WELCOME- Genetic Epidemiology Day 2

Be sure to download breakout activities worksheet from slack or from the course website – this includes all the instructions for the zoom breakout activities.

Hardy Weinberg Equilibrium
Linkage Disequilibrium

Section 4

THURSDAY			
8:30 – 9:15	Alie	HWE/LD	Hardy Weinberg Equilibrium, and Linkage Disequilibrium
BREAK			
9:30-10:15	Alie	Population Structure	Ancestry and Principal Component Analysis
BREAK			
10:30-11:15	Sara	Study Designs	Types of genetic epidemiology studies,imputation
LUNCH BREAK			
11:45 – 12:30	Alie	Association studies	Conducting association studies andcalculating odds ratios
BREAK			
12:45-1:30	Sara	GWAS	Genome wide association studies
BREAK			
1:45 – 2:30	Sara/Alie	Office Hours	Stop by to ask questions from the day, or schedule time to discuss your own project.

Learning objectives:

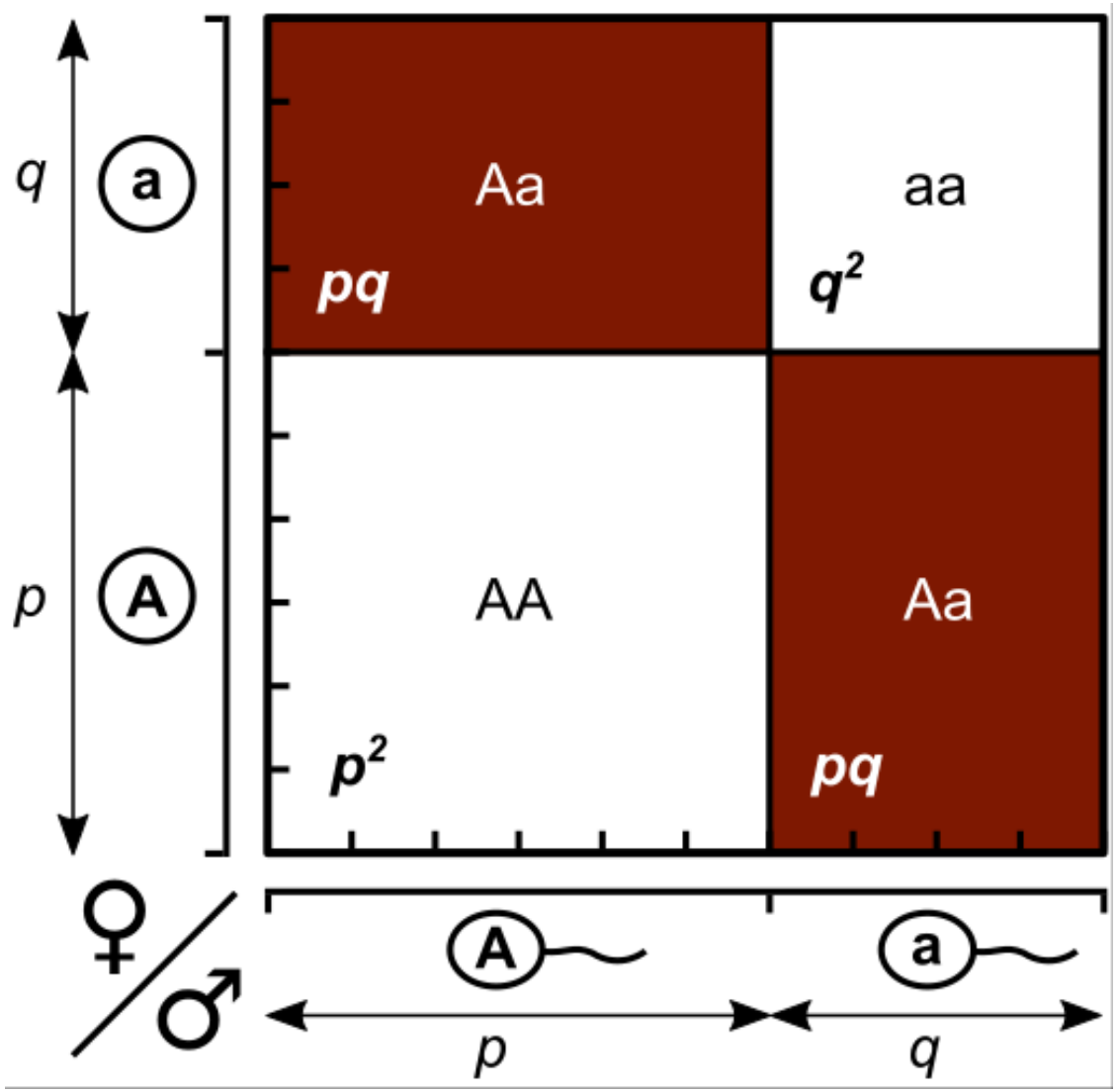
- Evaluate hardy-Weinberg equilibrium and describe its importance for genetic epidemiology studies.
- Interpret linkage disequilibrium and describe how it helps and hinders genetic epidemiology studies.

Introduction to genetics: single mating pair and offspring

	A	a
A	AA	Aa
a	Aa	aa

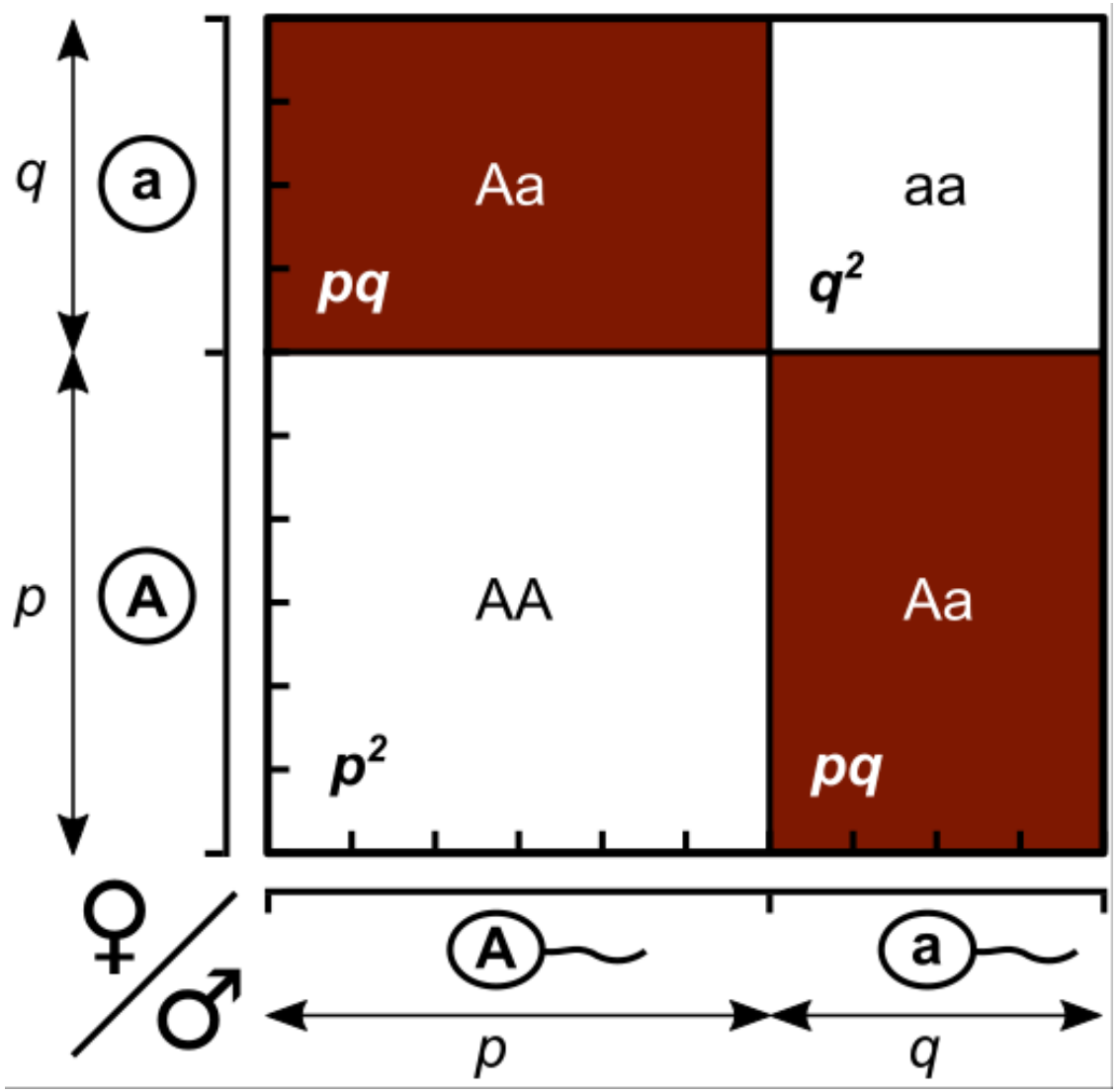
¼ (AA) + $^{2}/_{4}$ (Aa) + ¼ (aa)

Population scale expected genotype combinations



Hardy Weinberg Equilibrium is a probabilistic relationship between ALLELE frequencies and GENOTYPE frequencies

Population scale expected genotype combinations



Based on random mating:
Probability grab an “a” from the female is *q*
Probability grab an “a” from the male is *q*

So, probability grab an “a” from the female and an “a” from the male is *q*q*

The Hardy-Weinberg principle

- Assume that…
  - Population is large (coin flip likelihoods)
  - Mating is random (selective genotype matches)
  - No immigration or emigration
  - Natural selection is not occurring (all genotypes have an equal chance of surviving and reproducing)
  - No mutations

- If these assumptions are true, we say that a population is not evolving (allele frequencies stay the same) and in **Hardy-Weinberg Equilibrium**

The Hardy-Weinberg principle

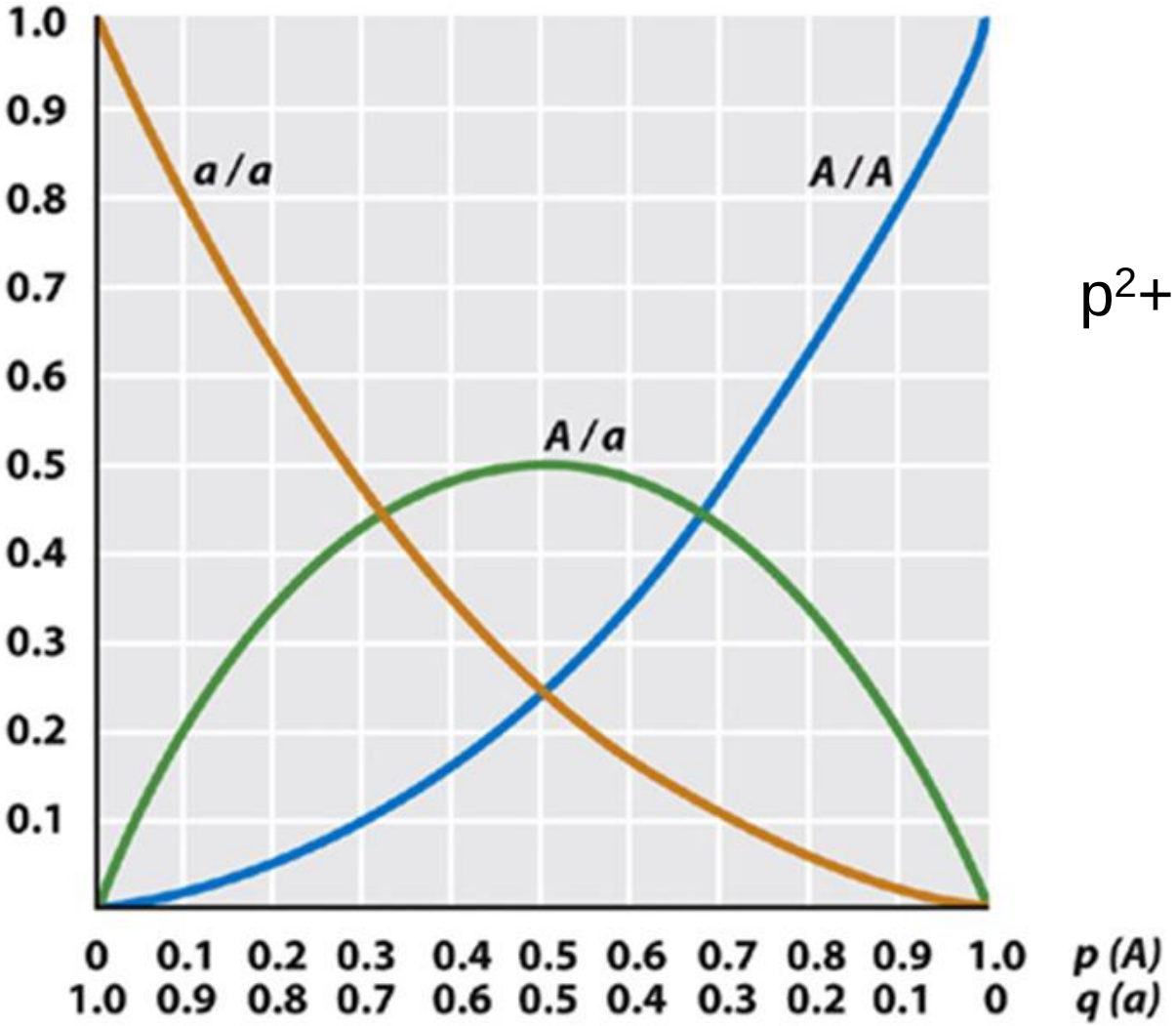


Figure 17-5
Introduction to Genetic Analysis, Ninth Edition

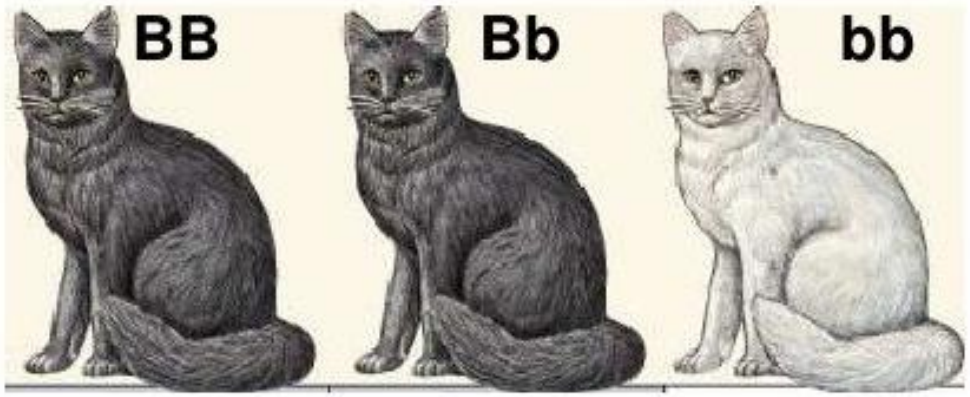
$p+q=1$ (allele frequencies)

$p^2+2qp+q^2=1$ (genotype frequencies)

HWE example

- Assume 100 cats (200 alleles) with alleles B and b. B allele is dominant and results in black coloring. 16 of the cats are white (genotype bb). If you assume HWE, what are the allele (B,b) and genotype (BB, Bb, bb) frequencies?

- $p+q=1$
- $p^2+2pq+q^2=1$



Assessing deviation from HWE

χ2-goodness-of-fit (GOF) tests with 1 degree of freedom

Sum of observed minus expected

- *O* = observed counts, *E* = expected counts, sum across genotypes

$$\chi^2 = \sum_i \frac{(O_i - E_i)^2}{E_i}.$$

Compare to chi-square distribution to determine whether the deviance is significant.

Deviation from Hardy Weinberg?

Check chi-square distribution with 1-degree of freedom:

Chi-Square (χ^2) Distribution

Degrees of Freedom	Area to the Right of Critical Value							
	0.99	0.975	0.95	0.90	0.10	0.05	0.025	0.01
1	—	0.001	0.004	0.016	2.706	3.841	5.024	6.635
2	0.020	0.051	0.103	0.211	4.605	5.991	7.378	9.210
3	0.115	0.216	0.352	0.584	6.251	7.815	9.348	11.345
4	0.297	0.484	0.711	1.064	7.779	9.488	11.143	13.277
5	0.554	0.831	1.145	1.610	9.236	11.071	12.833	15.086

Reasons to defy Hardy Weinberg equilibrium

- True selective pressures
- Genotyping error! (most common reason)
- Undetected population substructure (differences in ancestry)
- Non-random procreation

Many statistical tests rely on SNPs being in hardy weinberg equilibrium, so we test this chi-square test on every SNP in a study.

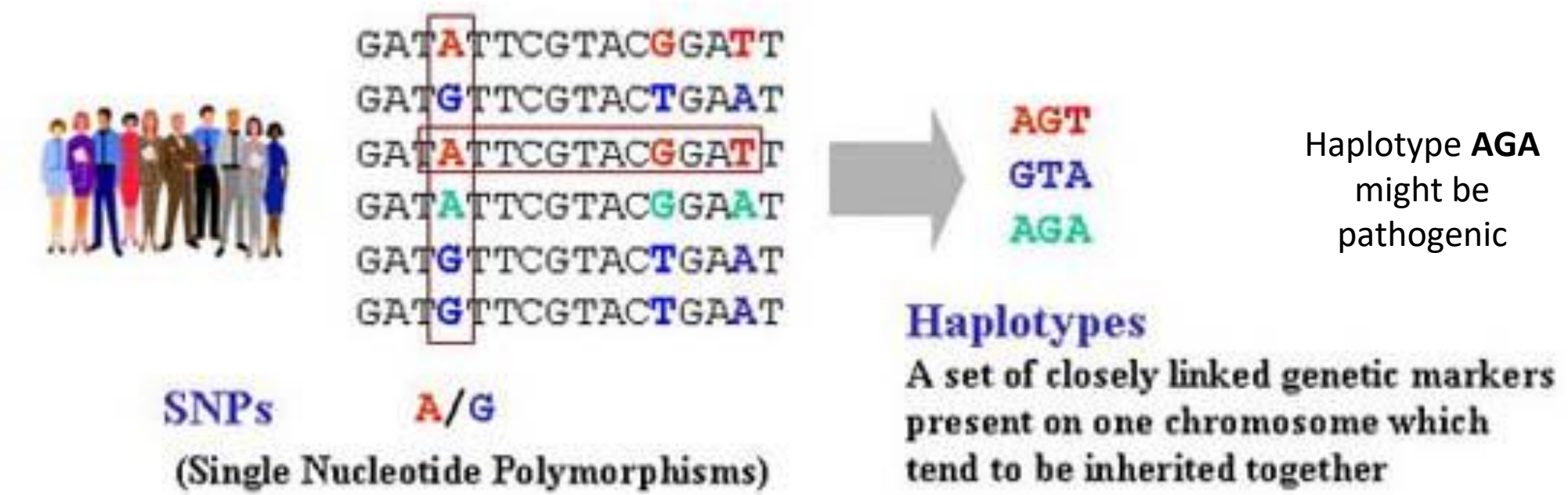
Zoom breakout Q1

- Be sure to introduce yourself (name, pronouns) to your new group before jumping into question 1!

Another important equilibrium…
Linkage Disequilibrium

Haplotypes

Specific combination of SNPs occurring on the same segment of chromosome. This depends on Linkage Disequilibrium, which we will discuss later

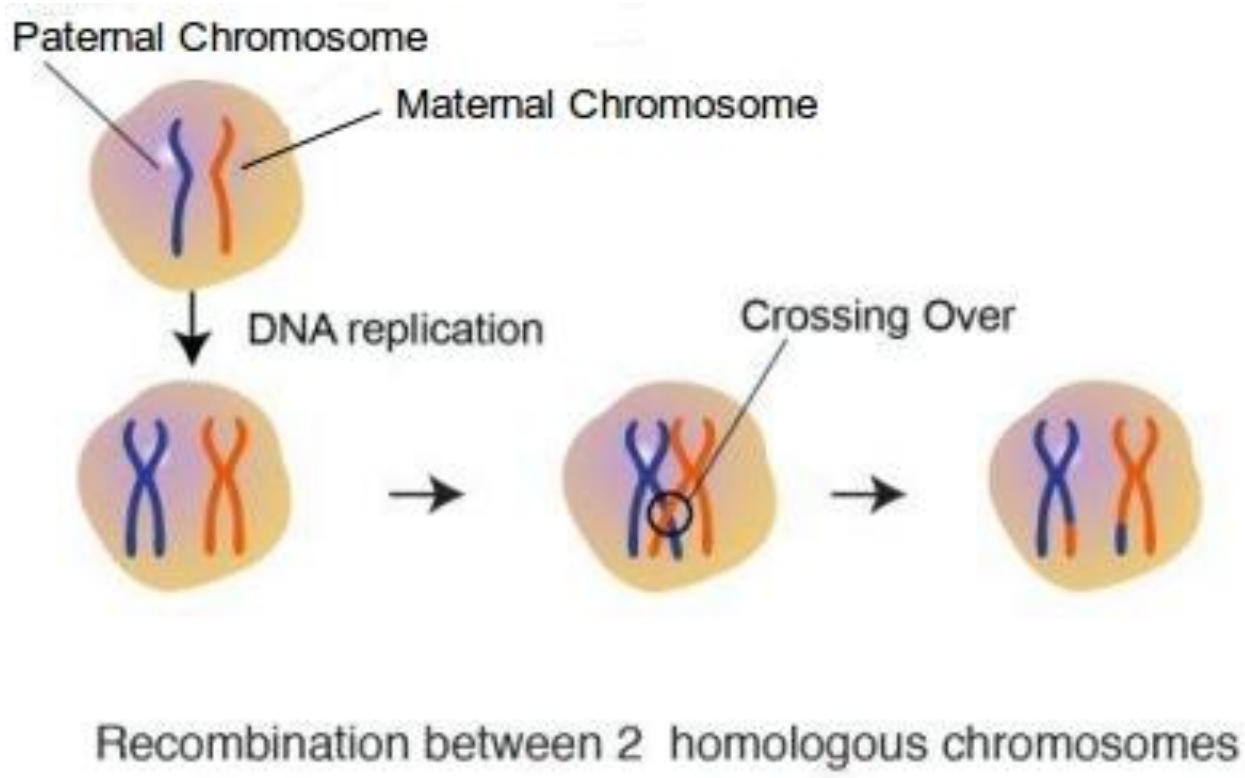


Haplotype **AGA** might be pathogenic

Recombination

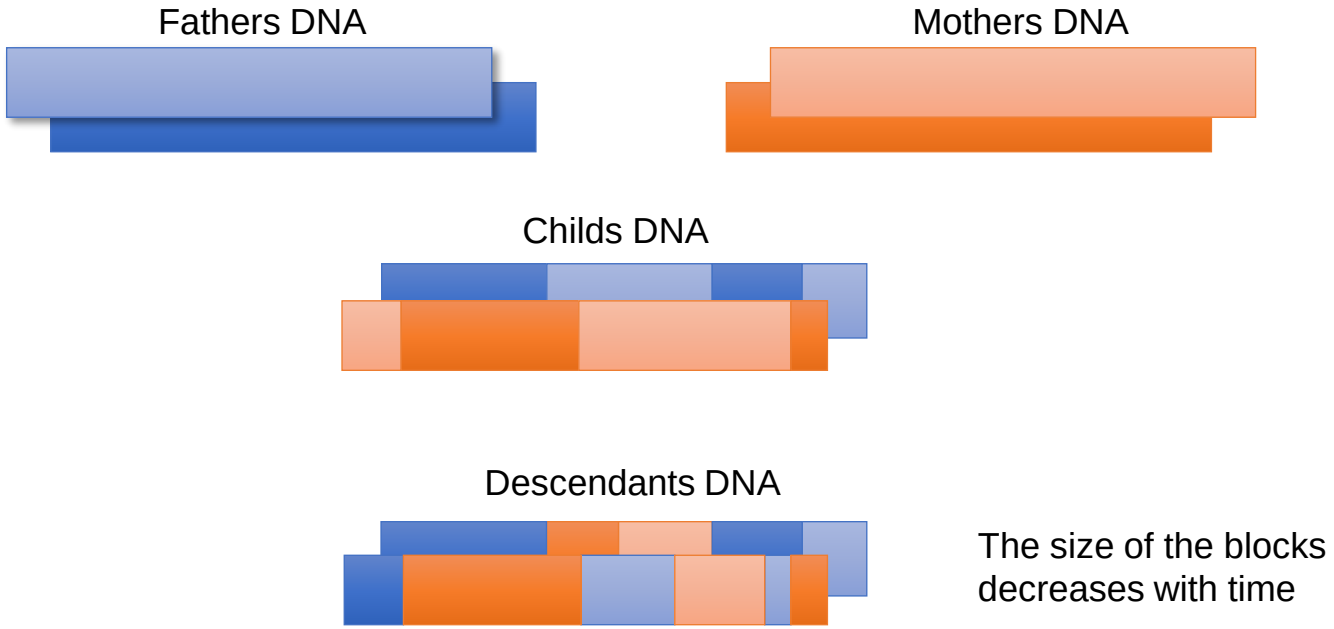
- Alleles on the same chromosome are inherited together unless *recombination (crossing over)* occurs

- The probability of recombination between two alleles increases with the distance between them



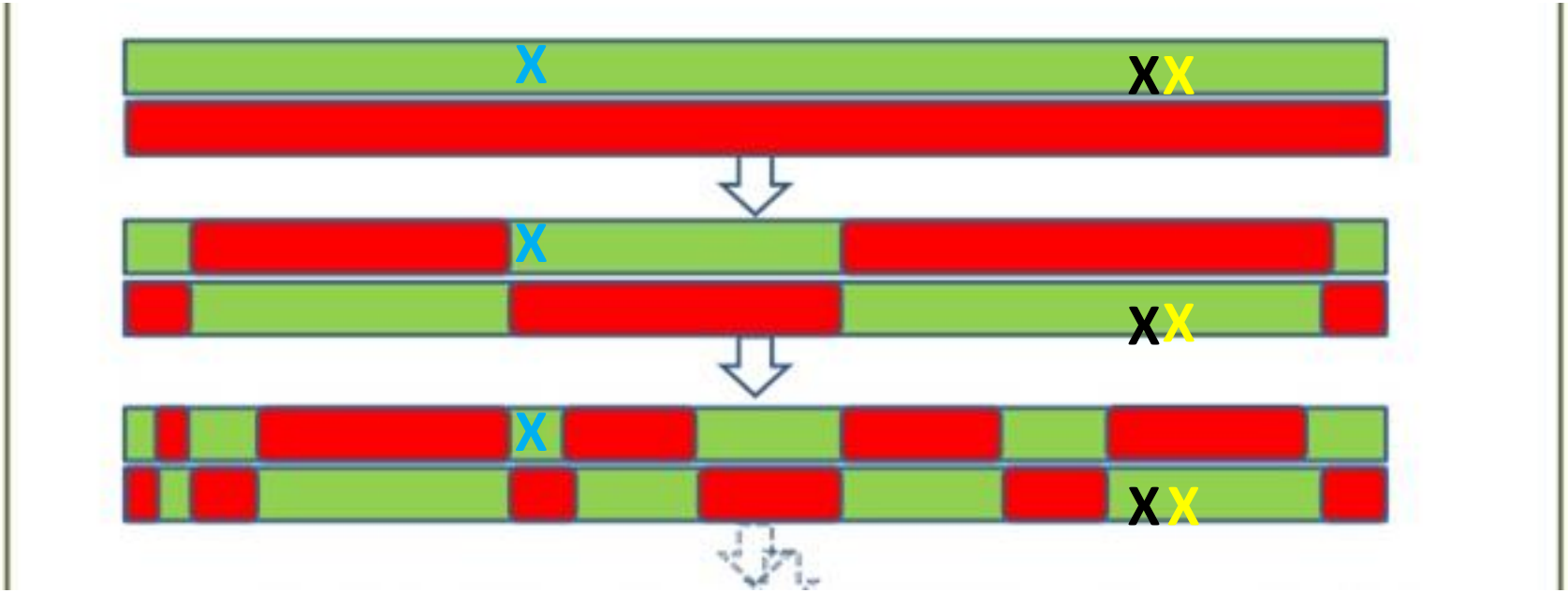
DNA inheritance breaks DNA into blocks

We inherit “blocks” of the genome from our parents (and not independent base-pairs)



The size of the blocks decreases with time

Recombination and linkage disequilibrium



How well can you tell me if the **X** is present if your chromosome also has the X?
How about if your chromosome has the X?

Linkage Disequilibrium

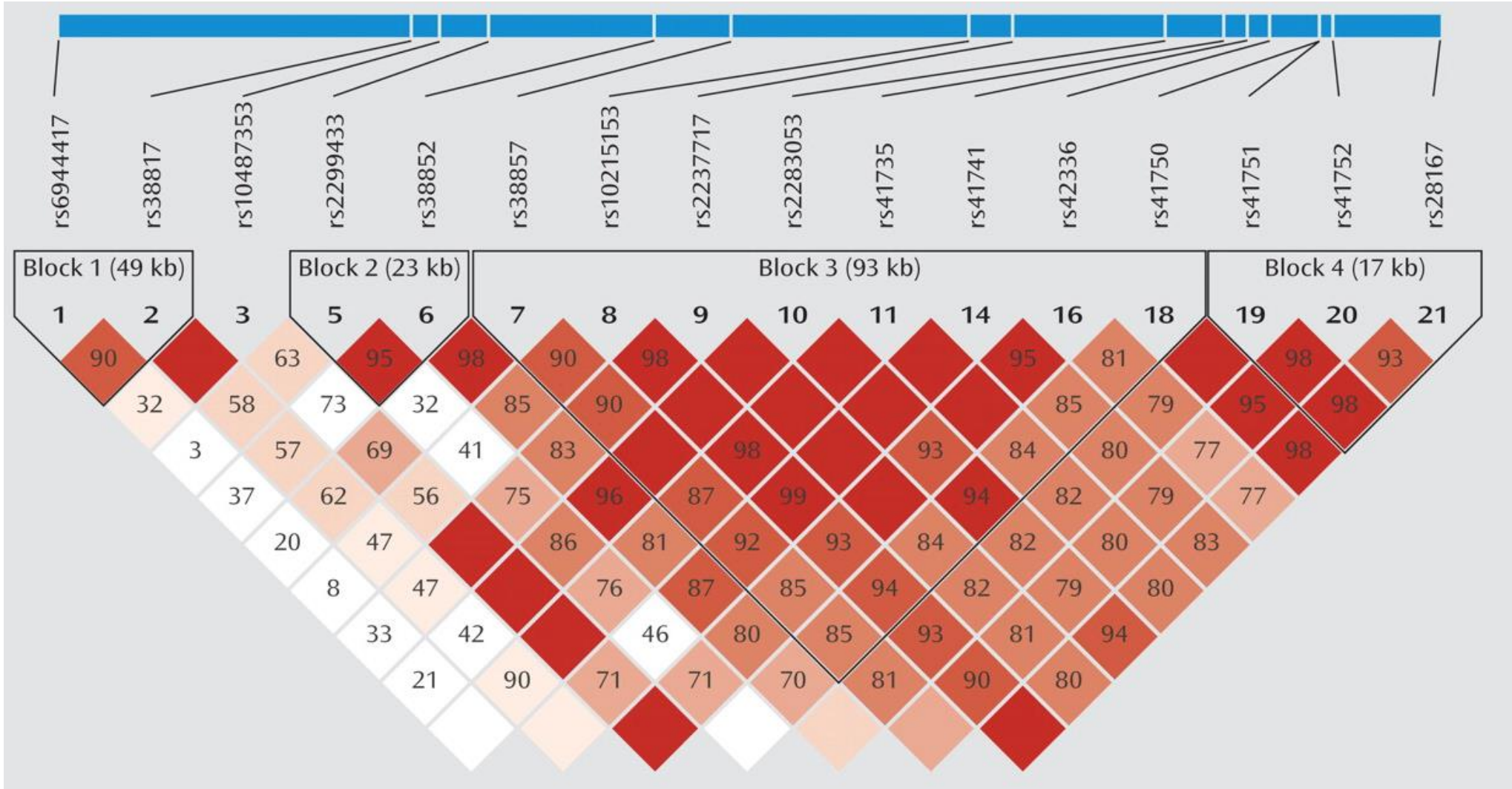
- Non-random association of alleles at different loci within a population
  - Alleles are found together more or less often than you would expect by chance if all alleles randomly and independently segregated during meiosis.
- Scores range from 0 (not in LD) to 1 (in complete LD).
- Two ways to measure LD: D’ and r2

- r^2 depends on allele frequencies. D’ is more difficult to compare across different allele frequencies. r^2 is used more often.
- How to calculate each in slides at the end of the slide deck PDF.

Factors that influence LD

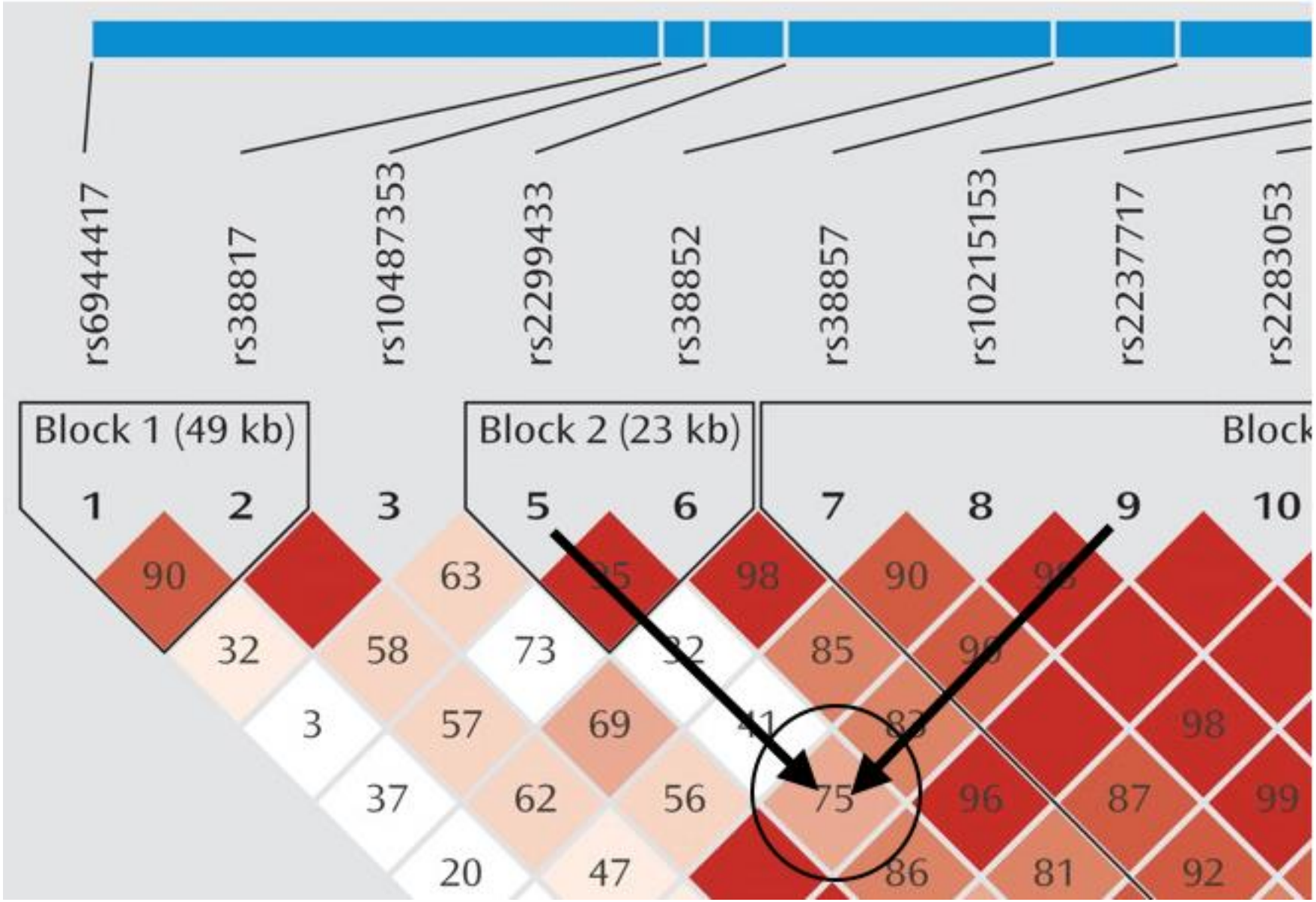
- New mutations
- Genetic drift
- Rapid population growth
- Admixture between populations
- Population structure – inbreeding
- Natural selection
  - Haplotypes that carry favorable mutations increase in frequency

Reading an LD map



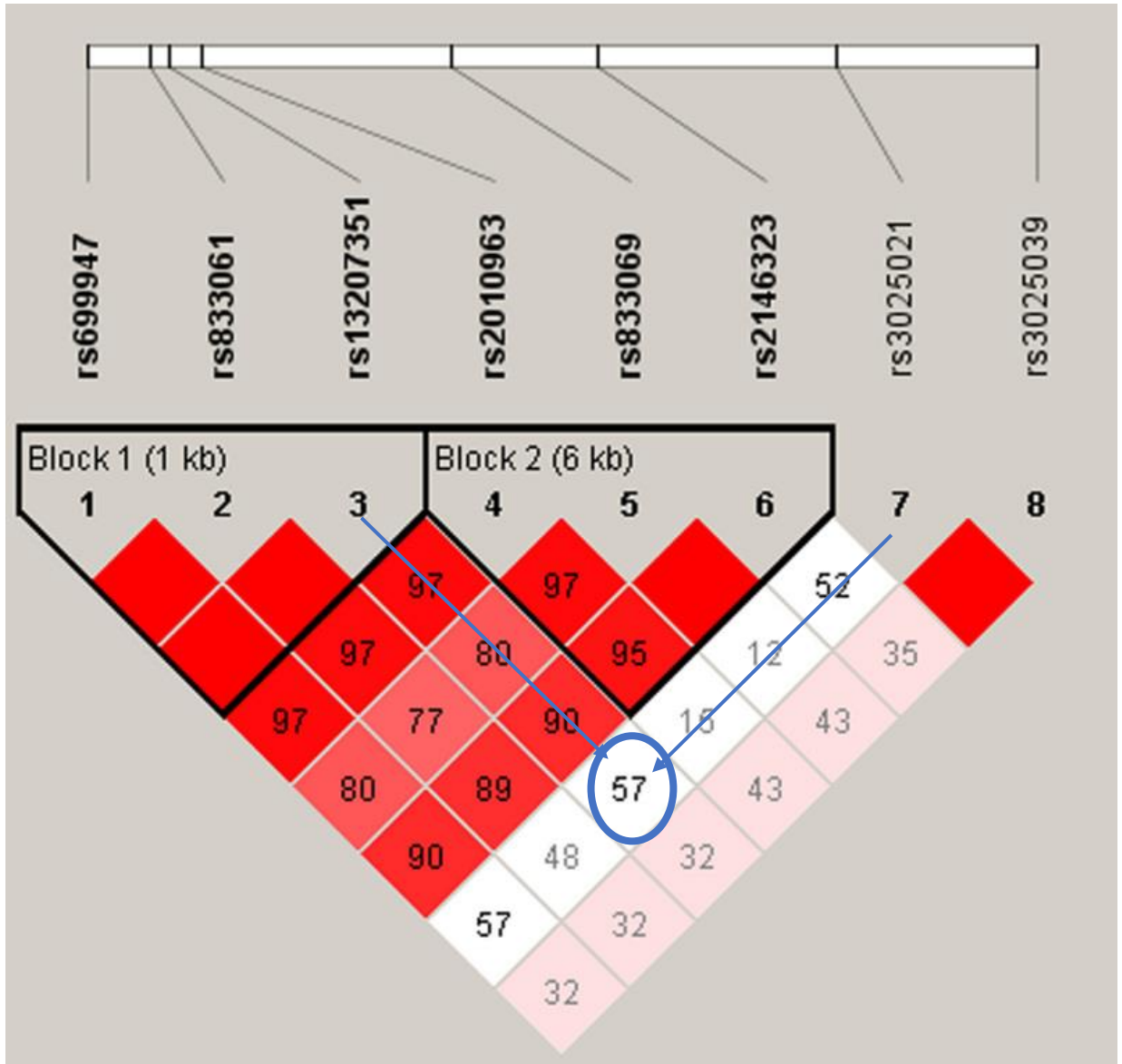
https://estrip.org/articles/read/tinypliny/44920/Linkage_Disequilibrium_Blocks_Triangles.html

Reading an LD map



What is the correlation between rs13207351 and rs3025021?

Zoom poll:



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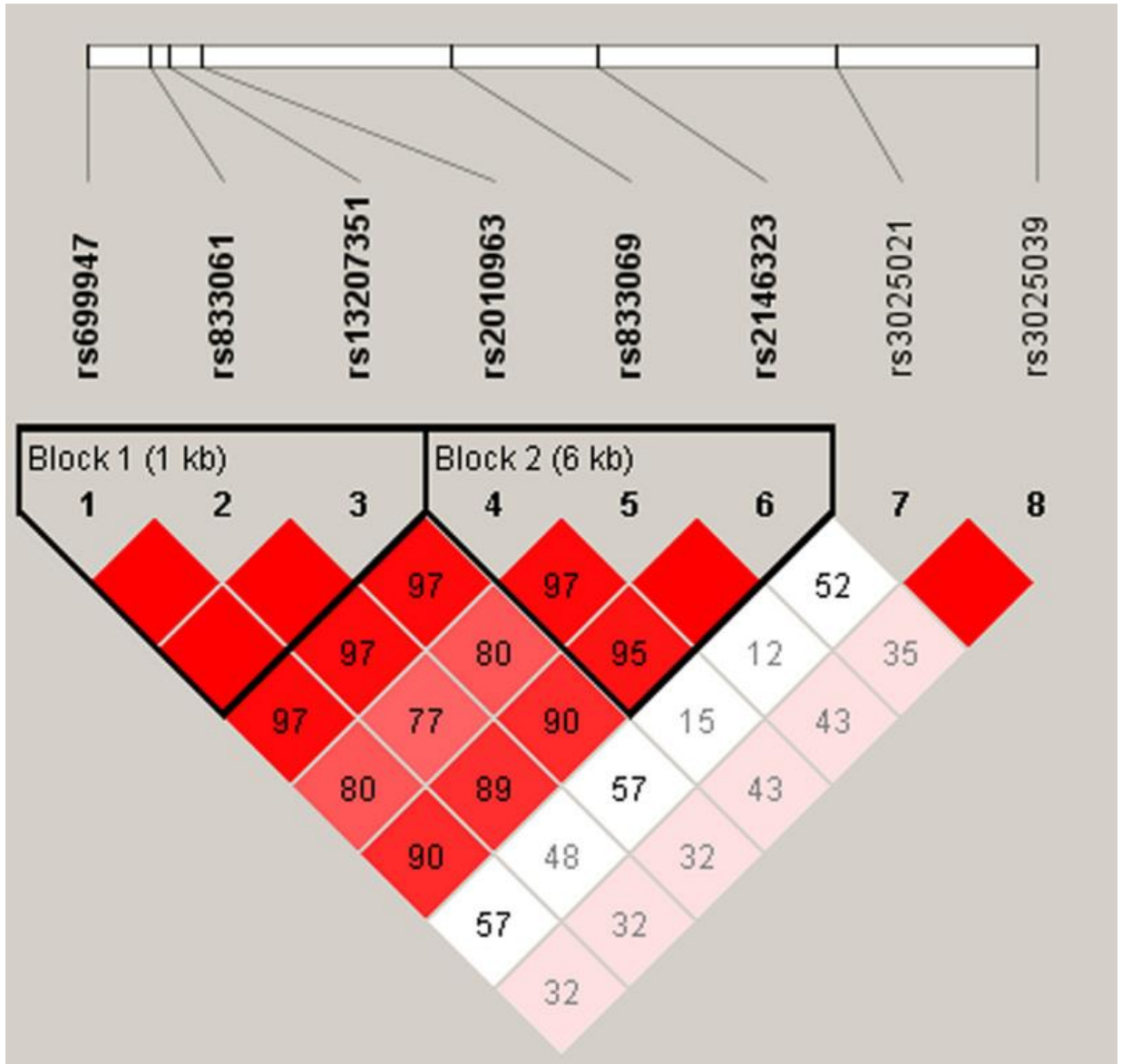

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Division of Cancer Epidemiology & Genetics

Home | LD Tools | API Access | Citations | Version History | Documentation

Welcome to LDlink

LDlink is a suite of web-based applications designed to easily and efficiently interrogate linkage disequilibrium in population groups. Each included application is specialized for querying and displaying unique aspects of linkage disequilibrium.

LDassoc

Interactively visualize association p-value results and linkage disequilibrium patterns for a genomic region of interest.

LDhap

Calculate population specific haplotype frequencies of all haplotypes observed for a list of query variants.

LDmatrix

Create an interactive heatmap matrix of pairwise linkage disequilibrium statistics.

LDpair

Investigate correlated alleles for a pair of variants in high LD.

LDpop

Investigate allele frequencies and linkage disequilibrium patterns across 1000G populations.

LDproxy

Interactively explore proxy and putatively functional variants for a query variant.

LDtrait

Search if a list of variants (or variants in LD with those variants) have previously been associated with a trait or disease.

SNPchip

Find commercial genotyping platforms for variants.

SNPclip

Prune a list of variants by linkage disequilibrium.

Navigate to ldlink.nci.nih.gov/?tab=home

- Click on “LDpop”
- Enter 2 snps: rs7412 and rs429358
- Select “all populations” , R^2
- Then ‘calculate’

Zoom breakout Q2 and Q3

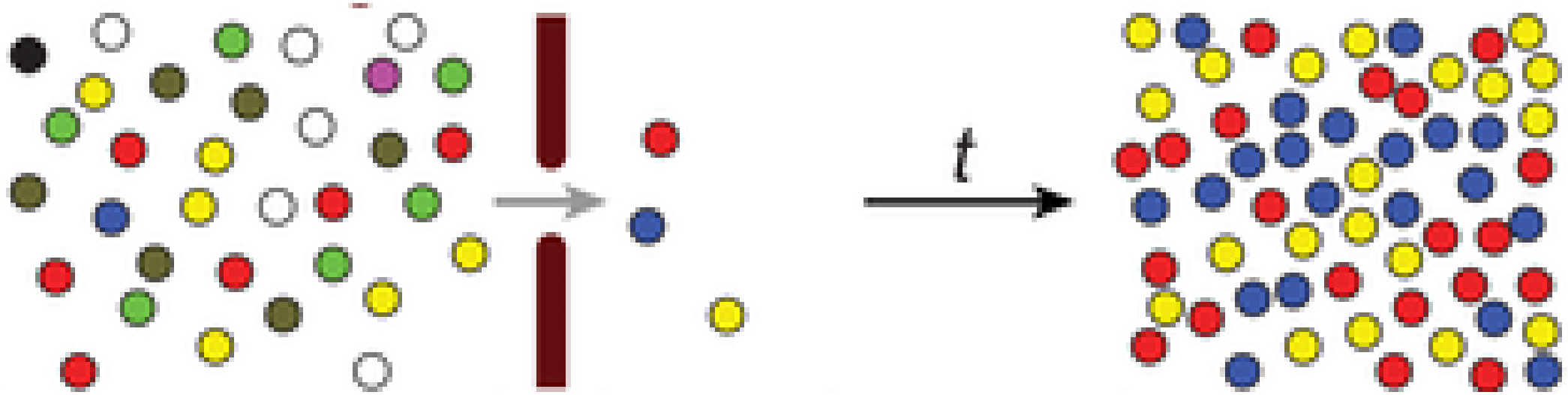
Benefit of LD in genetic studies?

- We don’t need to look at every location! Because of LD patterns, knowing an allele at one position can tell us about alleles are other positions. We can save $ and time by only looking at a subset.

Considerations of LD in genetic studies?

- We might not be able to tell if the SNP we are looking at actually CAUSES the phenotype, or if it is just in tight LD with something that does.
- Different populations have different LD patterns.

Founding populations reset LD



Abel, PLoS Pathogenics 2015

How does LD influence our study power? Misclassification

- If a SNP C and causal SNP G are in LD with **r^2**, then a study with N cases and controls which measures C (but not G) will have the same power to detect an association between C and disease as a study with **r^2 N** cases and controls that directly measured G.

- **r^2 N** is the “effective sample size”
  - If the r^2 between your measured SNP C and causal SNP G is 0.5 you need to double your sample size to obtain the same power as if you had measured (genotyped) G directly.

Pritchard and Przeworski (2001)

Summary

- Hardy Weinberg disequilibrium tests can indicate underlying population structure or selective pressure.
- Linkage disequilibrium is the nonrandom assortment of alleles in a genomic region.

Calculation of LD

Haplotypes frequencies if SNP1 (Aa) and SNP2 (Bb) are independent of each other. (This is called linkage equilibrium)

	SNP2 (Bb)		
SNP1 (Aa)	AB $p_{AB} = p_A p_B$	Ab $p_{Ab} = p_A p_b$	p_A
	aB $p_{aB} = p_a p_B$	ab $p_{ab} = p_a p_b$	p_a
	p_B	p_b	1

Calculation of LD

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	aB $p_{aB}= p_a p_B$	ab $p_{ab}= p_a p_b$	p_a
	p_B	p_b	1

What do we actually see?
We can infer LD as the deviation of observed haplotype frequency from its corresponding allele frequencies if SNP1 and SNP2 are independent of each other

	SNP2 (Bb)		
SNP1 (Aa)	AB $p_A p_B+D$	Ab $p_A p_b-D$	p_A
	aB $p_a p_B-D$	ab $p_a p_b+D$	p_a
	p_B	p_b	1

$$D=p_{AB}p_{ab}-p_{Ab}p_{aB}$$

Two measures of LD: D’ and r^2

$$D' = \frac{D}{D_{max}},$$

$$D_{max} = \begin{cases} max\{-p_A p_B, -(1-p_A)(1-p_B)\}, when\ D < 0 \\ min\{p_A(1-p_B), (1-p_A)p_B\}, when\ D > 0 \end{cases}$$

$$r^2 = \frac{D^2}{p_A p_a p_B p_b}$$

LD calculation exercise

SNPs rs6025 and rs4524 are both associated with venous thromboembolism (blood clot in a vein). The number of alleles for each SNP based on 503 individuals are displayed in the table below. Based on these numbers, calculate

a) **Frequencies of the four alleles (rs6025-C, rs6025-T, rs4524-G, rs4524-A)**
b) **Frequencies for the four haplotypes (C-G, C-A, T-G and T-A)**
c) **D' and r^2 between the two SNPs.**

Distribution of alleles for rs6025 and rs4524 across 503 individuals.

rs6025/rs4524	rs4524-G	rs4524-A	Total
rs6025-C	255	739	994
rs6025-T	0	12	12
Total	255	751	1006

Instead of D, we often express LD in terms of D’ (normalized D) or r^2 (correlation coefficient)

$$D' = \frac{D}{D_{max}},$$

$$D_{max} = \begin{cases} max\{-p_A p_B, -(1-p_A)(1-p_B)\}, when\ D < 0 \\ min\{p_A(1-p_B), (1-p_A)p_B\}, when\ D > 0 \end{cases}$$

$$r^2 = \frac{D^2}{p_A p_a p_B p_b}$$

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LD calculation exercise

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rs6025/rs4524	rs4524-G	rs4524-A	Total
rs6025-C	255	739	994
rs6025-T	0	12	12
Total	255	751	1006

rs6025/ rs4524	**G**	**A**		
C	**255**	**739**	**C=994**	**pC=0.988**
T	**0**	**12**	**T=12**	**pT=0.012**
	G=255	**A=751**	**1006**	**1**
	pG=0.253	**pA=0.747**	**1**	

LD calculation exercise

b) Frequencies for the four haplotypes (C-G, C-A, T-G and T-A)

rs6025/rs4524	rs4524-G	rs4524-A	Total
rs6025-C	255	739	994
rs6025-T	0	12	12
Total	255	751	1006

rs6025/rs4524	**G**	**A**
C	**pCG=255/1006=0.253**	**pCA=739/1006=0.735**
T	**pTG=0**	**pTA=12/1006=0.0119**

LD calculation exercise

c) D’ and r^2 between the two SNPs.

D=pCG*pTA-pCA*pTG
=0.253*0.0119-0.735*0
=0.0030

D’=D/Dmax
=0.003/min{0.253*(1-0.988), (1-0.253)*0.998)
=0.003/min{0.003, 0.746}
=0.003/0.003
=1

$r^2=D^2/(p_{rs6025\text{-}C}*p_{rs6025\text{-}T}*p_{rs4524\text{-}G}*p_{rs4524\text{-}A)}$
$=0.003^2/(0.988*0.012*0.253*0.747)$
$=9.217x10^{-6}/0.0022$
=0.0041