Lecture Outline 9/20

- Mapping genes in human pedigrees
- Mapping the centromere using tetrads

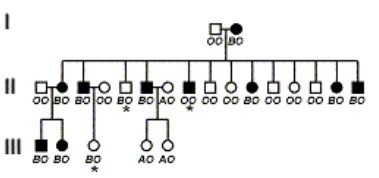
Announcements

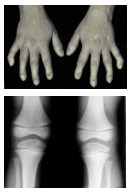
I have posted a new homework assignment on the web page

First Exam is next Wednesday (9/28).
Review session tomorrow at 4:30

Mapping Human Genes

- Here is a pedigree that shows blood types and "nail patella syndome"
  - NPS is a rare genetic disease occurring in only about 2/100,000 births, characterized by underdeveloped nails and kneecaps.





Nail-patella syndrome = ● or ■
Blood Types = *OO, BB, BO, AO*

NPS is a dominant trait

Lod scores

- Test to compare the likelihood that two loci are linked, vs the likelihood that the two loci are unlinked.

$$LOD = \log_{10} \frac{\text{likelihood if linked}}{\text{likelihood if the loci are unlinked}}$$

LOD = "logarithm of the odds"

Testcross BN/bn x bn/bn

Genotype	Expected if unlinked	Expected if "r" cM apart
BN/bn	0.25	(1-r)/2
Bn/bn	0.25	r/2
bN/bn	0.25	r/2
bn/bn	0.25	(1-r)/2

Prob(recombination) = r
Split that among two recombinant classes

Prob(no recombination) = 1-r
Split that among two parental classes

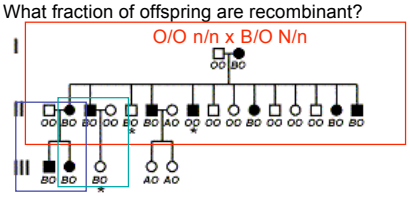
Lod score calculation

- Assume you have a testcross family with 8 non-recombinant offspring and zero recombinants between genes B and N
- Then, start with a hypothesis about linkage:
  - maybe they are 10 cM apart
- What is the probability of no recombinantion for each gamete?
  1-r = prob(no recombination) = 0.90
  Remember there are two different parental genotypes.
  If you keep track of individual genotypes, then each has probability (1-r)/2
  so, (1-r)/2 = 0.45
- Probability of 8 non-recombinants if genes are linked (at 10 cM) is: 0.45^8
- Probability if not linked is 0.25^8
  remember, r=0.5 when loci are not linked
- Lod score = $\log_{10} (0.45^8/0.25^8)$
  $= \log_{10} (110.19) = 2.04$ (LOD must be >3.0 to be "significant")

The pattern of 8 non-recombinants is 110 times more likely if the genes are linked at 10 cM than if they were unlinked

Human pedigree mapping

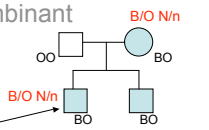
1. Determine genotypes in the pedigree
2. Find informative families (e.g. test crosses)
3. What fraction of offspring are recombinant?



Nail-patella syndrome = ● or ■
Blood Types = *OO, BB, BO, AO*

Calculate LOD score

- Fam 1 : 2/13 recombinant
- Fam 2: 0/2
- Fam 3 1/1



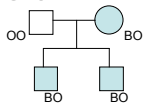
Both offspring are non-recombinant

Prob (seeing this non-recombinant genotype)
= (1-r)2
If r=0.5, then Prob = 0.25
If r=0.1, then Prob = 0.45

Log Odds Ratio =
Log (L(r=x) / L(r=0.5))

Calculate LOD score

- Fam 1 : 2/13 recombinant
- Fam 2: 0/2
- Fam 3 1/1



Family 2
Likelihood if independent? 0.25*0.25 =0.0625
Likelihood if linked at r=0.1? 0.45*0.45 =0.2025

Ratio = 3.24
LOD = 0.51

Prob(getting 2 non-recombinant)

Log Odds Ratio =
Log (L(r=x) / L(r=0.5))

Calculate LOD score

- Fam 1 : 2/13 recombinant
- Fam 2: 0/2
- Fam 3 1/1

Family 1
Likelihood if independent? $0.25^{11} * 0.25^{2}$ $=1.49 \times 10^{-8}$
Likelihood if linked at r=0.1? $0.45^{11} * 0.05^{2}$ $=3.83 \times 10^{-7}$

Ratio = 25.7
LOD = 1.41

11 non-recombinants

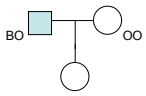
.45*.45*.45*.45* . . . *.45

2 recombinants

.05*.05

Calculate LOD score

- Fam 1 : 2/13 recombinant
- Fam 2: 0/2
- Fam 3 1/1



Family 3
Likelihood if independent? 0.25 =0.25
Likelihood if linked at r=0.1? 0.05 =0.05

Ratio = 0.20
LOD = -0.69

1 recombinant

BO

Calculate LOD score

- Fam 1: 2/13 LOD = 1.41
- Fam 2: 0/2 LOD = 0.51
- Fam 3 1/1 LOD = -0.69

Add the LOD scores from all 3 families:
1.41 + 0.51 + -0.69 = 1.22

Should be bigger than 3.0 for significant linkage

Now, do that whole exercise for other values of r!

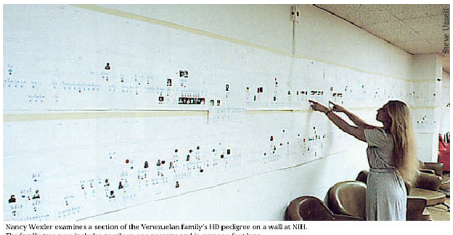
Calculate LOD score

R	LOD
0.01	-1.24
0.1	1.22
0.2	1.46
0.3	1.23
0.4	0.73
0.5	0

LOD is maximum around r=0.2, so that is the best estimate of linkage

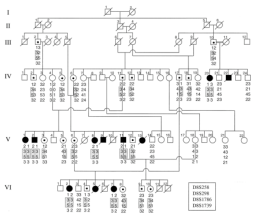
Human pedigree mapping

- It is hard to get big enough pedigrees, with enough informative families



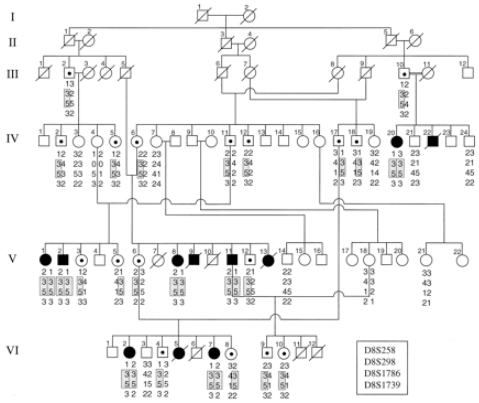
Nancy Wexler examines a section of the Venezuelan family's HD pedigree on a wall at NIH. The family tree now includes nearly 10,000 persons and is over 100 feet long.

Mapping a hair loss gene



- Allopecia universalis
- Found a large family in Pakistan with many affected individuals
- Looked for linkage between molecular markers and the trait
  - 300 highly polymorphic markers
  - Searched for markers that were homozygous in affected individuals and heterozygous otherwise
  - One such marker was found on chromosome 8p12

Source: Ahmed et al., 1998. Science 279:720

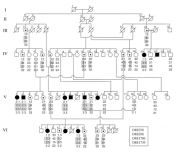


Finding the actual gene

- In other work, they had also mapped the "hairless" gene (*hr*) using somatic cell hybrid mapping
  - Used the mouse *hr* gene to amplify the human gene sequence
  - They fused bits of human chromosomes to hamster cells, to find the fragments that corresponded to the mouse hairless gene
  - It also mapped to 8p12

Finding the amino acid

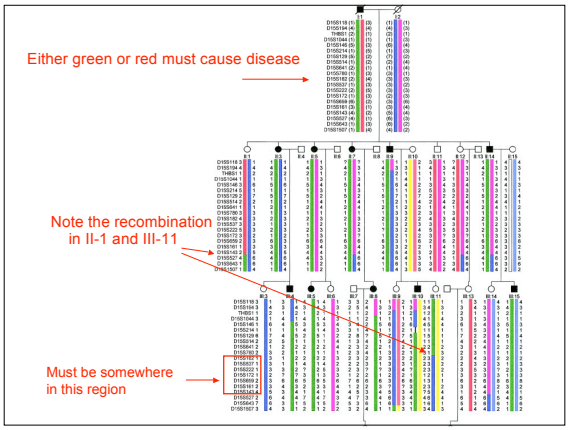
- The *hr* gene was a good candidate, so they sequenced the gene from individuals in this pedigree.
- All affected individuals had an allele of *hr* that differed in a single base, A to G
  - Changes threonine to alanine



But, it all started with this pedigree . . .

Another example

- Renal Fanconi syndrome
  - Lichter-Konecki et al, 2001 Am J Hum Gen
- RFS is a dominant disorder
- Used a big pedigree from Wisconsin and a lot of polymorphic markers
- Found a set of markers on Chromosome 15 that were associated with the disorder

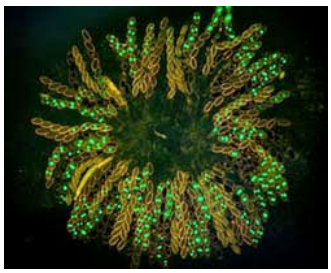


Tetrad analysis

- In some haploid fungi and algae, the meiotic products stay together in 'tetrads'
  - *Why are there four?*
- Because you see all products of meiosis, you can do more detailed analyses
  - like mapping the centromere
  - distinguishing various kinds of crossovers
- In many ways, everything is the same except Meiotic tetrads are counted, not individuals

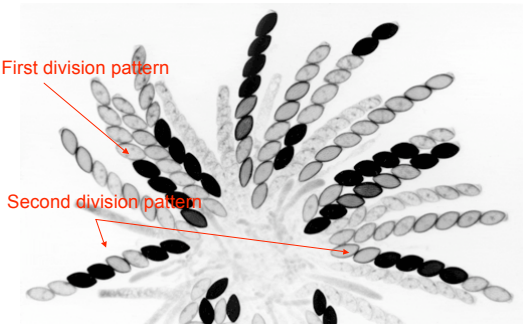
Tetrads can be either ordered or unordered

- The bread mold *Neurospora crassa* produces ordered tetrads.



Mapping the centromere

- Centromeres always segregate in Meiosis I
  - AAaa or aaAA
    Draw that out to convince yourself . . .
- If alleles segregate in meiosis II, then there must have been a crossover between that gene and the centromere.
  - AaAa or AaaA
    Again, draw this out to convince yourself . . .



http://www.stanford.edu/group/neurospora/RajuNcrassaWeb/Fig49.WTxcys3.Raju781308.gif

Tetrad example

- Tetrad types from a heterozygous Aa individual.
- What is the distance to the centromere?

Pattern	Number of ascii	
AAaa	36	
aaAA	44	How many show second-division segregation?
AaAa	4	
aAaA	6	What proportion of *gametes* will be recombinant?
AaaA	3	
aAAa	7	
Total:	100	

– *In Neurospora, each of these gametes would be doubled, producing ascii with 8 spores. Here I'm just showing the four meiotic products.*