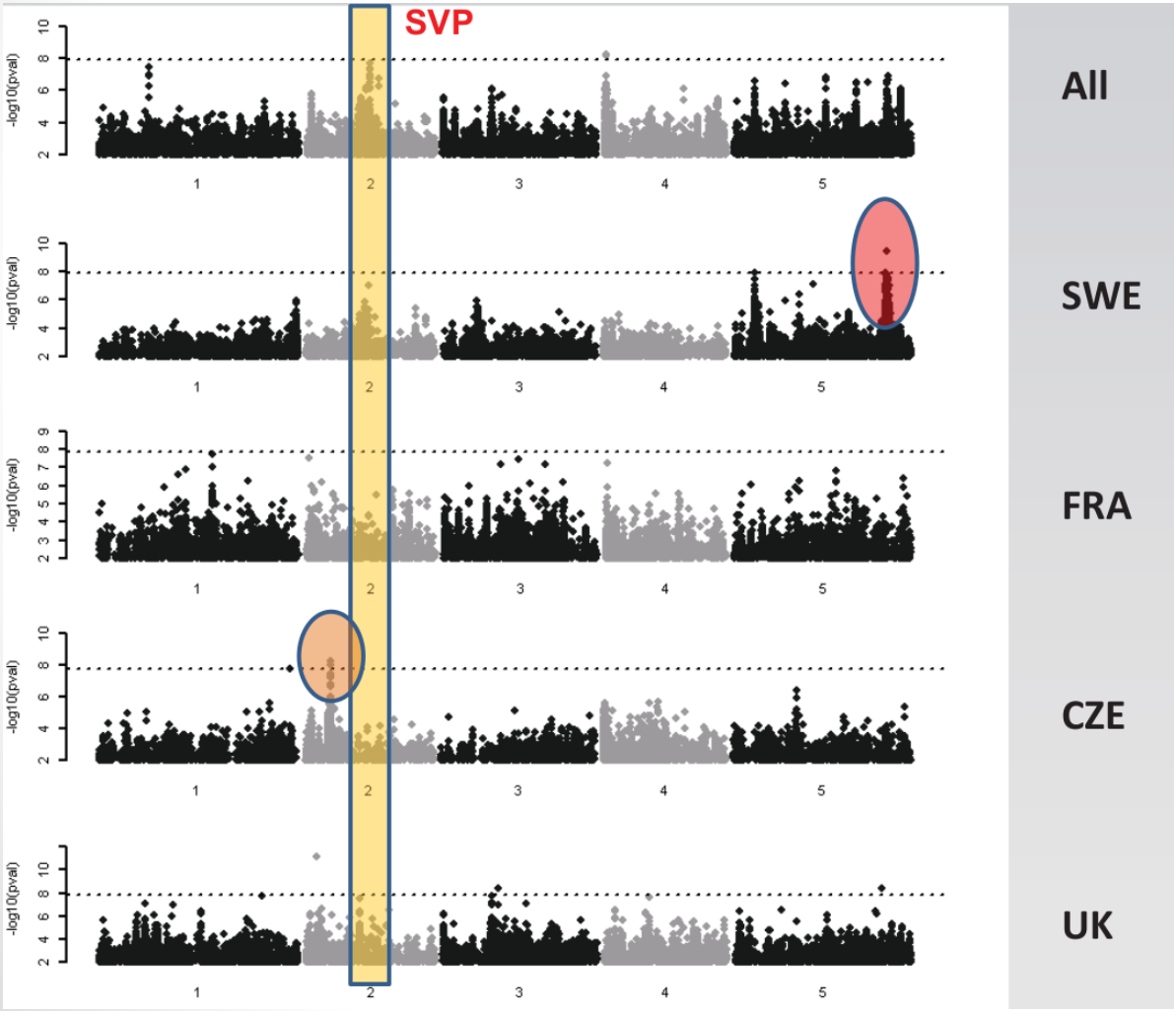
Caveats & Problems

Different associations for different subsets (i.e. Flowering time at 10 °C):

- Highly heritable, easy to measure, polygenic trait
- 925 worldwide accessions
- Flowering time greatly varies in different populations



Caveats & Problems



Significance and effect size differ dramatically in different subsets

Reasons:

- False positives
- Effect depends on genetic background (Epistasis)
- Differences in allele frequency of the causal marker
- Artefact of LMM

Caveats & Problems

Korte and Farlow *Plant Methods* 2013, **9**:29
http://www.plantmethods.com/content/9/1/29

PLANT METHODS

REVIEW Open Access

The advantages and limitations of trait analysis with GWAS: a review

Arthur Korte*† and Ashley Farlow†

Abstract

COMMENT

The nature of confounding in genome-wide association studies

Bjarni J. Vilhjálmsson[1,2] and Magnus Nordborg[3,4]

The authors argue that population structure per se is not a problem in genome-wide association studies — the true sources are the environment and the genetic background, and the latter is greatly underappreciated. They conclude that mixed models effectively address this issue.

Thanks to dramatically decreasing genotyping and sequencing costs, genome-wide association studies (GWASs) are becoming the default method for studying the genetics of natural variation. The increasing number and diversity of GWASs will require appropriate statistical analysis methods. The most basic problem is assessing the significance of an association in the light of confounding effects that may cause spurious associations.

The aspect of this problem that has received the most attention is the danger of false positives in structured populations. If the study population is a mixture of populations that differ with respect to allele frequencies as well as the trait of interest, spurious correlations in 'unrelated' individuals. Variation in relatedness is a basic property of natural populations, as is correlation between causative loci. This issue is familiar to quantitative geneticists[5] but has not been widely appreciated in other fields. It is important for GWASs and will become crucial as sample sizes increase.

To demonstrate this, let us return to the chopstick example but fast-forward to the era of millions of SNPs. Genetic differentiation between East Asians and other populations means that vast numbers of markers in addition to *HLA-A1* would be associated with chopstick skill. These markers would also be correlated with *HLA-A1*, with each other and with any trait (genetic or not) that

Review Highly accessed

Genome-wide association studies in plants: the missing heritability is in the field

Benjamin Brachi, **Geoffrey P Morris** and **Justin O Borevitz***

* Corresponding author: Justin O Borevitz borevitz@uchicago.edu ▾ Author Affiliations

Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, USA

For all author emails, please log on.

Genome Biology 2011, **12**:232 doi:10.1186/gb-2011-12-10-232

The electronic version of this article is the complete one and can be found online at:
http://genomebiology.com/2011/12/10/232

Published: 28 October 2011

Abstract

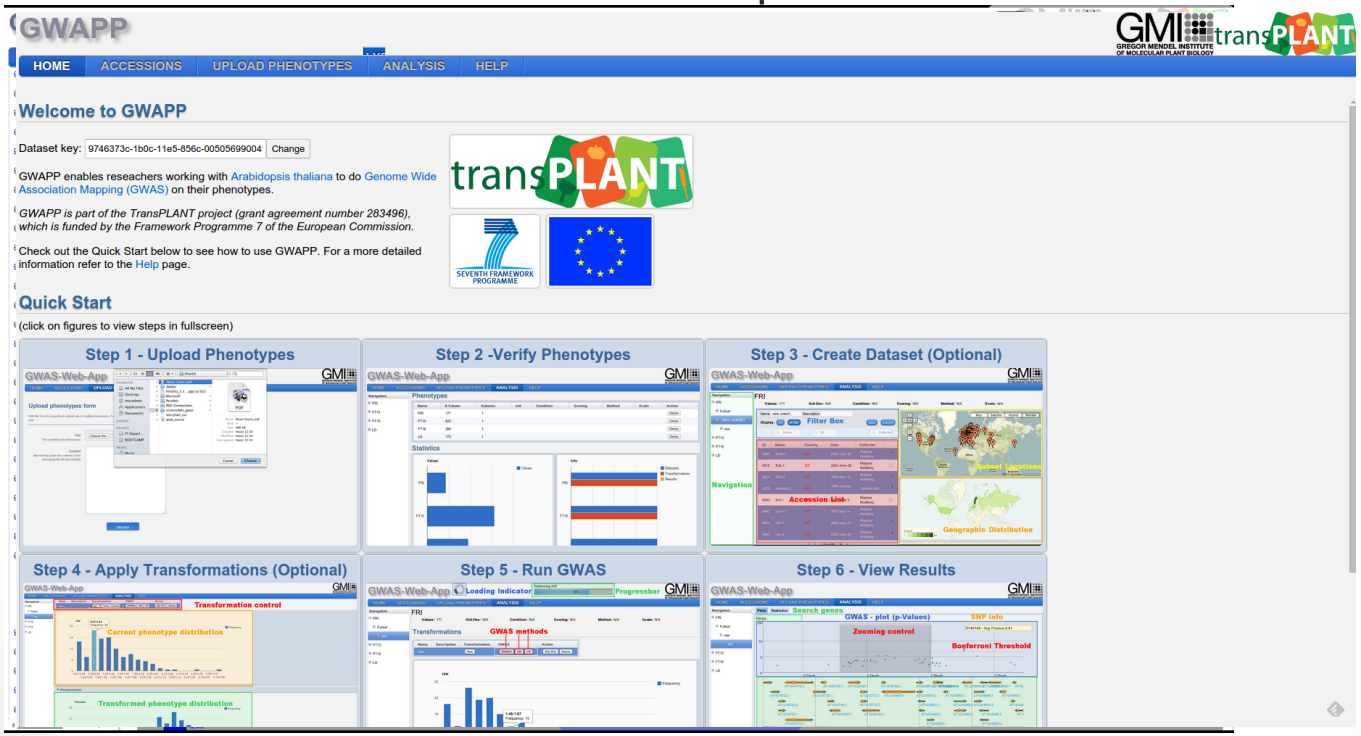
Genome-wide association studies (GWAS) have been even more successful in plants than in humans. Mapping approaches can be extended to dissect adaptive genetic variation from structured background variation in an ecological context.

Introduction to GWA-Portal, Step-by-step guide and Resources

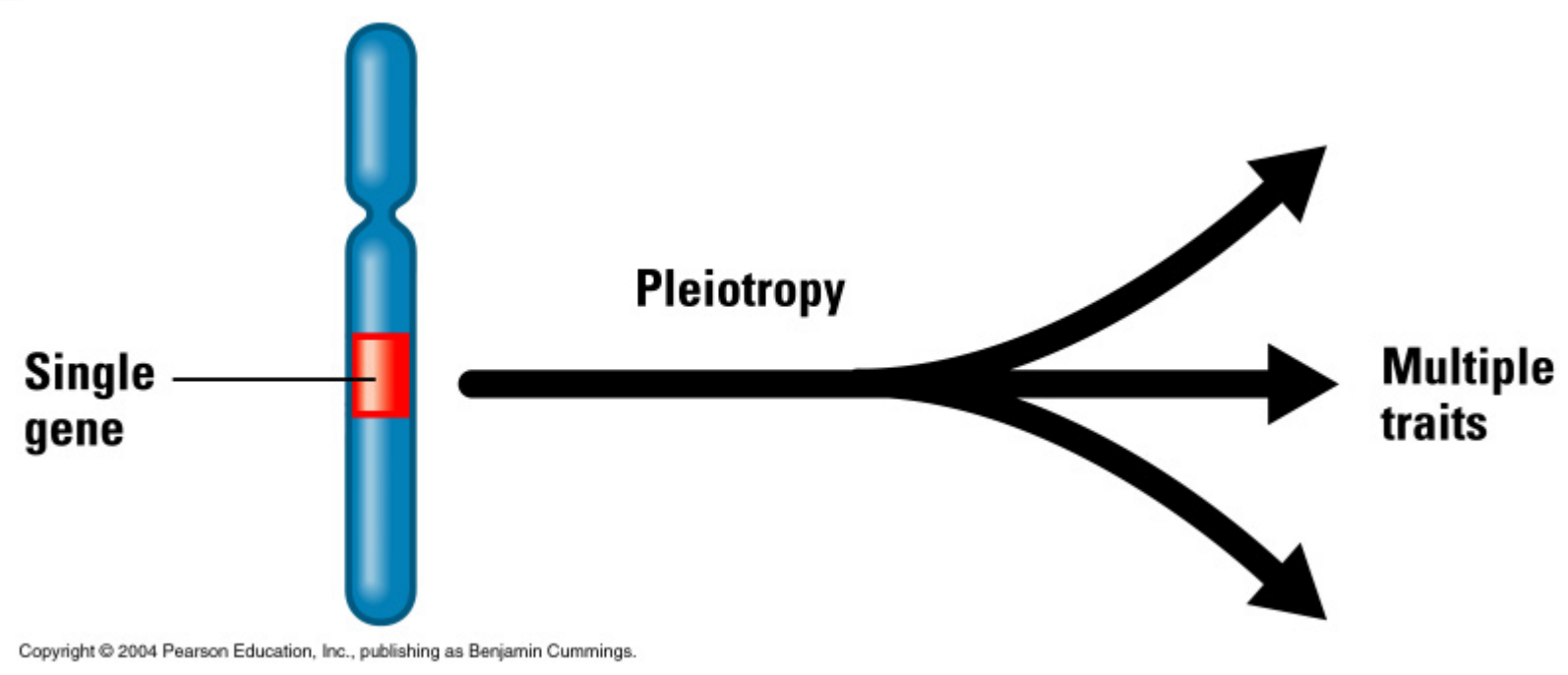
Hands-on tutorial

Introduction to GWA-Portal

- GWAPP (Seren *et al.*, 2012) was a case study to see if we can provide real-time on-the-fly LMM GWAS as a web-application
  - 250k genotype (Horton *et al.*, 2012)
  - 4 methods: LM, KW, EMMAX and MLMM
  - Interactive Manhattan and LD plots

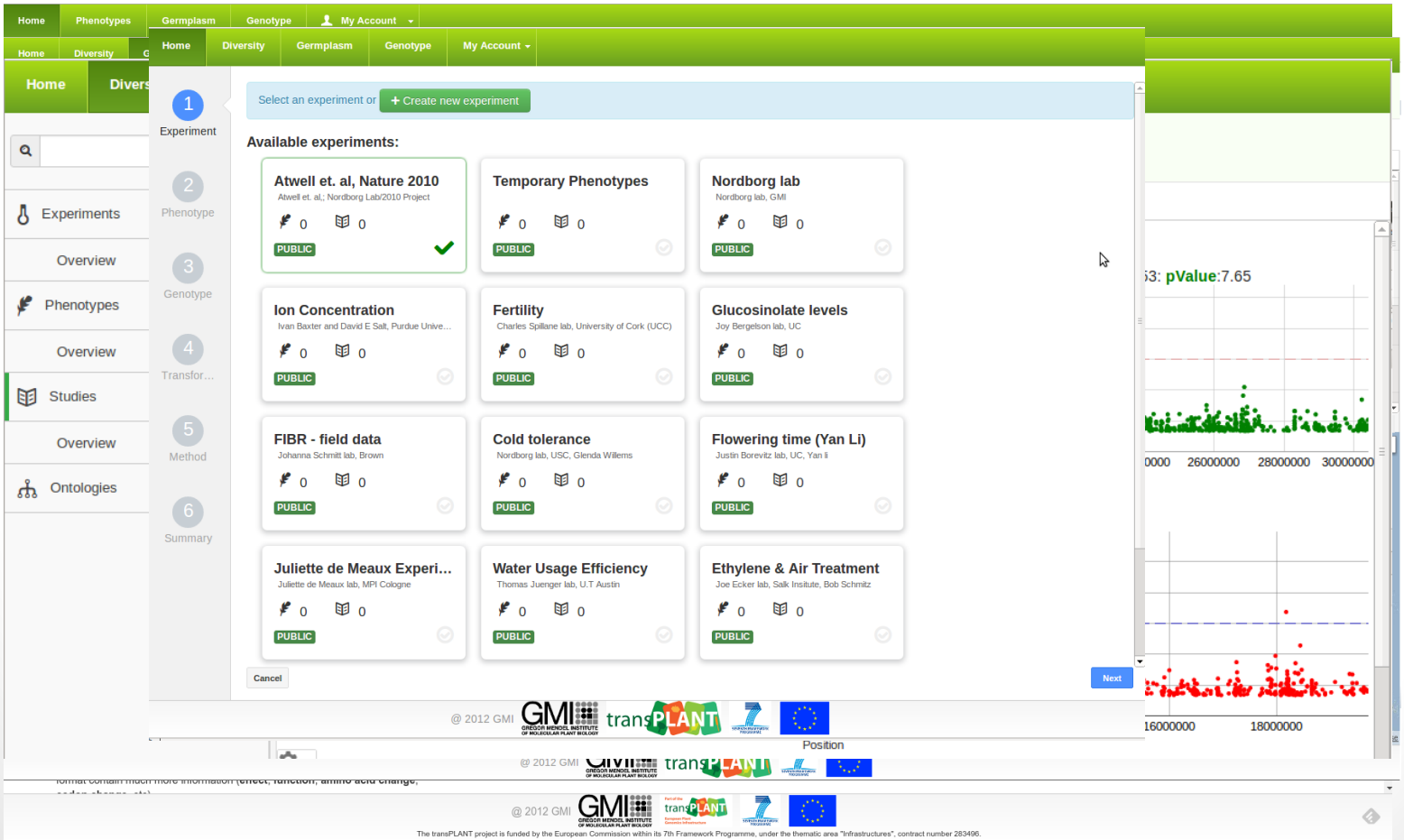


Pleiotropy analysis



Introduction to GWA-Portal

- Single resource for **phenotypes**, **GWAS analysis**, **germplasm** and **genotypes**.



Introduction to GWA-Portal

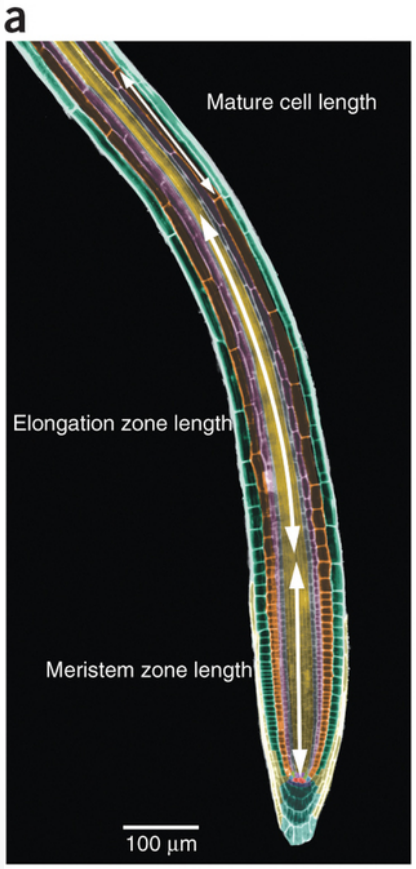
Features

- Genotype datasets:
  - 250k dataset (1386)
  - Swedish genomes (181)
  - 1001 genomes (1135)
  - Imputed data (2029)
- Permission system & sharing options for phenotypes and GWAS results
- Integrated search via fulltext search engine
- Interactive charts and visualizations
- **Analysis of Pleiotropy**:
  - Candidate gene list enrichment
  - Top-SNPs and Gene view
  - Detailed SNP information

Step-by-step guide

1. Groups of 2 - 3 users
2. Download phenotype file
3. Each groups creates a study
4. Upload the phenotype and create a GWAS analysis
5. 5-10 minute coffee break (until GWAS analysis is finished)
6. Interactive discovery using Manhattan plots (filtering, zooming, etc)
7. Display detailed SNP information
8. View candidate gene list enrichment analysis
9. Meta-analysis of pleiotropy

Cellular phenotype



nature genetics
Search Go
Advanced search
Home | Current issue | Comment | Research | Archive | Authors & referees | About the journal
home ▸ archive ▸ issue ▸ letter ▸ full text

NATURE GENETICS | LETTER
日本語要約

Genome-wide association study using cellular traits identifies a new regulator of root development in *Arabidopsis*

Mónica Meijón, Santosh B Satbhai, Takashi Tsuchimatsu & Wolfgang Busch
Affiliations | Contributions | Corresponding author

Nature Genetics **46**, 77–81 (2014) | doi:10.1038/ng.2824
Received 18 July 2013 | Accepted 11 October 2013 | Published online 10 November 2013
| Corrected online **26 November 2013**

With the increased availability of high-resolution sequence information, genome-wide association (GWA) studies have become feasible in a number of species[1, 2, 3, 4, 5, 6, 7, 8]. The vast majority of these studies are conducted in human populations, where it is difficult to provide strong evidence for the functional involvement of unknown genes that are identified using GWA. Here we used the model organism *Arabidopsis thaliana* to combine high-throughput confocal microscopy imaging of traits at the cellular level, GWA and expression analyses to identify genomic regions that are associated with developmental cell–type traits. We identify and characterize a new F-box gene, *KUK*, that regulates meristem and cell length. We further show that polymorphisms in the coding sequence are the major causes of *KUK* allele–dependent natural variation in root development. This work demonstrates the feasibility of GWA using cellular traits to identify causal genes for basic biological processes such as development.

Step-by-step guide

2. Download phenotype file:

Group A:

- Meristem zone length
- https://goo.gl/gKEIKe

Group B:

- Mature cell length
- https://goo.gl/qiq0oX

Step-by-step guide

Site: http://gwas.gmi.oeaw.ac.at

Login: gwas@workshop.org

Password: gwas

What did we learn?, Resources & Acknowledgements?

Summary

Summary

- GWAS is a powerful tool to understand the genetics of natural variation.
- Methods are fast enough to do GWAS on big sample sizes in reasonable time
- Population structure confounding can cause issues
  - Linear Mixed Model can help address this issue
- BUT GWAS is not without challenges to be aware of
  - Epistatic interaction
  - Allelic heterogeneity
  - GWAS on sub-samples
  - …
- Web-based tools like GWA-Portal allow to mine the GWAS data, look at the information from different perspectives and uncover previously unknown pleiotropic effects.

Summary

THE END

Acknowledgements

GMI:

- Radka Slovak
- Arthur Korte
- Magnus Nordborg
- Nordborg lab

BiRC:

- Bjarni Vilhjálmsson

BSC:

- Josep Lluis Gelpi
- Laia Codo

Part of the transPLANT European Plant Genomics Infrastructure

GMI GREGOR MENDEL INSTITUTE OF MOLECULAR PLANT BIOLOGY

The transPLANT project is funded by the European Commission within its 7th Framework Programme under the thematic area "Infrastructures", contract number 283496.

Resources

- GWAPP (Seren *et al.*):
  - URL: http://gwapp.gmi.oeaw.ac.at
  - Code: http://github.com/timeu/GWAPP
- GWA-Portal:
  - URL: http://gwas.gmi.oeaw.ac.at
  - Code: https://github.com/timeu/GWA-Portal
- Phenotypes:
  - Meijón *et al.*, 2013 (Nature Genetics)
  - http://www.nature.com/ng/journal/v46/n1/full/ng.2824.html
- PyGWAS:
  - https://pypi.python.org/pypi/PyGWAS/0.1.4
  - https://registry.hub.docker.com/u/timeu/pygwas/

References

- Estimating kinship
  - Weir, BS, Anderson, AD, & Hepler, AB. (2006) Genetic relatedness analysis: modern data and new challenges. *Nat Rev Genet.*
  - Kang, H, Zaitlen, N, *et al.* (2008). Efficient control of population structure in model organism association mapping. *Genetics.*
  - Kang, H. M., Sul, J. H., Service, S. K., et al. (2010) Variance component model to account for sample structure in genome-wide association studies. *Nat Genet.*
  - Powell, JE, Visscher, PM, & Goddard, ME. (2010) Reconciling the analysis of IBD and IBS in complex trait studies. *Nat Rev Genet.*

References

- Estimating heritability
  - Visscher, P. M., Hill, W. G., & Wray, N. R. (2008) Heritability in the genomics era - concepts and misconceptions. *Nat Rev Genet.*
  - Yang, J., Benyamin,B, et al. (2010) Common SNPs explain a large proportion of the heritability for human height. *Nat Genet.*
  - Yang, J., et al. (2011) Genome partitioning of genetic variation for complex traits using common SNPs. *Nat Genet.*
  - Deary, I. J., et al. (2012). Genetic contributions to stability and change in intelligence from childhood to old age. *Nature.*
  - Korte, A., Vilhjálmsson, B. J., Segura, V., *et al.* (2012) A mixed-model approach for genome-wide association studies of correlated traits in structured populations. *Nat Genet.*
  - Zaitlen, N., & Kraft, P. (2012) Heritability in the genome-wide association era. *Hum Genet.*

References

- Controlling for population structure in GWAS using mixed models.
  - Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., et al. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat Genet.*
  - Zhao, K, *et al.* (2007). An Arabidopsis example of association mapping in structured samples. *PLoS Genet.*
  - Kang, HM, *et al.* (2008) Efficient control of population structure in model organism association mapping. *Genetics.*
  - Zhang, Z, Ersoz, E, *et al.* (2010) Mixed linear model approach adapted for genome-wide association studies. *Nat Genet.*
  - Kang, H. M., et al. (2010) Variance component model to account for sample structure in genome-wide association studies. *Nat Genet.*

References

- Controlling for population structure in GWAS using mixed models.
  - Lippert, C., Listgarten, J., et al. (2011) FaST linear mixed models for genome-wide association studies. *Nat Meth.*
  - Segura, V., Vilhjálmsson, B. J., et al. (2012) An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. *Nat Genet.*
  - Listgarten, J., Lippert, C., et al. (2012) Improved linear mixed models for genome-wide association studies. *Nat Meth.*
  - Pirinen, M, *et al.* (http://arxiv.org/abs/1207.4886) Efficient computation with a linear mixed model on large-scale data sets with applications to genetic studies. *Submitted to the Annals of Applied Statistics*.
  - Zhou, X., & Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association studies. *Nat Genet.*

References

- Principal components
  - Price, A. L., Patterson, N. J., *et al.* (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet.*
  - Patterson, N., Price, A. L., & Reich, D. (2006). Population structure and eigenanalysis. *PLoS Genet.*
  - Novembre, J., & Stephens, M. (2008) Interpreting principal component analyses of spatial population genetic variation. *Nat Genet*.
  - Janss, L., de los Campos, G., Sheehan, N., & Sorensen, D. A. (2012). Inferences from Genomic Models in Stratified Populations. *Genetics.*

References

- Fisher's infinitesimal model
  - RA Fisher. (1918) The correlation between relatives on the supposition of Mendelian inheritance. *Trans Royal Soc Edinburgh.*
- Other interesting papers
  - Meuwissen, TH, et al. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics.*
  - Daetwyler, HD, *et al.* (2008) Accuracy of predicting the genetic risk of disease using a genome-wide approach. *PLoS One.*
  - de los Campos, G., Gianola, D., & Allison, D. B. (2010) Predicting genetic predisposition in humans: the promise of whole-genome markers. *Nat Rev Genet*.
  - Price, AL, et al. (2011) Single-tissue and cross-tissue heritability of gene expression via identity-by-descent in related or unrelated individuals. *PLoS Genet.*
  - Vazquez, A. I., Duarte, C. W., Allison, D. B., & de los Campos, G. (2011) Beyond Missing Heritability: Prediction of Complex Traits. *PLoS Genet*.

References

- Reviews on GWAS:
  - Hirschhorn, J. N., & Daly, M. J. (2005). Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet*.
  - Balding, D. J. (2006). A tutorial on statistical methods for population association studies. *Nat Rev Genet*.
  - McCarthy, M. I., Abecasis, G. R., Cardon, L. R., Goldstein, D. B., Little, J., Ioannidis, J. P. A., & Hirschhorn, J. N. (2008). Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet*.
  - Altshuler, D., Daly, M. J., & Lander, E. S. (2008). Genetic mapping in human disease. *Science.*
  - Stranger, B. E., Stahl, E. A., & Raj, T. (2011). Progress and Promise of Genome-Wide Association Studies for Human Complex Trait Genetics. *Genetics*.
  - PM Visscher, MA Brown, MI McCarthy, & J Yang (2012). Five Years of GWAS Discovery. *Am J Hum Genet.*

References

- Population structure
  - Pritchard, J. K., Stephens, M., & Rosenberg, N. A. (2000) Association mapping in structured populations. *Am J Hum Genet.*
  - Price, A. L., Patterson, N. J., Plenge, R. M., et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet.*
  - Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., et al. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat Genet.*
  - Novembre, J., Johnson, T., Bryc, K., et al. (2008) Genes mirror geography within Europe. *Nature.*
  - Yang, W.-Y., Novembre, J., et al. (2012) A model-based approach for analysis of spatial structure in genetic data. *Nat Genet.*

References

- Multiple markers approaches
  - Tibshirani, R. (1996) Regression Shrinkage and Selection via the Lasso. *JSTOR: Journal of the Royal Statistical Society.*
  - Hoggart, C. J., Whittaker, J. C., De Iorio, M., & Balding, D. J. (2008) Simultaneous analysis of all SNPs in genome-wide and re-sequencing association studies. *PLoS Genet.*
  - Ayers, K. L., & Cordell, H. J. (2010) SNP selection in genome-wide and candidate gene studies via penalized logistic regression. *Genet Epidem.*

References

- Synthetic associations
  - Platt, A., Vilhjálmsson, B. J., & Nordborg, M. (2010). Conditions under which genome-wide association studies will be positively misleading. *Genetics.*
  - Dickson, S. P., Wang, K., et al. (2010) Rare variants create synthetic genome-wide associations. *PLoS Genet*
  - Wang, K., Dickson, S. P., Stolle, C. A., *et al.* (2010) Interpretation of association signals and identification of causal variants from genome-wide association studies. *Am J Hum Genet*

References

- Stephens, M, Balding, DJ. (2009). Bayesian statistical methods for genetic association studies. *Nat Rev Genet*.
- Astle, W, Balding, D. (2009) Population Structure and Cryptic Relatedness in Genetic Association Studies. *Statistical Science*.
- Cordell, H. J. (2009) Detecting gene–gene interactions that underlie human diseases. *Nat Rev Genet.*
- Bansal, V, Libiger, O, Torkamani, A, & Schork, NJ. (2010) Statistical analysis strategies for association studies involving rare variants. *Nat Rev Genet.*
- Zaitlen, N, Pasaniuc, B, *et al.* (2012) Analysis of case-control association studies with known risk variants. *Bioinformatics*
- Shen, X., Pettersson, M., Rönnegård, L., & Carlborg, O. (2012) Inheritance Beyond Plain Heritability: Variance-Controlling Genes in Arabidopsis thaliana. *PLoS Genet.*