QTL mapping and GWAS

Bioinformatics Applications (PLPTH813)

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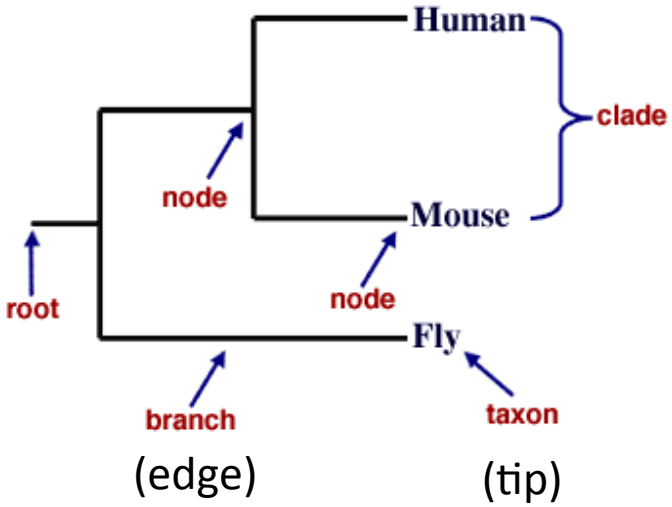
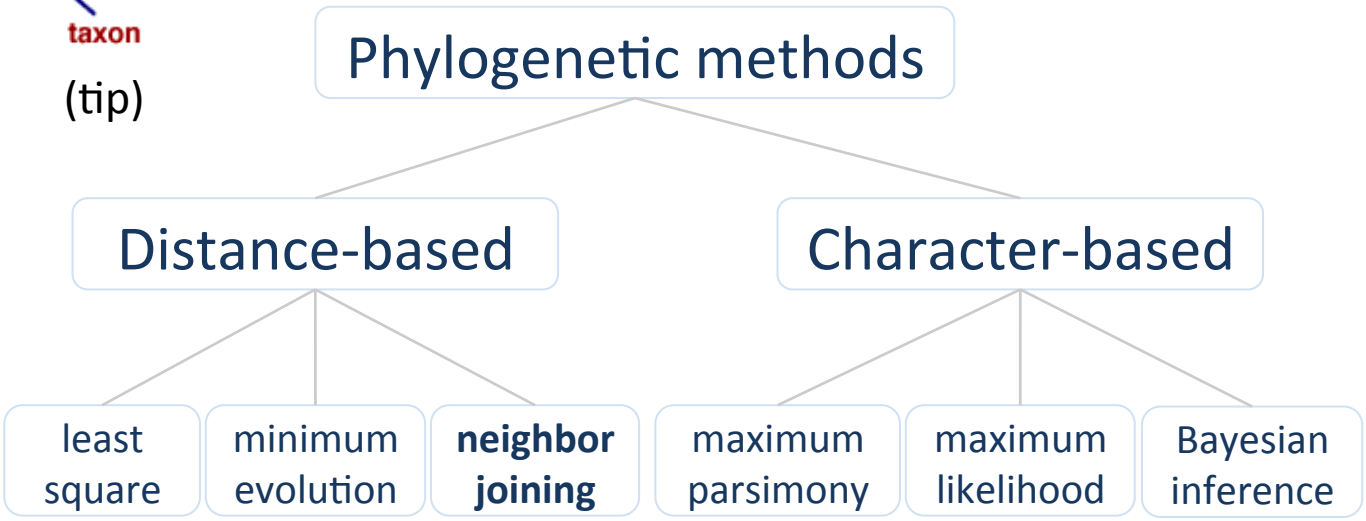
Phylogenic trees





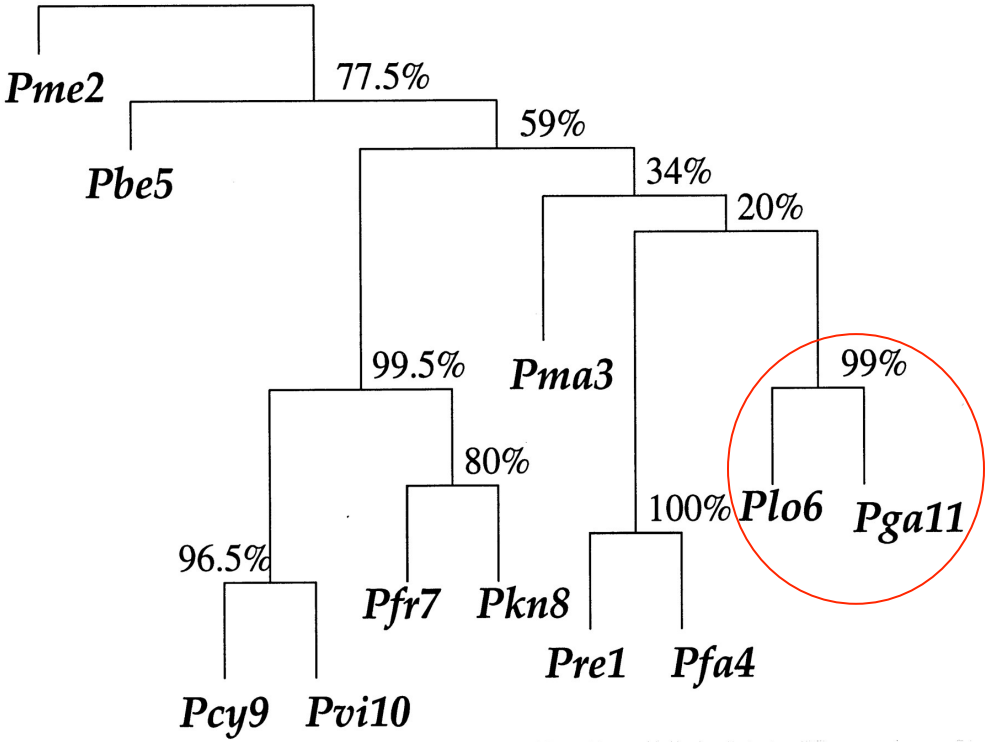
Outgroup rooting

- Many methods (e.g., NJ) construct unrooted tree. An outgroup can be introduced to identify the “root”. This strategy is called outgroup rooting.

- A good outgroup needs to satisfy:

1. not a member of the ingroup
2. close related to the ingroup

Tree evaluation: Bootstrap analysis



Boostrapping measures how consistently the data support given taxon bipartitions (Hedges, 1992).

Plo6 and Pga11 are grouped together in 99% bootstrap replicates.

* B = 200 bootstrap replications.

Bradley Efron et al. PNAS 1996;93:13429

Outline

- QTL mapping
- Genome-wide association study (GWAS)

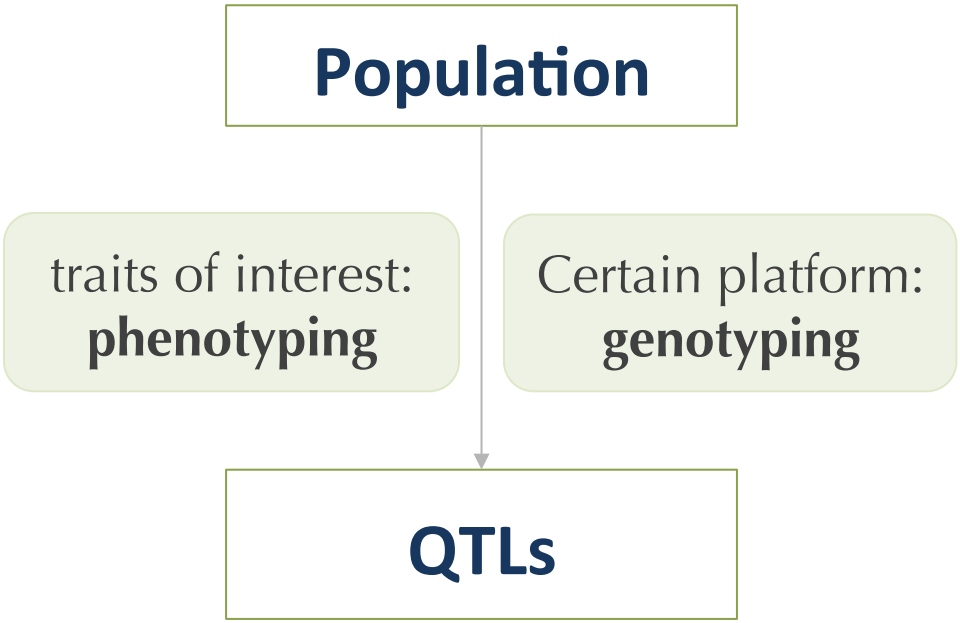
Acknowledgements: some slides were prepared by Dr. Lei Li.

What is the goal to perform

QTL or GWAS?

QTL mapping

A **Q**uantitative **T**rait **L**ocus (QTL) is **a genomic locus** that genetically influence variation in a phenotype of a quantitative trait.



Genetic linkage map or a physical map would be helpful to identify QTLs and locate the QTL on a map

Sequencing technology is an excellent tool to genotype many loci in parallel

```
ATCGCTGCCGATCTGCGTCATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTGATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTCATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTGATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTGATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTGATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTCATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTCATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTGATACGGAATCGTCGGCTTCAG
ATCGCTGCCGATCTGCGTCATACGGAATCGTCGGCTTCAG
```

Genotyping score

```
----------C/G----------
----------1/0----------
```

Marker

Phenotyping

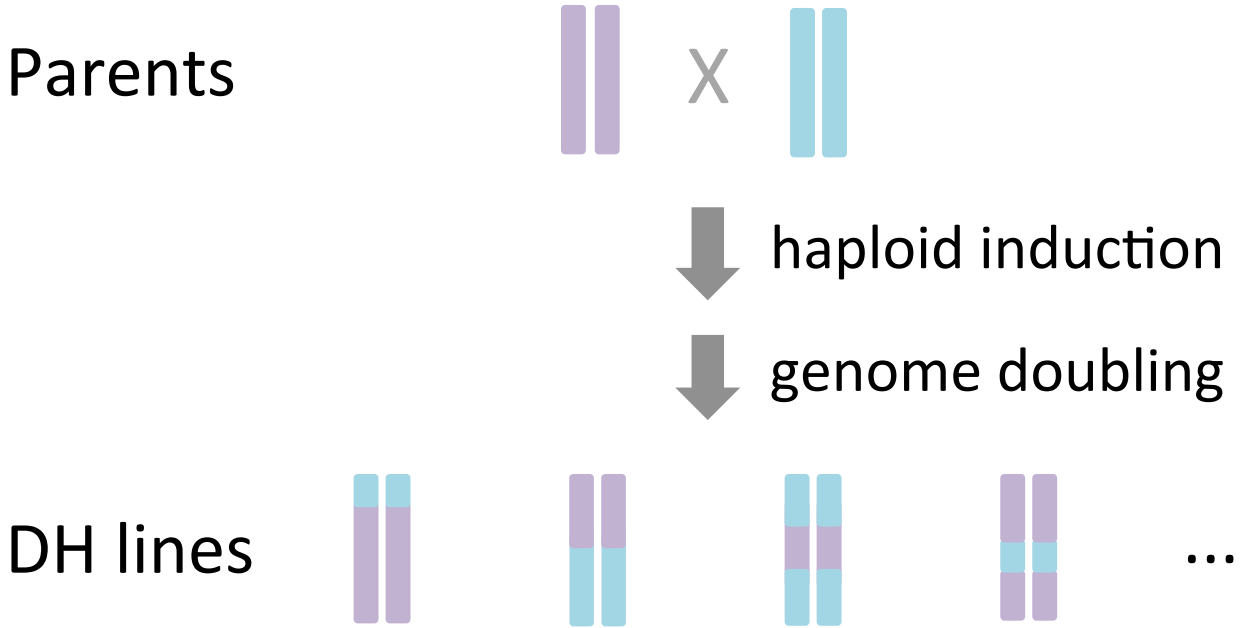
wikimedia.org

agphd.com

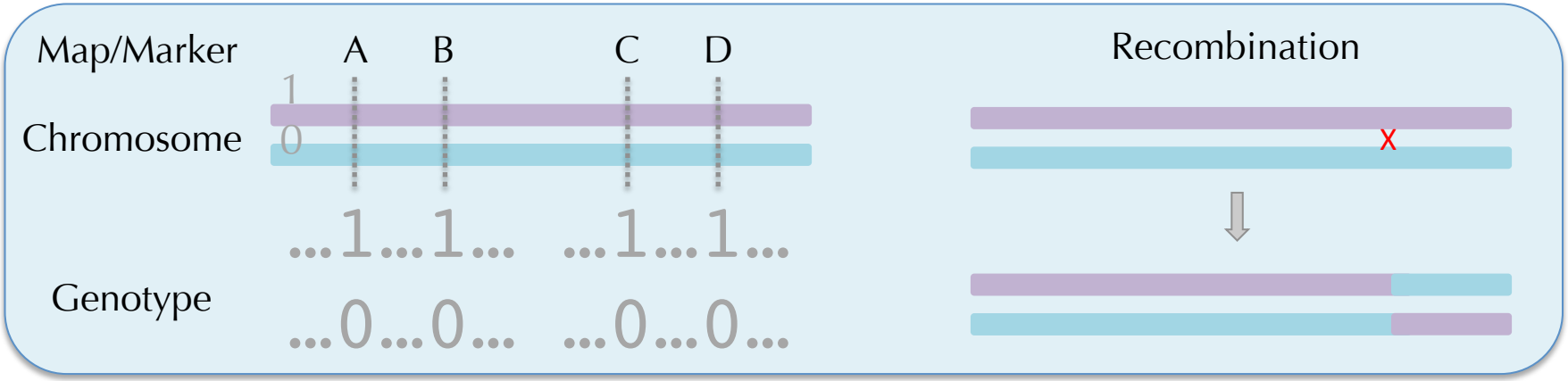
High-throughput phenotyping

Mapping populations

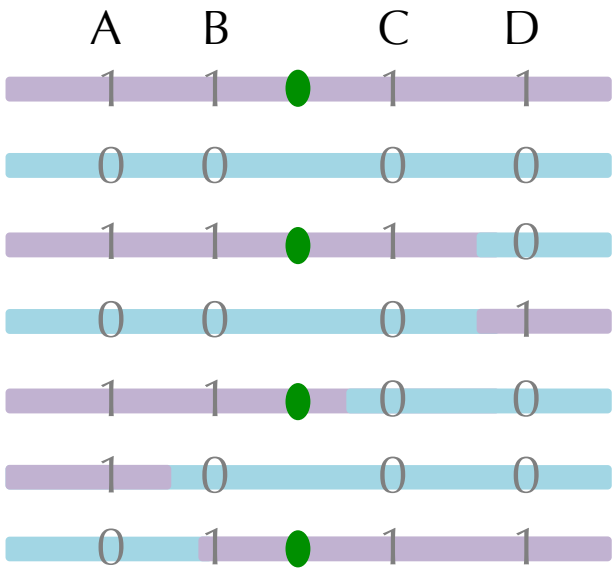
1. F1, F2
2. Recombinant Inbred Lines
3. Double haploid (DH) lines



Mapping a causal genetic controlling component (X)



Mapping population



Genotype

X
1
0
1
0
1
0
1

Phenotype

Mapping result

A B X C D

X′
35
3
24
12
45
18
20

Phenotype

Approach 1: t-test or ANOVA

1. Based on the genotype data, individuals are divided into groups
2. Perform t-test or ANOVA
3. Repeat for all markers

(use t-test if only two groups exist)

genotype	phenotype
1	35
1	24
1	20
1	45
0	3
0	18
0	12

Pros:

- Simple
- No genetic map required

Cons:

- Individuals with missing data are excluded
- Suffers in low density markers

Approach 2: Interval mapping (IM)

- Assume a single QTL model (QTL at a certain genetic position)
- Determine the ***confidence*** of each QTL model
- Scan the whole genome (interval by interval)

Interval mapping – estimate genotypes

Genotype			
	?	0	
	0	0	
	0	?	
	0	1	
	1	1	
	1	?	
	1	1	
	1	0	
	?	0	
	…	…	

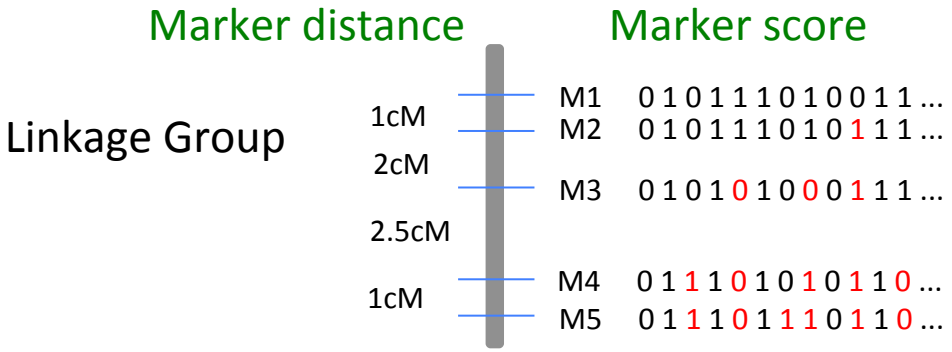
Estimate genotypes: each estimated genotype is associated with a certain probability

- Genetic linkage map

Assume a single QTL model (QTL at a certain genetic position)

Genetic linkage map

- Describe the linear order of markers within a linkage group



- **Recombination frequency**: the percentage of recombinant gametes produced in a cross

  Recombination frequency (*r*) = #recombinants / total x 100%

- 1 **centimorgan (cM)** apart on a genetic map indicates approximately 1% of recombination events.

Mapping function

- Conversion between recombination frequencies and genetic distances
- Different formula (Haldane and Kosambi)
- Haldane's mapping function

$$r = \frac{1}{2}\left(1 - e^{-2d}\right)$$

$$d = -\frac{1}{2}\ln\left(1 - 2r\right)$$

r = recombination rate (0-0.5)
d = distance in Morgans

Interval mapping – estimate genotypes

Genotype

?	**0**	0
0	**0**	0
0	**0**	?
0	**0**	1
1	**1**	1
1	**1**	?
1	**1**	1
1	**1**	0
?	**0**	0
…	**…**	…

each estimated genotype is associated with a certain probability

Estimate likelihood of a QTL model

- ***Maximum likelihood estimates (MLE)***

Prob(pheno data | geno data; a QTL at a given position)

e.g., EM algorithm, Haley-Knott regression (HK)

- ***No QTL Likelihood***

Prob(pheno data | geno data; no QTL)

LOD (logarithm of the odds)

$$LOD = log_{10} \frac{Prob(\text{pheno data} \mid \text{geno data; a QTL at a given position})}{Prob(\text{pheno data} \mid \text{geno data; no QTL})}$$

LOD = ***log*$_{10}$ likelihood ratio**, comparing a single-QTL model to the "no QTL anywhere".

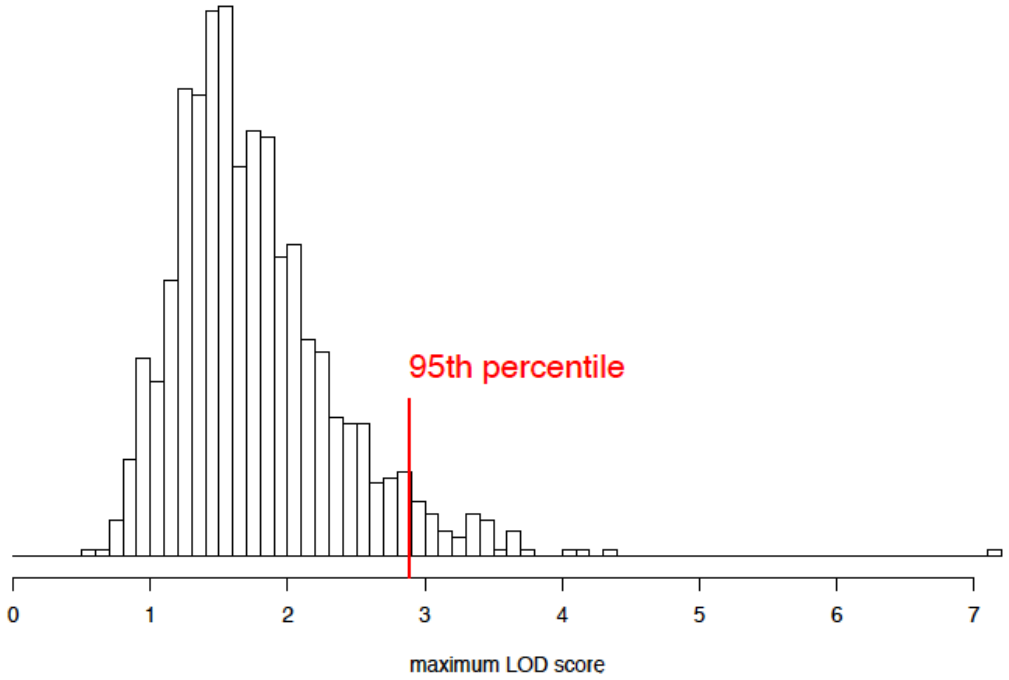
The **LOD score** is a measure of the strength of evidence for the presence of a QTL at a particular location.

LOD scores must be closer to 3 before they will generally be deemed interesting. - Broman, Lab Animal, 30(7):44–52, 2001

LOD = 3?

Permutation tests to infer a *LOD* threshold

- Permute/shuffle the phenotypes; keep the genotype data intact.
- QTL analysis and get the max(LOD) ($maxLOD_1$)
- Repeat 1000 times to have ($maxLOD_1$, $maxLOD_2$, … $maxLOD_{1000}$)
- The 95th percentile of MaxLOD is a genome-wide LOD threshold.



Question

Can we perform a QTL study on a human population?

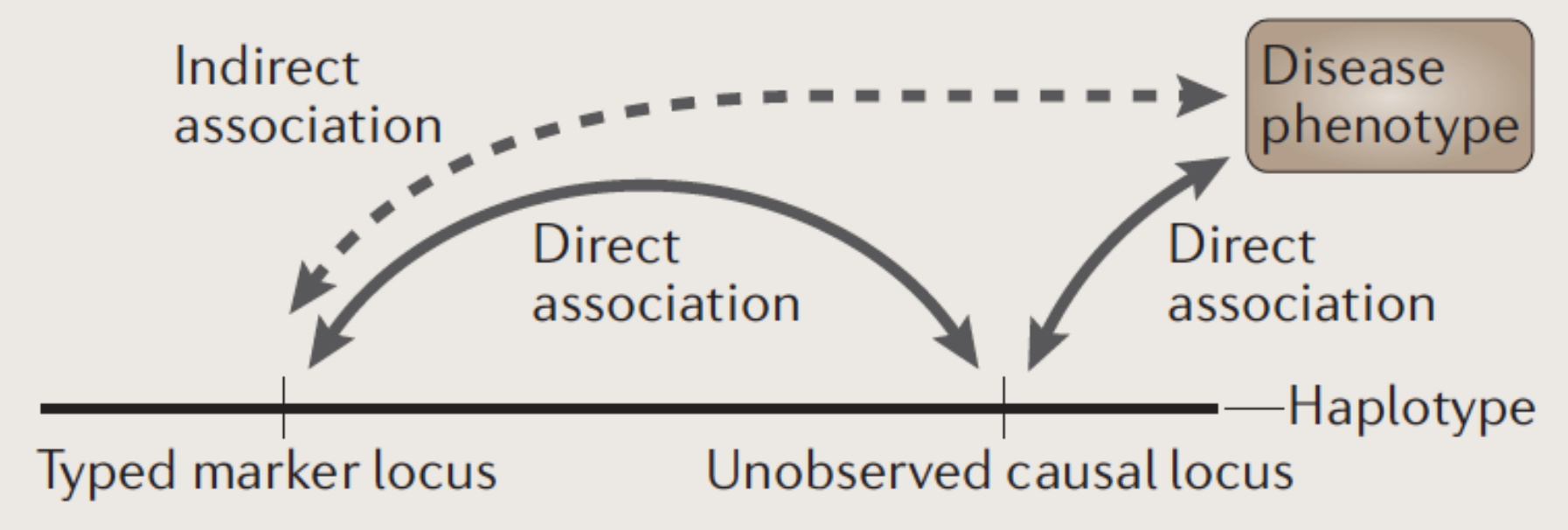
Genome-wide association study (GWAS)

GWAS is the study to correlate a great number of **genomic variants** with a large number of individuals to identify variants that are significantly associated with **the phenotype of interest**.

Goal: to identify causal variants

Linkage disequilibrium (LD)

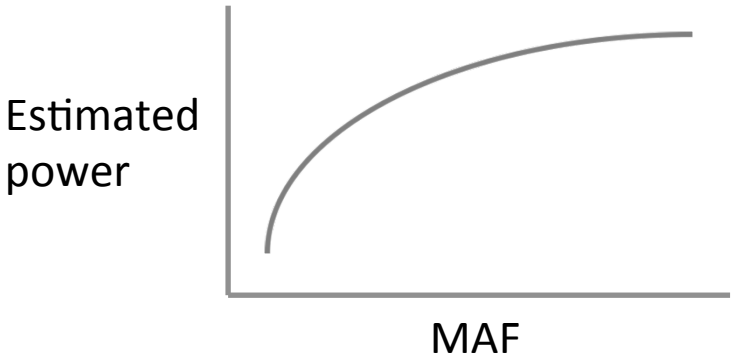
Linkage disequilibrium (LD): a non-random association of alleles at different loci; genotyping data at two loci have some level of correlations



Balding et al., Nature Review Genetics, 2006, 7:781

Genotyping data and filtering

- Typically only bi-allelic markers are used.
- Of two alleles, the allele with a smaller frequency is the minor allele. Its frequency is **minor allele frequency (MAF)**. A MAF cutoff is needed to filter SNPs (e.g., 1%).
- Filter out markers with high missing data (e.g., 30%).
- Imputation can reduce missing data.



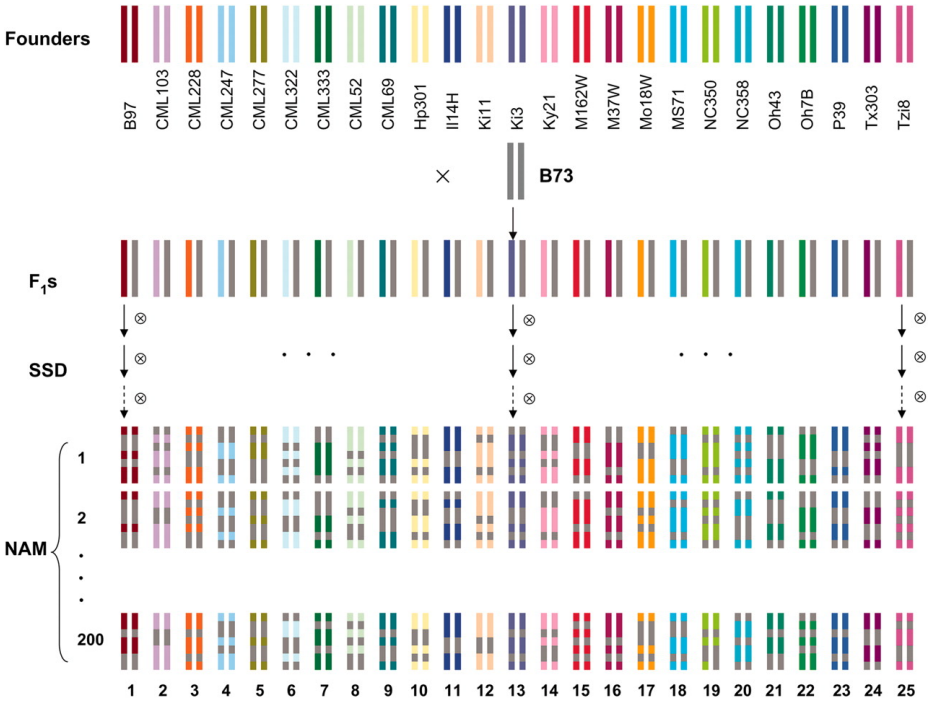
Mapping populations

- **Natural population**

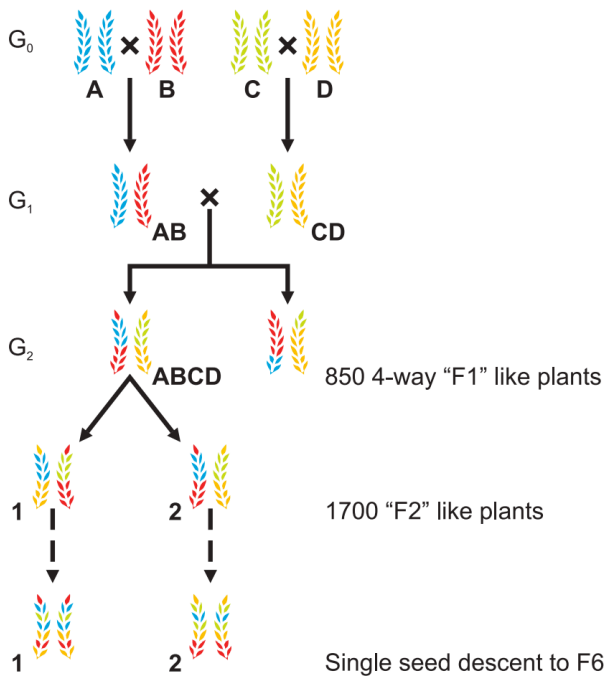
Diverse individual plant lines/animals/human beings.

- **Multi-parent crosses**

1. Nested association mapping lines (NAM)
2. Multiparent Advanced Generation Inter-Cross (MAGIC)



Yu et al., Genetics 2008;178:539-551



Huang et al., Plant Biotechnology Journal 2012; 10:826–839

Statistical test for each SNP

$$y \sim X\beta + S\alpha + e$$

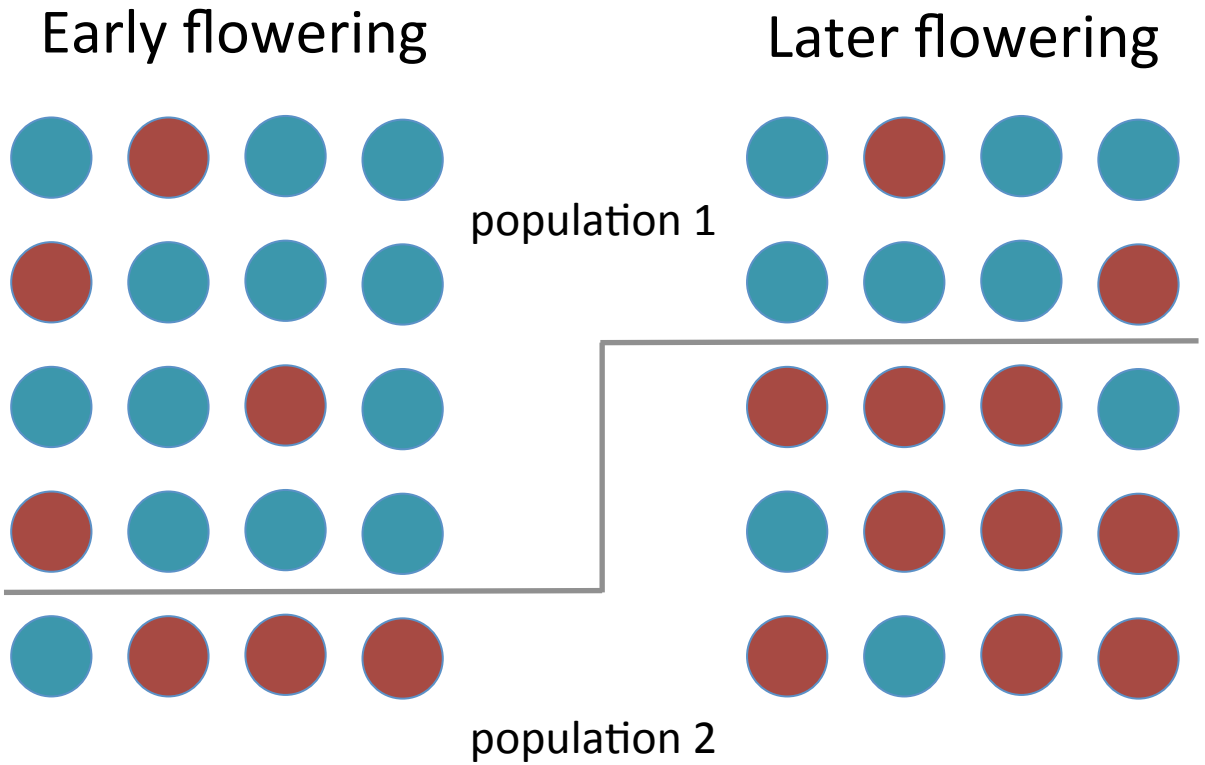
y: trait data

$X\beta$: all non-variant fixed effect

$S\alpha$: variant effects

This simple model is not sufficient to explain phenotypic data.

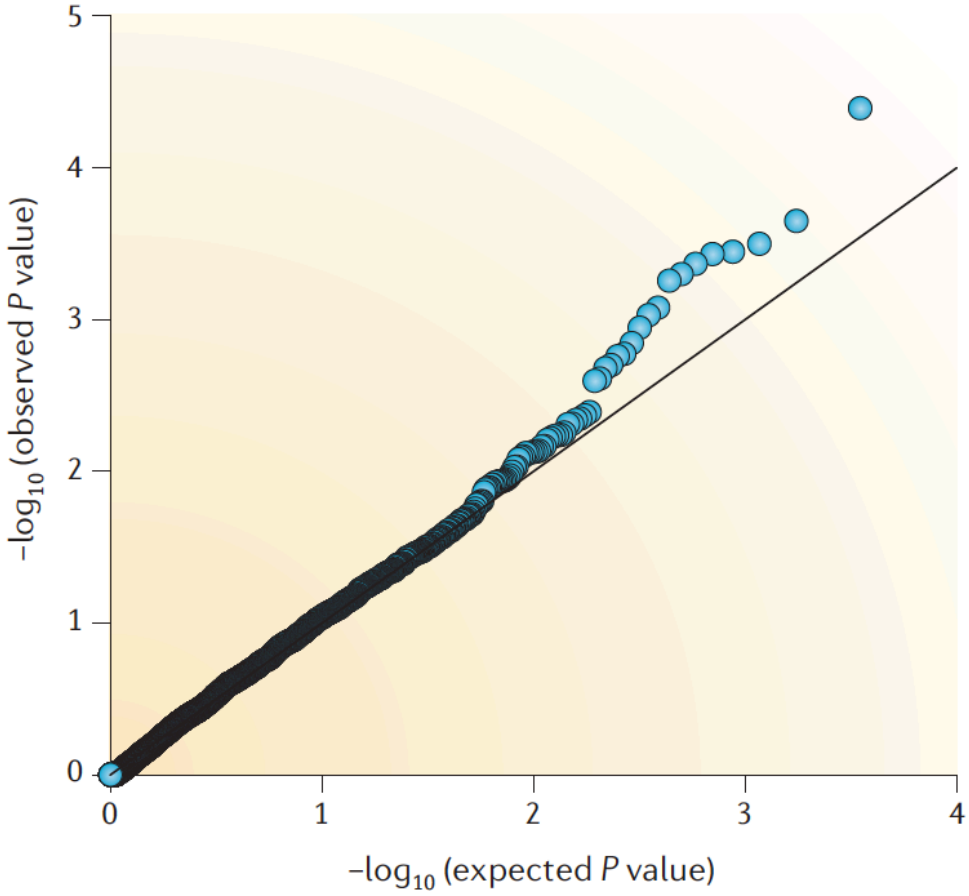
Spurious associations

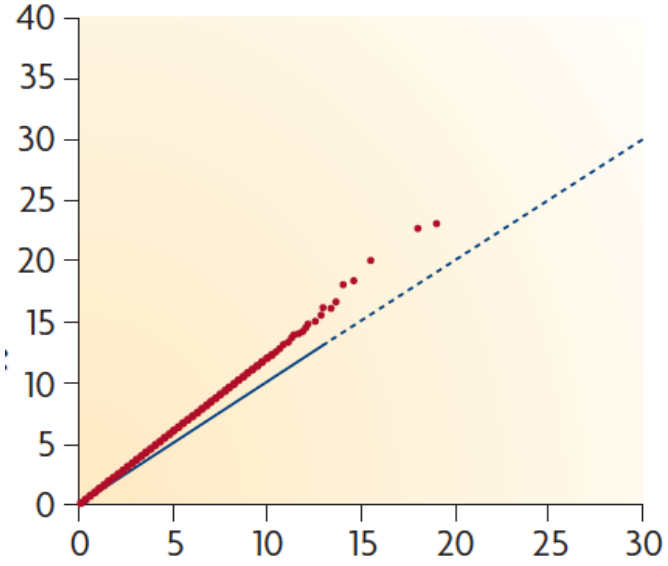


Flowering time is confounded with Populations

Modified from Balding et al., Nature Review Genetics, 2006, 7:781

quantile-quantile (Q-Q) p-value plot





Balding et al., Nature Review Genetics, 2006, 7:781

Population structure (Q)

Population structure (Q)

Confounding structure leads to false positive.

- Define a set of markers
- Population structure:

1. Principal Component Analysis (PCA) (EIGENSOFT)
2. Distance-based cluster (R/stats)
3. Model-based clustering (STRUCTURE)

$$y \sim \underbrace{X\beta + S\alpha + Qv} + e$$

Fixed effect

Q + K model explains more phenotypic variants

- **Population structure (Q)**

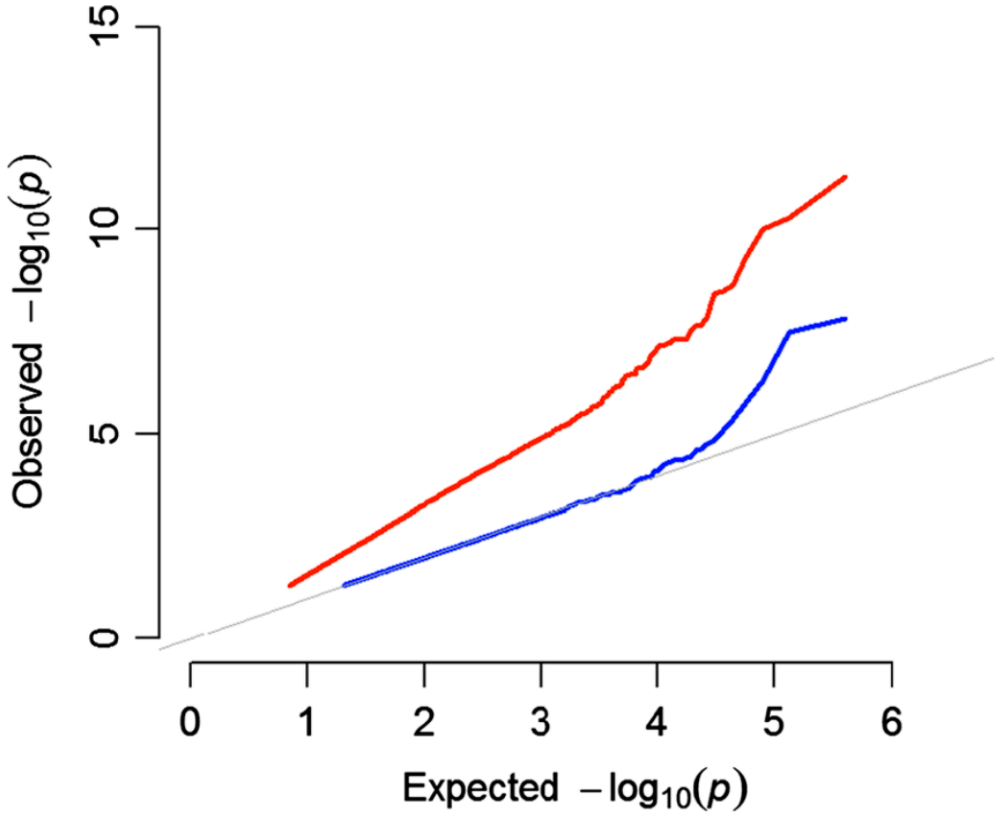
- **Kinship coefficient (K):** The probability that two homologous genes are identical by descent, estimated by using all genotyped markers

Mixed linear model (MLM)

$$y \sim X\beta + S\alpha + Qv + \boxed{Zu} + e$$

Random effect

Mixed linear model (Q+K)



The mixed model (blue) dramatically reduces inflation of p-values

GWAS w/o accounting for population structure

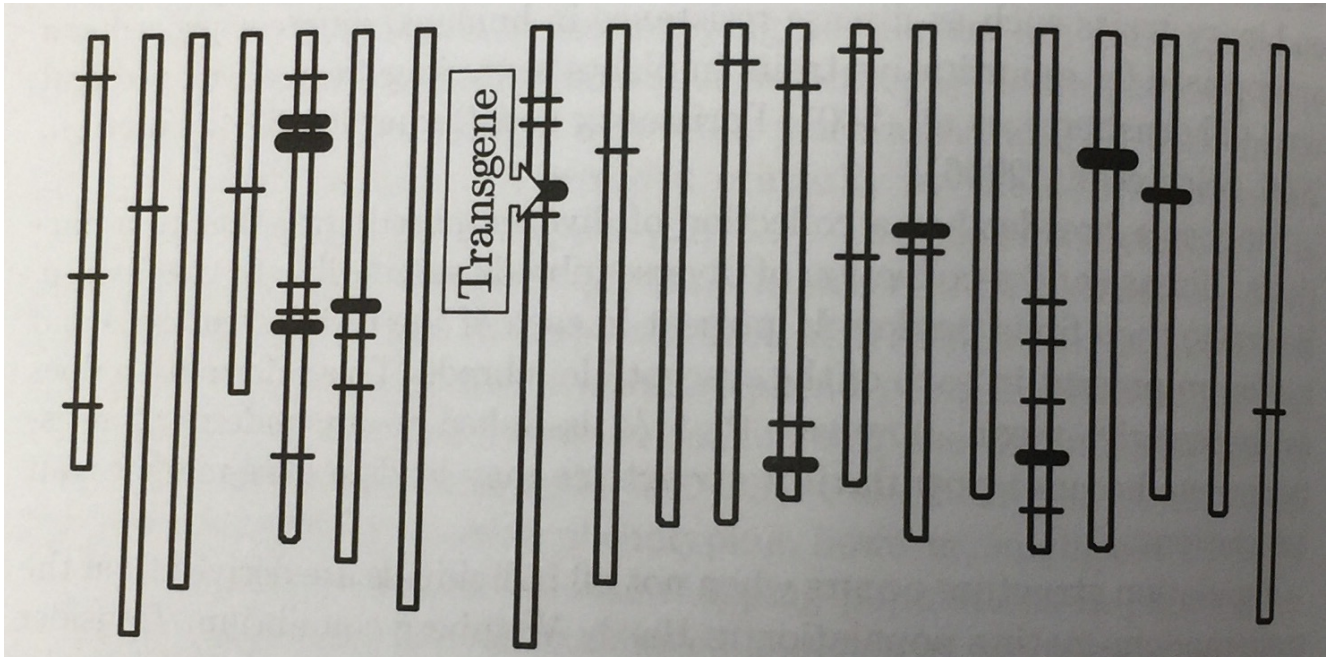


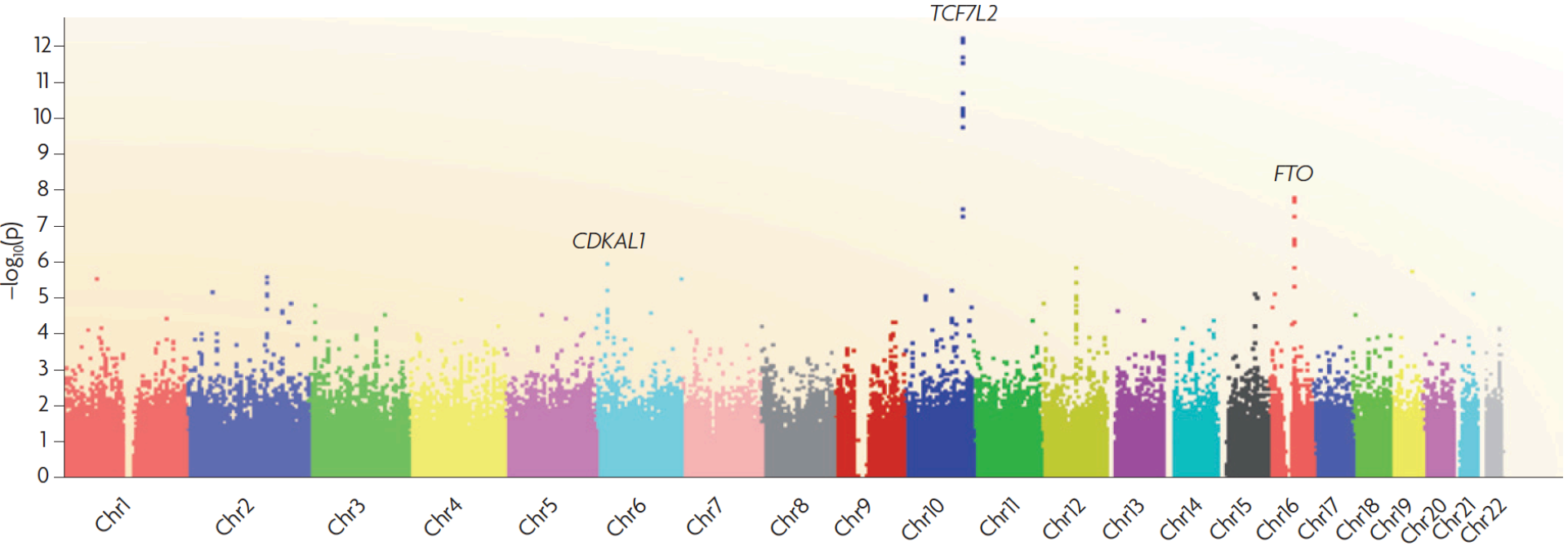
FIGURE 5.6. Markers (denoted by horizontal bars) found significant for glyphosate tolerance when population structure was ignored in association mapping (adapted from Eathington et al., 2007). Thicker bars indicate two or three markers.

750 soybean inbred lines

49 markers on 15 chromosomes

Bernardo, 2010, Breeding for quantitative traits in plants
Eathington *et al.*, 2007

Manhattan plot



McCarthy et al., Nature Review Genetics, 2008: 9:356-369

association does not imply causation

What is the difference between QTL and GWAS?

Comparison between QTL and GWAS

Attribute	**QTL mapping**	**Association genetics**
Populations	Typically from biparental lines; Limited recombination	from diverse lines, taking advantage of historic recombination
Markers for genome coverage	No high-density markers required	high-density markers required
Resolution	Limited	High