

# Population Genetic Data Analysis

Summer Institute in Statistical Genetics  
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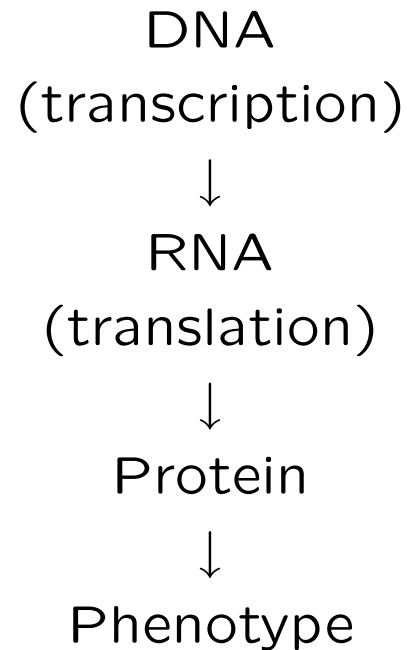
Lectures on these topics by Bruce Weir and David Balding will alternate with R exercises led by Jérôme Goudet.

The R material is at <http://www2.unil.ch/popgen/teaching/SISG16/>

# GENETIC DATA

# Central Dogma of Genetics

“Genes are perpetuated as sequences of nucleic acid, but function by being expressed as proteins.” (Lewin, *GENES*).



# Sources of Data

Phenotype Mendel's peas  
Blood groups

Protein Allozymes  
Amino acid sequences

DNA Restriction sites, RFLPs  
Length variants, VNTRs, STRs  
SNPs  
Nucleotide sequences

# Mendel's Data

Dominant Form		Recessive Form	
Seed characters			
5474	Round	1850	Wrinkled
6022	Yellow	2001	Green
Plant characters			
705	Grey-brown	224	White
882	Simply inflated	299	Constricted
428	Green	152	Yellow
651	Axial	207	Terminal
787	Long	277	Short

## Genetic Data

Human ABO blood groups discovered in 1900.

Elaborate mathematical theories constructed by Sewall Wright, R.A. Fisher, J.B.S. Haldane and others. This theory was challenged by data from new data from electrophoretic methods in the 1960's:

“For many years population genetics was an immensely rich and powerful theory with virtually no suitable facts on which to operate. ... Quite suddenly the situation has changed. The motherlode has been tapped and facts in profusion have been pored into the hoppers of this theory machine. ... The entire relationship between the theory and the facts needs to be reconsidered.”

Lewontin RC. 1974. The Genetic Basis of Evolutionary Change. Columbia University Press.

## STR markers: CTT set

([http://www.cstl.nist.gov/biotech/strbase/seq\\_info.htm](http://www.cstl.nist.gov/biotech/strbase/seq_info.htm))

Locus	Structure	Chromosome	Usual No. of repeats
CSF1PO	[AGAT] <sub>n</sub>	5q	6–16
TPOX	[AATG] <sub>n</sub>	2p	5–14
TH01*	[AATG] <sub>n</sub>	11p	3–14

\* “9.3” is [AATG]<sub>6</sub>ATG[AATG]<sub>3</sub>

Length variants detected by capillary electrophoresis.



# “CTT” Data - Forensic Frequency Database

CSF1P0		TPOX		TH01	
11	12	8	11	7	8
11	13	8	8	6	7
11	12	8	11	6	7
10	12	8	8	6	9
11	12	8	12	9	9.3
10	12	9	11	6	7
10	13	8	11	6	6
11	12	8	8	6	9.3
9	10	8	9	7	9.3
11	12	8	8	6	8
11	13	8	11	7	9
11	12	8	11	6	9.3
10	11	8	8	7	9.3
10	10	8	11	7	9.3
9	10	8	8	6	9.3
11	12	9	11	9	9.3
9	11	9	11	9	9.3
11	12	8	8	6	7
10	10	9	11	6	9.3
10	13	8	8	8	9.3

## Sequencing of STR Alleles

“STR typing in forensic genetics has been performed traditionally using capillary electrophoresis (CE). Massively parallel sequencing (MPS) has been considered a viable technology in recent years allowing high-throughput coverage at a relatively affordable price. Some of the CE-based limitations may be overcome with the application of MPS ... generate reliable STR profiles at a sensitivity level that competes with current widely used CE-based method.”

Zeng XP, King JL, Stoljarova M, Warshauer DH, LaRue BL, Sajtanta A, Patel J, Storts DR, Budowle B. 2015. High sensitivity multiplex short tandem repeat loci analyses with massively parallel sequencing. *Forensic Science International: Genetics* 16:38-47.

## Single Nucleotide Polymorphisms (SNPs)

“Single nucleotide polymorphisms (SNPs) are the most frequently occurring genetic variation in the human genome, with the total number of SNPs reported in public SNP databases currently exceeding 9 million. SNPs are important markers in many studies that link sequence variations to phenotypic changes; such studies are expected to advance the understanding of human physiology and elucidate the molecular bases of diseases. For this reason, over the past several years a great deal of effort has been devoted to developing accurate, rapid, and cost-effective technologies for SNP analysis, yielding a large number of distinct approaches. ”

Kim S. Misra A. 2007. SNP genotyping: technologies and biomedical applications. *Annu Rev Biomed Eng.* 2007;9:289-320.

# AMD SNP Data

SNP	Individual														
rs6424140	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
rs1496555	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2
rs1338382	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
rs10492936	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
rs10489589	3	1	1	1	2	2	1	2	1	1	1	3	1	1	1
rs10489588	3	1	1	1	2	2	1	2	1	1	1	3	1	1	1
rs4472706	1	3	3	3	2	2	3	2	3	3	3	1	3	3	3
rs4587514	3	3	3	3	3	2	2	3	2	2	2	3	3	1	3
rs10492941	3	3	3	3	3	3	3	3	2	3	3	2	3	3	1
rs1112213	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
rs4648462	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
rs2455122	2	1	1	0	1	2	1	1	1	1	1	1	1	1	2
rs2455124	2	1	1	2	1	2	1	1	1	1	1	1	1	1	2
rs10492940	2	1	1	1	1	2	1	2	1	1	1	2	1	1	2
rs10492939	1	2	1	1	1	1	3	2	1	2	3	2	2	1	1
rs10492938	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
rs10492937	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3
rs7546189	1	2	3	3	1	3	2	2	3	3	2	2	2	2	2
rs1128474	3	2	3	2	3	3	2	3	3	3	3	3	2	1	3

Genotype key: 0 –; 1 AA; 2 AB; 3 BB.

## Phase 3 1000Genomes Data

- 84.4 million variants
- 2504 individuals
- 26 population

[www.1000Genomes.org](http://www.1000Genomes.org)

# Whole-genome Sequence Studies

One current study is the NHLBI Trans-Omics for Precision Medicine (TOPMed) project. [www.nhlbiwgs.org](http://www.nhlbiwgs.org)

In the first data freeze of Phase 1 of this study:

Description	Variants Passing Filters
Total Number of SNPs	86,974,704
Singletons	35,883,567
% Singletons	41.3%
Nonsynonymous	599,883
Singletons	305,479
% Singletons	50.9%
Stop Gains	13,436
Singletons	8,067
% Singletons	60.0%
# in dbSNP (142)	43,141,344
% in dbSNP	49.6%

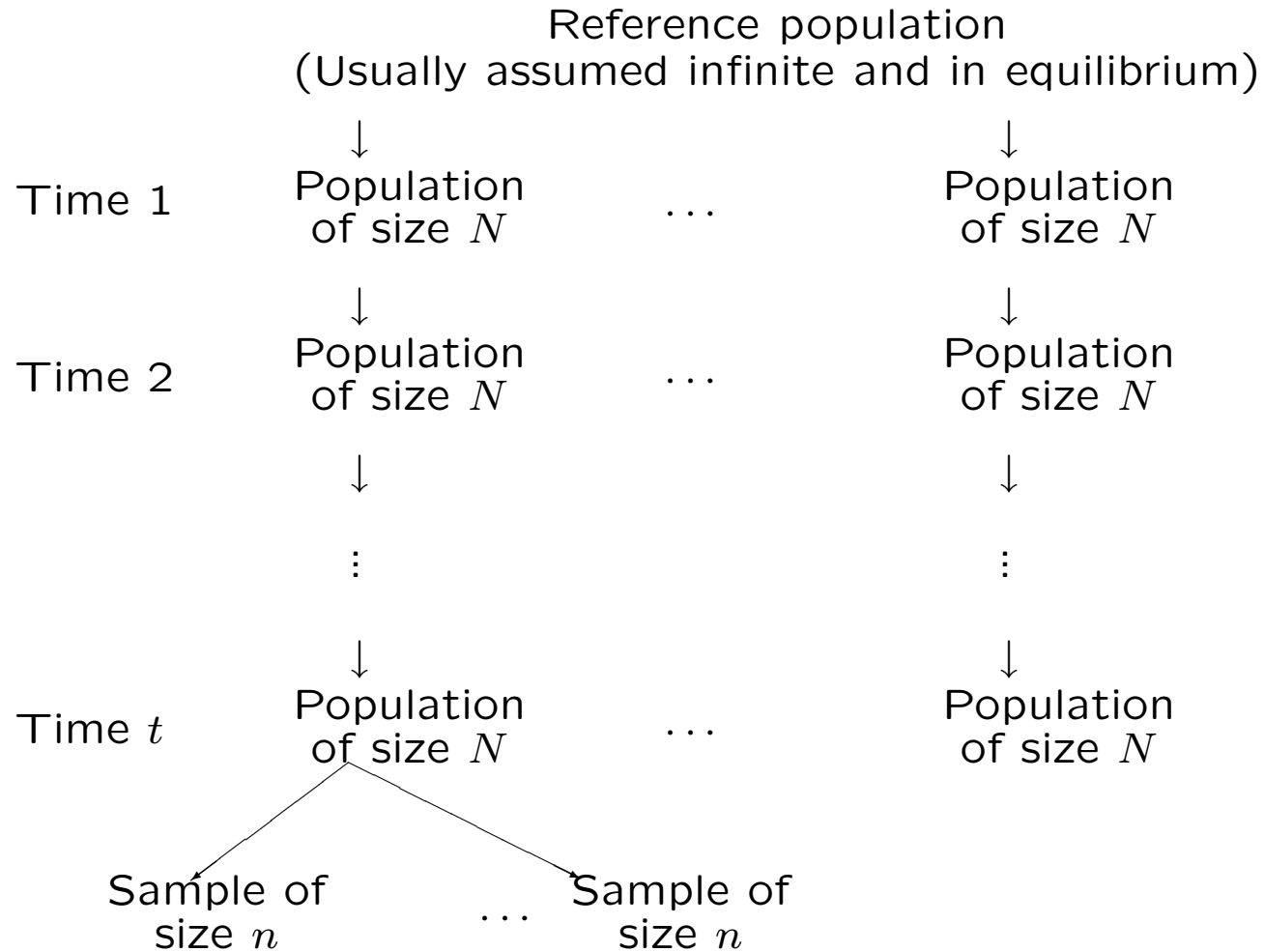
Abecasis et al. 2016. ASHG Poster

# Sampling

Statistical sampling: The variation among repeated samples from the same population is analogous to “fixed” sampling. Inferences can be made about that particular population.

Genetic sampling: The variation among replicate (conceptual) populations is analogous to “random” sampling. Inferences are made to all populations with the same history.

# Classical Model





## Coalescent Theory

An alternative framework works with genealogical history of a sample of alleles. There is a tree linking all alleles in a current sample to the “most recent common ancestral allele.” Allelic variation due to mutations since that ancestral allele.

The coalescent approach requires mutation and may be more appropriate for long-term evolution and analyses involving more than one species. The classical approach allows mutation but does not require it: within one species variation among populations may be due primarily to drift.

# Probability

Probability provides the language of data analysis.

*Equiprobable outcomes definition:*

Probability of event  $E$  is number of outcomes favorable to  $E$  divided by the total number of outcomes. e.g. Probability of a head =  $1/2$ .

*Long-run frequency definition:*

If event  $E$  occurs  $n$  times in  $N$  identical experiments, the probability of  $E$  is the limit of  $n/N$  as  $N$  goes to infinity.

*Subjective probability:*

Probability is a measure of belief.

## First Law of Probability

Law says that probability can take values only in the range zero to one and that an event which is certain has probability one.

$$\begin{cases} 0 \leq \Pr(E) \leq 1 \\ \Pr(E|E) = 1 \text{ for any } E \end{cases}$$

i.e. If event  $E$  is true, then it has a probability of 1. For example:

$$\Pr(\text{Seed is Round}|\text{Seed is Round}) = 1$$

## Second Law of Probability

If  $G$  and  $H$  are mutually exclusive events, then:

$$\Pr(G \text{ or } H) = \Pr(G) + \Pr(H)$$

For example,

$$\Pr(\text{Round or Wrinkled}) = \Pr(\text{Round}) + \Pr(\text{Wrinkled})$$

More generally, if  $E_i, i = 1, \dots, r$ , are mutually exclusive then

$$\begin{aligned} \Pr(E_1 \text{ or } \dots \text{ or } E_r) &= \Pr(E_1) + \dots + \Pr(E_r) \\ &= \sum_i \Pr(E_i) \end{aligned}$$

## Complementary Probability

If  $\Pr(E)$  is the probability that  $E$  is true then  $\Pr(\bar{E})$  denotes the probability that  $E$  is false. Because these two events are mutually exclusive

$$\Pr(E \text{ or } \bar{E}) = \Pr(E) + \Pr(\bar{E})$$

and they are also exhaustive in that between them they cover all possibilities – one or other of them must be true. So,

$$\begin{aligned}\Pr(E) + \Pr(\bar{E}) &= 1 \\ \Pr(\bar{E}) &= 1 - \Pr(E)\end{aligned}$$

The probability that  $E$  is false is one minus the probability it is true.

## Third Law of Probability

For any two events,  $G$  and  $H$ , the third law can be written:

$$\Pr(G \text{ and } H) = \Pr(G) \Pr(H|G)$$

There is no reason why  $G$  should precede  $H$  and the law can also be written:

$$\Pr(G \text{ and } H) = \Pr(H) \Pr(G|H)$$

For example

$$\begin{aligned} \Pr(\text{Seed is round \& is type AA}) &= \Pr(\text{Seed is round} | \text{Seed is type AA}) \\ &\quad \times \Pr(\text{Seed is type AA}) \\ &= 1 \times p_A^2 \end{aligned}$$

## Independent Events

If the information that  $H$  is true does nothing to change uncertainty about  $G$ , then

$$\Pr(G|H) = \Pr(G)$$

and

$$\Pr(H \text{ and } G) = \Pr(H) \Pr(G)$$

Events  $G, H$  are independent.

## Law of Total Probability

If  $G, H$  are two mutually exclusive and exhaustive events (so that  $H = \bar{G} = \text{not } - G$ ), then for any other event  $E$ , the law of total probability states that

$$\Pr(E) = \Pr(E|G) \Pr(G) + \Pr(E|H) \Pr(H)$$

This generalizes to any set of mutually exclusive and exhaustive events  $\{S_i\}$ :

$$\Pr(E) = \sum_i \Pr(E|S_i) \Pr(S_i)$$

For example

$$\begin{aligned} \Pr(\text{Seed is round}) &= \Pr(\text{Round}|\text{Type AA}) \Pr(\text{Type AA}) \\ &\quad + \Pr(\text{Round}|\text{Type Aa}) \Pr(\text{Type Aa}) \\ &\quad + \Pr(\text{Round}|\text{Type aa}) \Pr(\text{Type aa}) \\ &= 1 \times p_A^2 + 1 \times 2p_A p_a + 0 \times p_a^2 = p_A(1 + p_A) \end{aligned}$$



# Bayes' Theorem

Bayes' theorem relates  $\Pr(G|H)$  to  $\Pr(H|G)$ :

$$\begin{aligned}\Pr(G|H) &= \frac{\Pr(GH)}{\Pr(H)}, \text{ from third law} \\ &= \frac{\Pr(H|G) \Pr(G)}{\Pr(H)}, \text{ from third law}\end{aligned}$$

If  $\{G_i\}$  are exhaustive and mutually exclusive, Bayes' theorem can be written as

$$\Pr(G_i|H) = \frac{\Pr(H|G_i) \Pr(G_i)}{\sum_i \Pr(H|G_i) \Pr(G_i)}$$

## Bayes' Theorem Example

Suppose  $G$  is event that a man has genotype  $A_1A_2$  and  $H$  is the event that he transmits allele  $A_1$  to his child. Then  $\Pr(H|G) = 0.5$ .

Now what is the probability that a man has genotype  $A_1A_2$  given that he transmits allele  $A_1$  to his child?

$$\begin{aligned}\Pr(G|H) &= \frac{\Pr(H|G) \Pr(G)}{\Pr(H)} \\ &= \frac{0.5 \times 2p_1p_2}{p_1} = p_2\end{aligned}$$

## Mendel's Data

Model: seed shape governed by gene **A** with alleles  $A, a$ :

Genotype	Phenotype
$AA$	Round
$Aa$	Round
$aa$	Wrinkled

Cross two inbred lines:  $AA$  and  $aa$ . All offspring ( $F_1$  generation) are  $Aa$ , and so have round seeds.

## $F_2$ generation

Self an  $F_1$  plant: each allele it transmits is equally likely to be  $A$  or  $a$ , and alleles are independent, so for  $F_2$  generation:

$$\Pr(AA) = \Pr(A) \Pr(A) = 0.25$$

$$\Pr(Aa) = \Pr(A) \Pr(a) + \Pr(a) \Pr(A) = 0.5$$

$$\Pr(aa) = \Pr(a) \Pr(a) = 0.25$$

Probability that an  $F_2$  seed (observed on  $F_1$  parental plant) is round:

$$\begin{aligned} \Pr(\text{Round}) &= \Pr(\text{Round}|AA)\Pr(AA) \\ &\quad + \Pr(\text{Round}|Aa)\Pr(Aa) \\ &\quad + \Pr(\text{Round}|aa)\Pr(aa) \\ &= 1 \times 0.25 + 1 \times 0.5 + 0 \times 0.25 \\ &= 0.75 \end{aligned}$$

## $F_2$ generation

What are the proportions of  $AA$  and  $Aa$  among  $F_2$  plants with round seeds? From Bayes' Theorem:

$$\begin{aligned}\Pr(F_2 = AA|F_2 \text{ Round}) &= \frac{\Pr(F_2 \text{ Round}|AA) \Pr(F_2 AA)}{\Pr(F_2 \text{ round})} \\ &= \frac{1 \times \frac{1}{4}}{\frac{3}{4}} \\ &= \frac{1}{3}\end{aligned}$$

## Seed Characters

As an experimental check on this last result, and therefore on Mendel's theory, Mendel selfed a round-seeded  $F_2$  plant and noted the  $F_3$  seed shape (observed on the  $F_2$  parental plant).

If all the  $F_3$  seeds are round, the  $F_2$  must have been  $AA$ . If some  $F_3$  seeds are round and some are wrinkled, the  $F_2$  must have been  $Aa$ . Possible to observe many  $F_3$  seeds for an  $F_2$  parental plant, so no doubt that all seeds were round. Data supported theory: one-third of  $F_2$  plants gave only round seeds and so must have had genotype  $AA$ .

# Plant Characters

Model for stem length is

Genotype	Phenotype
$GG$	Long
$Gg$	Long
$gg$	Short

To check this model it is necessary to grow the  $F_3$  seed to observe the  $F_3$  stem length.

## $F_2$ Plant Character

Mendel grew only 10  $F_3$  seeds per  $F_2$  parent. If all 10 seeds gave long stems, he concluded they were all  $GG$ , and  $F_2$  parent was  $GG$ . This could be wrong. The probability of a  $Gg$   $F_2$  plant giving 10 long-stemmed  $F_3$  offspring ( $GG$  or  $Gg$ ), and therefore wrongly declared to be homozygous  $GG$  is  $(3/4)^{10} = 0.0563$ .



## Fisher's 1936 Criticism

The probability that a long-stemmed  $F_2$  plant is declared to be homozygous (event  $V$ ) is

$$\begin{aligned}\Pr(V) &= \Pr(V|U) \Pr(U) + \Pr(V|\bar{U}) \Pr(\bar{U}) \\ &= 1 \times (1/3) + 0.0563 \times (2/3) \\ &= 0.3709 \neq 0.3333\end{aligned}$$

where  $U$  is the event that a long-stemmed  $F_2$  is actually homozygous and  $\bar{U}$  is the event that it is actually heterozygous.

Fisher claimed Mendel's data closer to the 0.3333 probability appropriate for seed shape than to the correct 0.3709 value. Mendel's experiments were "a carefully planned demonstration of his conclusions."

## Weldon's 1902 Doubts

In *Biometrika*, Weldon said:

“Here are seven determinations of a frequency which is said to obey the law of Chance. Only one determination has a deviation from the hypothetical frequency greater than the probable error of the determination, and one has a deviation sensible equal to the probable error; so that a discrepancy between the hypothesis and the observations which is equal to or greater than the probable error occurs twice out of seven times, and deviations much greater than the probable error do not occur at all. These results then accord so remarkably with Mendel's summary of them that if they were repeated a second time, under similar conditions and on a similar scale, the chance that the agreement between observation and hypothesis would be worse than that actually obtained is about 16 to 1.”

“Run Mendel's experiments again at the same scale, Weldon reckoned, and the chance of getting worse results is 16 to 1.”  
Radick, *Science* 350:159-160, 2015.

## Edwards' 1986 Criticism

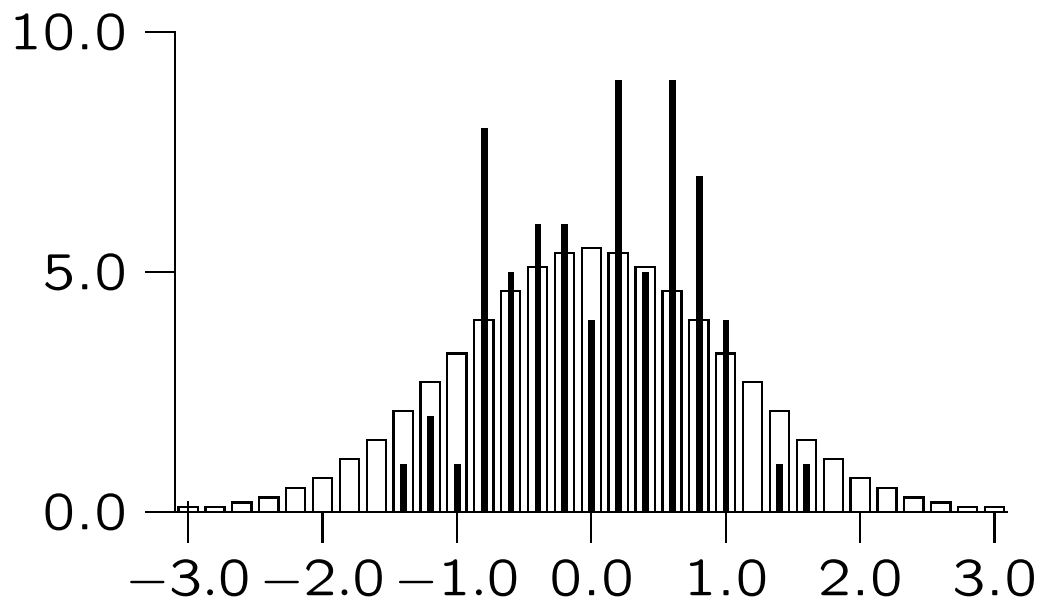
Mendel had 69 comparisons where the expected ratios were correct. Each set of data can be tested with a chi-square test:

		Category 1	Category 2	Total
Observed	(o)	a	n-a	n
Expected	(e)	b	n-b	n

$$\begin{aligned} X^2 &= \frac{(a - b)^2}{b} + \frac{[(n - a) - (n - b)]^2}{(n - b)} \\ &= \frac{n(a - b)^2}{b(n - b)} \end{aligned}$$

## Edwards' Criticism

If the hypothesis giving the expected values is true, the  $X^2$  values follow a chi-square distribution, and the  $X$  values follow a normal distribution. Edwards claimed Mendel's values were too small – not as many large values as would be expected by chance.



## Recent Discussions

Pires AM, Branco JA. 2010. A statistical model to explain the Mendel-Fisher controversy. *Statistical Science* 15:545-565, 2010.

Reid JB, Ross JJ. 2011. Mendel's genes: Toward a full molecular characterization. *Genetics* 189:3-10.

Franklin A, Edwards AWF, Fairbanks DJ, Hartl DL, Seidenfeld T. "Ending the Mendel-Fisher Controversy." University of Pittsburgh Press, Pittsburgh.

Smith MU, Gericke NM. 2015. Mendel in the modern classroom. *Science and Education* 24:151-172.

Radick G. 2015. Beyond the "Mendel-Fisher controversy." *Science* 350:159-160.

# ALLELE FREQUENCIES

# Properties of Estimators

Consistency	Increasing accuracy as sample size increases
Unbiasedness	Expected value is the parameter
Efficiency	Smallest variance
Sufficiency	Contains all the information in the data about parameter

## Binomial Distribution

Most population genetic data consists of numbers of observations in some categories. The values and frequencies of these counts form a *distribution*.

Toss a coin  $n$  times, and note the number of heads. There are  $(n + 1)$  outcomes, and the number of times each outcome is observed in many sets of  $n$  tosses gives the sampling distribution. Or: sample  $n$  alleles from a population and observe  $x$  copies of type  $A$ .



## Binomial distribution

If every toss has the same chance  $p$  of giving a head:

Probability of  $x$  heads in a row is

$$p \times p \times \dots \times p = p^x$$

Probability of  $n - x$  tails in a row is

$$(1 - p) \times (1 - p) \times \dots \times (1 - p) = (1 - p)^{n-x}$$

The number of ways of ordering  $x$  heads and  $n - x$  tails among  $n$  outcomes is  $n!/[x!(n - x)!]$ .

The binomial probability of  $x$  successes in  $n$  trials is

$$\Pr(x|p) = \frac{n!}{x!(n - x)!} p^x (1 - p)^{n-x}$$

## Binomial Likelihood

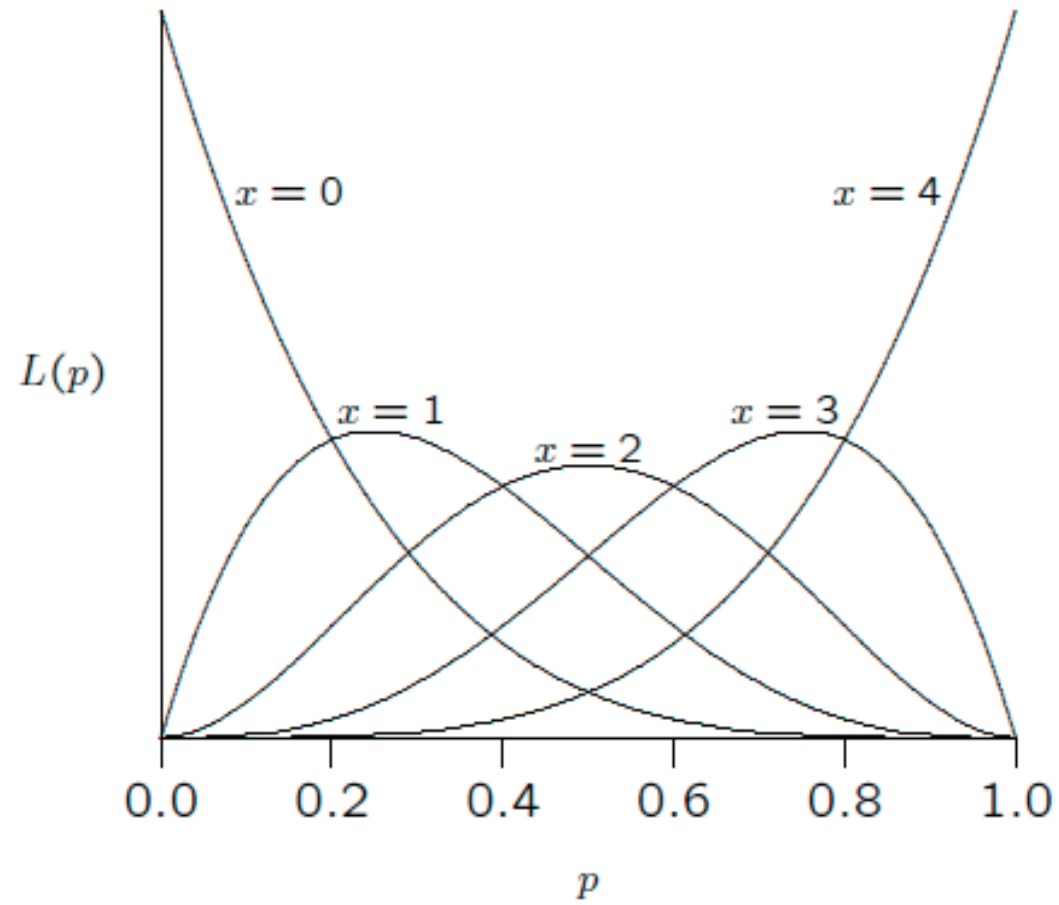
The quantity  $\Pr(x|p)$  is the *probability of the data*,  $x$  successes in  $n$  trials, when each trial has probability  $p$  of success.

The same quantity, written as  $L(p|x)$ , is the *likelihood of the parameter*,  $p$ , when the value  $x$  has been observed. The terms that do not involve  $p$  are not needed, so

$$L(p|x) \propto p^x (1 - p)^{(n-x)}$$

Each value of  $x$  gives a different likelihood curve, and each curve points to a  $p$  value with maximum likelihood. This leads to *maximum likelihood estimation*.

# Likelihood $L(p|x, n = 4)$



## Binomial Mean

If there are  $n$  trials, each of which has probability  $p$  of giving a success, the *mean* or the *expected number* of successes is  $np$ .

The *sample proportion* of successes is

$$\tilde{p} = \frac{x}{n}$$

(This is also the maximum likelihood estimate of  $p$ .)

The expected, or *mean*, value of  $\tilde{p}$  is  $p$ .

$$\mathcal{E}(\tilde{p}) = p$$

## Binomial Variance

The expected value of the squared difference between the number of successes and its mean,  $(x - np)^2$ , is  $np(1 - p)$ . This is the *variance* of the number of successes in  $n$  trials, and indicates the spread of the distribution.

The variance of the sample proportion  $\tilde{p}$  is

$$\text{Var}(\tilde{p}) = \frac{p(1 - p)}{n}$$

## Normal Approximation

Provided  $np$  is not too small (e.g. not less than 5), the binomial distribution can be approximated by the normal distribution with the same mean and variance. In particular:

$$\tilde{p} \sim N\left(p, \frac{p(1-p)}{n}\right)$$

To use the normal distribution in practice, change to the *standard normal* variable  $z$  with a mean of 0, and a variance of 1:

$$z = \frac{\tilde{p} - p}{\sqrt{p(1-p)/n}}$$

For a standard normal, 95% of the values lie between  $\pm 1.96$ . The normal approximation to the binomial therefore implies that 95% of the values of  $\tilde{p}$  lie in the range

$$p \pm 1.96\sqrt{p(1-p)/n}$$

# Confidence Intervals

A 95% confidence interval is a variable quantity. It has endpoints which vary with the sample. Expect that 95% of samples will lead to an interval that includes the unknown true value  $p_c$ .

The standard normal variable  $z$  has 95% of its values between  $-1.96$  and  $+1.96$ . This suggests that a 95% confidence interval for the binomial parameter  $p$  is

$$\tilde{p} \pm 1.96 \sqrt{\frac{\tilde{p}(1 - \tilde{p})}{n}}$$

# Confidence Intervals

For samples of size 10, the 11 possible confidence intervals are:

$\tilde{p}_c$	Confidence Interval	
0.0	$0.0 \pm 0.00$	0.00, 0.00
0.1	$0.1 \pm 2\sqrt{0.009}$	0.00, 0.29
0.2	$0.2 \pm 2\sqrt{0.016}$	0.00, 0.45
0.3	$0.3 \pm 2\sqrt{0.021}$	0.02, 0.58
0.4	$0.4 \pm 2\sqrt{0.024}$	0.10, 0.70
0.5	$0.5 \pm 2\sqrt{0.025}$	0.19, 0.81
0.6	$0.6 \pm 2\sqrt{0.024}$	0.30, 0.90
0.7	$0.7 \pm 2\sqrt{0.021}$	0.42, 0.98
0.8	$0.8 \pm 2\sqrt{0.016}$	0.55, 1.00
0.9	$0.9 \pm 2\sqrt{0.009}$	0.71, 1.00
1.0	$1.0 \pm 0.00$	1.00, 1.00

Can modify interval a little by extending it by the “continuity correction”  $\pm 1/2n$  in each direction.



## Confidence Intervals

To be 95% sure that the estimate is no more than 0.01 from the true value,  $1.96\sqrt{p(1-p)/n}$  should be less than 0.01. The widest confidence interval is when  $p = 0.5$ , and then need

$$0.01 \geq 1.96\sqrt{0.5 \times 0.5/n}$$

which means that  $n \geq 10,000$ . For a width of 0.03 instead of 0.01,  $n \approx 1,000$ .

If the true value of  $p$  was about 0.05, however,

$$\begin{aligned} 0.01 &\geq 2\sqrt{0.05 \times 0.95/n} \\ n &\geq 1,900 \approx 2,000 \end{aligned}$$

## Exact Confidence Intervals: One-sided

The normal-based confidence intervals are constructed to be symmetric about the sample value, unless the interval goes outside the interval from 0 to 1. They are therefore less satisfactory the closer the true value is to 0 or 1.

More accurate confidence limits follow from the binomial distribution exactly. For events with low probabilities  $p$ , how large could  $p$  be for there to be at least a 5% chance of seeing no more than  $x$  (i.e.  $0, 1, 2, \dots, x$ ) occurrences of that event among  $n$  events. If this upper bound is  $p_U$ ,

$$\sum_{k=0}^x \Pr(k) \geq 0.05$$

$$\sum_{k=0}^x \binom{n}{k} p_U^k (1 - p_U)^{n-k} \geq 0.05$$

If  $x = 0$ , then  $(1 - p_U)^n \geq 0.05$  or  $p_U \leq 1 - 0.05^{1/n}$  and this is 0.0295 if  $n = 100$ . More generally  $p_U \approx 3/n$  when  $x = 0$ .

## Exact Confidence Intervals: Two-sided

Now want to know how large  $p$  could be for there to be at least a 2.5% chance of seeing no more than  $x$  (i.e.  $0, 1, 2, \dots, x$ ) occurrences, and in knowing how small  $p$  could be for there to be at least a 2.5% chance of seeing at least  $x$  (i.e.  $x, x+1, x+2, \dots, n$ ) occurrences then we need

$$\sum_{k=0}^x \binom{n}{k} p_U^k (1 - p_U)^{n-k} \geq 0.025$$
$$\sum_{k=x}^n \binom{n}{k} p_L^k (1 - p_L)^{n-k} \geq 0.025$$

The second of these equations may be written as

$$\sum_{k=0}^{x-1} \binom{n}{k} p_L^k (1 - p_L)^{n-k} \leq 0.975$$

If  $x = n$ , then  $p_L^n \leq 0.975$  or  $p_L \leq 0.975^{1/n}$  and this is 0.9997 if  $n = 100$ . Interval is not symmetric if  $p \neq 0.5$ .

# Bootstrapping

An alternative method for constructing confidence intervals uses *numerical resampling*. A set of samples is drawn, with replacement, from the original sample to mimic the variation among samples from the original population. Each new sample is the same size as the original sample, and is called a *bootstrap sample*.

The middle 95% of the sample values  $\tilde{p}$  from a large number of bootstrap samples provides a 95% confidence interval.

## Multinomial Distribution

Toss two coins  $n$  times. For each double toss, the probabilities of the three outcomes are:

$$\begin{array}{ll} 2 \text{ heads} & p_{HH} = 1/4 \\ 1 \text{ head, 1 tail} & p_{HT} = 1/2 \\ 2 \text{ tails} & p_{TT} = 1/4 \end{array}$$

The probability of  $x$  lots of 2 heads is  $(p_{HH})^x$ , etc.

The numbers of ways of ordering  $x, y, z$  occurrences of the three outcomes is  $n!/[x!y!z!]$  where  $n = x + y + z$ .

The multinomial probability for  $x$  of  $HH$ , and  $y$  of  $HT$  or  $TH$  and  $z$  of  $TT$  in  $n$  trials is:

$$\Pr(x, y, z) = \frac{n!}{x!y!z!} (p_{HH})^x (p_{HT})^y (p_{TT})^z$$

## Multinomial Variances and Covariances

If  $\{p_i\}$  are the probabilities for a series of categories, the sample proportions  $\tilde{p}_i$  from a sample of  $n$  observations have these properties:

$$\begin{aligned}\mathcal{E}(\tilde{p}_i) &= p_i \\ \text{Var}(\tilde{p}_i) &= \frac{1}{n}p_i(1 - p_i) \\ \text{Cov}(\tilde{p}_i, \tilde{p}_j) &= -\frac{1}{n}p_i p_j, \quad i \neq j\end{aligned}$$

The covariance is defined as  $\mathcal{E}[(\tilde{p}_i - p_i)(\tilde{p}_j - p_j)]$ .

For the sample counts:

$$\begin{aligned}\mathcal{E}(n_i) &= np_i \\ \text{Var}(n_i) &= np_i(1 - p_i) \\ \text{Cov}(n_i, n_j) &= -np_i p_j, \quad i \neq j\end{aligned}$$

# Allele Frequency Sampling Distribution

If a locus has alleles  $A$  and  $a$ , in a sample of size  $n$  the allele counts are sums of genotype counts:

$$\begin{aligned}n &= n_{AA} + n_{Aa} + n_{aa} \\n_A &= 2n_{AA} + n_{Aa} \\n_a &= 2n_{aa} + n_{Aa} \\2n &= n_A + n_a\end{aligned}$$

Genotype counts in a random sample are multinomially distributed. What about allele counts? Approach this question by calculating variance of  $n_A$ .

## Within-population Variance

$$\begin{aligned}\text{Var}(n_A) &= \text{Var}(2n_{AA} + n_{Aa}) \\ &= \text{Var}(2n_{AA}) + 2\text{Cov}(2n_{AA}, n_{Aa}) + \text{Var}(n_{Aa}) \\ &= 2np_A(1 - p_A) + 2n(P_{AA} - p_A^2)\end{aligned}$$

This is not the same as the binomial variance  $2np_A(1 - p_A)$  unless  $P_{AA} = p_A^2$ . In general, the allele frequency distribution is not binomial.

The variance of the sample allele frequency  $\tilde{p}_A = n_A/(2n)$  can be written as

$$\text{Var}(\tilde{p}_A) = \frac{p_A(1 - p_A)}{2n} + \frac{P_{AA} - p_A^2}{2n}$$



## Within-population Variance

It is convenient to reparameterize genotype frequencies with the (within-population) *inbreeding coefficient*  $f$ :

$$P_{AA} = p_A^2 + fp_A(1 - p_A)$$

$$P_{Aa} = 2p_Ap_a - 2fp_Ap_a$$

$$P_{aa} = p_a^2 + fp_a(1 - p_a)$$

Then the variance can be written as

$$\text{Var}(\tilde{p}_A) = \frac{p_A(1 - p_A)(1 + f)}{2n}$$

This variance is different from the binomial variance of  $p_A(1 - p_A)/2n$ .

## Bounds on $f$

Since

$$\begin{aligned} p_A \geq P_{AA} &= p_A^2 + fp_A(1 - p_A) \geq 0 \\ p_a \geq P_{aa} &= p_a^2 + fp_a(1 - p_a) \geq 0 \end{aligned}$$

there are bounds on  $f$ :

$$\begin{aligned} -p_A/(1 - p_A) &\leq f \leq 1 \\ -p_a/(1 - p_a) &\leq f \leq 1 \end{aligned}$$

or

$$\max\left(-\frac{p_A}{p_a}, -\frac{p_a}{p_A}\right) \leq f \leq 1$$

This range of values is  $[-1, 1]$  when  $p_A = p_a$ .

## Indicator Variables

A very convenient way to derive many statistical genetic results is to define an indicator variable  $x_{ij}$  for allele  $j$  in individual  $i$ :

$$x_{ij} = \begin{cases} 1 & \text{if allele is } A \\ 0 & \text{if allele is not } A \end{cases}$$

Then

$$\begin{aligned} \mathcal{E}(x_{ij}) &= p_A \\ \mathcal{E}(x_{ij}^2) &= p_A \\ \mathcal{E}(x_{ij}x_{i'j'}) &= P_{AA} \end{aligned}$$

If there is random sampling, individuals are independent, and

$$\mathcal{E}(x_{ij}x_{i'j'}) = \mathcal{E}(x_{ij})\mathcal{E}(x_{i'j'}) = p_A^2$$

## Intraclass Correlation

The inbreeding coefficient is the correlation of the indicator variables for the two alleles at a locus carried by an individual. This is because:

$$\begin{aligned}\text{Var}(x_{ij}) &= \mathcal{E}(x_{ij}^2) - [\mathcal{E}(x_{ij})]^2 \\ &= p_A(1 - p_A) \\ &= \text{Var}(x_{ij'}), \quad j \neq j'\end{aligned}$$

and

$$\begin{aligned}\text{Cov}(x_{ij}, x_{ij'}) &= \mathcal{E}(x_{ij}x_{ij'}) - [\mathcal{E}(x_{ij})][\mathcal{E}(x_{ij'})], \quad j \neq j' \\ &= P_{AA} - p_A^2 \\ &= fp_A(1 - p_A)\end{aligned}$$

so

$$\text{Corr}(x_{ij}, x_{ij'}) = \frac{\text{Cov}(x_{ij}, x_{ij'})}{\sqrt{\text{Var}(x_{ij})\text{Var}(x_{ij'})}} = f$$

## Maximum Likelihood Estimation: Binomial

For binomial sample of size  $n$ , the likelihood of  $p_A$  for  $n_A$  alleles of type  $A$  is

$$L(p_A|n_A) = C(p_A)^{n_A}(1 - p_A)^{n - n_A}$$

and is maximized when

$$\frac{\partial L(p_A|n_A)}{\partial p_A} = 0 \quad \text{or when} \quad \frac{\partial \ln L(p_A|n_A)}{\partial p_A} = 0$$

Now

$$\ln L(p_A|n_A) = \ln C + n_A \ln(p_A) + (n - n_A) \ln(1 - p_A)$$

so

$$\frac{\partial \ln L(p_A|n_A)}{\partial p_A} = \frac{n_A}{p_A} - \frac{n - n_A}{1 - p_A}$$

and this is zero when  $p_A = \hat{p}_A = n_A/n$ .

## Maximum Likelihood Estimation: Multinomial

If  $\{n_i\}$  are multinomial with parameters  $n$  and  $\{Q_i\}$ , then the MLE's of  $Q_i$  are  $n_i/n$ . This will always hold for genotype proportions, but not always for allele proportions.

For two alleles, the MLE's for genotype proportions are:

$$\begin{aligned}\hat{P}_{AA} &= n_{AA}/n \\ \hat{P}_{Aa} &= n_{Aa}/n \\ \hat{P}_{aa} &= n_{aa}/n\end{aligned}$$

Does this lead to estimates of allele proportions and the within-population inbreeding coefficient?

$$\begin{aligned}P_{AA} &= p_A^2 + fp_A(1 - p_A) \\ P_{Aa} &= 2p_A(1 - p_A) - 2fp_A(1 - p_A) \\ P_{aa} &= (1 - p_A)^2 + fp_A(1 - p_A)\end{aligned}$$

# Maximum Likelihood Estimation

The likelihood function for  $p_A, f$  is

$$L(p_A, f) = \frac{n!}{n_{AA}!n_{Aa}!n_{aa}!} [p_A^2 + p_A(1 - p_A)f]^{n_{AA}} \\ \times [2p_A(1 - p_A)f]^{n_{Aa}} [(1 - p_A)^2 + p_A(1 - p_A)f]^{n_{aa}}$$

and it is difficult to find, analytically, the values of  $p_A$  and  $f$  that maximize this function or its logarithm.

There is an alternative way of finding maximum likelihood estimates in this case: equating the observed and expected values of the genotype frequencies.

## Bailey's Method

Because the number of parameters (2) equals the number of degrees of freedom in this case, we can just equate observed and expected (using the estimates of  $p_A$  and  $f$ ) genotype proportions

$$\begin{aligned}n_{AA}/n &= \hat{p}_A^2 + \hat{f}\hat{p}_A(1 - \hat{p}_A) \\n_{Aa}/n &= 2\hat{p}_A(1 - \hat{p}_A) - 2\hat{f}\hat{p}_A(1 - \hat{p}_A) \\n_{aa}/n &= (1 - \hat{p}_A)^2 + \hat{f}\hat{p}_A(1 - \hat{p}_A)\end{aligned}$$

Solving these equations (e.g. by adding the first equation to half the second equation) for  $\hat{p}_A$  and  $\hat{f}$ :

$$\begin{aligned}\hat{p}_A &= \frac{2n_{AA} + n_{Aa}}{2n} = \tilde{p}_A \\ \hat{f} &= \frac{4n_{AA}n_{aa} - n_{Aa}^2}{(2n_{AA} + n_{Aa})(2n_{aa} + n_{Aa})} = 1 - \frac{\tilde{P}_{Aa}}{2\tilde{p}_A\tilde{p}_a}\end{aligned}$$



## Three-allele Case

With three alleles, there are six genotypes and 5 df. To use Bailey's method, would need five parameters: 2 allele frequencies and 3 inbreeding coefficients:

$$P_{11} = p_1^2 + f_{12}p_1p_2 + f_{13}p_1p_3$$

$$P_{12} = 2p_1p_2 - 2f_{12}p_1p_2$$

$$P_{22} = p_2^2 + f_{12}p_1p_2 + f_{23}p_2p_3$$

$$P_{13} = 2p_1p_3 - 2f_{13}p_1p_3$$

$$P_{23} = 2p_2p_3 - 2f_{23}p_2p_3$$

$$P_{33} = p_3^2 + f_{13}p_1p_3 + f_{23}p_2p_3$$

We would generally prefer to have only one inbreeding coefficient  $f$ . It is a difficult numerical problem to find the MLE for  $f$ .

## Method of Moments

An alternative to maximum likelihood estimation is the method of moments (MoM) where observed values of statistics are set equal to their expected values. In general, this does not lead to unique estimates or to estimates with variances as small as those for maximum likelihood. (Bailey's method is for the special case where the MLEs are also MoM estimates.)

## Method of Moments

For the inbreeding coefficient at loci with  $m$  alleles, two different MoM estimates are

$$\begin{aligned}\hat{f}_W &= \frac{\sum_{u=1}^m (\tilde{P}_{uu} - \tilde{p}_u^2) + \frac{1}{2n} \sum_{u=1}^m (\tilde{p}_u - \tilde{P}_{uu})}{\sum_{u=1}^m \tilde{p}_u (1 - \tilde{p}_u) - \frac{1}{2n} \sum_{u=1}^m (\tilde{p}_u - \tilde{P}_{uu})} \\ &\approx \frac{\sum_{u=1}^m (\tilde{P}_{uu} - \tilde{p}_u^2)}{\sum_{u=1}^m \tilde{p}_u (1 - \tilde{p}_u)} \\ \hat{f}_H &= \frac{1}{m-1} \sum_{u=1}^m \left( \frac{\tilde{P}_{uu} - \tilde{p}_u^2}{\tilde{p}_u} \right)\end{aligned}$$

For loci with two alleles,  $m = 2$ , the two moment estimates are equal to each other and to the maximum likelihood estimate:

$$\hat{f}_W = \hat{f}_H = 1 - \frac{\tilde{P}_{Aa}}{2\tilde{p}_A\tilde{p}_a}$$

## Expectations of Moment Estimates

The expected values of the estimated inbreeding coefficients can be found by using the results

$$\begin{aligned}\mathcal{E}(\tilde{P}_{uu}) &= P_{uu} = p_u^2 + fp_u(1 - p_u) \\ \mathcal{E}(\tilde{p}_u) &= p_u \\ \mathcal{E}(\tilde{p}_u^2) &= p_u^2 + \frac{1}{2n}p_u(1 - p_u)(1 + f)\end{aligned}$$

Then, approximating the expectation of a ratio by the ratio of expectations:

$$\begin{aligned}\mathcal{E}(\hat{f}_W) &\approx \frac{f \frac{n-1}{n} (1 - \sum_{u=1}^m p_u^2)}{\frac{n-1}{n} (1 - \sum_{u=1}^m p_u^2)} = f \\ \mathcal{E}(\hat{f}_H) &\approx \frac{1}{m-1} \sum_{u=1}^m \left( \frac{p_u(1 - p_u)(f - \frac{1+f}{2n})}{p_u} \right) \\ &= f - \frac{1+f}{2n} \approx f\end{aligned}$$

## MLE for Recessive Alleles

Suppose allele  $a$  is recessive to allele  $A$ . If there is Hardy-Weinberg equilibrium, the likelihood for the two phenotypes is

$$\begin{aligned}L(p_a) &= (1 - p_a^2)^{n - n_{aa}} (p_a^2)^{n_{aa}} \\ \ln(L(p_a)) &= (n - n_{aa}) \ln(1 - p_a^2) + 2n_{aa} \ln(p_a)\end{aligned}$$

where there are  $n_{aa}$  individuals of type  $aa$  and  $n - n_{aa}$  of type  $A$ . Differentiating wrt  $p_a$ :

$$\frac{\partial \ln L(p_a)}{\partial p_a} = -\frac{2p_a(n - n_{aa})}{1 - p_a^2} + \frac{2n_{aa}}{p_a}$$

Setting this to zero leads to an equation that can be solved explicitly:  $p_a^2 = n_{aa}/n$ . No need for iteration.

## EM Algorithm for Recessive Alleles

An alternative way of finding maximum likelihood estimates when there are “missing data” involves *Estimation* of the missing data and then *Maximization* of the likelihood. For a locus with allele  $A$  dominant to  $a$  the missing information is the frequencies  $(1 - p_a)^2$  of  $AA$ , and  $2p_a(1 - p_a)$  of  $Aa$  genotypes. Only the joint frequency  $(1 - p_a^2)$  of  $AA + Aa$  can be observed.

**Estimate** the missing genotype counts (assuming independence of alleles):

$$n_{AA} = \frac{(1 - p_a)^2}{1 - p_a^2}(n - n_{aa}) = \frac{(1 - p_a)(n - n_{aa})}{(1 + p_a)}$$
$$n_{Aa} = \frac{2p_a(1 - p_a)}{1 - p_a^2}(n - n_{aa}) = \frac{2p_a(n - n_{aa})}{(1 + p_a)}$$

## EM Algorithm for Recessive Alleles

**Maximize** the likelihood (using Bailey's method):

$$\begin{aligned}\hat{p}_a &= \frac{n_{Aa} + 2n_{aa}}{2n} \\ &= \frac{1}{2n} \left( \frac{2p_a(n - n_{aa})}{(1 + p_a)} + 2n_{aa} \right) \\ &= \frac{2(np_a + n_{aa})}{2n(1 + p_a)}\end{aligned}$$

An initial estimate  $p_a$  is put into the right hand side to give an updated estimated  $\hat{p}_a$  on the left hand side. This is then put back into the right hand side to give an iterative equation for  $p_a$ .

This procedure also has explicit solution  $\hat{p}_a = \sqrt{(n_{aa}/n)}$ .

## EM Algorithm for Two Loci

For two loci with two alleles each, the ten two-locus frequencies are:

Genotype	Actual	Expected	Genotype	Actual	Expected
$AB/AB$	$P_{AB}^{AB}$	$p_{AB}^2$	$AB/Ab$	$P_{Ab}^{AB}$	$2p_{AB}p_{Ab}$
$AB/aB$	$P_{aB}^{AB}$	$2p_{AB}p_{aB}$	$AB/ab$	$P_{ab}^{AB}$	$2p_{AB}p_{ab}$
$Ab/Ab$	$P_{Ab}^{Ab}$	$p_{Ab}^2$	$Ab/aB$	$P_{aB}^{Ab}$	$2p_{Ab}p_{aB}$
$Ab/ab$	$P_{ab}^{Ab}$	$2p_{Ab}p_{ab}$	$aB/aB$	$P_{aB}^{aB}$	$p_{aB}^2$
$aB/ab$	$P_{ab}^{aB}$	$2p_{aB}p_{ab}$	$ab/ab$	$P_{ab}^{ab}$	$p_{ab}^2$



## EM Algorithm for Two Loci

Gamete frequencies are marginal sums:

$$p_{AB} = P_{AB}^{AB} + \frac{1}{2}(P_{Ab}^{AB} + P_{aB}^{AB} + P_{ab}^{AB})$$

$$p_{Ab} = P_{Ab}^{Ab} + \frac{1}{2}(P_{AB}^{Ab} + P_{ab}^{Ab} + P_{aB}^{Ab})$$

$$p_{aB} = P_{aB}^{aB} + \frac{1}{2}(P_{AB}^{aB} + P_{ab}^{aB} + P_{Ab}^{aB})$$

$$p_{ab} = P_{ab}^{ab} + \frac{1}{2}(P_{Ab}^{ab} + P_{aB}^{ab} + P_{AB}^{ab})$$

Can arrange gamete frequencies as two-way table to show that only one of them is unknown when the allele frequencies are known:

$p_{AB}$	$p_{Ab}$	$p_A$
$p_{aB}$	$p_{ab}$	$p_a$
$p_B$	$p_b$	$1$

## EM Algorithm for Two Loci

The two double heterozygote frequencies  $P_{ab}^{AB}$ ,  $P_{aB}^{Ab}$  are “missing data.”

Assume initial value of  $p_{AB}$  and *Estimate* the missing counts:

$$n_{ab}^{AB} = \frac{p_{AB}p_{ab}}{p_{AB}p_{ab} + p_{Ab}p_{aB}} n_{AaBb}$$
$$n_{aB}^{Ab} = \frac{p_{Ab}p_{aB}}{p_{AB}p_{ab} + p_{Ab}p_{aB}} n_{AaBb}$$

and then *Maximize* the likelihood by setting

$$p_{AB} = \frac{1}{2n} (2n_{AB}^{AB} + n_{Ab}^{AB} + n_{aB}^{AB} + n_{ab}^{AB})$$

## Example

As an example, consider the data

	<i>BB</i>	<i>Bb</i>	<i>bb</i>	Total
<i>AA</i>	$n_{AABB} = 5$	$n_{AABb} = 3$	$n_{AAbb} = 2$	$n_{AA} = 10$
<i>Aa</i>	$n_{AaBB} = 3$	$n_{AaBb} = 2$	$n_{Aabb} = 0$	$n_{Aa} = 5$
<i>aa</i>	$n_{aaBB} = 0$	$n_{aaBb} = 0$	$n_{aabb} = 0$	$n_{aa} = 0$
Total	$n_{BB} = 8$	$n_{Bb} = 5$	$n_{bb} = 2$	$n = 15$

There is one unknown gamete count  $x = n_{AB} = 2np_{AB}$  for *AB*:

	<i>B</i>	<i>b</i>	Total
<i>A</i>	$n_{AB} = x$	$n_{Ab} = 25 - x$	$n_A = 25$
<i>a</i>	$n_{aB} = 21 - x$	$n_{ab} = x - 16$	$n_a = 5$
Total	$n_B = 21$	$n_b = 9$	$2n = 30$

$$21 \geq x \geq 16$$

## Example

EM iterative equation:

$$\begin{aligned}x' &= 2n_{AABB} + n_{AABb} + n_{AaBB} + n_{AB/ab} \\&= 2n_{AABB} + n_{AABb} + n_{AaBB} + \frac{2p_{AB}p_{ab}}{2p_{AB}p_{ab} + 2p_{Ab}p_{aB}} n_{AaBb} \\&= 10 + 3 + 3 + 2 \times \frac{2x(x - 16)}{2x(x - 16) + 2(25 - x)(21 - x)} \\&= 16 + \frac{x(x - 16)}{x(x - 16) + (25 - x)(21 - x)}\end{aligned}$$

## Example

A good starting value would assume independence of  $A$  and  $B$  alleles:  $x = 2n * p_A * p_B = (25 \times 21/30) = 17.5$ .

Successive iterates are:

Iterate	$x$ value
0	17.5000
1	17.0000
2	16.6939
3	16.4893
4	16.3473
5	16.2472
...	...

The solution is actually  $x = 16$ . This particular example does not have convergence to the MLE for some starting values for  $x$ .

# ALLELIC ASSOCIATION

## Hardy-Weinberg Law

For a random mating population, expect that genotype frequencies are products of allele frequencies.

For a locus with two alleles,  $A, a$ :

$$P_{AA} = (p_A)^2$$

$$P_{Aa} = 2p_A p_a$$

$$P_{aa} = (p_a)^2$$

These are also the results of setting the inbreeding coefficient  $f$  to zero.

For a locus with several alleles  $A_i$ :

$$P_{A_i A_i} = (p_{A_i})^2$$

$$P_{A_i A_j} = 2p_{A_i} p_{A_j}$$

## Inference about HWE

Departures from HWE can be described by the within-population inbreeding coefficient  $f$ . This has an MLE that can be written as

$$\hat{f} = \frac{4n_{AA}n_{aa} - n_{Aa}^2}{(2n_{AA} + n_{Aa})(2n_{aa} + n_{Aa})}$$

and we can use “Delta method” to find

$$\begin{aligned}\mathcal{E}(\hat{f}) &= f \\ \text{Var}(\hat{f}) &\approx \frac{1}{2np_Ap_a}(1-f)[2p_Ap_a(1-f)(1-2f) + f(2-f)]\end{aligned}$$

If  $\hat{f}$  is assumed to be normally distributed then,  $(\hat{f}-f)/\sqrt{\text{Var}(\hat{f})} \sim N(0,1)$ . When  $H_0$  is true, the square of this quantity has a chi-square distribution.



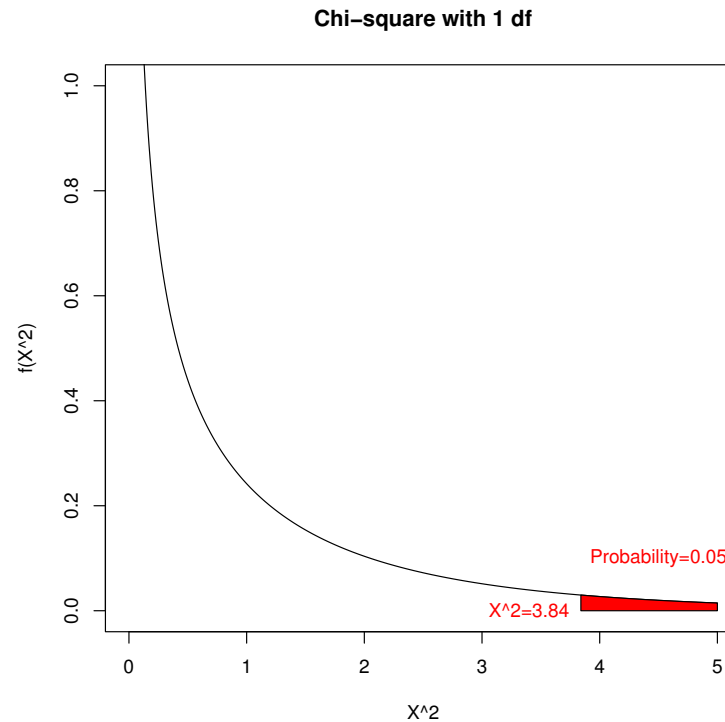
## Inference about HWE

Since  $\text{Var}(\hat{f}) = 1/n$  when  $f = 0$ :

$$\begin{aligned} X^2 &= \left( \frac{\hat{f} - f}{\sqrt{\text{Var}(\hat{f})}} \right)^2 \\ &= \frac{\hat{f}^2}{1/n} \\ &= n\hat{f}^2 \end{aligned}$$

is appropriate for testing  $H_0 : f = 0$ . When  $H_0$  is true,  $X^2 \sim \chi^2_{(1)}$ .  
Reject HWE if  $X^2 > 3.84$ .

# Significance level of HWE test



The area under the chi-square curve to the right of  $X^2 = 3.84$  is the probability of rejecting HWE when HWE is true. This is the significance level of the test.

## Goodness-of-fit Test

An alternative, but equivalent, test is the goodness-of-fit test.

Genotype	Observed	Expected	$\frac{(\text{Obs.} - \text{Exp.})^2}{\text{Exp.}}$
<i>AA</i>	$n_{AA}$	$n\tilde{p}_A^2$	$n\tilde{p}_a^2\tilde{f}^2$
<i>Aa</i>	$n_{Aa}$	$2n\tilde{p}_A\tilde{p}_a$	$2n\tilde{p}_A\tilde{p}_a\tilde{f}^2$
<i>aa</i>	$n_{aa}$	$n\tilde{p}_a^2$	$n\tilde{p}_A^2\tilde{f}^2$

The test statistic is

$$X^2 = \sum \frac{(\text{Obs.} - \text{Exp})^2}{\text{Exp.}} = n\tilde{f}^2$$

## Goodness-of-fit Test

Does a sample of 6 *AA*, 3 *Aa*, 1 *aa* support Hardy-Weinberg?

First need to estimate allele frequencies:

$$\tilde{p}_A = \tilde{P}_{AA} + \frac{1}{2}\tilde{P}_{Aa} = 0.75$$

$$\tilde{p}_a = \tilde{P}_{aa} + \frac{1}{2}\tilde{P}_{Aa} = 0.25$$

Then form “expected” counts:

$$n_{AA} = n(\tilde{p}_A)^2 = 5.625$$

$$n_{Aa} = 2n\tilde{p}_A\tilde{p}_a = 3.750$$

$$n_{aa} = n(\tilde{p}_a)^2 = 0.625$$

## Goodness-of-fit Test

Perform the chi-square test:

Genotype	Observed	Expected	(Obs. – Exp.) <sup>2</sup> /Exp.
<i>AA</i>	6	5.625	0.025
<i>Aa</i>	3	3.750	0.150
<i>aa</i>	1	0.625	0.225
Total	10	10	0.400

Note that  $\hat{f} = 1 - 0.3/(2 \times 0.75 \times 0.25) = 0.2$  and  $X^2 = n\hat{f}^2$ .

## Sample size determination

Although Fisher's exact test (below) is generally preferred for small samples, the normal or chi-square test has the advantage of simplifying power calculations.

Assuming that  $\hat{f}$  is normally distributed, form the test statistic

$$z = \frac{\hat{f} - f}{\sqrt{\text{Var}(\hat{f})}}$$

Under the null hypothesis  $H_0 : f = 0$  this is  $z_0 = \sqrt{n}\hat{f}$ . For a two-sided test, reject at the  $\alpha\%$  level if  $z_0 \leq z_{\alpha/2}$  or  $z_0 \geq z_{1-\alpha/2} = -z_{\alpha/2}$ . For a 5% test, reject if  $z_0 \leq -1.96$  or  $z_0 \geq 1.96$ .

## Sample size determination

If the hypothesis is false, the normal test statistic is

$$z = \frac{\hat{f} - f}{\sqrt{\text{Var}(\hat{f})}} \approx \sqrt{n}(\hat{f} - f) = z_0 - \sqrt{n}f$$

(using the null-hypothesis value of the variance in the denominator). Suppose  $\hat{f} > 0$  so rejection occurs when  $z_0 \geq -z_{\alpha/2}$ . With this rejection region, the probability of rejecting is  $\geq (1 - \beta)$  if the rejection region amounts to  $z = z_0 - \sqrt{n}f \geq z_{\beta}$ . i.e.

$$\begin{aligned} -z_{\alpha/2} - \sqrt{n}f &= z_{\beta} \\ nf^2 &= (z_{\alpha/2} + z_{\beta})^2 \end{aligned}$$

For 5% significance level  $-z_{\alpha/2} = 1.96$ , and for 90% power  $z_{\beta} = -1.28$  so we need  $nf^2 \geq (-1.96 - 1.28)^2 = 10.5$ . i.e.  $n$  has to be over 100,000 when  $f = 0.01$ .

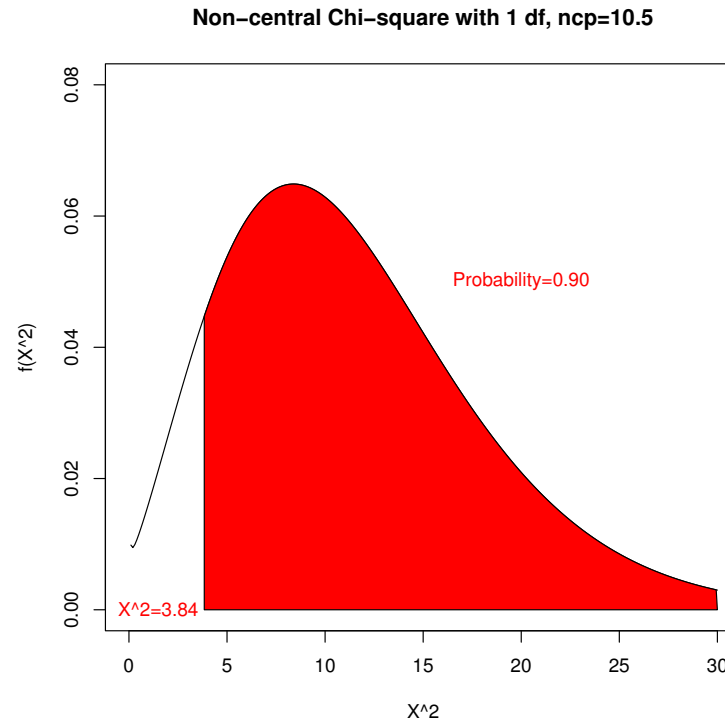
## Sample size determination

More directly, when the Hardy-Weinberg hypothesis is not true, the test statistic  $nf^2$  has a non-central chi-square distribution with one degree of freedom (df) and non-centrality parameter  $\lambda = nf^2$ . To reach 90% power with a 5% significance level, for example, it is necessary that  $\lambda \geq 10.5$ .

In this one-df case, the non-centrality value follows from percentiles of the standard normal distribution. If  $z_x$  is the  $x$ th percentile of the standard normal, then for significance level  $\alpha$  and power  $1 - \beta$ ,  $\lambda = (z_{\alpha/2} + z_\beta)^2$ .



# Power of HWE test



The area under the non-central chi-square curve to the right of  $X^2 = 3.84$  is the probability of rejecting HWE when HWE is false. This is the power of the test. In this plot, the non-centrality parameter is  $\lambda = 10.5$ .

## Significance Levels and $p$ -values

The *significance level*  $\alpha$  of a test is the probability of a false rejection. It is specified by the user, and along with the null hypothesis, it determines the rejection region. The specified, or “nominal” value may not be achieved for an actual test.

Once the test has been conducted on a data set, the probability of the observed test statistic, *or a more extreme value*, if the null hypothesis is true is the  *$p$ -value*. The chi-square and normal tests shown above give approximate  $p$ -values because they use a continuous distribution for discrete data.

An alternative class of tests, “exact tests,” use a discrete distribution for discrete data and provide accurate  $p$ -values. It may be difficult to construct an exact test with a particular nominal significance level.

## Exact HWE Test

The preferred test for HWE is an exact one. The test rests on the assumption that individuals are sampled randomly from a population so that genotype counts have a multinomial distribution:

$$\Pr(n_{AA}, n_{Aa}, n_{aa}) = \frac{n!}{n_{AA}!n_{Aa}!n_{aa}!} (P_{AA})^{n_{AA}} (P_{Aa})^{n_{Aa}} (P_{aa})^{n_{aa}}$$

This equation is always true, and when there is HWE ( $P_{AA} = p_A^2$  etc.) there is the additional result that the allele counts have a binomial distribution:

$$\Pr(n_A, n_a) = \frac{(2n)!}{n_A!n_a!} (p_A)^{n_A} (p_a)^{n_a}$$

## Exact HWE Test

Putting these together gives the conditional probability

$$\begin{aligned}\Pr(n_{AA}, n_{Aa}, n_{aa} | n_A, n_a, \text{HWE}) &= \frac{\Pr(n_{AA}, n_{Aa}, n_{aa}, n_A, n_a | \text{HWE})}{\Pr(n_A, n_a | \text{HWE})} \\ &= \frac{\frac{n!}{n_{AA}!n_{Aa}!n_{aa}!} (p_A^2)^{n_{AA}} (2p_A p_a)^{n_{Aa}} (p_a^2)^{n_{aa}}}{\frac{(2n)!}{n_A!n_a!} (p_A)^{n_A} (p_a)^{n_a}} \\ &= \frac{n!}{n_{AA}!n_{Aa}!n_{aa}!} \frac{2^{n_{Aa}} n_A! n_a!}{(2n)!}\end{aligned}$$

Reject the Hardy-Weinberg hypothesis if this quantity, the probability of the genotypic array conditional on the allelic array under HWE, is among the smallest of its possible values.

## Exact HWE Test

For convenience, write the probability of the genotypic array, conditional on the allelic array and HWE, as  $\Pr(n_{Aa}|n, n_A)$ . Reject the HWE hypothesis for a data set if this value is among the smallest probabilities.

As an example, consider  $(n_{AA} = 1, n_{Aa} = 0, n_{aa} = 49)$ . The allele counts are  $(n_A = 2, n_a = 98)$  and there are only two possible genotype arrays with those allele counts:

<i>AA</i>	<i>Aa</i>	<i>aa</i>	$\Pr(n_{Aa} n, n_A)$
1	0	49	$\frac{50!}{1!0!49!} \frac{2^0 2!98!}{100!} = \frac{1}{99}$
0	2	48	$\frac{50!}{0!2!48!} \frac{2^2 2!98!}{100!} = \frac{98}{99}$

## Exact HWE Test

The probability of the data ( $n_{AA} = 1, n_{Aa} = 0, n_{aa} = 49$ ), conditional on the allele frequencies and on HWE, is  $1/99 = 0.01$  which is not nearly as small as the value suggested by the chi-square statistic of 50. (Because  $\tilde{P}_{Aa} = 0, \hat{f} = 1, X^2 = n.$ )

Traditionally, the  $p$ -value is the (conditional) probability of the data plus the probabilities of all the less-probable datasets. The probabilities are all calculated assuming HWE is true. More recently (Graffelman and Moreno, *Statistical Applications in Genetics and Molecular Biology* 2013; 12:433-448) it has been shown that the test has a significance value closer to the nominal value if the  $p$ -value is half the probability of the data plus the probabilities of all datasets that are less probable under the null hypothesis. For the previous slide then, the  $p$ -value is  $1/198=0.005$ . A 5% exact significance level is not achievable.

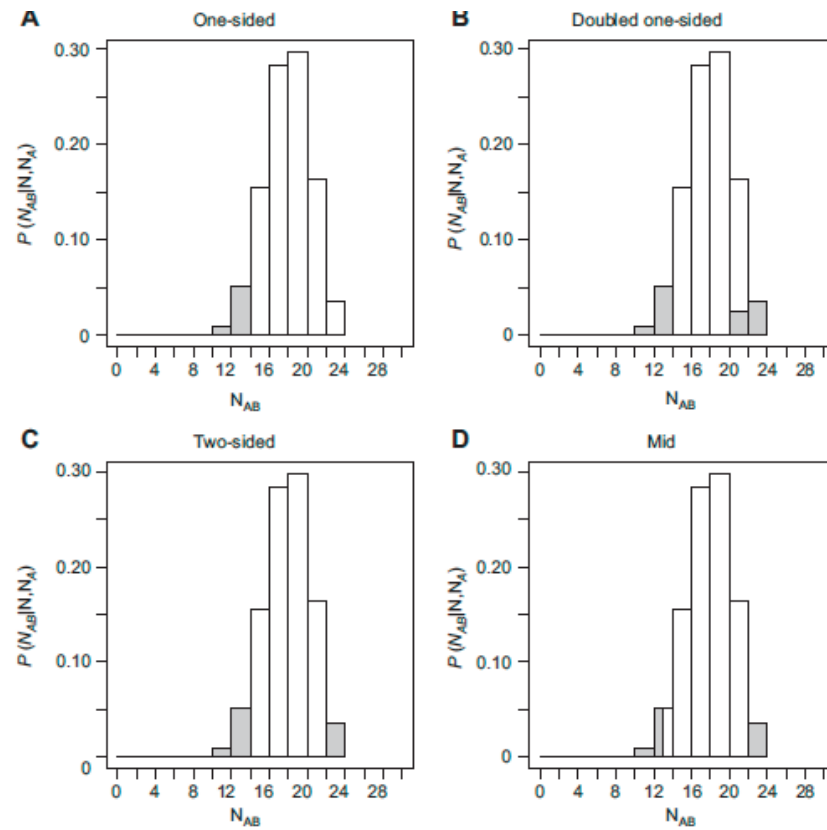
## Mid $p$ -value

For this example:

$AA$	$Aa$	$aa$	$\Pr(n_{Aa} n, n_A)$	$p$ -value	mid $p$ -value
1	0	49	$\frac{50!}{1!0!49!} \frac{2^0 2!98!}{100!} = \frac{1}{99}$	0.0101	0.0051
0	2	48	$\frac{50!}{0!2!48!} \frac{2^2 2!98!}{100!} = \frac{98}{99}$	1.0000	0.5051

The average mid- $p$  value is 0.5.

# Mid-p values



**Figure 1** Computation of the  $p$ -value in an exact test for HWP, for a sample of 50 individuals with a minor allele count of 23, for which 13 heterozygotes were observed. (A) One-sided  $p$ -value in a test for heterozygote death. (B)  $p$ -value obtained by doubling the one-sided tail. (C) Standard two-sided  $p$ -value, (D) Mid  $p$ -value based on half the probability of the observed sample.



## Example

For a sample of size  $n = 100$  with minor allele frequency of 0.07, there are 8 sets of possible genotype counts:

$n_{AA}$	$n_{Aa}$	$n_{aa}$	Exact		Chi-square	
			Prob.	Mid $p$ -value	$X^2$	$p$ -value
93	0	7	0.0000	0.0000*	100.00	0.0000*
92	2	6	0.0000	0.0000*	71.64	0.0000*
91	4	5	0.0000	0.0000*	47.99	0.0000*
90	6	4	0.0002	0.0001*	29.07	0.0000*
89	8	3	0.0051	0.0028*	14.87	0.0001*
88	10	2	0.0602	0.0354*	5.38	0.0204*
87	12	1	0.3209	0.2260	0.61	0.4348
86	14	0	0.6136	0.6932	0.57	0.4503

So, for a nominal 5% significance level, the actual significance level is 0.0204 for a chi-square test that rejects when  $n_{Aa} \leq 10$  and is 0.0354 for an exact test that also rejects when  $n_{Aa} \leq 10$ .

## Effect of Minor Allele Frequency

The minor allele frequency (MAF) in the previous example was  $14/200 = 0.07$ . How does the exact test behave with other MAF values?

In particular, what is the size of the actual significance level when the nominal value is  $\alpha = 0.05$ ? In other words, we decide to reject HWE for any sample with a  $p$ -value of 0.05 or less and choose the rejection region accordingly, what are the probabilities of rejecting? We would hope that the empirical significance level would be close to the nominal value, but we find that it may not be.

## $n_a = 16$ minor alleles

When the minor allele frequency is 0.08, for a nominal 5% significance level, the actual significance level is 0.0070 for an exact test that rejects when  $n_{Aa} \leq 10$ .

$n_{AA}$	$n_{Aa}$	$n_{aa}$	$\Pr(n_{Aa} n_a)$	Mid $p$ -value
92	0	8	.0000	.0000
91	2	7	.0000	.0000
90	4	6	.0000	.0000
89	6	5	.0000	.0000
88	8	4	.0008	.0004
87	10	3	.0123	.0070
86	12	2	.0974	.0618
85	14	1	.3681	.2946
84	16	0	.5215	.7392

## $n_a = 15$ minor alleles

When the minor allele frequency is 0.075, for a nominal 5% significance level, the actual significance level is 0.0474 for an exact test that rejects when  $n_{Aa} \leq 11$ .

$n_{AA}$	$n_{Aa}$	$n_{aa}$	$\Pr(n_{Aa} n_a)$	Mid $p$ -value
92	1	7	.0000	.0000
91	3	6	.0000	.0000
90	5	5	.0000	.0000
89	7	4	.0004	.0002
88	9	3	.0081	.0045
87	11	2	.0776	.0474
86	13	1	.3464	.2594
85	15	0	.5675	.7163

## $n_a = 13$ minor alleles

When the minor allele frequency is 0.065, for a nominal 5% significance level, the actual significance level is 0.0483 for an exact test that rejects when  $n_{Aa} \leq 9$ .

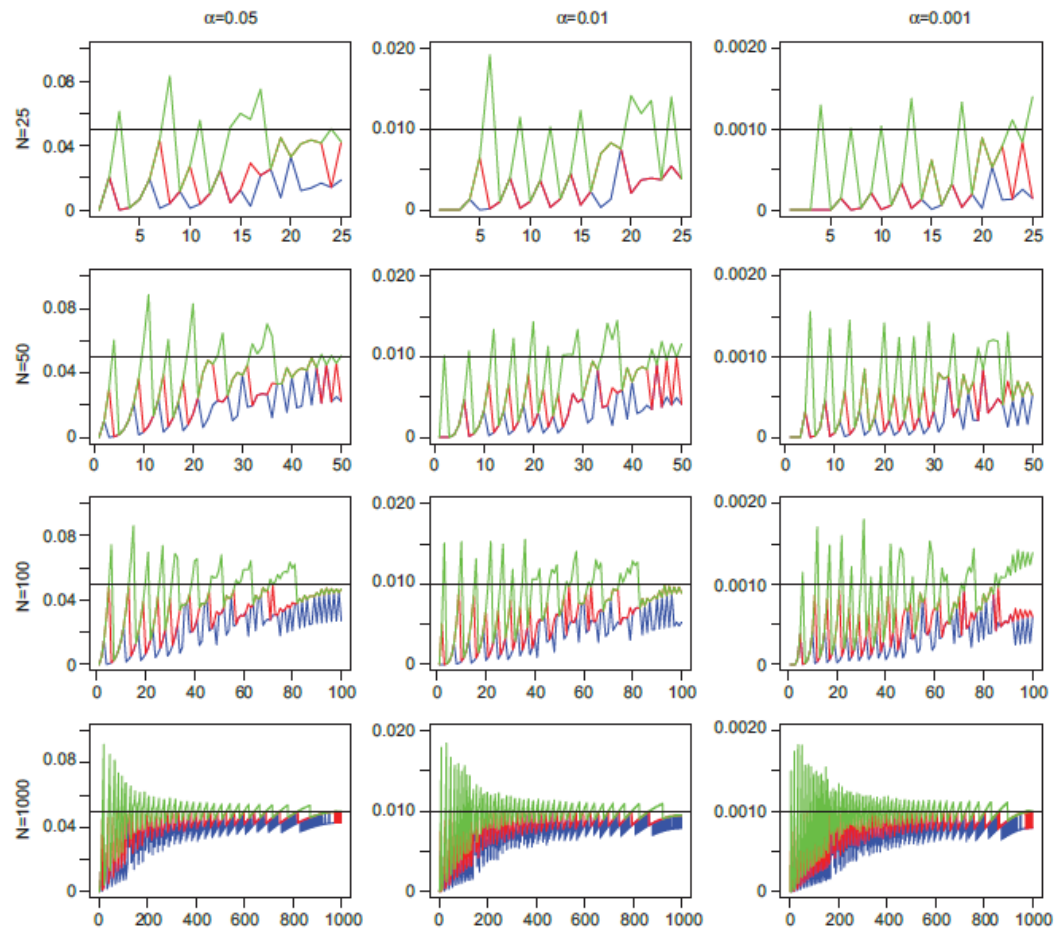
$n_{AA}$	$n_{Aa}$	$n_{aa}$	$\Pr(n_{Aa} n_a)$	Mid $p$ -value
93	1	6	.0000	.0000
92	3	5	.0000	.0000
91	5	4	.0001	.0000
90	7	3	.0030	.0016
89	9	2	.0452	.0257
88	11	1	.2923	.1945
87	13	0	.6595	.6704

## $n_a = 12$ minor alleles

When the minor allele frequency is 0.06, for a nominal 5% significance level, the actual significance level is 0.0344 for an exact test that rejects when  $n_{Aa} \leq 8$ .

$n_{AA}$	$n_{Aa}$	$n_{aa}$	$\Pr(n_{Aa} n_a)$	Mid $p$ -value
94	0	6	.0000	.0000
93	2	5	.0000	.0000
92	4	4	.0000	.0000
91	6	3	.0017	.0009
90	8	2	.0327	<b>.0181</b>
89	10	1	.2612	.1650
88	12	0	.7045	.6479

# Graffelman and Moreno, 2013



**Figure 2** Type I error rate against minor allele count for different sample sizes (25, 50, 100 and 1000) and significance levels (0.05, 0.01, and 0.001) for exact tests with standard two-sided (red), doubled one-sided (blue) and mid  $p$ -values (green).

## Power of Exact Test

If there is not HWE:

$$\begin{aligned}
 \Pr(n_{Aa}|n_A, n_a) &= \frac{n!}{n_{AA}!n_{Aa}!n_{aa}!} (P_{AA})^{n_{AA}} (P_{Aa})^{n_{Aa}} (P_{aa})^{n_{aa}} \\
 &= \frac{n!}{n_{AA}!n_{Aa}!n_{aa}!} (P_{AA})^{\frac{n_A - n_{Aa}}{2}} (P_{Aa})^{n_{Aa}} (P_{aa})^{\frac{n_a - n_{Aa}}{2}} \\
 &= \frac{n!}{n_{AA}!n_{Aa}!n_{aa}!} (\sqrt{P_{AA}})^{n_A} (\sqrt{P_{aa}})^{n_a} \left( \frac{P_{Aa}}{\sqrt{P_{AA}P_{aa}}} \right)^{n_{Aa}} \\
 &= \frac{C\psi^{n_{Aa}}}{n_{AA}!n_{Aa}!n_{aa}!}
 \end{aligned}$$

where  $\psi = P_{Aa}/(\sqrt{P_{AA}P_{aa}})$  measures the departure from HWE. The constant  $C$  makes the probabilities sum to one over all possible  $n_{Aa}$  values:  $C = 1/[\sum_{n_{Aa}} \psi^{n_{Aa}}/(n_{AA}!n_{Aa}!n_{aa}!)]$ .



## Power of Exact Test

Once the rejection region has been determined, the power of the test (the probability of rejecting) can be found by adding these probabilities for all sets of genotype counts in the region. HWE corresponds to  $\psi = 2$ . What is the power to detect HWE when  $\psi = 1$ , the sample size is  $n = 10$  and the sample allele counts are  $n_A = 15, n_a = 5$ . Note that  $C = 1/[1/(5!5!0!) + 1/(6!3!1!) + 1/(7!1!2!)]$ .

$n_{AA}$	$n_{Aa}$	$n_{aa}$	$\Pr(n_{Aa} n_A, n)$	
			$\psi = 2$	$\psi = 1$
5	5	0	0.520	0.262
6	3	1	0.433	0.364
7	1	2	0.047	0.374

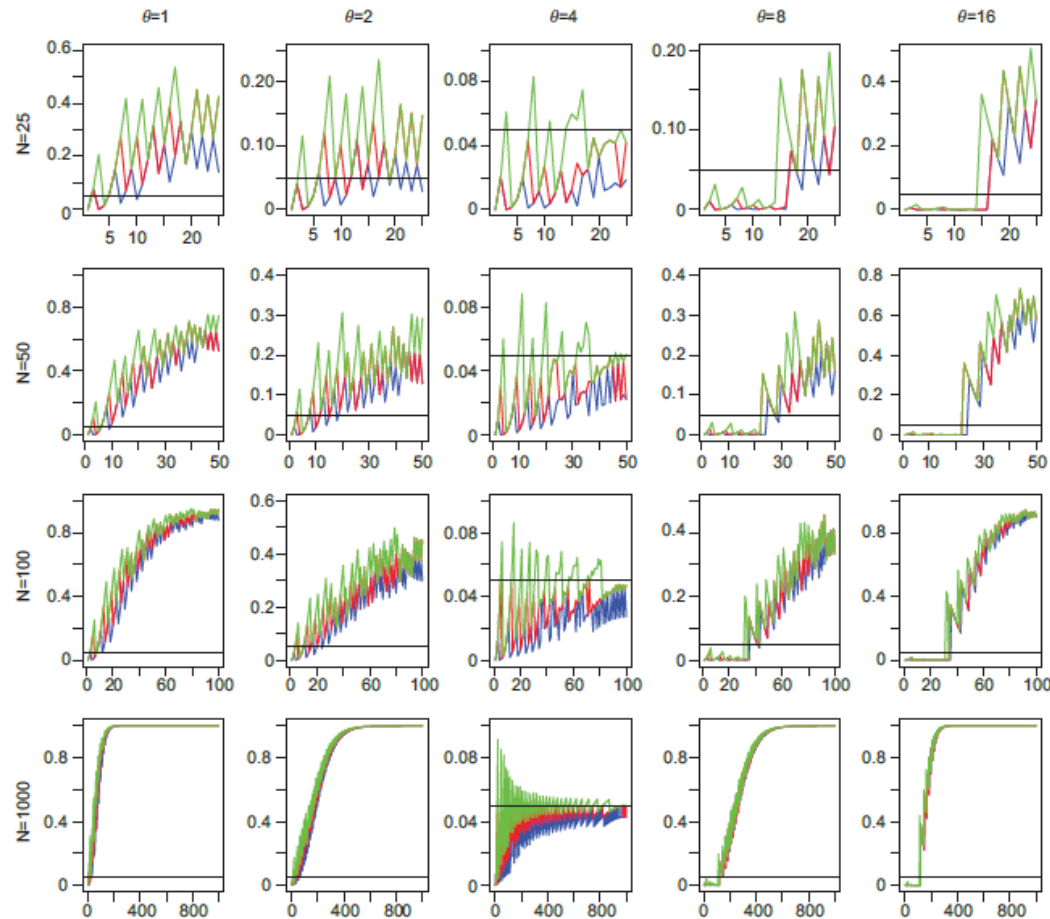
The  $\psi = 2$  column (i.e. HWE) shows that the rejection region is  $n_{Aa} = 1$ . The  $\psi = 1$  column (not HWE) shows that the power (the probability  $n_{Aa} = 1$  when  $\psi = 1$ ) is 37.4%.

## Power when $n_a = 16$

The rejection region of  $n_{Aa} \leq 10$  is determined from null hypothesis, and the power is determined from the multinomial distribution. Rejection is unlikely if  $f < 0$ .

		Pr( $n_{Aa}   n_a = 16, n = 100$ )							
		$\psi$	.250	.500	1.000	2.000	4.000	8.000	16.000
$n_{Aa}$	$f$		.631	.398	.157	.000	-.062	-.081	-.085
0		.0042	.0000	.0000	.0000	.0000	.0000	.0000	.0000
2		.0956	.0026	.0000	.0000	.0000	.0000	.0000	.0000
4		.3172	.0349	.0003	.0000	.0000	.0000	.0000	.0000
6		.3568	.1569	.0056	.0000	.0000	.0000	.0000	.0000
8		.1772	.3116	.0441	.0008	.0000	.0000	.0000	.0000
10		.0433	.3047	.1725	.0123	.0003	.0000	.0000	.0000
12		.0054	.1506	.3411	.0974	.0098	.0007	.0000	.0000
14		.0003	.0356	.3223	.3681	.1485	.0422	.0109	.0109
16		.0000	.0032	.1142	.5214	.8414	.9571	.9890	.9890
Power		.9943	.8107	.2225	.0131	.0003	.0000	.0000	.0000

# Graffelman and Moreno, 2013



**Figure 3** Power of HWP exact tests against minor allele count for different sample sizes (25, 50, 100 and 1000) and degree of disequilibrium (1, 2, 4, 8 and 16). Standard two-sided (red), doubled one-sided (blue) and mid  $p$ -values (green).

$\theta$  in this plot is  $\psi^2$ .

## Multiple Testing

When multiple tests are performed, each at significance level  $\alpha$ , a proportion  $\alpha$  of the tests are expected to cause rejection even if all the hypotheses are true.

Bonferroni correction makes the overall (experimentwise) significance level equal to  $\alpha$  by adjusting the level for each individual test to  $\alpha'$ . If  $\alpha$  is the probability that at least one of the  $L$  tests causes rejection, it is also 1 minus the probability that none of the tests causes rejection:

$$\begin{aligned}\alpha &= 1 - (1 - \alpha')^L \\ &\approx L\alpha'\end{aligned}$$

provided the  $L$  tests are independent.

If  $L = 15$ , need  $\alpha' = 0.0033$  in order for  $\alpha = 0.05$ .

## Combining $p$ -values

There is also the issue that if the same hypothesis is tested  $L$  times and just fails to cause rejection each time, there is some overall evidence against the hypothesis.

Suppose that tests have been conducted for each of  $L$  hypotheses  $H_i, i = 1, 2, \dots, L$ . For each test the  $p$ -value  $p_i$  is calculated: if  $H_i$  is true, this is the probability of observing a test statistic as extreme as or more extreme than the observed value in the direction of rejection.

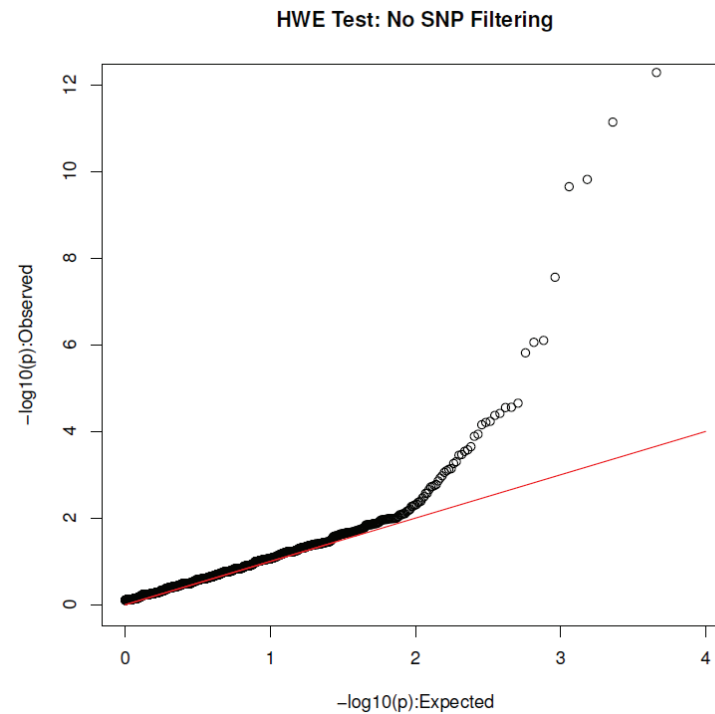
Methods for combining  $p$ -values rest on  $p$  having a uniform distribution when the hypothesis is true.

## QQ-Plots

A convenient approach to considering multiple-testing issues is to use QQ-plots. If all the hypotheses being tested are true then the resulting  $p$ -values are uniformly distributed between 0 and 1.

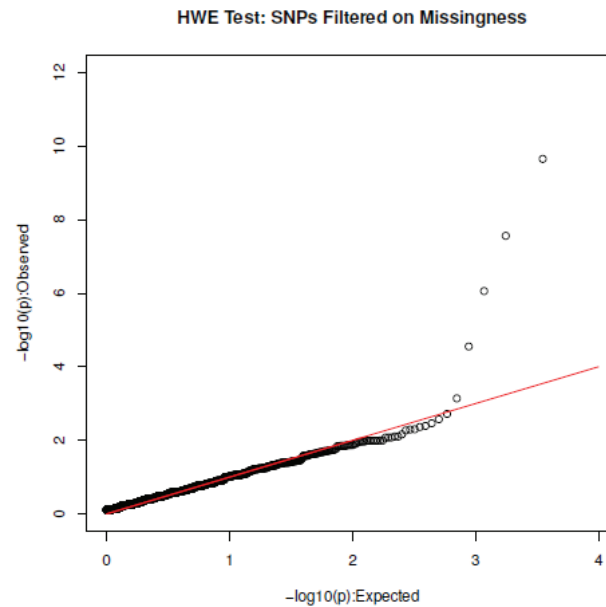
For a set of  $n$  tests, we would expect to see  $p$  values at  $1/(n + 1), 2/(n + 1), \dots, n/(n + 1)$ . We plot the observed  $p$ -values against these expected values: the smallest against  $1/(n + 1)$  and the largest against  $n/(n + 1)$ . It is more convenient to transform to  $-\log_{10}(p)$  to accentuate the extremely small  $p$  values. The point at which the observed values start departing from the expected values is an indication of “significant” values in a way that takes into account the number of tests.

# QQ-Plots



The results for 9208 SNPs on human chromosome 1 for the AMD controls. Bonferroni would suggest rejecting HWE when  $p \leq 0.05/9205 = 5.4 \times 10^{-6}$  or  $-\log_{10}(p) \geq 5.3$ .

# QQ-Plots



The same set of results as on the previous slide except now that any SNP with any missing data was excluded. Now 7446 SNPs and Bonferroni would reject if  $-\log_{10}(p) \geq 5.2$ . All five outliers had zero counts for the minor allele homozygote and at least 32 heterozygotes in a sample of size 50.



## HWE Testing for X-linked Markers

Usual procedure is to ignore males and test for HWE with female data only.

If there is HWE, the allele frequencies at X-linked markers are the same in males and females.

Different allele frequencies for males and females suggest a departure from HWE.

## HWE Testing for X-linked Markers

Sample sizes:  $n_m$  males,  $n_f$  females,  $n = n_m + n_f$ .

Male allele counts:  $m_A, m_B$ .

Female genotype counts:  $f_{AA}, f_{AB}, f_{BB}$ .

Total sample allele counts:  $n_A, n_B$ .

Probability of data, under HWE for females and equal male and female allele frequencies:

$$\Pr(m_A, f_{AB} | n, n_A, n_m) = \frac{n_A! n_B! n_m! n_f! 2^{f_{AB}}}{m_A! m_B! f_{AA}! f_{AB}! f_{BB}!}$$

An exact test for the joint hypotheses of female HWE and equal male and female allele frequencies was constructed from this probability, as described in version 1.5.6 of the *HardyWeinberg* package.

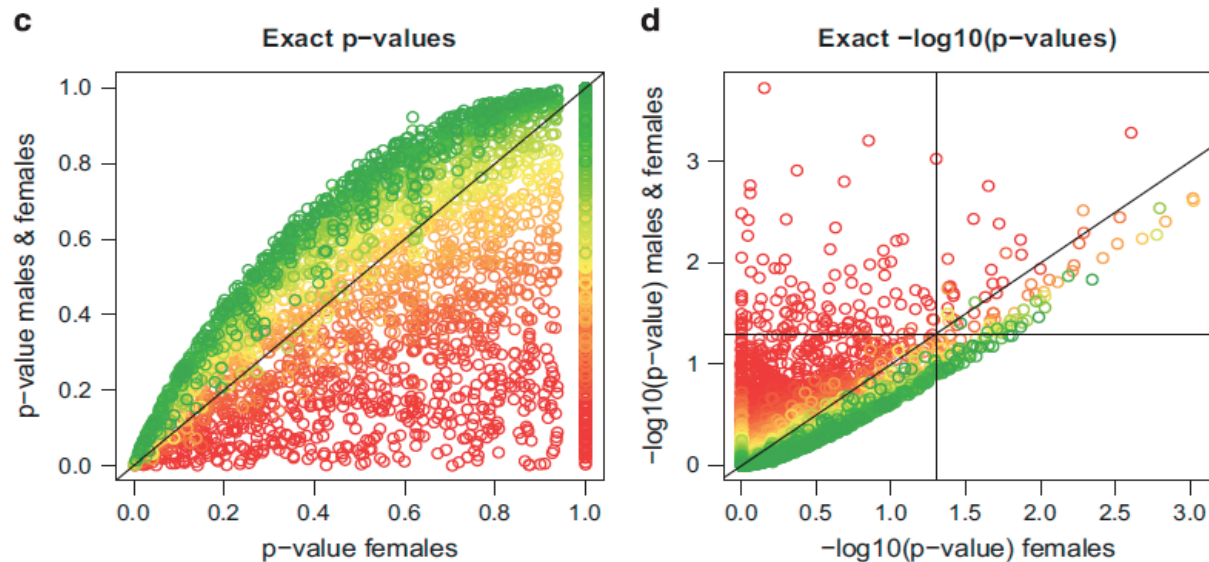
# rGaffelman and Weir, 2016

Table 2 All possible samples for a set of 20 individuals (10 males and 10 females) with a total of 6 A alleles

	$m_A$	$m_B$	$f_{AA}$	$f_{AB}$	$f_{BB}$	<i>Prob</i>
1	0	10	3	0	7	0.0002
2	0	10	2	2	6	0.0085
3	0	10	1	4	5	0.0340
4	0	10	0	6	4	0.0226
5	1	9	2	1	7	0.0121
6	1	9	1	3	6	0.1132
7	1	9	0	5	5	0.1358
8	2	8	2	0	8	0.0034
9	2	8	1	2	7	0.1091
10	2	8	0	4	6	0.2546
11	3	7	1	1	8	0.0364
12	3	7	0	3	7	0.1940
13	4	6	1	0	9	0.0035
14	4	6	0	2	8	0.0637
15	5	5	0	1	9	0.0085
16	6	4	0	0	10	0.0004

Heredity 116:558–568.

# Gaffelman and Weir, 2016



Scatter plots of P-values in original and  $-\log_{10}$  scale for exact test for HWE using females only and using both males and females for 4158 SNPs at the X chromosome of the venous thrombosis database. The horizontal and vertical black lines correspond to a significance level of 5%. Points colored according to their significance level in Fisher's test for equality of allele frequencies (range 0-1 from red to green).

## Linkage Disequilibrium

This term reserved for association between pairs of alleles – one at each of two loci.

When gametic data are available, could refer to gametic disequilibrium.

When genotypic data are available, but gametes can be inferred, can make inferences about gametic and non-gametic pairs of alleles.

When genotypic data are available, but gametes cannot be inferred, can work with composite measures of disequilibrium.

## Linkage Disequilibrium

For alleles  $A$  and  $B$  are two loci, the usual measure of linkage disequilibrium is

$$D_{AB} = P_{AB} - p_A p_B$$

Whether or not this is zero does not provide a direct statement about linkage between the two loci. For example, consider marker YFM and disease DTD:

		A	N	Total
YFM	+	1	24	25
	-	0	75	75
Total		1	99	100

$$D_{A+} = \frac{1}{100} - \frac{1}{100} \frac{25}{100} = 0.0075, \text{ (maximum possible value)}$$

## Gametic Linkage Disequilibrium

For loci **A**, **B** define indicator variables  $x, y$  that take the value 1 for allele  $A, B$  and 0 for any other alleles. If gametes within individuals are indexed by  $j$ ,  $j = 1, 2$  then for expectations over samples from the same population

$$\begin{aligned}\mathcal{E}(x_j) &= p_A, \quad j = 1, 2 \quad , \quad \mathcal{E}(y_j) = p_B \quad j = 1, 2 \\ \mathcal{E}(x_j^2) &= p_A, \quad j = 1, 2 \quad , \quad \mathcal{E}(y_j^2) = p_B \quad j = 1, 2 \\ \mathcal{E}(x_1x_2) &= P_{AA} \quad , \quad \mathcal{E}(y_1y_2) = P_{BB} \\ \mathcal{E}(x_1y_1) &= P_{AB} \quad , \quad \mathcal{E}(x_2y_2) = P_{AB}\end{aligned}$$

The variances of  $x_j, y_j$  are  $p_A(1 - p_A), p_B(1 - p_B)$  for  $j = 1, 2$  and the covariance and correlation coefficients for  $x$  and  $y$  are

$$\begin{aligned}\text{Cov}(x_1, y_1) &= \text{Cov}(x_2, y_2) = P_{AB} - p_A p_B = D_{AB} \\ \text{Corr}(x_1, y_1) &= \text{Corr}(x_2, y_2) = D_{AB} / \sqrt{[p_A(1 - p_A)p_B(1 - p_B)]} = \rho_{AB}\end{aligned}$$

## Estimation of LD

With random sampling of gametes, gamete counts have a multinomial distribution:

$$\begin{aligned} \Pr(n_{AB}, n_{Ab}, n_{aB}, n_{ab}) &= \frac{n!(P_{AB})^{n_{AB}}(P_{Ab})^{n_{Ab}}(P_{aB})^{n_{aB}}(P_{ab})^{n_{ab}}}{n_{AB}!n_{Ab}!n_{aB}!n_{ab}!} \\ &= \frac{n!(p_A p_B + D_{AB})^{n_{AB}}(p_A p_b - D_{AB})^{n_{Ab}}}{n_{AB}!n_{Ab}!n_{aB}!n_{ab}!} \\ &\quad \times (p_a p_B - D_{AB})^{n_{aB}}(p_a p_b + D_{AB})^{n_{ab}} \end{aligned}$$

and this provides the maximum likelihood estimates of  $D_{AB}$  and  $\rho_{AB}$ :

$$\begin{aligned} \hat{D}_{AB} &= \frac{n_{AB}}{n} - \frac{n_{AB} + n_{Ab}}{n} \times \frac{n_{AB} + n_{aB}}{n} = \tilde{P}_{AB} - \tilde{p}_A \tilde{p}_B \\ \hat{\rho}_{AB} = r_{AB} &= \frac{\hat{D}_{AB}}{\sqrt{\tilde{p}_A \tilde{p}_a \tilde{p}_B \tilde{p}_b}} \end{aligned}$$



## Testing LD

Write MLE of  $D_{AB}$  as

$$\hat{D}_{AB} = \frac{n_{AB}n_{ab} - n_{Ab}n_{aB}}{(n_{AB} + n_{Ab})(n_{aB} + n_{ab})(n_{AB} + n_{aB})(n_{Ab} + n_{ab})}$$

and use “Delta method” to find

$$\begin{aligned} \text{Var}(\hat{D}_{AB}) \approx & \frac{1}{n} [p_A(1 - p_A)p_B(1 - p_B) \\ & + (1 - 2p_A)(1 - 2p_B)D_{AB} - D_{AB}^2] \end{aligned}$$

When  $D_{AB} = 0$ ,  $\text{Var}(\hat{D}_{AB}) = p_A(1 - p_A)p_B(1 - p_B)/n$ .

If  $\hat{D}_{AB}$  is assumed to be normally distributed then

$$X_{AB}^2 = \frac{\hat{D}_{AB}^2}{\text{Var}(\hat{D}_{AB})} = n\hat{\rho}_{AB}^2 = nr_{AB}^2$$

is appropriate for testing  $H_0 : D_{AB} = 0$ . When  $H_0$  is true,  $X_{AB}^2 \sim \chi_{(1)}^2$ . Note the analogy to the test statistic for Hardy-Weinberg equilibrium:  $X^2 = nf^2$ .

## Goodness-of-fit Test

The test statistic for the  $2 \times 2$  table

$$\begin{array}{cc|c} n_{AB} & n_{Ab} & n_A \\ n_{aB} & n_{ab} & n_a \\ \hline n_B & n_b & n \end{array}$$

has the value

$$X^2 = \frac{n(n_{AB}n_{ab} - n_{Ab}n_{aB})^2}{n_A n_a n_B n_b}$$

For DTD/YFM example,  $X^2 = 3.03$ . This is not statistically significant, even though disequilibrium was maximal.

## Composite Disequilibrium

When genotypes are scored, it is often not possible to distinguish between the two double heterozygotes  $AB/ab$  and  $Ab/aB$ , so that gametic frequencies cannot be inferred.

Under the assumption of random mating, in which genotypic frequencies are assumed to be the products of gametic frequencies, it is possible to estimate gametic frequencies with the EM algorithm. To avoid making the random-mating assumption, however, it is possible to work with a set of composite disequilibrium coefficients.

## Composite Disequilibrium

Although the separate digenic frequencies  $p_{AB}$  (one gamete) and  $p_{A,B}$  (two gametes) cannot be observed, their sum can be since

$$\begin{aligned}p_{AB} &= P_{AB}^{AB} + \frac{1}{2}P_{Ab}^{AB} + \frac{1}{2}P_{aB}^{AB} + \frac{1}{2}P_{ab}^{AB} \\p_{A,B} &= P_{AB}^{AB} + \frac{1}{2}P_{Ab}^{AB} + \frac{1}{2}P_{aB}^{AB} + \frac{1}{2}P_{aB}^{Ab} \\p_{AB} + p_{A,B} &= 2P_{AB}^{AB} + P_{Ab}^{AB} + P_{aB}^{AB} + \frac{P_{ab}^{AB} + P_{aB}^{Ab}}{2}\end{aligned}$$

Digenic disequilibrium is measured with a composite measure  $\Delta_{AB}$  defined as

$$\begin{aligned}\Delta_{AB} &= p_{AB} + p_{A,B} - 2p_A p_B \\ &= D_{AB} + D_{A,B}\end{aligned}$$

which is the sum of the gametic ( $D_{AB} = p_{AB} - p_A p_B$ ) and nongametic ( $D_{A,B} = p_{A,B} - p_A p_B$ ) coefficients.

## Composite Disequilibrium

If the counts of the nine genotypic classes are

	<i>BB</i>	<i>Bb</i>	<i>bb</i>
<i>AA</i>	$n_1$	$n_2$	$n_3$
<i>Aa</i>	$n_4$	$n_5$	$n_6$
<i>aa</i>	$n_7$	$n_8$	$n_9$

the count for pairs of alleles in an individual being *A* and *B*, whether received from the same or different parents, is

$$n_{AB} = 2n_1 + n_2 + n_4 + \frac{1}{2}n_5$$

and the MLE for  $\Delta$  is

$$\hat{\Delta}_{AB} = \frac{1}{n}n_{AB} - 2\tilde{p}_A\tilde{p}_B$$

## Composite Linkage Disequilibrium

For loci **A**, **B** define indicator variables  $x, y$  that take the value 1 for allele  $A, B$  and 0 for any other alleles. If gametes within individuals are indexed by  $j$ ,  $j = 1, 2$  then for expectations over samples from the same population

$$\mathcal{E}(x_j) = p_A, \quad j = 1, 2 \quad , \quad \mathcal{E}(y_j) = p_B \quad j = 1, 2$$

$$\mathcal{E}(x_j^2) = p_A, \quad j = 1, 2 \quad , \quad \mathcal{E}(y_j^2) = p_B \quad j = 1, 2$$

$$\mathcal{E}(x_1 x_2) = P_{AA} \quad , \quad \mathcal{E}(y_1 y_2) = P_{BB}$$

$$\mathcal{E}(x_1 y_1) = P_{AB} \quad , \quad \mathcal{E}(x_2 y_2) = P_{AB}$$

$$\mathcal{E}(x_1 y_2) = P_{A,B} \quad , \quad \mathcal{E}(x_2 y_1) = P_{A,B}$$

Write

$$D_A = P_{AA} - p_A^2 \quad , \quad D_B = P_{BB} - p_B^2$$

$$D_{AB} = P_{AB} - p_A p_B \quad , \quad D_{A,B} = P_{A,B} - p_A p_B$$

$$\Delta_{AB} = D_{AB} + D_{A,B}$$

## Composite Linkage Disequilibrium

Now set  $X = x_1 + x_2, Y = y_1 + y_2$  to get

$$\mathcal{E}(X) = 2p_A \quad , \quad \mathcal{E}(Y) = 2p_B$$

$$\mathcal{E}(X^2) = 2(p_A + P_{AA}) \quad , \quad \mathcal{E}(Y^2) = 2(p_B + P_{BB})$$

$$\text{Var}(X) = 2p_A(1 - p_A)(1 + f_A) \quad , \quad \text{Var}(Y) = 2p_B(1 - p_B)(1 + f_B)$$

and

$$\mathcal{E}(XY) = 2(P_{AB} + P_{A,B})$$

$$\text{Cov}(X, Y) = 2(P_{AB} - p_A p_B) + 2(P_{A,B} - p_A p_B)$$

$$= 2(D_{AB} + D_{A,B}) = 2\Delta_{AB}$$

$$\text{Corr}(X, Y) = \frac{\Delta_{AB}}{\sqrt{p_A(1 - p_A)(1 + f_A)p_B(1 - p_B)(1 + f_B)}}$$

## Composite Linkage Disequilibrium

$$\hat{\Delta}_{AB} = n_{AB}/n - 2\tilde{p}_A\tilde{p}_B$$

where

$$n_{AB} = 2n_{AABB} + n_{AABb} + n_{AaBB} + \frac{1}{2}n_{AaBb}$$

This does not require phased data.

By analogy to the gametic linkage disequilibrium result, a test statistic for  $\Delta_{AB} = 0$  is

$$X_{AB}^2 = \frac{n\hat{\Delta}_{AB}^2}{\tilde{p}_A(1 - \tilde{p}_A)(1 + \hat{f}_A)\tilde{p}_B(1 - \tilde{p}_B)(1 + \hat{f}_B)}$$

This is assumed to be approximately  $\chi_{(1)}^2$  under the null hypothesis.



## Example

For the data on slide 75:

	<i>BB</i>	<i>Bb</i>	<i>bb</i>	Total
<i>AA</i>	$n_{AABB} = 5$	$n_{AABb} = 3$	$n_{AAbb} = 2$	$n_{AA} = 10$
<i>Aa</i>	$n_{AaBB} = 3$	$n_{AaBb} = 2$	$n_{Aabb} = 0$	$n_{Aa} = 5$
<i>aa</i>	$n_{aaBB} = 0$	$n_{aaBb} = 0$	$n_{aabb} = 0$	$n_{aa} = 0$
Total	$n_{BB} = 8$	$n_{Bb} = 5$	$n_{bb} = 2$	$n = 15$

$$n_{AB} = 2 \times 5 + 3 + 3 + \frac{1}{2}(2) = 17$$

$$n_A = 25, \tilde{p}_A = 5/6$$

$$n_B = 21, \tilde{p}_B = 7/10$$

## Example

The estimated composite disequilibrium coefficient is

$$\hat{\Delta}_{AB} = \frac{17}{15} - 2\frac{2521}{3030} = -\frac{1}{30} = -0.033$$

Previous work on EM algorithm estimated  $p_{AB}$  as  $16/30$  so

$$\hat{D}_{AB} = \frac{16}{30} - \frac{2521}{3030} = -\frac{1}{20} = -0.050$$

# LD vs Composite LD Estimates

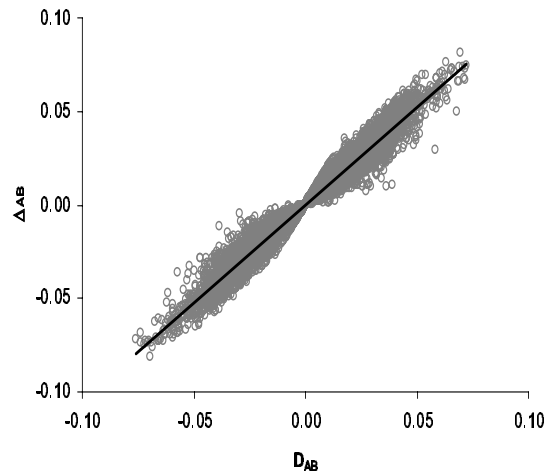


Figure 6: Linkage disequilibrium estimates

A comparison of gametic LD estimates from the EM algorithm assuming HWE vs composite LD with no HWE assumption.

# LD vs Composite LD Tests

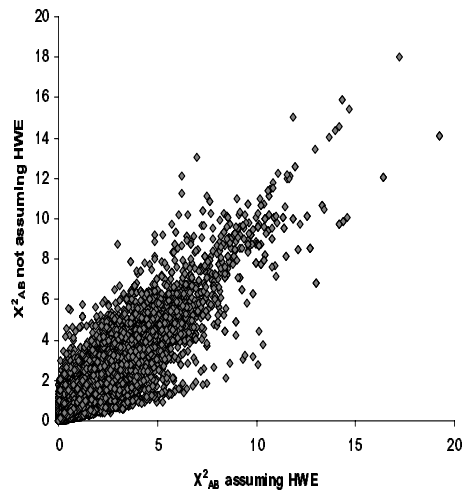


Figure 5: Linkage disequilibrium test statistics (Caucasian)

A comparison of gametic LD tests from the EM algorithm assuming HWE vs composite LD with no HWE assumption.

# POPULATION STRUCTURE

## Population Data

Individuals from several populations are scored at a series of marker loci. At each locus, an individual has two alleles, one from each parent, and these can be identified. For example, at locus D3S1358:

Allele	AFC	NSC	QLC	SAC	TAC	VIA	WAB
11	.000	.001	.002	.001	.000	.000	.000
12	.004	.003	.001	.001	.000	.000	.010
13	.008	.003	.002	.002	.000	.000	.001
14	.123	.098	.159	.125	.152	.008	.075
15	.261	.264	.365	.252	.244	.385	.353
16	.250	.270	.250	.265	.241	.277	.242
17	.187	.198	.123	.202	.197	.246	.190
18	.154	.152	.091	.144	.157	.077	.122
19	.012	.011	.006	.007	.010	.008	.007
20	.002	.000	.000	.000	.000	.000	.000

# Questions of Interest

- How much genetic variation is there? (animal conservation)
- How much migration (gene flow) is there between populations? (molecular ecology)
- How does the genetic structure of populations affect tests for linkage between genetic markers and human disease genes? (human genetics)
- How should the evidence of matching marker profiles be quantified? (forensic science)
- What is the evolutionary history of the populations sampled? (evolutionary genetics)

## Statistical Analysis

Possible to approach these data from purely statistical viewpoint.

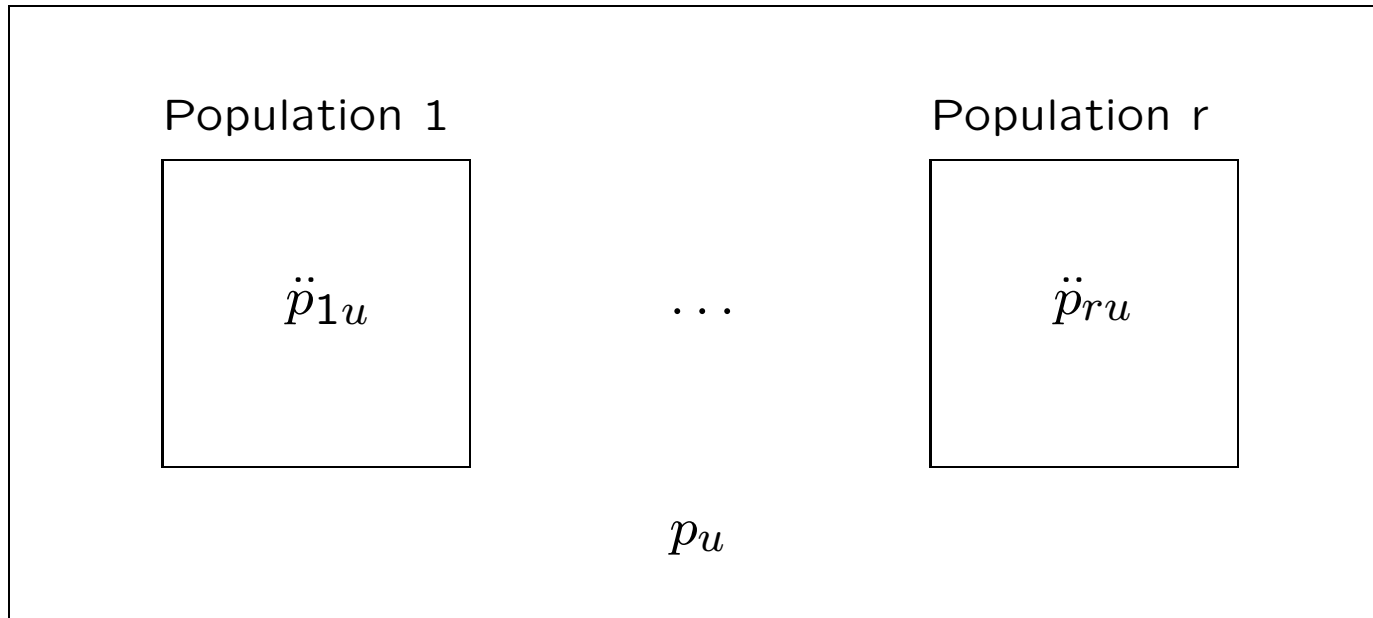
Could test for differences in allele frequencies among populations.

Could use various multivariate techniques to cluster populations.

These analyses may not answer the biological questions.



## Genetic Analysis: Frequencies of Allele $A_u$

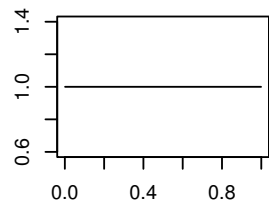


Among samples from population  $i$ : counts for allele  $u$  follow a binomial distribution with mean  $\check{p}_{iu}$ .

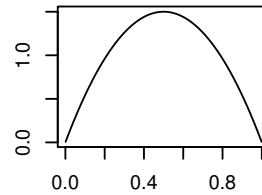
Among replicates of population  $i$ :  $\check{p}_{iu}$  values follow a Beta distribution with mean  $p_u$  and variance  $p_u(1 - p_u)\theta_i$ .

# Beta distribution: Theoretical

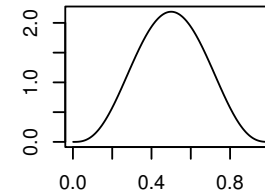
The beta distribution can take a variety of shapes.



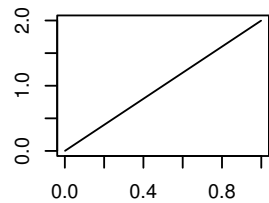
$v=1, w=1$



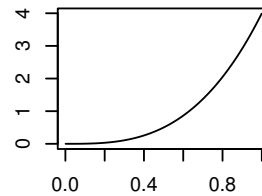
$v=2, w=2$



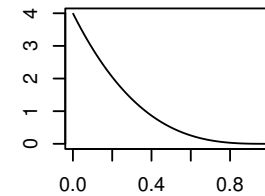
$v=4, w=4$



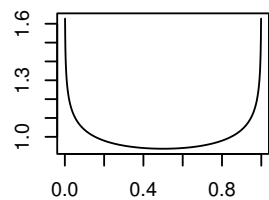
$v=2, w=1$



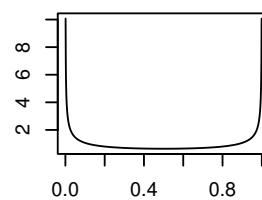
$v=4, w=1$



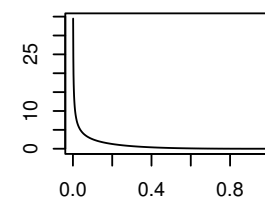
$v=1, w=4$



$v=0.9, w=0.9$



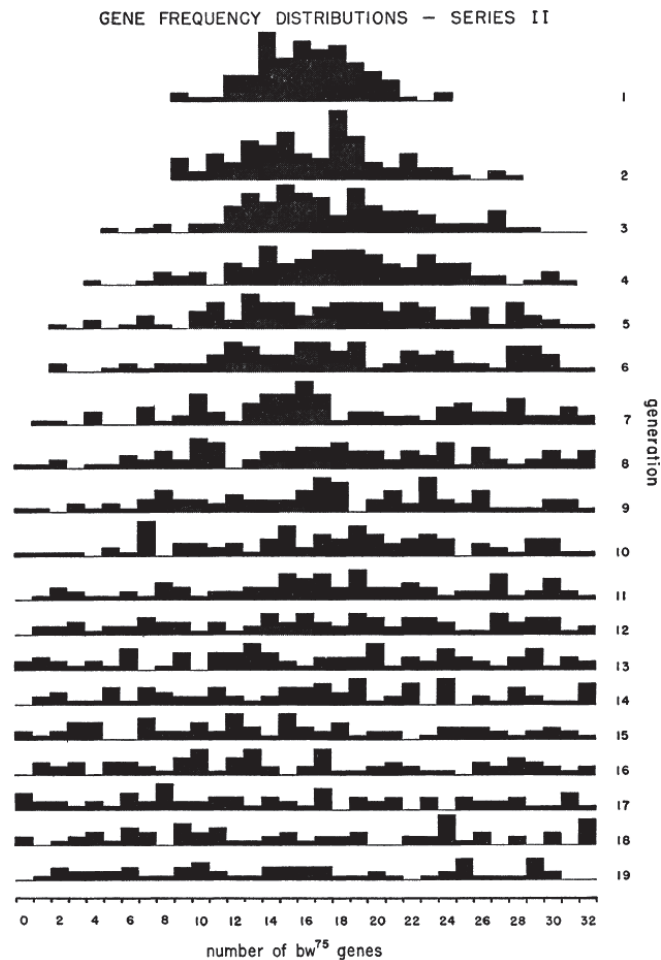
$u=0.5, w=0.5$



$u=0.5, w=4$

# Beta distribution: Experimental

The beta distribution is suggested by a *Drosophila* cage experiment by P. Buri (Evolution 10:367, 1956).



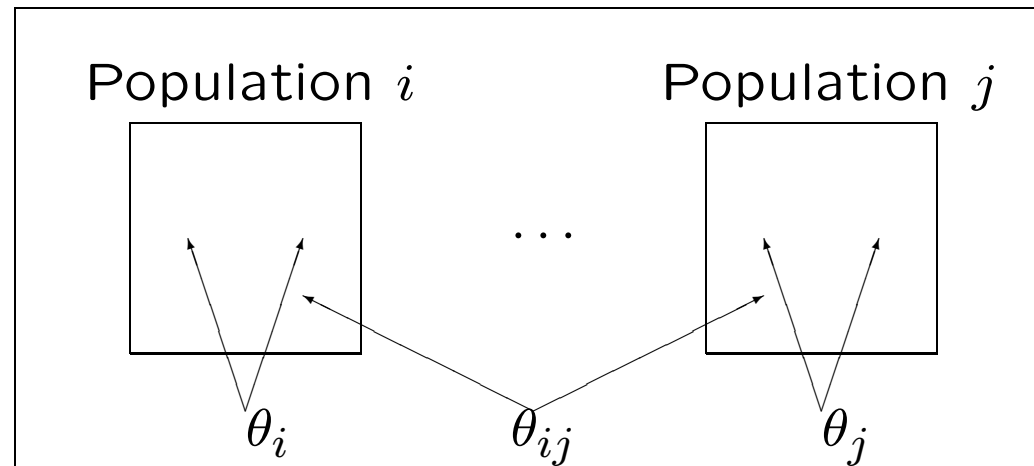
## What is $\theta$ ?

Two ways of thinking about  $\theta$ .

It measures the extra degree of relatedness of individuals because they belong to the same population. We think of this reflecting long-term evolutionary history, but can use the same logic for people related because they are in the same family: first cousins have a  $\theta$  value of 0.0625.

$\theta$  also helps to measure the variance of allele frequencies over populations.

## What is $\theta$ ?



$\theta$ 's are statements about pairs of alleles: the probabilities the pairs are identical by descent.

$\theta_W$  is the average of the within-population coancestries  $\theta_i$ .

$\theta_B$  is the average of the between-population-pair coancestries  $\theta_{ij}$ .

## Predicted Values of the $\theta$ 's: Pure Drift

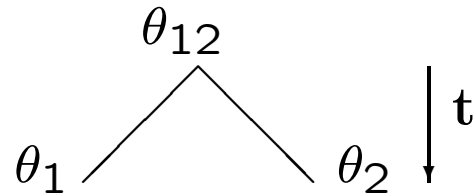
Our estimation procedure for the  $\theta$ 's holds for all evolutionary scenarios, but the theoretical values of the  $\theta$ 's do depend on the history of the sampled populations.

However, in the case of pure drift, where population  $i$  has constant size  $N_i$  and there is random mating,  $t$  generations after the population began drifting from an ancestral population in which  $\beta = 0$

$$\beta_W(t) = 1 - \frac{1}{r} \sum_i \left(1 - \frac{1}{2N_i}\right)^t$$

If  $t$  is small relative to large  $N_i$ 's,  $\beta_W(t) \approx t/(2N_h)$  where  $N_h$  is the harmonic mean of the  $N_i$ .

## Drift Model: Two Populations



$$\theta_1 = 1 - (1 - \theta_{12}) \left( \frac{2N_1 - 1}{2N_1} \right)^t, \quad \theta_2 = 1 - (1 - \theta_{12}) \left( \frac{2N_2 - 1}{2N_2} \right)^t$$

Therefore

$$\frac{\theta_i - \theta_{12}}{1 - \theta_{12}} = 1 - \left( \frac{2N_i - 1}{2N_i} \right)^t \approx \frac{t}{2N_i}$$

showing the advantage of working with ibd within populations relative to ibd between populations:  $\beta_i = (\theta_i - \theta_{12}) / (1 - \theta_{12})$ .

## Drift Model: Two Populations

The unweighted within-population average estimator  $\hat{\beta}_W$  has expectation

$$\begin{aligned}\beta_W &= \frac{\theta_W - \theta_B}{1 - \theta_B} = \frac{\frac{\theta_1 + \theta_2}{2} - \theta_{12}}{1 - \theta_{12}} \\ &\approx \frac{1}{2} \left( \frac{1}{2N_1} + \frac{1}{2N_2} \right)^t = \frac{t}{2N_h}\end{aligned}$$

so time is being estimated in terms of the harmonic mean of the two population sizes.



## Drift, Mutation and Migration

For populations 1 or 2 with sizes  $N_1$  or  $N_2$ , if  $m_1$  or  $m_2$  are the proportions of alleles from population 2 or 1, the changes in the  $\theta$ 's from generation  $t$  to  $t + 1$  are

$$\begin{aligned}\theta_{1,t+1} &= (1 - \mu)^2 \left[ (1 - m_1)^2 \theta_{1,t}^* + 2m_1(1 - m_1)\theta_{12,t} + m_1^2 \theta_{2,t}^* \right] \\ \theta_{2,t+1} &= (1 - \mu)^2 \left[ m_2^2 \theta_{1,t}^* + 2m_2(1 - m_2)\theta_{12,t} + (1 - m_2)^2 \theta_{2,t}^* \right] \\ \theta_{12,t+1} &= (1 - \mu)^2 \left[ (1 - m_1)m_2 \theta_{1,t}^* + [(1 - m_1)(1 - m_2) \right. \\ &\quad \left. + m_1m_2] \theta_{12,t} + m_1(1 - m_2)\theta_{2,t}^* \right]\end{aligned}$$

where  $\theta_{i_t}^* = 1/(2N_i) + (2N_i - 1)\theta_{i_t}/(2N_i)$  and  $\mu$  is the infinite-allele mutation rate.

## Drift and Mutation

If there is no migration, the  $\theta$ 's tend to equilibrium values of

$$\hat{\theta}_1 \approx \frac{1}{1 + 4N_1\mu}$$

$$\hat{\theta}_2 \approx \frac{1}{1 + 4N_2\mu}$$

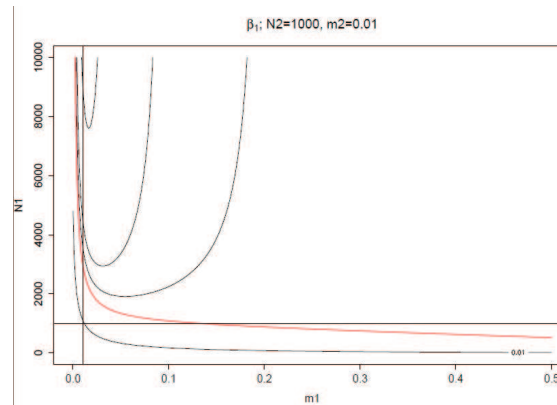
$$\hat{\theta}_{12} = 0$$

so  $\beta_1 = \theta_1, \beta_2 = \theta_2$ .

# Drift, Mutation and Migration

The  $\theta$ 's are all positive, and  $\theta_1 + \theta_2 \geq 2\theta_{12}$ , so that  $\beta_W \geq 0$ .

It is possible that  $\theta_1 > \theta_{12} > \theta_2$  or  $\theta_1 < \theta_{12} < \theta_2$ . In that case  $\beta_1 > 0 > \beta_2$  or  $\beta_1 < 0 < \beta_2$ .



The region above and to the right of the red line has equilibrium values of  $\theta_1 \leq \theta_{12}$ , i.e.  $\beta_1 \leq 0$ . In that region a pair of alleles within population 1 has a smaller probability of ibd than does an allele from population 1 with an allele from population 2.

## Variation in allele frequencies

From Weir & Hill. 2002. Annual Review of Genetics:

The sample frequencies for allele  $A_u$  for populations  $i$  have variances and covariances over samples from populations and over replicates of populations:

$$\text{Var}(\tilde{p}_{iu}) = p_u(1 - p_u) \left( \theta_i + \frac{1 - \theta_i}{n_i} \right)$$

$$\text{Cov}(\tilde{p}_{iu}, \tilde{p}_{iv}) = -p_u p_v \left( \theta_i + \frac{1 - \theta_i}{n_i} \right), \quad u \neq v$$

$$\text{Cov}(\tilde{p}_{iu}, \tilde{p}_{ju}) = p_u(1 - p_u)\theta_{ij}, \quad i \neq j$$

$$\text{Cov}(\tilde{p}_{iu}, \tilde{p}_{ju}) = -p_u p_v \theta_{ij}, \quad u \neq v, i \neq j$$

## Sample Heterozygosities

Population genetic literature makes use of “heterozygosities”

$$\begin{aligned}\tilde{H}_i &= \frac{n_i}{n_i - 1} \sum_u \tilde{p}_{iu}(1 - \tilde{p}_{iu}) = \frac{n_i}{n_i - 1} (1 - \sum_u \tilde{p}_{iu}^2) \\ &\approx 1 - \sum_u \tilde{p}_{iu}^2\end{aligned}$$

$$\tilde{H}_{ij} = 1 - \sum_u \tilde{p}_{iu}\tilde{p}_{ju}$$

Unweighted averages of these quantities over  $r$  populations are

$$\begin{aligned}\tilde{H}_W &= \frac{1}{r} \sum_{i=1}^r \tilde{H}_i \\ \tilde{H}_B &= \frac{1}{r(r-1)} \sum_{i \neq j} \tilde{H}_{ij}\end{aligned}$$

## Sample Match Proportions

If a sample of  $n_i$  alleles from population  $i$  has  $n_{iu}$  copies of allele  $u$ , then the proportion of pairs of alleles that match within this population is

$$\tilde{M}_i = \frac{1}{n_i(n_i - 1)} \sum_u n_{iu}(n_{iu} - 1) = 1 - \tilde{H}_i$$

Similarly, the proportion of pairs of alleles, one from each of populations  $i$  and  $j$ , that match is

$$\tilde{M}_{ij} = \frac{1}{n_i n_j} \sum_u n_{iu} n_{ju} = 1 - \tilde{H}_{ij}$$

Sample match proportions and heterozygosities may therefore be used interchangeably:  $\tilde{M} = 1 - \tilde{H}$ .

# Population Genetic Model

Under our model, we can predict the values of sample match proportions:

$$\mathcal{E}(\tilde{M}_i) = \theta_i + (1 - \theta_i)M_T$$

$$\mathcal{E}(\tilde{M}_{ij}) = \theta_{ij} + (1 - \theta_{ij})M_T$$

$$\mathcal{E}(\tilde{M}_W) = \theta_W + (1 - \theta_W)M_T$$

$$\mathcal{E}(\tilde{M}_B) = \theta_B + (1 - \theta_B)M_T$$

where  $M_T = \sum_u p_u^2$ .

These suggest that we can manipulate sample matching proportions to make statements about  $\theta_W$  and  $\theta_B$ .

## Estimators of $\beta$ 's

We can estimate  $\theta_i$  or  $\theta_W$  only relative to  $\theta_B$ . Matching within a population has meaning only when compared to matching between populations. The estimates do not require us to know the true allele frequencies.

$$\hat{\beta}_i = \frac{\tilde{M}_i - \tilde{M}_B}{1 - \tilde{M}_B} , \quad \mathcal{E}(\hat{\beta}_i) = \beta_i = \frac{\theta_i - \theta_B}{1 - \theta_B}$$

$$\hat{\beta}_W = \frac{\tilde{M}_W - \tilde{M}_B}{1 - \tilde{M}_B} , \quad \mathcal{E}(\hat{\beta}_W) = \beta_W = \frac{\theta_W - \theta_B}{1 - \theta_B}$$

These *unweighted* estimates are “relative to”  $\theta_B$ , but they allow comparisons among populations. Estimation of  $\theta_B$  is not possible:  $\theta_B$  can also refer to the ancestral/reference population.

It is  $\hat{\beta}_W$  that corresponds to  $F_{ST}$ .



## Drift, Mutation and Migration

The  $\theta$ 's are all positive, and  $\theta_1 + \theta_2 \geq 2\theta_{12}$ , so that  $\beta_W \geq 0$ .

It is possible that  $\theta_1 > \theta_{12} > \theta_2$  or  $\theta_1 < \theta_{12} < \theta_2$ . In that case  $\beta_1 > 0 > \beta_2$  or  $\beta_1 < 0 < \beta_2$ .

Similarly, the  $\tilde{M}$ 's are all positive, and  $\tilde{M}_1 + \tilde{M}_2 \geq 2\tilde{M}_{12}$ , so that  $\hat{\beta}_W \geq 0$ .

It is possible that  $\tilde{M}_1 > \tilde{M}_{12} > \tilde{M}_2$  or  $\tilde{M}_1 < \tilde{M}_{12} < \tilde{M}_2$ . In that case  $\hat{\beta}_1 > 0 > \hat{\beta}_2$  or  $\hat{\beta}_1 < 0 < \hat{\beta}_2$ .

## Weir & Cockerham 1984 Restricted Model

If populations have equal evolutionary histories ( $\theta_i = \theta$ , all  $i$ ) and are independent ( $\theta_{ij} = 0$ , all  $i \neq j$ )

$$\text{Var}(\tilde{p}_{iu}) = p_u(1 - p_u) \left( \theta + \frac{1 - \theta}{n_i} \right)$$

$$\text{Cov}(\tilde{p}_{iu}, \tilde{p}_{iv}) = -p_u p_v \left( \theta + \frac{1 - \theta}{n_i} \right), \quad u \neq v$$

$$\text{Cov}(\tilde{p}_{iu}, \tilde{p}_{ju}) = 0, \quad i \neq j$$

$$\text{Cov}(\tilde{p}_{iu}, \tilde{p}_{jv}) = 0, \quad u \neq v, i \neq j$$

W&C worked with overall allele frequencies that were weighted by sample sizes

$$\bar{p}_u = \frac{1}{\sum_i n_i} \sum_i n_i \tilde{p}_{iu}$$

If  $\theta = 0$ , these weighted means have minimum variance.

## Weir & Cockerham 1984 Restricted Model

Two mean squares were constructed for each allele:

$$\text{MSB}_u = \frac{1}{r-1} \sum_{i=1}^r n_i (\tilde{p}_{iu} - \bar{p}_u)^2$$

$$\text{MSW}_u = \frac{1}{\sum_i (n_i - 1)} \sum_i n_i \tilde{p}_{iu} (1 - \tilde{p}_{iu})$$

These have expected values

$$\mathcal{E}(\text{MSB}_u) = p_u(1 - p_u)[(1 - \theta) + n_c\theta]$$

$$\mathcal{E}(\text{MSW}_u) = p_u(1 - p_u)(1 - \theta)$$

where  $n_c = (\sum_i n_i - \sum_i n_i^2 / \sum_i n_i) / (r - 1)$ . The Weir & Cockerham *weighted* estimator of  $\theta$  (or  $F_{ST}$ ) is

$$\hat{\theta}_{WC} = \frac{\sum_u (\text{MSB}_u - \text{MSW}_u)}{\text{MSB}_u + (n_c - 1)\text{MSW}_u}$$

## Weir & Cockerham Estimator

Under the Weir and Hill model, the Weir and Cockerham estimator has expectation

$$\mathcal{E}(\hat{\theta}_{WC}) = \frac{\theta_W^c - \theta_B^c + Q}{1 - \theta_B^c + Q} \quad \text{instead of} \quad \frac{\theta_W - \theta_B}{1 - \theta_B}$$

where

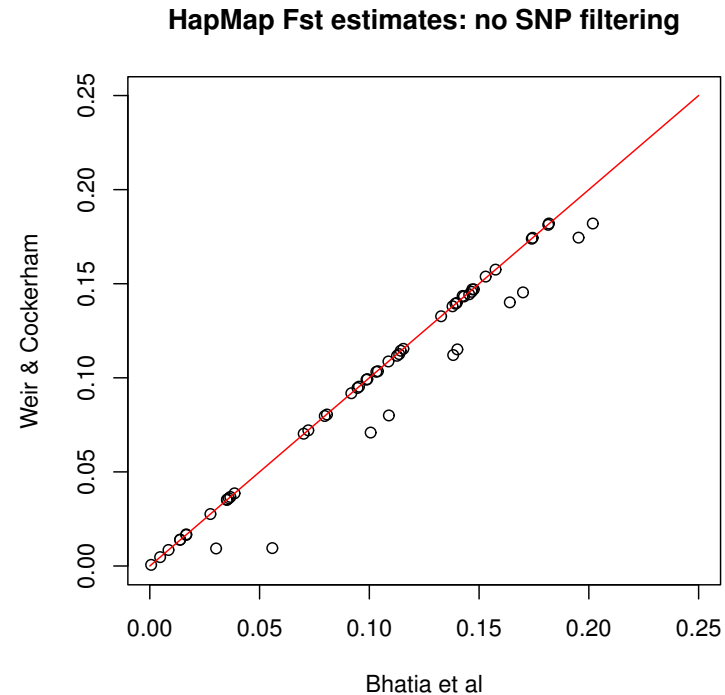
$$\theta_W^c = \frac{\sum_i n_i^c \theta_i}{\sum_i n_i^c}, \quad \theta_B^c = \frac{\sum_{i \neq i'} n_i n_{i'} \theta_{ii'}}{\sum_{i \neq i'} n_i n_{i'}}$$
$$n_i^c = n_i - \frac{n_i^2}{\sum_i n_i}, \quad n_c = \frac{1}{r-1} \sum_i n_i^c$$
$$Q = \frac{1}{(r-1)n_c} \sum_i \left( \frac{n_i}{\bar{n}} - 1 \right) \theta_i$$

If the Weir and Cockerham model holds ( $\theta_i = \theta$ ), or if  $n_i = n$ , or if  $n_c$  is large, then  $Q = 0$ .

## HapMap III SNP Data

Code	Population Description	Sample size	Number of SNPs typed
ASW	African ancestry in Southwest USA	142	61566
CEU	Utah residents with Northern and Western European ancestry from CEPH collection	324	56218
CHB	Han Chinese in Beijing, China	160	51946
CHD	Chinese in Metropolitan Denver, Colorado	140	50517
GIH	Gujarati Indians in Houston, Texas	166	55710
JPT	Japanese in Tokyo, Japan	168	53020
LWK	Luhya in Webuye, Kenya	166	60026
MXL	Mexican ancestry in Los Angeles, California	142	57937
MKK	Maasai in Kinyawa, Kenya	342	61057
TSI	Toscani in Italia	154	55881
YRI	Yoruba in Ibadan, Nigeria	326	58559

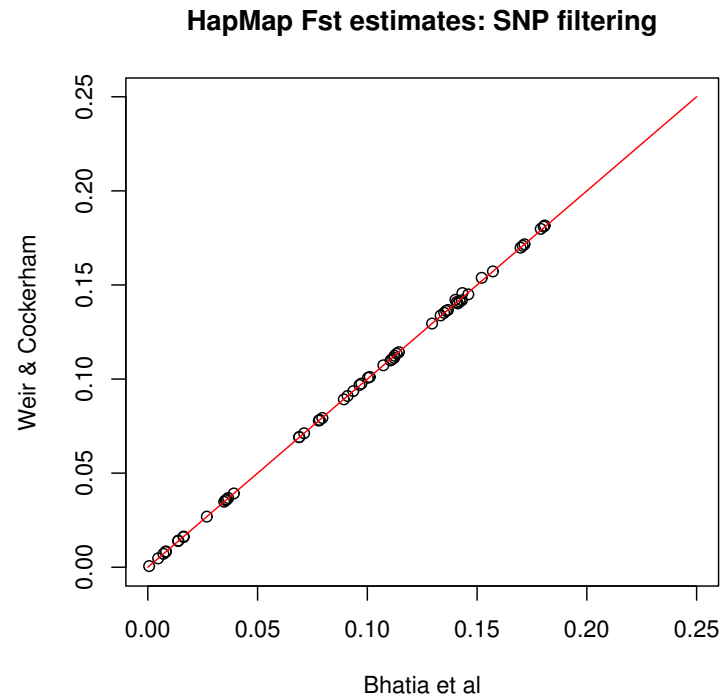
# WC vs Unweighted Estimator



$F_{ST}$  estimates for HapMap III, using all 87,592 SNPs on chromosome 1.

(Bhatia et al, 2013, Genome Research 23:1514-1521.)

# WC vs Unweighted Estimator



$F_{ST}$  estimates for HapMap III, using the 42,463 SNPs on chromosome 1 that have at least five copies of the minor allele in samples from all 11 populations.

(Bhatia et al, 2013, Genome Research 23:1514-1521.)

## Worldwide Autosomal-STR Survey

Buckleton et al, Forensic Sci Int, 2016 compiled a survey of 250 published papers showing allele frequencies at 24 forensic STR markers from 446 populations in 8 ancestral groups. Represents data from 494,473 individuals.

The ancestral groups were identified by a combination of clustering and geographic criteria.

Moment estimates were obtained for each locus  $l$  in each population  $i$  from

$$\hat{\beta}_{il} = \frac{\tilde{M}_{il} - \tilde{M}_{Bl}}{1 - \tilde{M}_{Bl}}$$



## STR Survey: $\hat{\beta}_W$ Values for Groups and Loci

Locus	Gepgraphic Region								Aver.
	Africa	AusAb	Asian	Cauc	Hisp	IndPK	NatAm	Poly	
CSF1PO	0.003	0.002	0.008	0.008	0.002	0.007	0.055	0.026	0.011
D1S1656	0.000	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.011
D2S441	0.000	0.000	0.002	0.003	0.021	0.000	0.000	0.000	0.020
D2S1338	0.009	0.004	0.011	0.017	0.013	0.003	0.023	0.005	0.031
D3S1358	0.004	0.010	0.009	0.006	0.012	0.040	0.079	0.001	0.025
D5S818	0.002	0.013	0.009	0.008	0.014	0.018	0.044	0.007	0.029
D6S1043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016
D7S820	0.004	0.021	0.010	0.007	0.007	0.046	0.030	0.005	0.026
D8S1179	0.003	0.007	0.012	0.006	0.002	0.031	0.020	0.008	0.019
D10S1248	0.000	0.000	0.000	0.002	0.004	0.000	0.000	0.000	0.007
D12S391	0.000	0.000	0.000	0.003	0.020	0.000	0.000	0.000	0.010
D13S317	0.015	0.016	0.013	0.008	0.014	0.025	0.050	0.014	0.038
D16S539	0.007	0.002	0.015	0.006	0.009	0.005	0.048	0.004	0.021
D18S51	0.011	0.012	0.014	0.006	0.004	0.010	0.033	0.003	0.018
D19S433	0.009	0.001	0.009	0.010	0.014	0.000	0.022	0.014	0.023
D21S11	0.014	0.012	0.013	0.007	0.006	0.023	0.067	0.018	0.021
D22S1045	0.000	0.000	0.007	0.001	0.000	0.000	0.000	0.000	0.015
FGA	0.002	0.009	0.012	0.004	0.007	0.016	0.021	0.006	0.013
PENTAD	0.008	0.000	0.012	0.012	0.002	0.017	0.000	0.000	0.022
PENTAE	0.002	0.000	0.017	0.006	0.003	0.012	0.000	0.000	0.020
SE33	0.000	0.000	0.012	0.001	0.000	0.000	0.000	0.000	0.004
TH01	0.022	0.001	0.022	0.016	0.018	0.014	0.071	0.017	0.071
TPOX	0.019	0.087	0.016	0.011	0.007	0.018	0.064	0.031	0.035
VWA	0.009	0.007	0.017	0.007	0.012	0.022	0.028	0.005	0.023
All Loci	0.006	0.014	0.010	0.007	0.008	0.018	0.043	0.011	0.022

# Variation Over Populations for Each Group

See Buckleton et al, 2016, Figure 1.

# Variation Over Loci for Each Population

See Buckleton et al, 2016, Figure 2.

Color code: Black=African, Grey=AusAb, Yellow=Asian, Blue=Caucn, Purple=Hispanic, Brown=IndPk, Red=NatAm, Orange=Inuit, Brown=Andam, Green=Polyn.

## Multiple Loci

The unweighted estimators for locus  $l$  are of the form

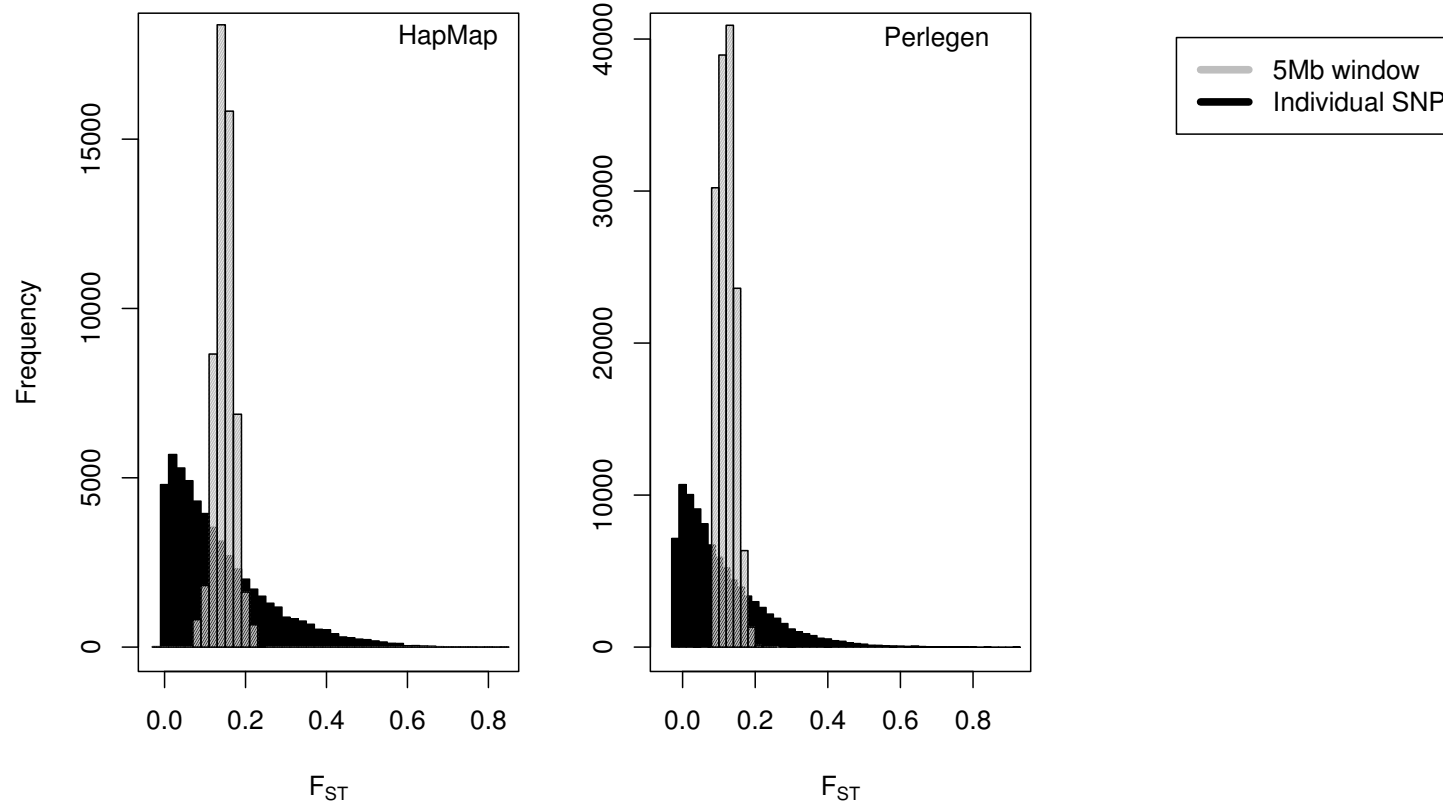
$$\text{Estimator}_l = \frac{\tilde{M}_{xl} - \tilde{M}_{Bl}}{1 - \tilde{M}_{Bl}}, \quad x = i, W, ij$$

Here  $x$  can refer to population  $i$ , the average  $W$  over populations, or the pair of populations  $i, j$ . With several loci, these can be extended to

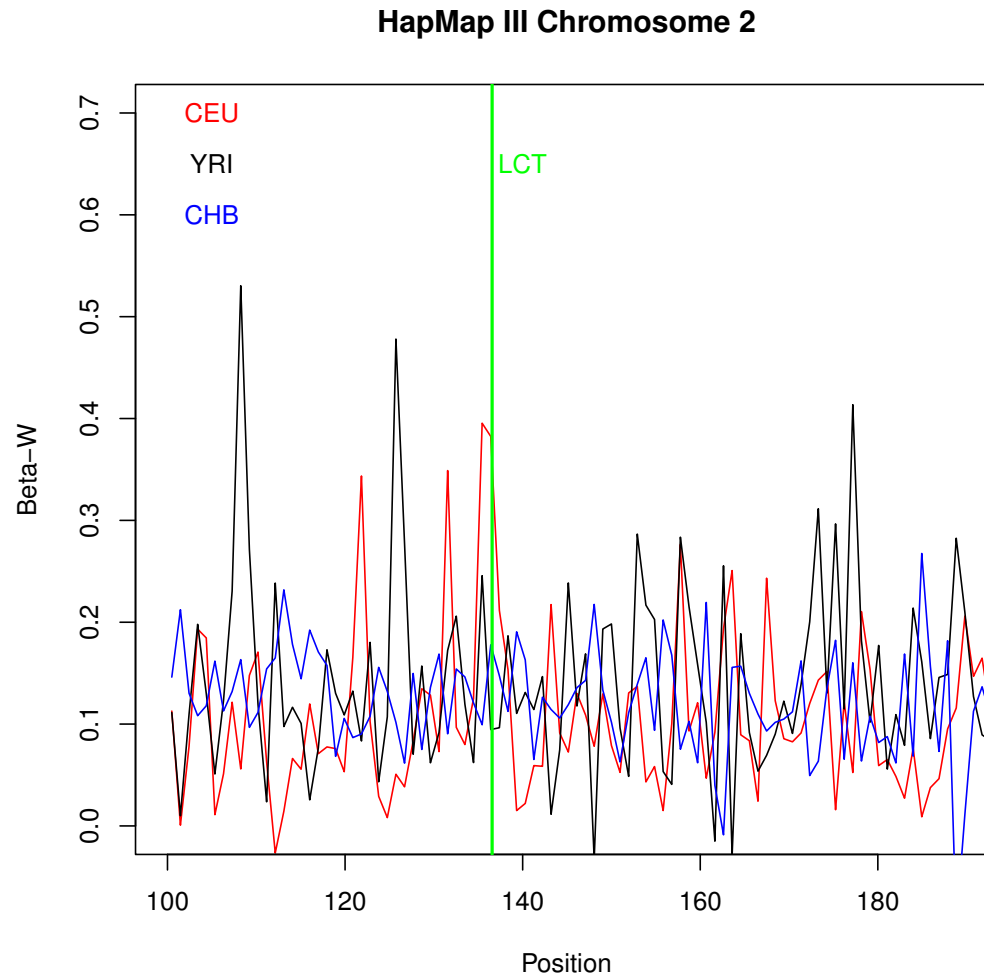
$$\text{Estimator} = \frac{\sum_l (\tilde{M}_{xl} - \tilde{M}_{Bl})}{\sum_l (1 - \tilde{M}_{Bl})} \quad x = i, ij, W$$

and these estimate  $(\theta_x - \theta_B)/(1 - \theta_B)$  if each locus has the same value of the  $\theta$ 's. Otherwise they estimate a weighted average of the different  $\theta$  values, where the weights are functions of the allele frequencies at the loci in the sum.

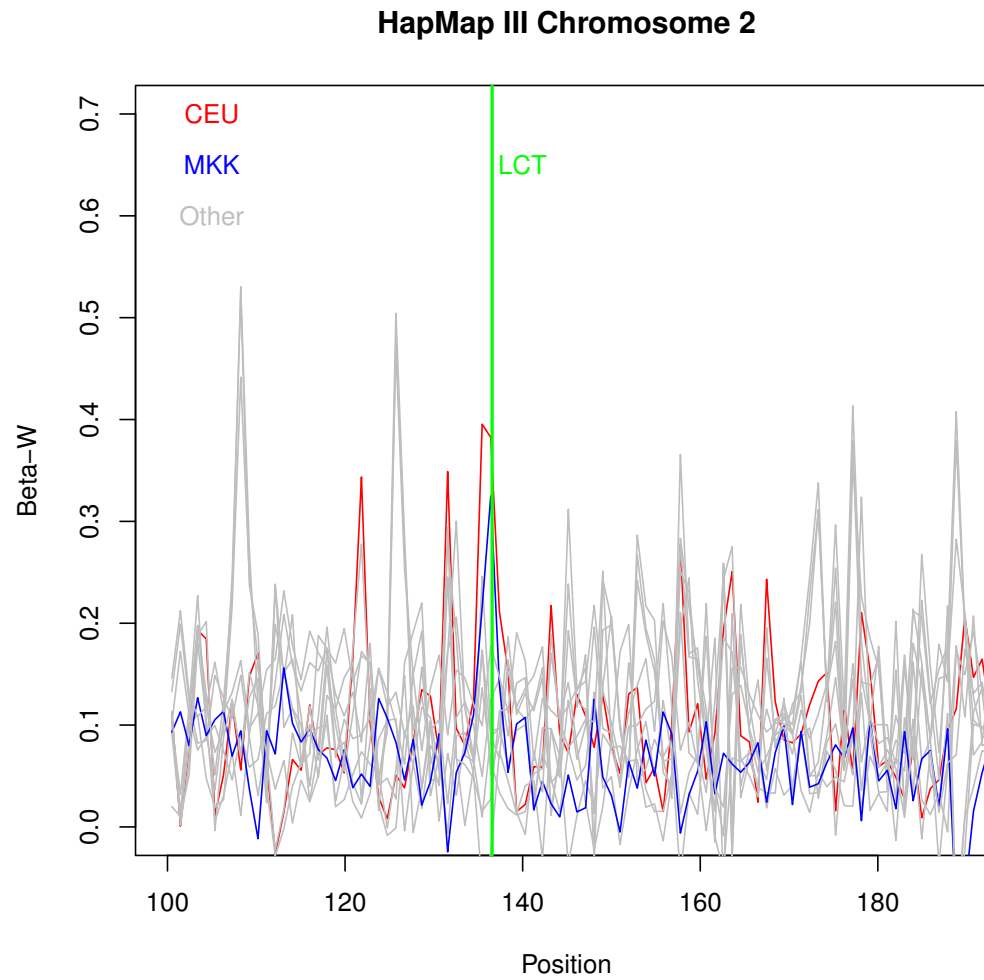
# Effect of Number of Loci



# $\hat{\beta}_W$ in LCT Region: 3 Populations



# $\hat{\beta}_W$ in LCT Region: 11 Populations



## MKK Population

“The Maasai are a pastoral people in Kenya and Tanzania, whose traditional diet of milk, blood and meat is rich in lactose, fat and cholesterol. In spite of this, they have low levels of blood cholesterol, and seldom suffer from gallstones or cardiac diseases.

Analysis of HapMap 3 data using Fixation Index (Fst) identified genomic regions and single nucleotide polymorphisms (SNPs) as strong candidates for recent selection for lactase persistence and cholesterol regulation in 143156 founder individuals from the Maasai population in Kinyawa, Kenya (MKK). The strongest signal identified by all three metrics was a 1.7 Mb region on Chr2q21. This region contains the gene LCT (Lactase) involved in lactase persistence.”

Wagh et al., PLoS One 7: e44751, 2012



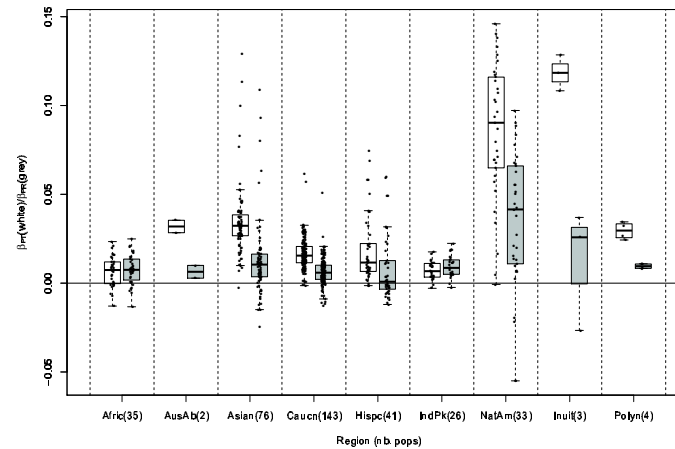
## Region vs World Reference Populations

In general, consider populations in some region  $R$ . For population  $i$  in the region,  $\beta_i$  relative to the region can be estimated by comparing the within-population- $i$  matching proportion  $\tilde{M}_i$  for that population to the average between-population-pair matching proportion  $\tilde{M}_R$  for populations in the region:  $\hat{\beta}_{i_R} = (\tilde{M}_i - \tilde{M}_R)/(1 - \tilde{M}_R)$ .

Can also estimate  $\beta_i$  relative to the world by comparing the population- $i$  matching proportion  $\tilde{M}_i$  for that population to the average between-population-pair matching proportion  $\tilde{M}_T$  for all populations in the world:  $\hat{\beta}_{i_T} = (\tilde{M}_i - \tilde{M}_T)/(1 - \tilde{M}_T)$ .

The regional-reference estimate  $\hat{\beta}_{i_R}$  is greater than the world-reference estimate  $\hat{\beta}_{i_T}$  if the between-population-pair matching proportion is greater across the world than it is across the region:  $\tilde{M}_{B_T} > \tilde{M}_{B_R}$ . More global matching than regional matching: more regional diversity than global diversity.

# STR Data: World vs Regional Reference Population



Population-specific  $\beta$ 's, for each geographic group, using all loci. Each box plot indicates the inter-quartile variation among populations within the group. For each geographic group, the left hand plot (white box plot) compares each within-population matching proportion to the average matching proportion among all pairs of populations. The right hand plot (grey box plot) compares each within-population matching proportion to the average matching proportion among all pairs of populations in that region.

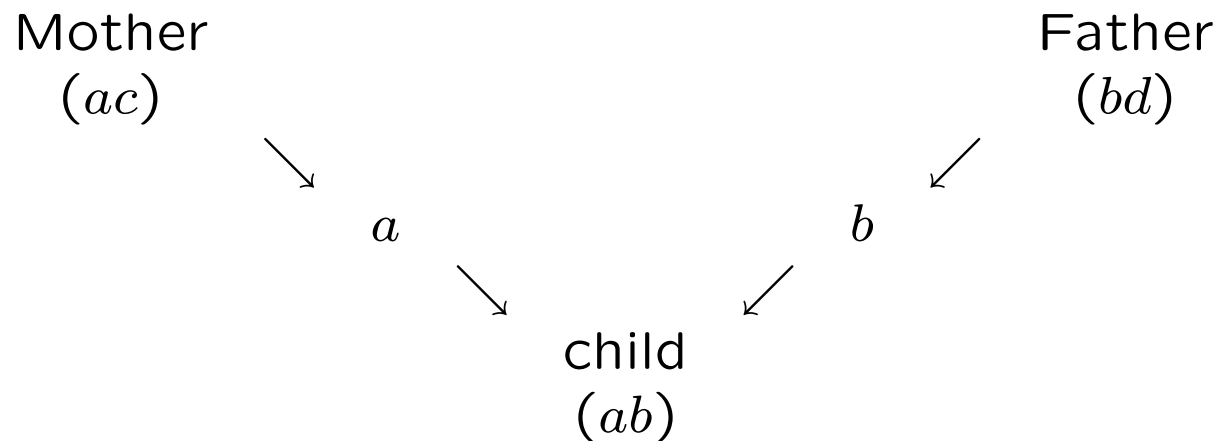
# **INBREEDING AND RELATEDNESS**

## Inbreeding

Two alleles that descend from the same allele are said to be **identical by descent (ibd)**.

The probability that two parents transmit ibd alleles to a child is the **inbreeding coefficient  $F$**  of the child.

Use small letters for alleles, and capital letters for their particular type: i.e. parents might transmit alleles  $a$  and  $b$  to their child, and these alleles might both be type  $A$ .



## Relatedness

Two individuals that have ibd alleles are said to be **related**.

The probability that an allele taken at random from one individual is ibd to an allele taken at random from another individual is the **coancestry coefficient**  $\theta$  of those two individuals.

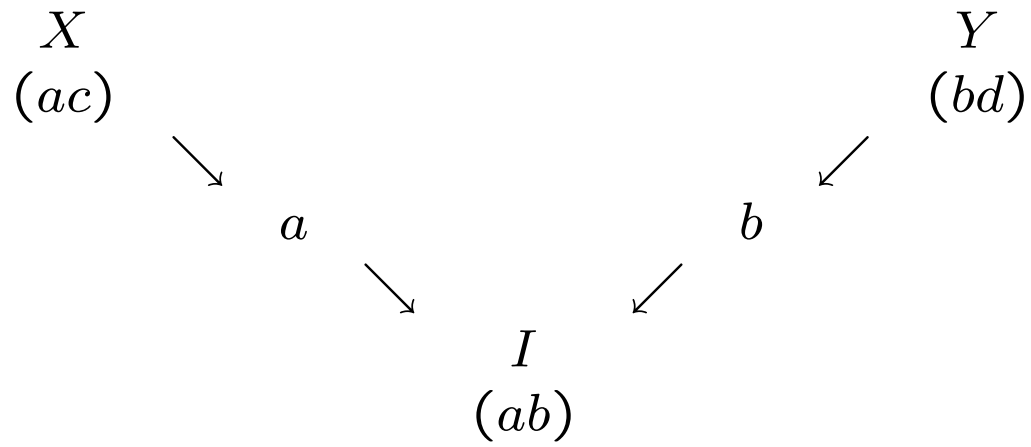
The inbreeding coefficient of an individual is the coancestry of its parents.

## Inbreeding

The within-population inbreeding coefficient  $f$  (or  $F_{IS}$ ) was introduced to describe the departures from Hardy-Weinberg equilibrium in a population. This coefficient is a correlation coefficient and it can be negative. There does not need to be a genetic interpretation of the quantity: it measures how homozygote proportions in a population, for example, differ from squares of allele proportions in a population:  $P_{AA} = p_A^2 + fp_A(1 - p_A)$ .

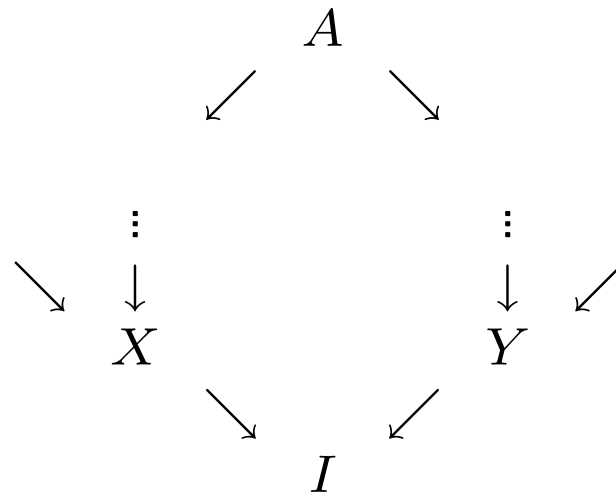
The total inbreeding coefficient  $F$  (or  $F_{IT}$ ) has an explicit genetic interpretation: it is the probability an individual receives alleles that are identical by descent (ibd) from its two parents. There is a requirement to consider events in previous generations. If  $p_A$  is the probability that either allele an individual receives is of type  $A$ , and  $F$  is the probability the two alleles are ibd, then the probability the individual is homozygous  $AA$  is  $P_{AA} = Fp_A + (1 - F)p_A^2$  or  $P_{AA} = p_A^2 + Fp_A(1 - p_A)$ .

# Relatedness



$$F_I = \theta_{XY}$$

# Path Counting



Identify the path linking the parents of  $I$  to their common ancestor(s).



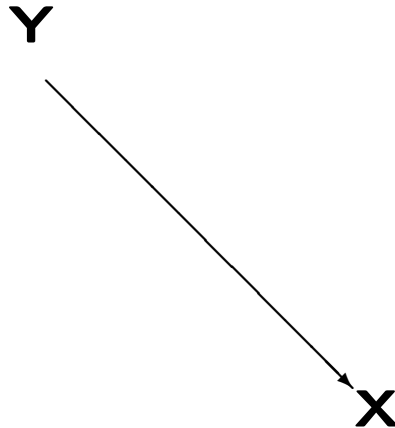
## Path Counting

If the parents  $X, Y$  of an individual  $I$  have ancestor  $A$  in common, and if there are  $n$  individuals (including  $X, Y, I$ ) in the path linking the parents through  $A$ , then the inbreeding coefficient of  $I$ , or the coancestry of  $X$  and  $Y$ , is

$$F_I = \theta_{XY} = \left(\frac{1}{2}\right)^n (1 + F_A)$$

If there are several ancestors, this expression is summed over all the ancestors.

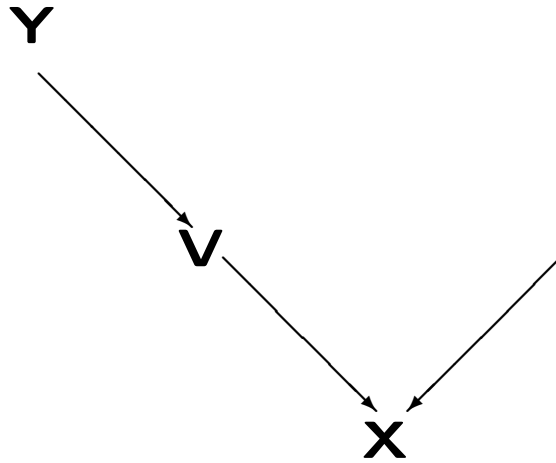
## Parent-Child



The common ancestor of parent  $X$  and child  $Y$  is  $X$ . The path linking  $X, Y$  to their common ancestor is  $YX$  and this has  $n = 2$  individuals. Therefore

$$\theta_{XY} = \left(\frac{1}{2}\right)^2 = \frac{1}{4}$$

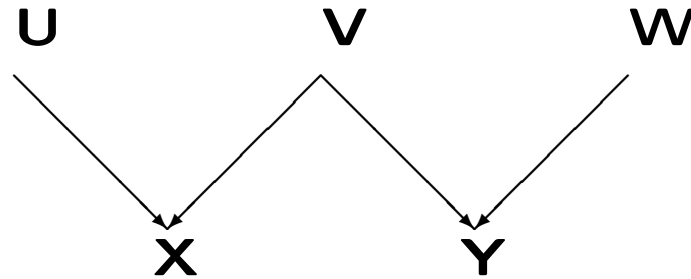
## Grandparent-grandchild



The common ancestor of grandparent  $X$  and grandchild  $Y$  is  $X$ . The path linking  $X, Y$  to their common ancestor is  $YVX$  and this has  $n = 3$  individuals. Therefore

$$\theta_{XY} = \left(\frac{1}{2}\right)^3 = \frac{1}{8}$$

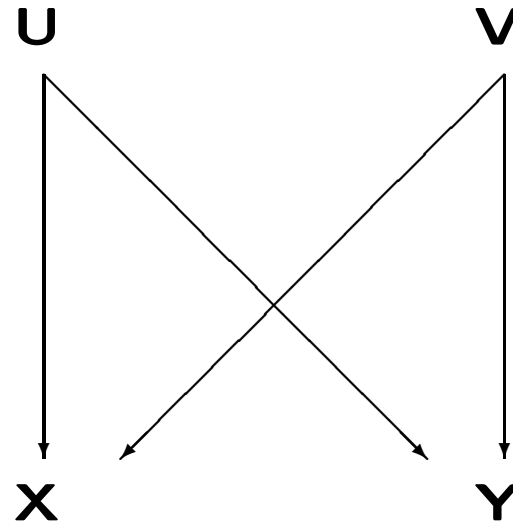
## Half sibs



The common ancestor of half sibs  $X$  and  $Y$  is  $V$ . The path linking  $X, Y$  to their common ancestor is  $XVY$  and this has  $n = 3$  individuals. Therefore

$$\theta_{XY} = \left(\frac{1}{2}\right)^3 = \frac{1}{8}$$

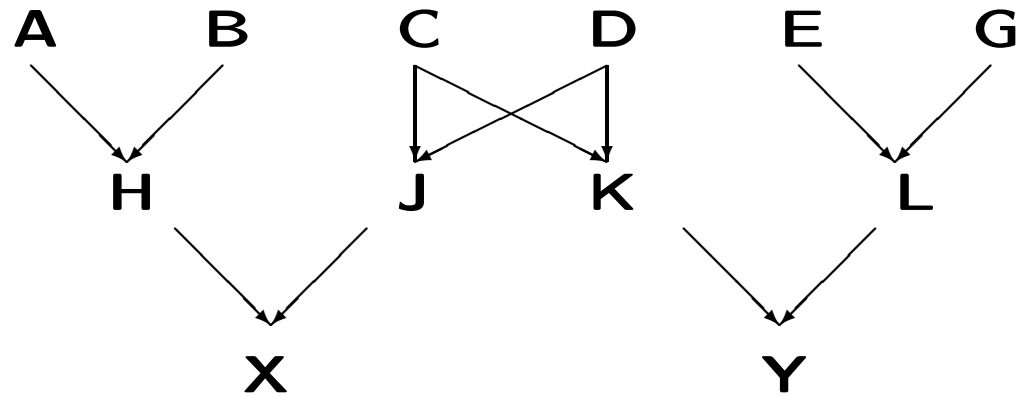
## Full sibs



The common ancestors of full sibs  $X$  and  $Y$  are  $U$  and  $V$ . The paths linking  $X, Y$  to their common ancestors are  $XUY$  and  $XVY$  and these each have  $n = 3$  individuals. Therefore

$$\theta_{XY} = \left(\frac{1}{2}\right)^3 + \left(\frac{1}{2}\right)^3 = \frac{1}{4}$$

## First cousins

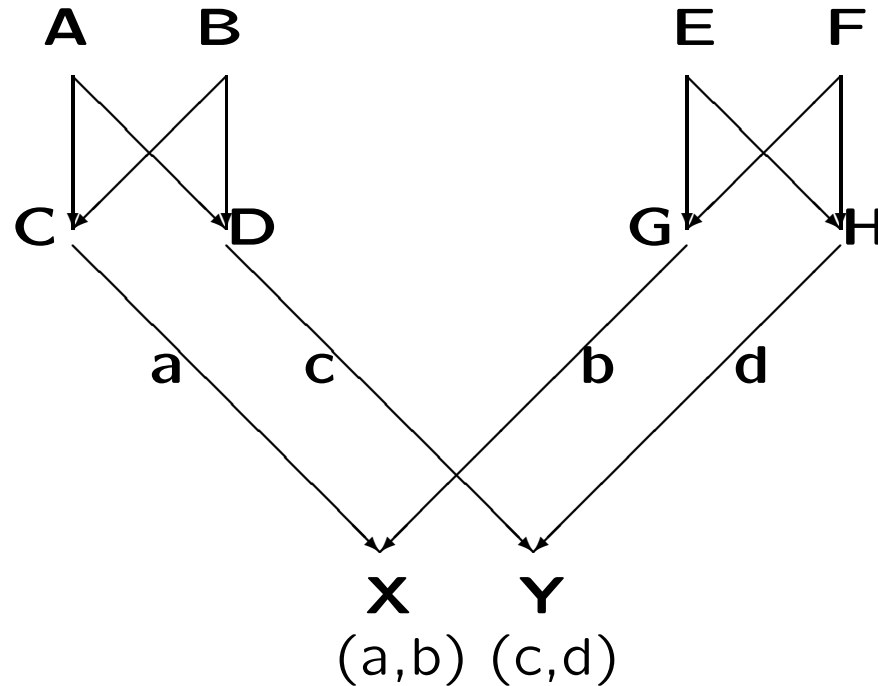


The common ancestors of cousins  $X$  and  $Y$  are  $C$  and  $D$ . The paths linking  $X, Y$  to their common ancestors are  $XJCKY$  and  $XJDKY$  and these each have  $n = 5$  individuals. Therefore

$$\theta_{XY} = \left(\frac{1}{2}\right)^5 + \left(\frac{1}{2}\right)^5 = \frac{1}{16}$$

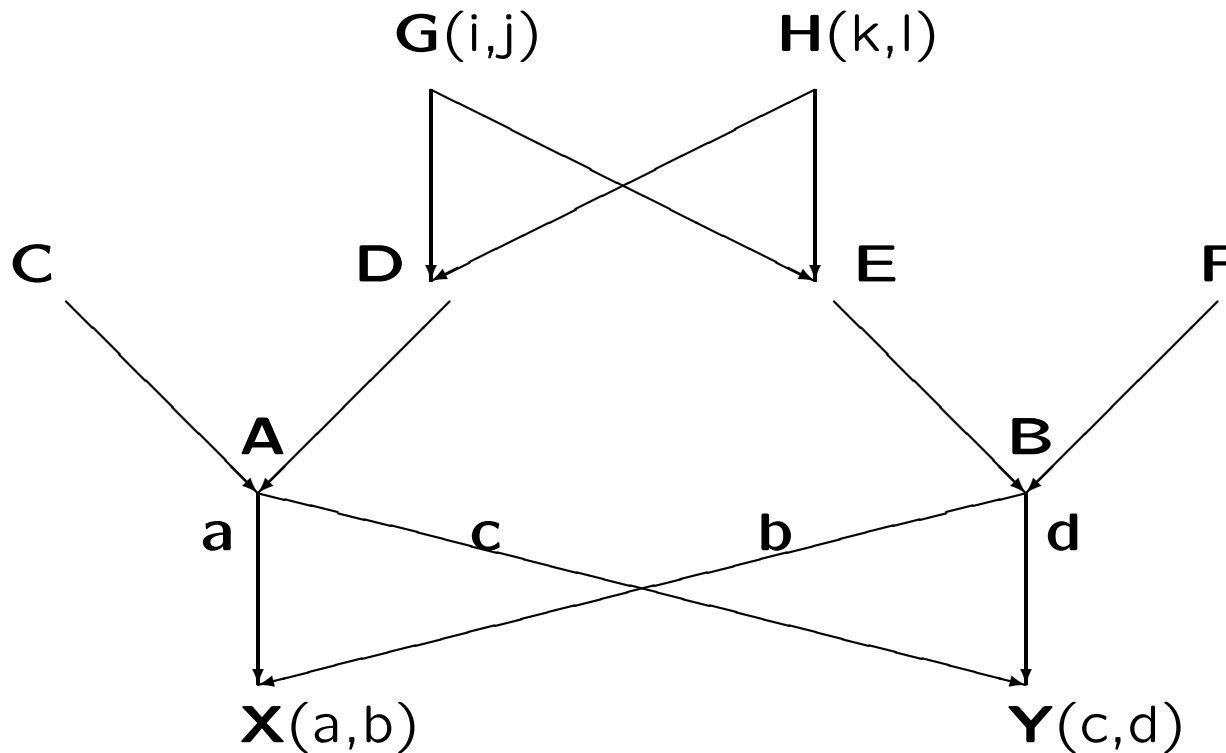
## Double First Cousins

If two brothers  $C, D$  marry two sisters  $G, H$ , their children  $X, Y$  are both maternal and paternal first cousins: i.e. they are double first cousins. What is the coancestry coefficient of double first cousins?



## Siblings whose Parents are Cousins

If two first cousins,  $A, B$ , marry and have two children  $X, Y$ , what is the coancestry coefficient of those children?





## Genotype frequencies

Consider an individual with inbreeding coefficient  $F$ . The probability it has two ibd alleles is  $F$ , and the probability of two non-ibd alleles is  $1 - F$ . The probability that any allele is type  $A$  is  $p_A$ , the population allele frequency. So, the probability the individual is homozygous is

$$\begin{aligned} P_{AA} &= \Pr(AA|\text{ibd}) \Pr(\text{ibd}) + \Pr(AA|\text{not ibd}) \Pr(\text{not ibd}) \\ &= p_A \times F + p_A^2 \times (1 - F) \end{aligned}$$

There is an evolutionary perspective here:  $F$  reflects the history that leads to alleles being ibd. Identity by descent has meaning only with reference to previous generations. The allele frequency  $p_A$  is the expected value over evolutionary histories.

## Genotype frequencies

To emphasize the difference from Hardy-Weinberg:

$$P_{AA} = p_A^2 + Fp_A(1 - p_A)$$

Because heterozygous individuals must have non-ibd alleles:

$$\begin{aligned} P_{Aa} &= 2(1 - F)p_Ap_a \\ &= 2p_Ap_a - 2Fp_Ap_a \end{aligned}$$

For individuals with parents who were first cousins,  $F = 1/16 = 0.0625$ . For a locus with allele frequencies  $\{p_i\}$  that were all 0.10:

$$P_{ii} = (0.1)^2 + 0.0625(0.1)(0.10) = 0.015625$$

$$P_{ij} = 2(0.1)(0.1) - 2(0.0625)(0.1)(0.1) = 0.018750$$

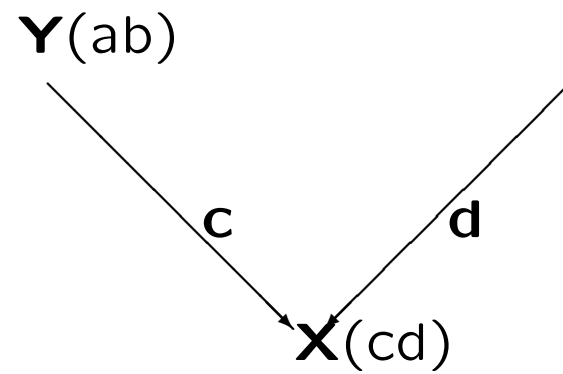
## ***k*-coefficients**

The coancestry coefficient is the probability of one pair of alleles being ibd.

For joint genotypic frequencies, and for a more detailed characterization of relatedness of two non-inbred individuals, we need the probabilities that they carry 0, 1, or 2 pairs of ibd alleles. For example: their two maternal alleles may be ibd or not ibd, and their two paternal alleles may be ibd or not.

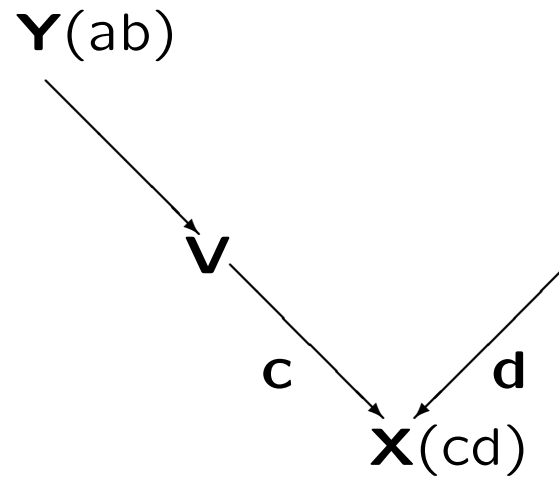
The probabilities of two individuals having 0, 1 or 2 pairs of ibd alleles are written as  $k_0, k_1, k_2$  and  $\theta = \frac{1}{2}k_2 + \frac{1}{4}k_1$ .

# Parent-Child



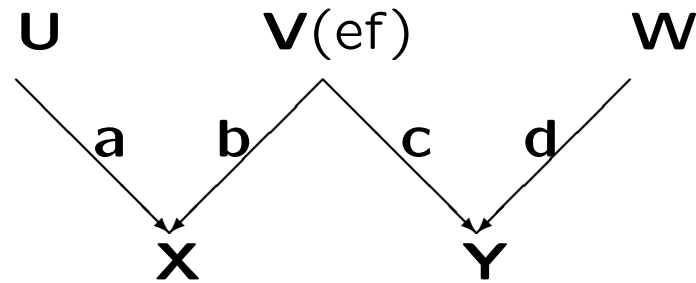
$$\Pr(c \equiv a) = 0.5, \quad \Pr(c \equiv b) = 0.5, \quad k_1 = 1$$

# Grandparent-grandchild



$$\Pr(c \equiv a) = 0.25, \quad \Pr(c \equiv b) = 0.25, \quad k_1 = 0.5 \& k_0 = 0.5$$

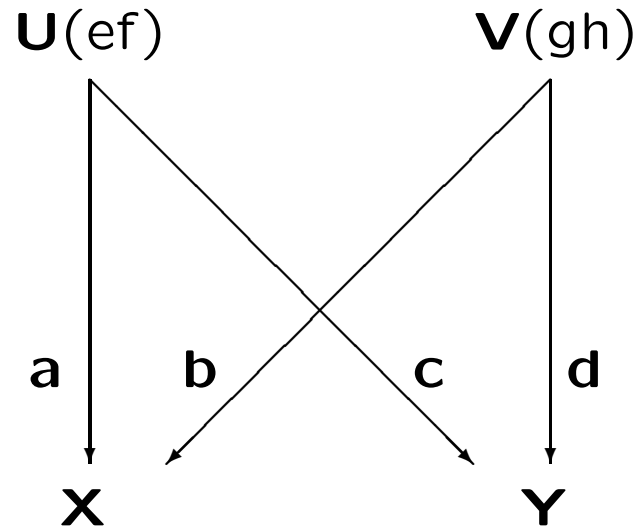
# Half sibs



		0.5	0.5
		$c \equiv e$	$c \equiv f$
		0.25	0.25
		0.25	0.25

Therefore  $k_1 = 0.5$  so  $k_0 = 0.5$ .

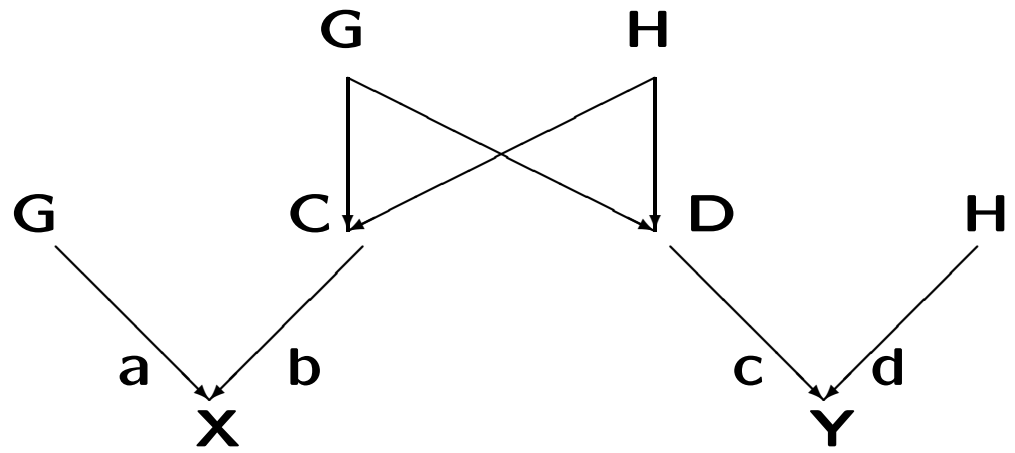
# Full sibs



		0.5	0.5
		$b \equiv d$	$b \not\equiv d$
	0.5	$a \equiv c$	0.25
	0.5	$a \not\equiv c$	0.25

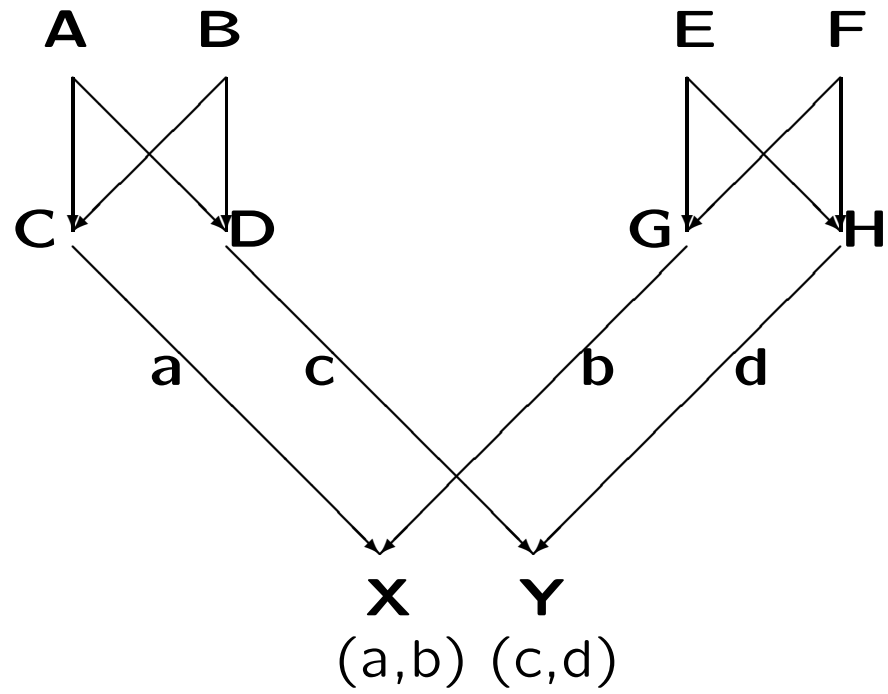
$$k_0 = 0.25, k_1 = 0.50, k_2 = 0.25$$

# First cousins





# Double First Cousins



## Non-inbred Relatives

Relationship	$k_2$	$k_1$	$k_0$	$\theta = \frac{1}{2}k_2 + \frac{1}{4}k_1$
Identical twins	1	0	0	$\frac{1}{2}$
Full sibs	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$
Parent-child	0	1	0	$\frac{1}{4}$
Double first cousins	$\frac{1}{16}$	$\frac{3}{8}$	$\frac{9}{16}$	$\frac{1}{8}$
Half sibs*	0	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{8}$
First cousins	0	$\frac{1}{4}$	$\frac{3}{4}$	$\frac{1}{16}$
Unrelated	0	0	1	0

\* Also grandparent-grandchild and avuncular (e.g. uncle-niece).

## Estimating Individual Inbreeding Coefficients

Individual-specific inbreeding coefficients  $F$  can be estimated under the assumption that all loci have the same coefficient, interpreted as the probability of identity by descent (IBD). Many loci are needed.

At marker locus  $l$ , write  $p_l$  for the frequency of  $M_l$  and code the genotypes  $M_lM_l, M_lm_l, m_lm_l$  as  $x_l = 2, 1, 0$ . These new variables have the properties  $\mathcal{E}(x_l) = 2p_l, \text{Var}(x_l) = 2p_l(1 - p_l)(1 + F)$ .

These follow from

$$\begin{aligned}\mathcal{E}(x) &= 2P_{AA} + P_{Aa} \\ \mathcal{E}(x^2) &= 4p_{AA} + P_{Aa} \\ \text{Var}(x) &= \mathcal{E}(x^2) - [\mathcal{E}(x)]^2\end{aligned}$$

## Individual Inbreeding Coefficients

Then one moment estimator (Price et al, Nature Genetics 38:904-909, 2006) is formed by averaging over loci  $l, l = 1, 2, \dots, L$ :

$$\hat{F}_1 = \frac{1}{L} \sum_{l=1}^L \frac{(x_l - 2p_l)^2}{2p_l(1 - p_l)} - 1$$

Another one (Yang et al, Nature Genetics 42:565-569, 2010) is

$$\begin{aligned} \hat{F}_2 &= \frac{1}{L} \sum_{l=1}^L \frac{x_l^2 - (1 + 2p_l)x_l + 2p_l^2}{2p_l(1 - p_l)} \\ &= \frac{1}{L} \sum_{l=1}^L \frac{(x_l - 2p_l)^2 - (1 - 2p_l)(x_l - 2p_l)}{2p_l(1 - p_l)} - 1 \end{aligned}$$

If the  $p_l$  are known, both these are unbiased. The second one has a smaller variance.

## Alternative MOMs for Individual Inbreeding Coefficients

The variances can be reduced by an alternative weighting over loci:

$$\hat{F}_3 = \frac{\sum_{l=1}^L (x_l - 2p_l)^2}{\sum_{l=1}^L 2p_l(1 - p_l)} - 1$$

$$\hat{F}_4 = \frac{\sum_{l=1}^L [x_l^2 - (1 + 2p_l)x_l + 2p_l^2]}{\sum_{l=1}^L 2p_l(1 - p_l)}$$

## Toy Example

Suppose five loci have genotypes

$$MM, Mm, mm, Mm, MM$$

.

The preferred moment estimate  $\hat{F}_3$  is

$$\hat{F}_3 = \frac{(2 - 2p_1)^2 + (1 - 2p_2)^2 + (2p_3)^2 + (1 - 2p_4)^2 + (2 - 2p_5)^2}{2[p_1(1 - p_1) + p_2(1 - p_2) + p_3(1 - p_3) + p_4(1 - p_4) + p_5(1 - p_5)]} - 1$$

If all five  $M$  allele frequencies were 0.5,

$$\hat{F}_3 = \frac{1 + 0 + 1 + 0 + 1}{2[1/4 + 1/4 + 1/4 + 1/4 + 1/4]} - 1 = 0.2$$

## MLE for Individual Inbreeding Coefficients

To avoid having to choose among MOMs can set up an MLE although there is more numerical work needed. An iterative method makes use of Bayes' theorem. If  $F$  represents the probability the individual in question has two IBD alleles at a locus, i.e. is inbred at that locus,

$$\Pr(A_l A_l | \text{inbred}) = p_l \quad , \quad \Pr(A_l A_l | \text{Not inbred}) = p_l^2$$

$$\Pr(A_l a_l | \text{inbred}) = 0 \quad , \quad \Pr(A_l a_l | \text{Not inbred}) = 2p_l(1 - p_l)$$

$$\Pr(a_l a_l | \text{inbred}) = 1 - p_l \quad , \quad \Pr(a_l a_l | \text{Not inbred}) = (1 - p_l)^2$$

From Bayes' theorem then

$$\Pr(\text{inbred} | A_l A_l) = \frac{\Pr(A_l A_l | \text{inbred}) \Pr(\text{inbred})}{\Pr(A_l A_l)} = \frac{p_l F}{p_l^2 + F p_l (1 - p_l)}$$

$$\Pr(\text{inbred} | A_l a_l) = \frac{\Pr(A_l a_l | \text{inbred}) \Pr(\text{inbred})}{\Pr(A_l a_l)} = 0$$

$$\Pr(\text{inbred} | a_l a_l) = \frac{\Pr(a_l a_l | \text{inbred}) \Pr(\text{inbred})}{\Pr(a_l a_l)} = \frac{(1 - p_l) F}{(1 - p_l)^2 + F p_l (1 - p_l)}$$

## MLE for Individual Inbreeding Coefficients

This suggests an iterative scheme: assign an initial value to  $F$ , and then average the updated values over loci. If  $G_l$  is the genotype at locus  $l$ , the updated value  $F'$  is

$$F' = \frac{1}{L} \sum_{l=1}^L \text{Pr}(\text{inbred} | G_l)$$

This value is then substituted into the right hand side and the process continues until convergence.



## Toy Example

Suppose 5 loci have genotypes

$$MM, Mm, mm, Mm, MM$$

.

Then the updated estimate is

$$F' = \frac{1}{5} \left( \frac{F}{p_1 + F(1 - p_1)} + 0 + \frac{F}{(1 - p_3) + Fp_3} + 0 + \frac{F}{p_5 + (1 - p_5)F} \right)$$

If all the  $p_l = 0.5$ ,

$$F' = \frac{1}{5} \left( \frac{2F}{1 + F} + 0 + \frac{2F}{1 + F} + 0 + \frac{2F}{1 + F} \right) = \frac{6F}{5(1 + F)}$$

and this converges to  $F = 0.2$ .

## Realistic Example

Using 1369 SNPs spread out along chromosome 22, Xiuwen Zheng found that

Minimum MAF	.000		
Average of ratios MOM mean and sd	.0484	.2851	
Ratio of averages MOM mean and sd	.0272	.0874	
MLE mean and sd	.0381	.0576	
Minimum MAF	.050		
Average of ratios MOM mean and sd	.0359	.1516	
Ratio of averages MOM mean and sd	.0264	.0860	
MLE mean and sd	.0380	.0576	
Minimum MAF	.100		
Average of ratios MOM mean and sd	.0358	.1585	
Ratio of averages MOM mean and sd	.0260	.0864	
MLE mean and sd	.0382	.0577	

## Method of Moments for Relatedness Coefficients

PLINK (Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., & Sham, P.C. 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* 81,559–575.) uses MOM to estimate three IBD coefficients  $k_0, k_1, k_2$  for non-inbred relatives. Two individuals are scored as being in IBS states 0,1,2.

State : Genotypes	Probability
2 : $(MM, MM), (mm, mm), (Mm, Mm)$	$(p_M^4 + 4p_M^2p_m^2 + p_m^4)k_0 + k_1(p_M^3 + p_Mp_m + p_m^3) + k_2$
1 : $(MM, Mm), (Mm, MM), (mm, Mm), (Mm, mm)$	$4p_Mp_m(p_M^2 + p_m^2)k_0 + 2p_Mp_mk_1$
0 : $(MM, mm), (mm, MM)$	$2p_M^2p_m^2k_0$

## MOM Approach: $k_0$

Count the number of loci in IBS state  $i$ ;  $i = 0, 1, 2$ . These numbers are  $N_0, N_1, N_2$ . The previous table gives the probabilities of IBS state  $i$  given IBD state  $j$ . From

$$\Pr(\text{IBS} = 0) = \Pr(\text{IBS} = 0 | \text{IBD} = 0) \Pr(\text{IBD} = 0)$$

sum over loci  $l$  to get

$$N_0 = \sum_l 2p_l^2(1 - p_l)^2 \Pr(\text{IBD} = 0)$$

This gives a moment estimate

$$\Pr(\text{IBD} = 0) = \frac{N_0}{\sum_l 2p_l^2(1 - p_l)^2}$$

## MOM Approach: $k_1$

From

$$\begin{aligned}\Pr(\text{IBS} = 1) &= \Pr(\text{IBS} = 1 | \text{IBD} = 0) \Pr(\text{IBD} = 0) \\ &\quad + \Pr(\text{IBS} = 1 | \text{IBD} = 1) \Pr(\text{IBD} = 1)\end{aligned}$$

sum over loci to get

$$\begin{aligned}N_1 &= \Pr(\text{IBD} = 0) \sum_l 4p_l(1 - p_l)[p_l^2 + (1 - p_l)^2] \\ &\quad + \Pr(\text{IBD} = 1) \sum_l 2p_l(1 - p_l)\end{aligned}$$

but we already have an estimate of  $\Pr(\text{IBD} = 0)$ . Therefore

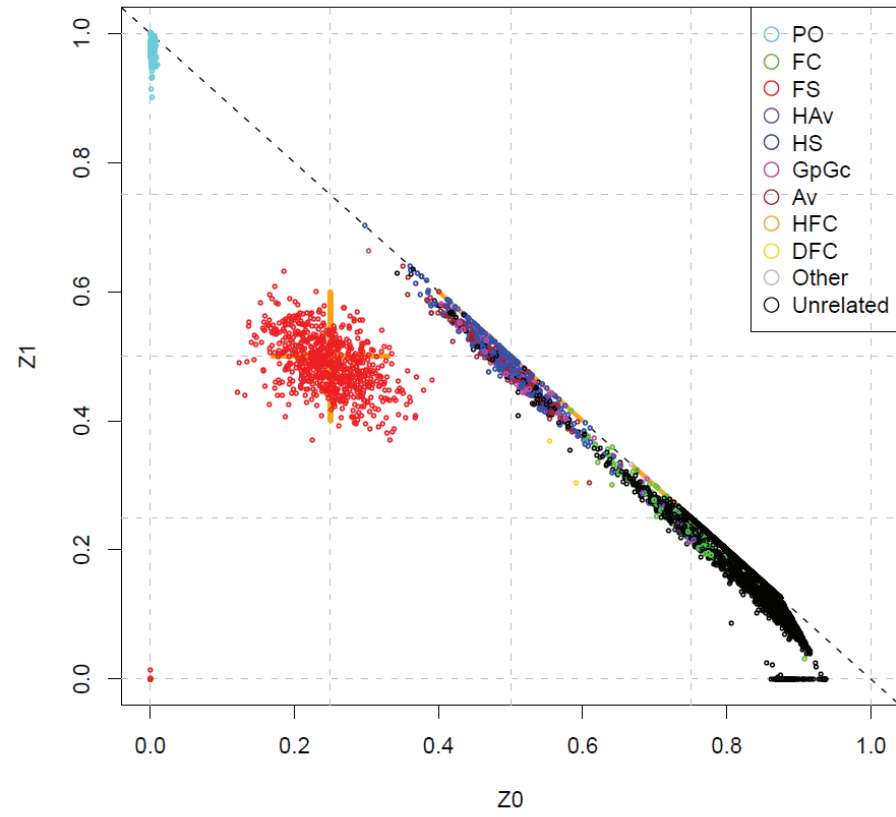
$$\Pr(\text{IBD} = 1) = \frac{N_1 - \sum_l 4p_l(1 - p_l)[p_l^2 + (1 - p_l)^2] \Pr(\text{IBD} = 0)}{\sum_l 2p_l(1 - p_l)}$$

## MOM Approach: $k_2$

Having estimated  $k_0$  and  $k_1$ , find  $\hat{k}_2$  as  $1 - \hat{k}_0 - \hat{k}_1$ .

Could then estimate  $\theta$  as  $\hat{k}_2/2 + \hat{k}_1/4$  or could go to a direct estimate.

# PLINK Example



## MOM for Coancestry Coefficient

One moment estimator (Price et al., 2006; Yang et al., 2010) for the coancestry between individuals  $X$  and  $Y$  is formed by summing over loci  $l, l = 1, 2, \dots, L$ :

$$\hat{\theta}_{XY} = \frac{1}{L} \sum_{l=1}^L \frac{(x_l - 2p_l)(y_l - 2p_l)}{4p_l(1 - p_l)}$$

where  $x_l, y_l$  are 2, 1, 0 if  $X, Y$  are  $(AA, Aa, aa)$ , respectively, at locus  $l$ . An estimate with smaller variance is

$$\hat{\theta}_{XY} = \frac{\sum_{l=1}^L (x_l - 2p_l)(y_l - 2p_l)}{\sum_{l=1}^L 2p_l(1 - p_l)}$$

In practice, sample allele frequencies will need to be used.



## MLE for Relatedness Coefficients

For any SNP there are six distinct pairs of genotypes with probabilities depending on allele frequencies for that SNP and on a set of three  $k$  parameters that are assumed to be the same for all SNPs. If  $G$  is the observed pair of genotypes, we know the conditional probabilities  $\Pr(G|D_i)$  where the  $D_i$  represent the identity states (with probabilities  $k_i$ ).

$G$	$\Pr(G) = \sum_i \Pr(G D_i)k_i$
$MM, MM$	$k_2p_M^2 + k_1p_M^3 + k_0p_M^4$
$mm, mm$	$k_2p_m^2 + k_1p_m^3 + k_0p_m^4$
$MM, mm$	$2k_0p_M^2p_m^2$
$MM, Mm$	$2k_1p_M^2p_m + 4k_0p_M^3p_m$
$mm, Mm$	$2k_1p_Mp_m^2 + 4k_0p_Mp_m^3$
$Mm, Mm$	$2k_2p_Mp_m + k_1p_Mp_m + 4k_0p_M^2p_m^2$

## MLE for Relatedness Coefficients

An iterative algorithm for estimating the  $k$ 's from observed genotypes  $G_l$  at SNP  $l$  is based on Bayes' theorem for the probability of descent state  $D_i, i = 0, 1, 2$ :

$$\Pr(D_i|G_l) = \frac{\Pr(G_l|D_i) \Pr(D_i)}{\Pr(G_l)}$$

The procedure begins with initial estimates of the  $k_i = \Pr(D_i)$ 's.

The updated estimates are obtained by averaging over  $L$  loci:

$$k'_i = \frac{1}{L} \sum_{l=1}^L \left( \frac{\Pr(G_l|D_i)k_i}{\sum_j \Pr(G_l|D_j)k_j} \right), \quad i = 0, 1, 2$$

These updated values are then substituted into the right hand side until they no longer change (or change by less than some specified small amount).

## Toy Example

Suppose the 5-locus genotypes for  $U, V$  are:

$$MM, Mm, mm, Mm, MM$$

and

$$MM, mm, Mm, Mm, Mm$$

The updating equations are:

$$k'_2 = \frac{1}{5} \left( \frac{p_1^2 k_2}{p_1^2 k_2 + p_2^3 k_1 + p_1^4 k_0} + 0 + 0 \right. \\ \left. + \frac{2p_4(1-p_4)k_2}{2p_4(1-p_4)k_2 + p_4(1-p_4)k_1 + 4p_4^2(1-p_4)^2 k_0} + 0 \right)$$

## Toy Example

$$k'_1 = \frac{1}{5} \left( \frac{p_1^3 k_1}{p_1^2 k_2 + p_2^3 k_1 + p_1^4 k_0} + \frac{2p_2(1-p_2)^2 k_1}{2p_2(1-p_2)^2 k_1 + 4p_2(1-p_2)^3 k_0} \right. \\ \left. + \frac{2p_3(1-p_3)^2 k_1}{2p_3(1-p_3)^2 k_1 + 4p_3(1-p_3)^3 k_0} \right. \\ \left. + \frac{p_4(1-p_4)k_1}{2p_4(1-p_4)k_2 + p_4(1-p_4)k_1 + 4p_4^2(1-p_4)^2 k_0} \right. \\ \left. + \frac{2p_5^2(1-p_5)k_1}{2p_5^2(1-p_5)k_1 + 4p_5^3(1-p_5)k_0} \right)$$

## Toy Example

$$k'_0 = \frac{1}{5} \left( \frac{p_1^4 k_0}{p_1^2 k_2 + p_2^3 k_1 + p_1^4 k_0} + \frac{4p_2(1-p_2)^3 k_0}{2p_2(1-p_2)^2 k_1 + 4p_2(1-p_2)^3 k_0} \right. \\ \left. + \frac{4p_3(1-p_3)^3 k_0}{2p_3(1-p_3)^2 k_1 + 4p_3(1-p_3)^3 k_0} \right. \\ \left. + \frac{4p_4^2(1-p_4)k_0}{2p_4(1-p_4)k_2 + p_4(1-p_4)k_1 + 4p_4^2(1-p_4)^2 k_0} \right. \\ \left. + \frac{4p_5^3(1-p_5)k_0}{2p_5^2(1-p_5)k_1 + 4p_5^3(1-p_5)k_0} \right)$$

## “RELPAIR” calculations

This approach compares the probabilities of two genotypes under alternative hypotheses;  $H_0$ : the individuals have a specified relationship, versus  $H_1$ : the individuals are unrelated. The alternative is that  $k_0 = 1, k_1 = k_2 = 0$  so the likelihood ratios for the two hypotheses are:

$$\text{LR}(MM, MM) = k_0 + k_1/p_M + k_2/p_M^2$$

$$\text{LR}(mm, mm) = k_0 + k_1/p_m + k_2/p_m^2$$

$$\text{LR}(Mm, Mm) = k_0 + k_1/(4p_M p_m) + k_2/(2p_M p_m)$$

$$\text{LR}(MM, Mm) = k_0 + k_1/(2p_M)$$

$$\text{LR}(mm, Mm) = k_0 + k_1/(2p_m)$$

$$\text{LR}(MM, mm) = k_0$$

## Testing relationship

Hypotheses about alternative pairs of relationships may be tested with likelihood ratio test statistics. These ratios are the probability of the observed pair of genotypes under one hypothesis divided by the probability under the alternative hypothesis. These ratios are multiplied over (independent) loci.

Each hypothesis is described by a set of  $k$ 's and there are three hypotheses likely to be of interest:

1. individuals are unrelated ( $k_0 = 1$ )
2. individuals have a specified (or annotated) relationship ( $k$ 's specified)
- or 3. individuals are related to an extent measured by the estimated  $k$ 's.

## Relatedness as Population Structure

The allelic-matching proportions within and between populations provided estimates of identity by descent within versus between populations.

If the sample sizes were only one individual (two alleles) per population, then the population-structure measures reduce to individual measures. Identity by descent within a population (now an individual) is the inbreeding coefficient for an individual. Identity by descent between a pair of populations (now between two individuals) is the coancestry for two individuals.



## Allelic Matching Inbreeding and Relatedness

If  $g_i$  is the number of reference alleles at locus for individual  $i$ , the proportion of pairs of alleles drawn randomly from individual  $i$  that match is  $\tilde{M}_i = [1 + (1 - g_i)^2]/2$  and this has expectation  $[1 - 2p(1 - p)(1 - \theta_{ii})]$ . Note that  $M_i$  is 1 for  $AA$  and  $BB$  individuals and 0.5 for  $AB$  individuals. Also,  $\theta_{ii} = (1 + F_i)/2$ .

The proportion of pairs of alleles drawn randomly, one from individual  $i$  and from individual  $j$ , that match is  $\tilde{M}_{ij} = [1 + (1 - g_i)(1 - g_j)]/2$  and this has expectation  $[1 - 2p(1 - p)(1 - \theta_{ij})]$ . Note that  $M_{ij}$  is 1 for  $AA, AA; AB, AB; BB, BB$ . It is 0.5 for  $AA, AB; BB, AB; AB, AA; AB, BB$  and it is 0 for  $AA, BB; BB, AA$ .

## Allelic Matching Estimates

Average over pairs of individuals to give an average between-individual-pair matching proportion  $\tilde{M}_B = \sum_i \sum_{j \neq i} \tilde{M}_{ij} / n(n-1)$  with expectation  $[1 - p(1-p)(1 - \theta_B)]$ .

Allelic matching inbreeding and coancestry estimates are

$$\hat{\beta}_{ii} = \frac{\tilde{M}_i - \tilde{M}_B}{1 - \tilde{M}_B} \quad , \quad \hat{\beta}_{ij} = \frac{\tilde{M}_{ij} - \tilde{M}_B}{1 - \tilde{M}_B}$$

Taking expectations:

$$\mathcal{E}(\hat{\beta}_{ii}) = \frac{\theta_{ii} - \theta_B}{1 - \theta_B} \quad , \quad \mathcal{E}(\hat{\beta}_{ij}) = \frac{\theta_{ij} - \theta_B}{1 - \theta_B}$$

These estimates provide clarity that inbreeding and pairwise coancestry can only be estimated “relative to” pairs of alleles whose ibd status is not estimated separately. In this case, individual inbreeding and pairwise coancestry is estimated relative to the average coancestry of all pairs of individuals in the sample.

## Comparison with EIGENSTRAT Estimates

The standard inbreeding and coancestry coefficient estimates use sample allele frequencies:

$$\hat{\theta}_{ij} = \frac{1}{L} \sum_{l=1}^L \frac{(g_{il} - 2\tilde{p}_l)(g_{j'l} - 2\tilde{p}_l)}{4\tilde{p}_l(1 - \tilde{p}_l)}$$

Here  $i$  can be the same or different from  $j$ . Approximating the expectation of a ratio by the expectation of ratios, for large sample sizes, gives

$$\begin{aligned} \mathcal{E}(\hat{\theta}_{ii}) &= \frac{(\theta_{ii} - \theta_B) - 2(\psi_i - \theta_B)}{1 - \theta_B} \\ \mathcal{E}(\hat{\theta}_{ij}) &= \frac{(\theta_{ij} - \theta_B) - (\psi_i - \theta_B) - (\psi_j - \theta_B)}{1 - \theta_B} \end{aligned}$$

where  $\psi_i = \sum_{j \neq i} \theta_{ij} / (n - 1)$ . These expectations are not  $\theta_{ii}$  and  $\theta_{ij}$ .

## KING-robust Estimates

Manichaikul et al, Bioinformatics 26:2867-2873, 2010 gave coancestry estimates that did not use allele frequencies:

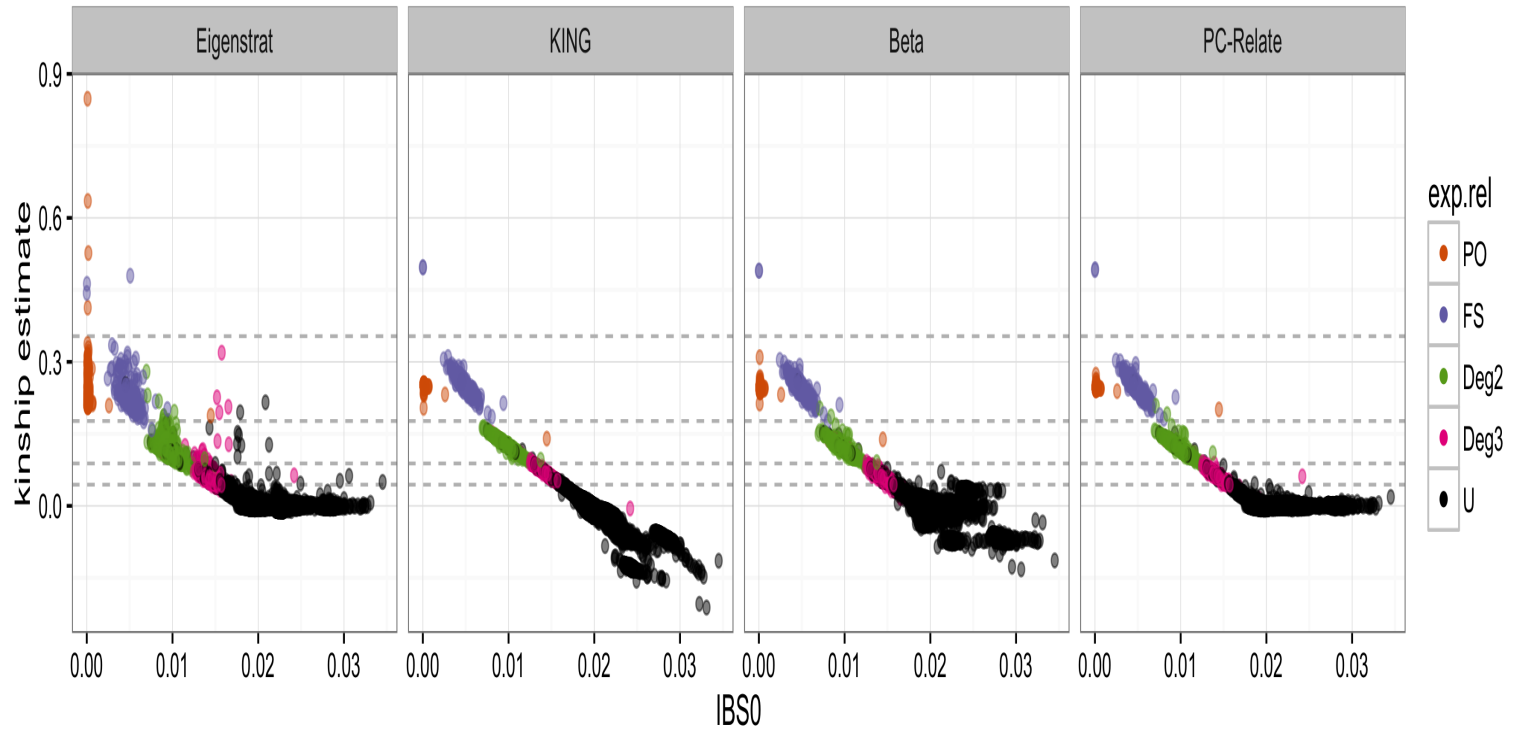
$$\hat{\theta}_{ij} = \frac{N(AB, AB)_{ij} - 2N(AA, BB)_{ij}}{N(AB)_i + N(AB)_j}$$

where the  $N$ 's are the numbers of loci with the indicated genotypes for individuals  $i$  and individual pairs  $i, j$ . From Table 1, the expected values of these estimates are

$$\mathcal{E}(\hat{\theta}_{ii'}^K) = \frac{\theta_{ij} - \frac{1}{2}(F_i + F_j)}{1 - \frac{1}{2}(F_i + F_j)}$$

The KING-robust estimator is for ibd between individuals relative to that within individuals. We could just as well estimate ibd within individuals relative to that between individuals. In either case it does not seem appropriate to estimate one by regarding the other as zero.

# Example



Comparison of Four Estimates of Coancestry.

# ASSOCIATION MAPPING

## Association Mapping

Association methods use random samples from a population and are alternatives to methods based on pedigrees or crosses between inbred lines. The associations depend on linkage disequilibrium between marker and trait loci instead of depending on linkage between those loci as in pedigree or line cross methods.

A quantitative trait locus **T** contributes to a trait of interest. The QTL genotype cannot be observed but maybe it can be inferred, and the location of the QTL be estimated, from observations on the trait and the genotype at a genetic marker **M**.

## Marker-Trait Genotype Frequencies

Each marker genotypic class  $M_iM_j$  is composed of a mixture of elements from each of the QTL classes,  $T_rT_s$ , where the proportion of QTL class  $T_rT_s$  contained within marker class  $M_iM_j$  is  $Pr(T_rT_s|M_iM_j)$ . With random mating, genotype frequencies are products of gamete frequencies. For example

$$\begin{aligned}Pr(T_rT_r, M_iM_i) &= Pr(T_rM_i)^2 \\Pr(T_rT_r|M_iM_i) &= Pr(T_rM_i)^2 / Pr(M_i)^2\end{aligned}$$

and gamete frequencies involve allele frequencies and linkage disequilibria:

$$Pr(T_rM_i) = p_r p_i + D_{ri}$$



## Two-allele Genotypes

	$TT$	$Tt$	$tt$
$MM$	$P_{MT}^2$	$2P_{MT}P_{Mt}$	$P_{Mt}^2$
$Mm$	$2P_{MT}P_{mT}$	$2P_{MT}P_{mt} + 2P_{Mt}P_{mT}$	$2P_{Mt}P_{mt}$
$mm$	$P_{mT}^2$	$2P_{mT}P_{mt}$	$P_{mt}^2$

## Two-allele Gametes

	$T$	$t$
$M$	$P_{MT} = p_M p_T + D_{MT}$	$P_{Mt} = p_M p_t - D_{MT}$
$m$	$P_{mT} = p_m p_T - D_{MT}$	$P_{mt} = p_m p_t + D_{MT}$

$$\rho_{MT} = \frac{D_{MT}}{\sqrt{p_M p_m p_T p_t}}$$

$$\rho_{MT}^2 = \frac{D_{MT}^2}{p_M p_m p_T p_t}$$

## Marker and Trait Variables

Introduce variables  $X$  and  $G$  for loci  $\mathbf{M}$  and  $\mathbf{T}$ . The values of  $X$  will be assigned for the marker whereas the values  $G$  represent the genetic contributions to measured trait variables or to disease status. In either case, the Hardy-Weinberg assumption provides the following expressions for the means and variances:

$$\mathcal{E}(X) = \mu_X = p_M^2 X_{MM} + 2p_M p_m X_{Mm} + p_m^2 X_{mm}$$

$$\mathcal{E}(G) = \mu_G = p_T^2 G_{TT} + 2p_T p_t G_{Tt} + p_t^2 G_{tt}$$

$$\text{Var}(X) = \sigma_{A_M}^2 + \sigma_{D_M}^2$$

$$\text{Var}(G) = \sigma_{A_T}^2 + \sigma_{D_T}^2$$

## Components of Variance

The “additive” and “dominance” components of variance are

$$\sigma_{A_M}^2 = 2p_M p_m [p_M (X_{MM} - X_{Mm}) + p_m (X_{Mm} - X_{mm})]^2$$

$$\sigma_{A_T}^2 = 2p_T p_t [p_T (G_{TT} - G_{Tt}) + p_t (G_{Tt} - G_{tt})]^2$$

$$\sigma_{D_M}^2 = p_M^2 p_m^2 (X_{MM} - 2X_{Mm} + X_{mm})^2$$

$$\sigma_{D_T}^2 = p_T^2 p_t^2 (G_{TT} - 2G_{Tt} + G_{tt})^2$$

and these lead to the following expression for the covariance of  $X$  and  $G$ :

$$\text{Cov}(G, X) = \rho_{MT} \sigma_{A_T} \sigma_{A_M} + \rho_{MT}^2 \sigma_{D_T} \sigma_{D_M}$$

## Correlation of Trait and Marker Variables

$$\text{Cov}(G, X) = \rho_{MT}\sigma_{A_T}\sigma_{A_M} + \rho_{MT}^2\sigma_{D_T}\sigma_{D_M}$$

If either  $X$  or  $G$  are purely additive, then their covariance is

$$\text{Cov}(G, X) = \rho_{MT}\sigma_{A_T}\sigma_{A_M}$$

If both  $X$  and  $G$  are purely additive, then their correlation is

$$\rho_{GX} = \rho_{MT}$$

If either  $X$  or  $G$  are purely non-additive, then their covariance is

$$\text{Cov}(G, X) = \rho_{MT}^2\sigma_{D_T}\sigma_{D_M}$$

If both  $X$  and  $G$  are purely non-additive, then their correlation is

$$\rho_{GX} = \rho_{MT}^2$$

## Measured Traits

Suppose  $Y = G + E$  where  $G$  is the genetic effect of locus **T** and  $E$  are all other effects. These other effects are supposed to have mean zero and to be independent of both  $G$  and the marker variable  $X$ . Then

$$\begin{aligned}\mathcal{E}(Y) &= \mathcal{E}(G) \\ \text{Cov}(X, Y) &= \text{Cov}(X, G) \\ \text{Var}(Y) &= \sigma_{A_T}^2 + \sigma_{D_T}^2 + V_E\end{aligned}$$

Trait values  $Y$  may be regressed on marker variables  $X$ . The regression coefficient is

$$\beta_{YX} = \frac{\text{Cov}(X, Y)}{\text{Var}(X)} = \frac{\rho_{MT}\sigma_{A_T}\sigma_{A_M} + \rho_{MT}^2\sigma_{D_T}\sigma_{D_M}}{\sigma_{A_M}^2 + \sigma_{D_M}^2}$$

## Marker Variable

Variable  $X$  may be chosen to be additive, e.g.  $X_{MM} = 2, X_{Mm} = 1, X_{mm} = 0$  so that  $\sigma_{A_M}^2 = 2p_M p_m, \sigma_{D_M}^2 = 0$ , and then

$$\beta_{YX} = \rho_{MT} \frac{\sigma_{A_T}}{\sigma_{A_M}}$$

The marker variable can also be made to have a zero additive variance, e.g.  $X_{MM} = p_m, X_{Mm} = 0, X_{mm} = p_M$  so that  $\sigma_{A_M}^2 = 0, \sigma_{D_M}^2 = p_M^2 p_m^2$ , and

$$\beta_{YX} = \rho_{MT}^2 \frac{\sigma_{D_T}}{\sigma_{D_M}}$$

A significant regression coefficient implies a significant linkage disequilibrium measure  $\rho_{MT}$  between marker and disease loci. The signal is expected to be stronger with an additive marker as  $\rho_{MT} \geq \rho_{MT}^2$  and it is usual that  $\sigma_{A_T}^2 \geq \sigma_{D_T}^2$ .

## Analysis of Variance

Instead of regressing trait on marker, the trait means could be compared among marker classes. The expected trait means follow as

$$\begin{aligned}\mathcal{E}(Y|M_iM_j) &= \sum_{r,s} G_{rs} \Pr(T_rT_s|M_iM_j) \\ &= \sum_{r,s} G_{rs} \Pr(T_rM_i, T_sM_j) / \Pr(M_iM_j)\end{aligned}$$

in general.

For a trait locus with only two alleles,  $T, t$ , for marker homozygote  $MM$ :

$$\mathcal{E}(Y|MM) = (G_{TT}P_{MT}^2 + 2G_{Tt}P_{MT}P_{Mt} + G_{tt}P_{Mt}^2) / p_M^2$$



## Trait Means in Marker Classes

This last expression can be manipulated to show the effects of linkage disequilibrium.

Trait means among the three marker genotype classes:

$$\mathcal{E}(Y|MM) = \mu_G + 2\rho_{MT}\mathcal{A}/p_M + \rho_{MT}^2\mathcal{D}/p_M^2$$

$$\mathcal{E}(Y|Mm) = \mu_G + \rho_{MT}\mathcal{A}(1/p_M - 1/p_m) - \rho_{MT}^2\mathcal{D}/(p_M p_m)$$

$$\mathcal{E}(Y|mm) = \mu_G - 2\rho_{MT}\mathcal{A}/p_m + \rho_{MT}^2\mathcal{D}/p_m^2$$

where  $\mathcal{A} = \sigma_{A_T}\sqrt{(p_M p_m)}$ ,  $\mathcal{D} = \sigma_{D_T}(p_M p_m)$ , so that an analysis of variance will also test that  $\rho_{MT} = 0$  and the test will be affected by both additive and dominance effects at the trait locus.

## Dichotomous Traits: Case Only

The case-control approach starts with independent samples of people who are either affected or not affected with a disease and compares marker frequencies between the two groups. The  $MM$  marker frequency among cases is

$$\Pr(MM|\text{Case}) = p_M^2 + \frac{1}{\mu_G} [p_M \rho_{MT} \mathcal{A} + \rho_{MT}^2 \sigma_{D_T} \mathcal{D}]$$

$$\Pr(Mm|\text{Case}) = 2p_M p_m + \frac{1}{\mu_G} [(p_m - p_M) \rho_{MT} \mathcal{A} - 2\rho_{MT}^2 \mathcal{D}]$$

$$\Pr(mm|\text{Case}) = p_m^2 + \frac{1}{\mu_G} [-p_m \rho_{MT} \mathcal{A} + \rho_{MT}^2 \mathcal{D}]$$

Note that these three probabilities sum to one.

## Case Allele Frequencies

Combining the genotypic frequencies to give allele frequencies:

$$\begin{aligned}\Pr(M|\text{Case}) &= p_M + \frac{\rho_{MT}\sigma_{A_T}}{2\mu_G} \sqrt{2p_M p_m} \\ \Pr(m|\text{Case}) &= p_m - \frac{\rho_{MT}\sigma_{A_T}}{2\mu_G} \sqrt{2p_M p_m}\end{aligned}$$

and these two sum to one.

The inbreeding coefficient at the marker locus in the case population follows from

$$\Pr(MM|\text{Case}) = \Pr(M|\text{Case})^2 + f_{\text{Case}} \Pr(M|\text{Case})[1 - \Pr(M|\text{Case})]$$

or

$$f_{\text{Case}} = \frac{\rho_{MT}^2(2\mu_G\sigma_{D_T} - \sigma_{A_T}^2)}{(\mu_G\sqrt{2p_M/p_m} + \rho_{MT}\sigma_{A_T})(\mu_G\sqrt{2p_m/p_M} - \rho_{MT}\sigma_{A_T})}$$

## Case-only HWE Testing

The power of this test depends on  $nf_{\text{Case}}^2$  which is proportional to  $\rho_{MT}^4$  so the power will decrease quickly as  $\rho_{MT}$  decreases.

## Dichotomous Traits: Case-Control

An argument similar to that above provides the marker genotype frequencies among controls:

$$\Pr(MM|\text{Control}) = p_M^2 - \frac{1}{1 - \mu_G} [p_M \rho_{MT} \mathcal{A} + \rho_{MT}^2 \mathcal{D}]$$

$$\Pr(Mm|\text{Control}) = 2p_M p_m - \frac{1}{1 - \mu_G} [(p_m - p_M) \rho_{MT} \mathcal{A} - 2\rho_{MT}^2 \mathcal{D}]$$

$$\Pr(mm|\text{Control}) = p_m^2 - \frac{1}{1 - \mu_G} [-p_m \rho_{MT} \mathcal{A} + \rho_{MT}^2 \mathcal{D}]$$

$$\Pr(M|\text{Control}) = p_M - \frac{\rho_{MT} \mathcal{A}}{2(1 - \mu_G)}$$

$$\Pr(m|\text{Control}) = p_m + \frac{\rho_{MT} \mathcal{A}}{2(1 - \mu_G)}$$

## Case-control Test

The simplest case-control test compares marker allele frequencies between the two samples and it is clearly equivalent to testing that  $\rho_{MT} = 0$  since

$$\Pr(M|\text{Case}) - \Pr(M|\text{Control}) \propto \rho_{MT}\sigma_{A_T}\sqrt{2p_Mp_m}$$

The test is not affected by non-additivity at the disease locus. If the allelic counts for  $M, m$  in cases and controls are laid out in a  $2 \times 2$  table, the contingency-table chi-square test statistic has 1 df. An alternative is to work with the  $3 \times 2$  table of marker genotype counts in cases and controls and calculate a 2 df chi-square test statistic. This test is affected by both additivity and non-additivity at the disease locus but it is sensitive to errors in genotype calls for rare alleles.

## Allelic Case Control Test

Write the marker genotype counts in random samples of cases and controls as

Genotype	$MM$	$Mm$	$mm$	Total
Case counts	$r_0$	$r_1$	$r_2$	$R$
Control counts	$s_0$	$s_1$	$s_2$	$S$
Total counts	$n_0$	$n_1$	$n_2$	$N$

The allelic test statistic uses the allele counts

Observed	$M$	$m$	Total
Case counts	$2r_0 + r_1$	$2r_2 + r_1$	$2R$
Control counts	$2s_0 + s_1$	$2s_2 + s_1$	$2S$
Total counts	$2n_0 + n_1$	$2n_2 + n_1$	$2N$

## Allelic Case Control Test

A contingency table test for independence of marker allele and disease status compares the observed allelic counts with the products of the marginal totals divided by the overall total:

Expected	$M$	$m$	Total
Case counts	$2R(2n_0 + n_1)/2N$	$2R(2n_2 + n_1)/2N$	$2R$
Control counts	$2S(2n_0 + n_1)/2N$	$2S(2n_2 + n_1)/2N$	$2S$
Total counts	$2n_0 + n_1$	$2n_2 + n_1$	$2N$

The test statistic is

$$\begin{aligned} X_A^2 &= \sum \frac{(\text{Obs.} - \text{Exp.})^2}{\text{Exp.}} \\ &= \frac{2N[N(r_1 + 2r_2) - R(n_1 + 2n_2)]^2}{SR[2N(n_1 + 2n_2) - (n_1 + 2n_2)^2]} \end{aligned}$$



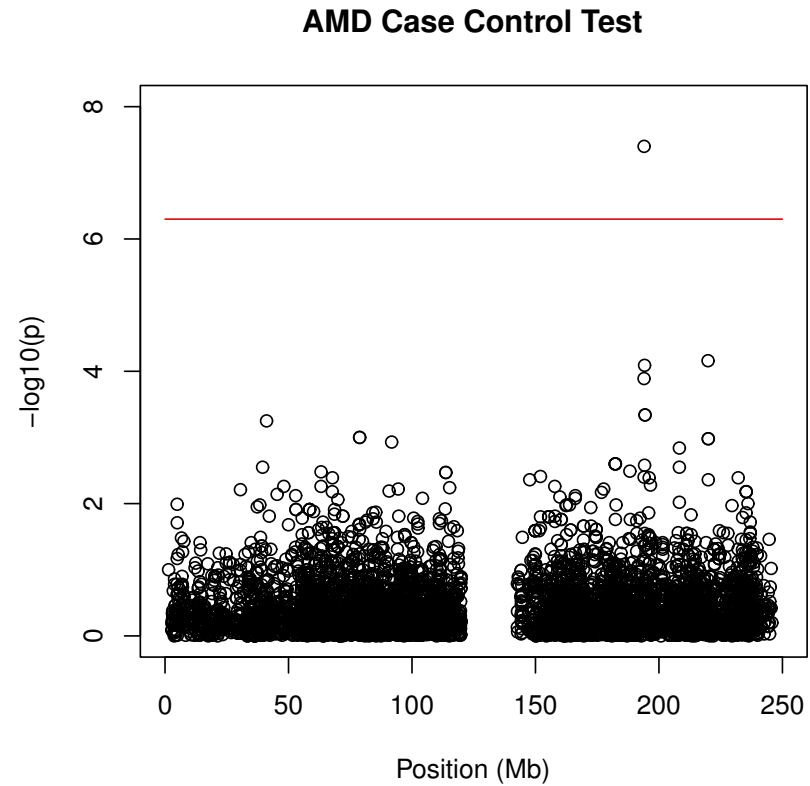
## Allelic Case Control Test

Approximating the expectation of  $X_A^2$  by the ratio of the expectations of the numerator and denominator:

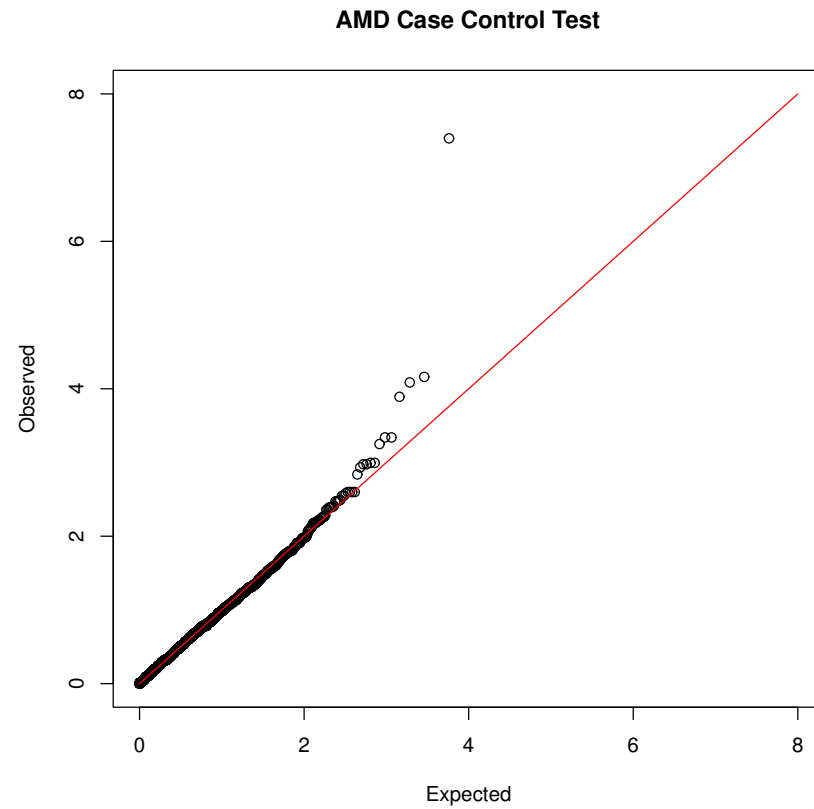
$$\mathcal{E}(X_A^2) \approx (1 + f)$$

showing an inflation factor of  $(1 + f)$  when there is inbreeding. The expected value is 1 when  $f = 0$  and the test statistic has a chi-square distribution with 1 df.

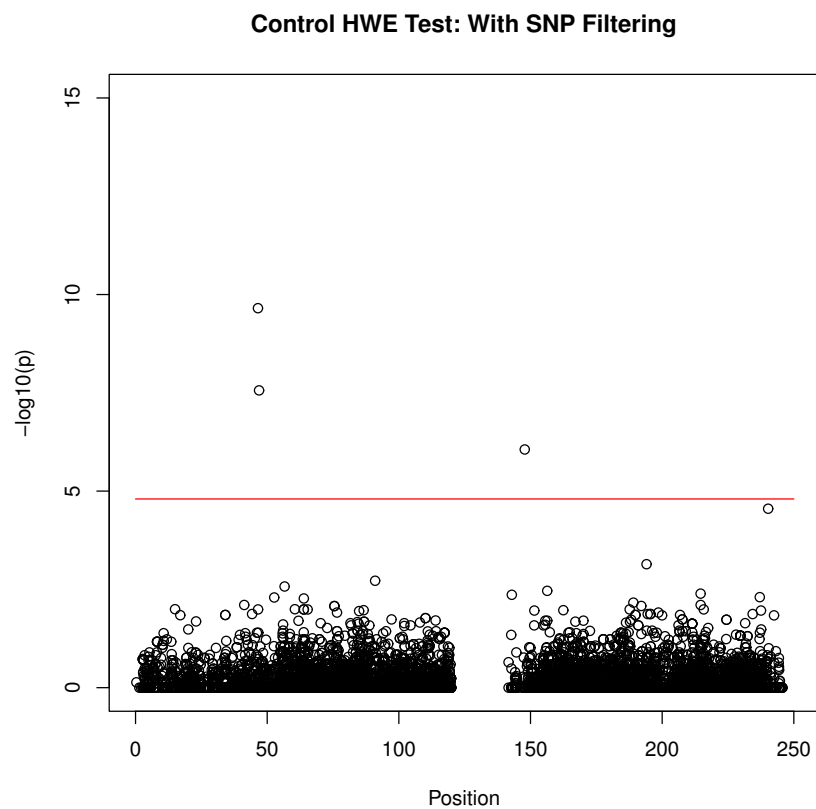
# AMD Example: Case-control test statistics on chromosome 1



# AMD Example: Case-control test statistics QQ plot



# AMD Example: HWE test statistics on chromosome 1



## Trend Test

		$i = 0$	$i = 1$	$i = 2$	
Marker Genotype		$MM$	$Mm$	$mm$	Total
Marker Variable		$X_0$	$X_1$	$X_2$	
Case counts	$Y = 1$	$r_0$	$r_1$	$r_2$	$R$
Control counts	$Y = 0$	$s_0$	$s_1$	$s_2$	$S$
Total counts		$n_0$	$n_1$	$n_2$	$N$

The Armitage trend test is based on a score statistic  $U$ :

$$U = \sum_{i=0}^2 X_i \left( \frac{S}{N} r_i - \frac{R}{N} s_i \right)$$

## Trend Test for Additivity

Assuming normality for  $U$ , the test statistic

$$X_T^2 = \frac{U^2}{\widehat{\text{Var}}(U)} = \frac{N(N \sum_i r_i X_i - R \sum_i n_i X_i)^2}{SR[N \sum_i n_i X_i^2 - (\sum_i n_i X_i)^2]}$$

is distributed as  $\chi_{(1)}^2$  under the hypothesis  $H_0 : \rho_{MT} = 0$ .

Usual to consider a linear trend test, with  $X_0 = 0, X_1 = 1, X_2 = 2$ , so that  $\sigma_{D_M}^2 = 0$  and

$$X_T^2 = \frac{N[N(r_1 + 2r_2) - R(n_1 + 2n_2)]^2}{SR[N(n_1 + 4n_2) - (n_1 + 2n_2)^2]}$$

This will provide a test for additive effects at the disease locus.

## Case-control vs Trend Tests

The allelic case-control test statistic is

$$X_A^2 = \frac{2N[N(r_1 + 2r_2) - R(n_1 + 2n_2)]^2}{SR[N(2n_1 + 4n_2) - (n_1 + 2n_2)^2]}$$

and the linear trend test statistic is

$$X_T^2 = \frac{N[N(r_1 + 2r_2) - R(n_1 + 2n_2)]^2}{SR[N(n_1 + 4n_2) - (n_1 + 2n_2)^2]}$$

In both cases,  $\sigma_{D_M}^2 = 0$  and the test is for linkage disequilibrium  $\rho_{MT}$  between trait and marker alleles, and is affected only by additive trait effects.

Unlike the allelic case-control test, the trend statistic has an expected value of 1 even when there are departures from Hardy-Weinberg equilibrium.

## Trend Test for Non-Additivity

Setting  $X_0 = p_m, X_1 = 0, X_2 = p_M$  gives  $\sigma_{A_M}^2 = 0$  and a test for non-additive effects. There is not an obvious simplification of the equation for the test statistic.