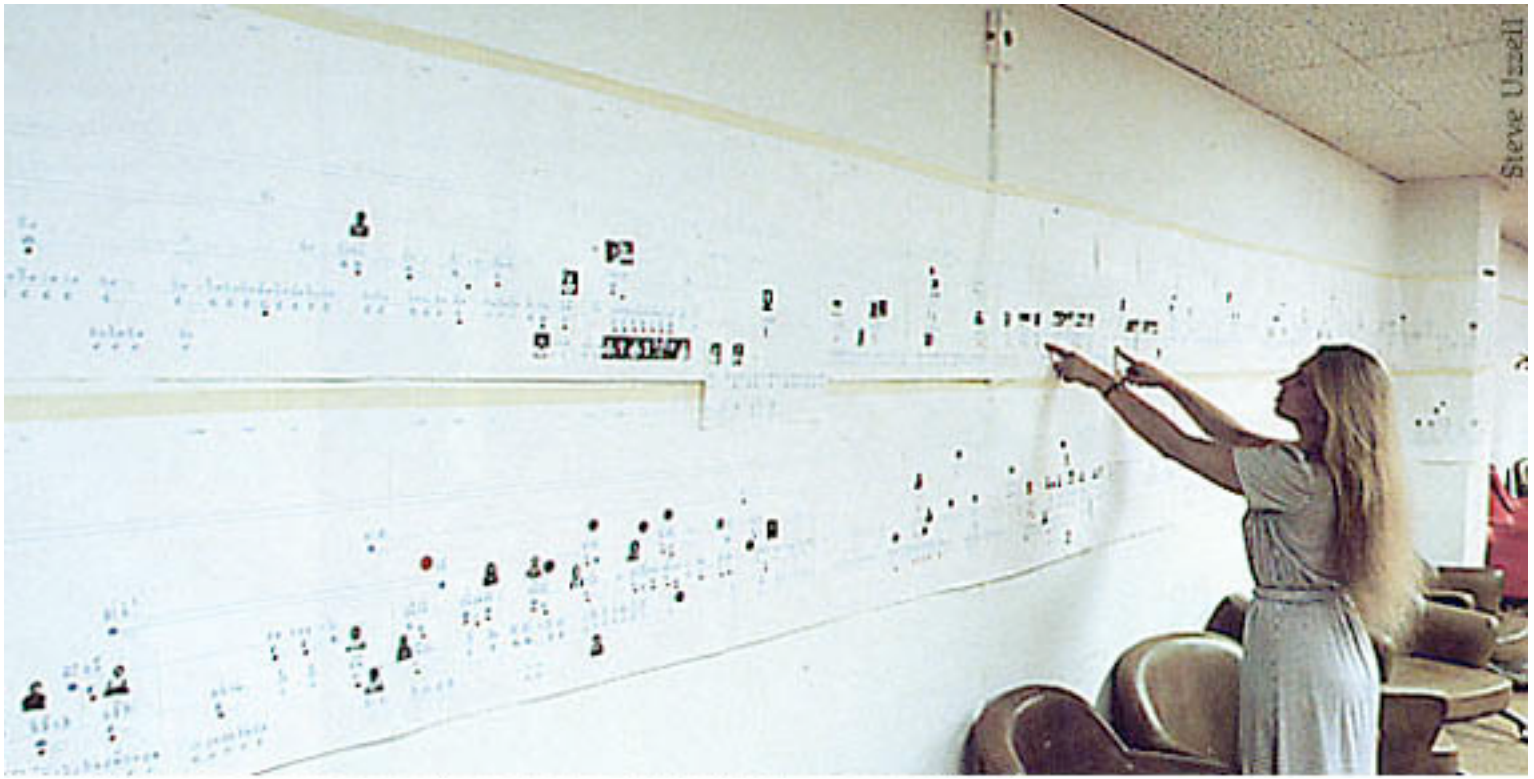
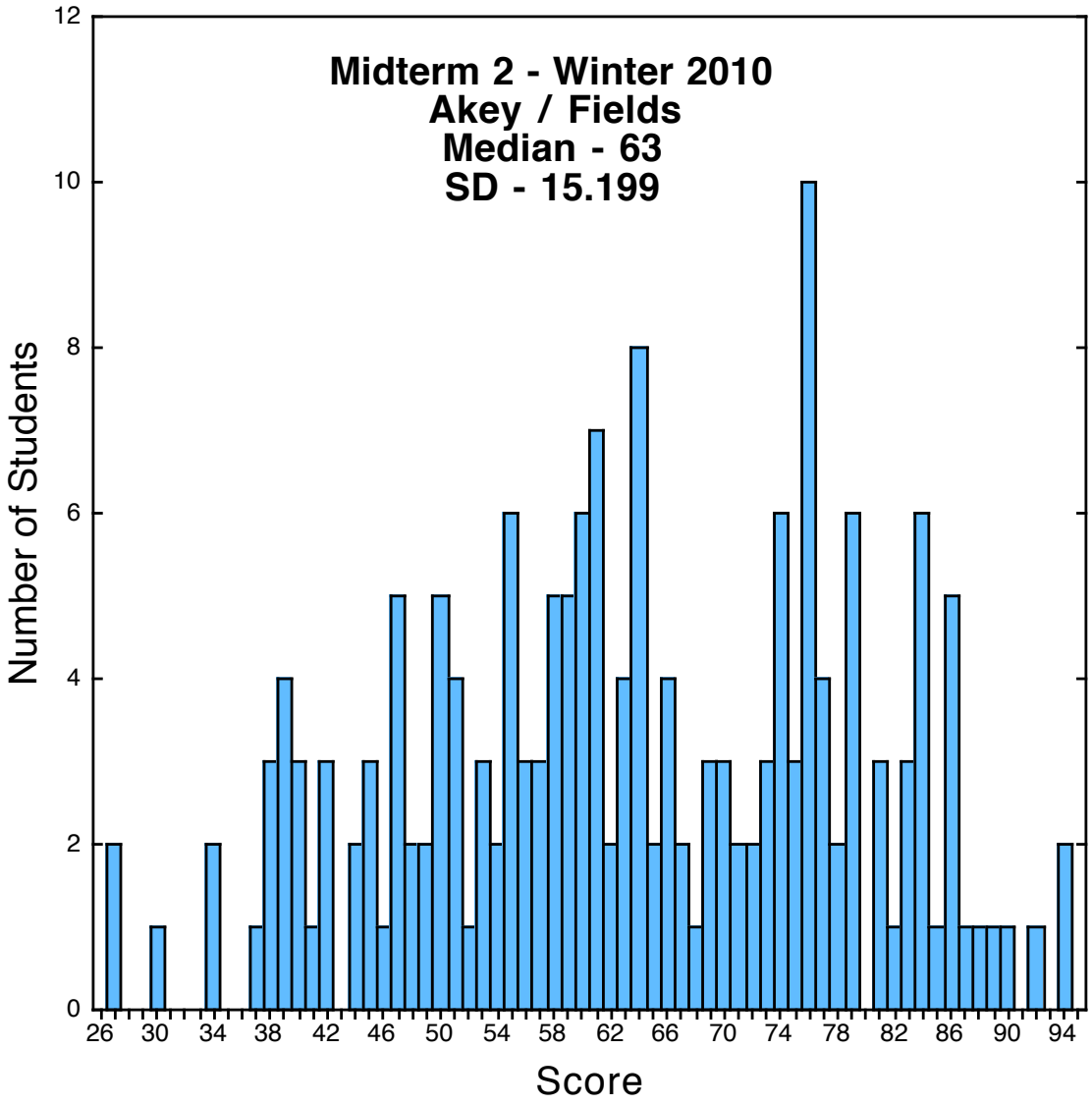
LOD Score Analysis in Humans

Gene mapping in humans

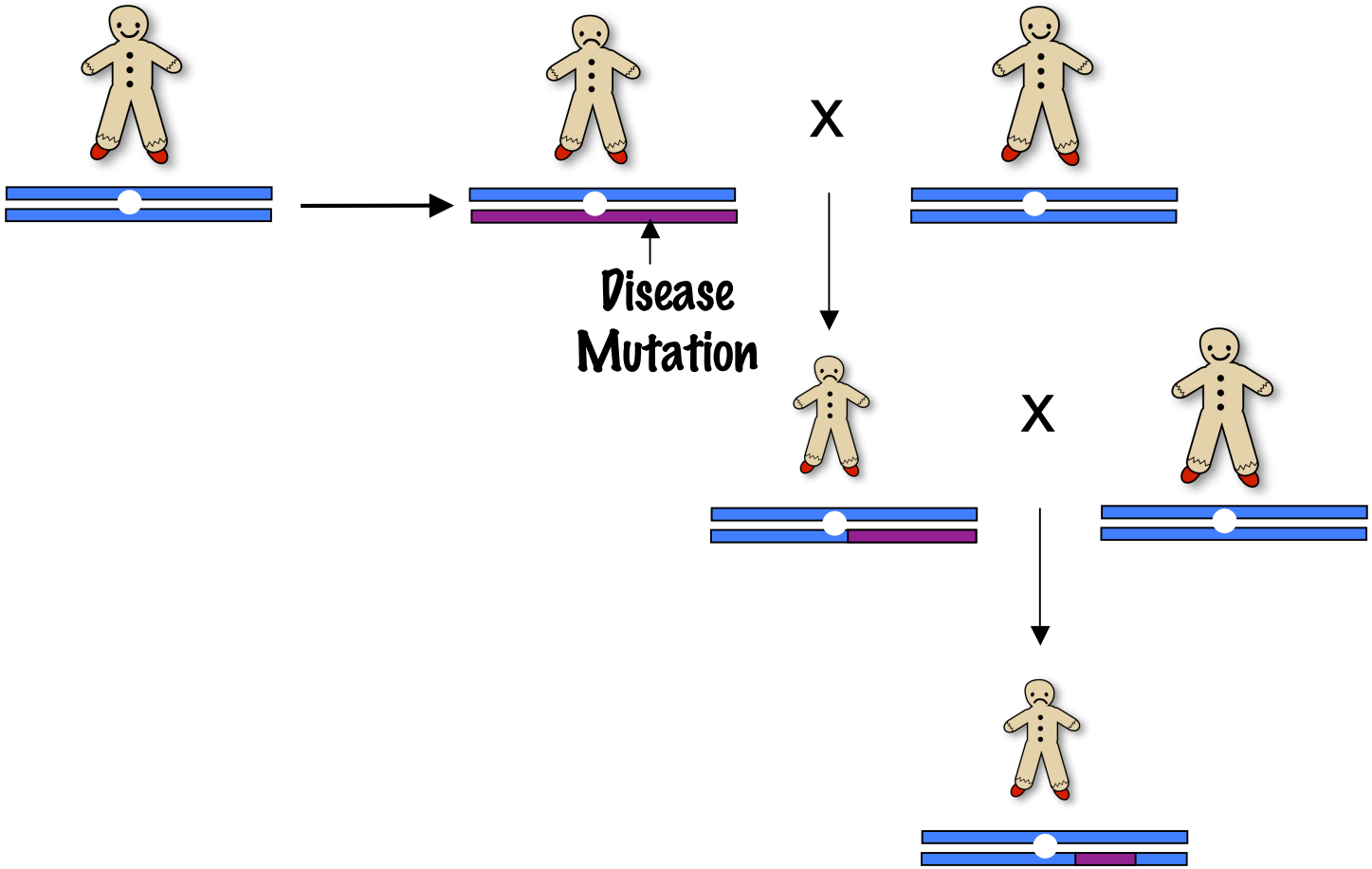
»Mapping using pedigrees—LOD score analysis, haplotype analysis



Midterm 2 distribution…



What creative liberties have I taken in this drawing?



Do as I say not as I do...

Not all matings are informative…

A test for informative vs. non-informative meiosis: can we tell if the gamete was recombinant?

If we can tell: the meiosis is informative

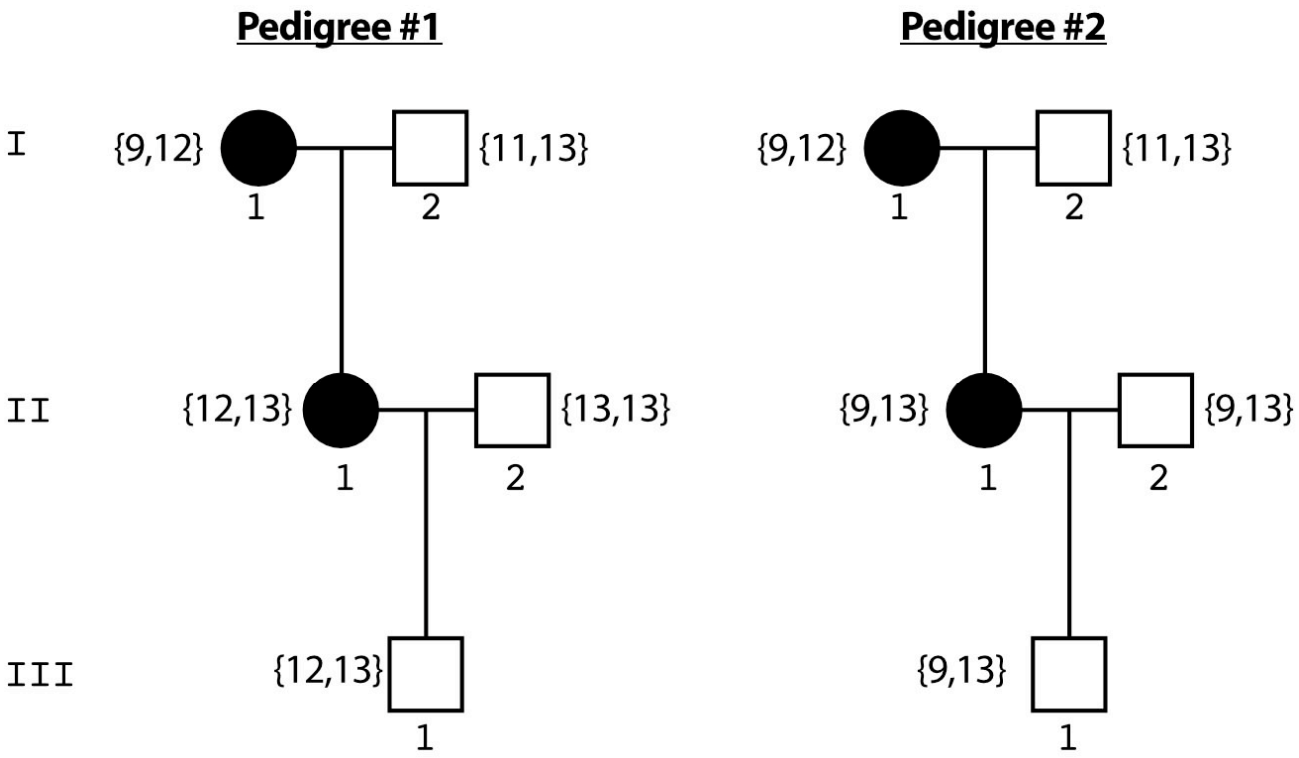
- yes, gamete was recombinant
  or
  no, not recombinant

} both are informative

cannot tell if the gamete was recombinant } → non-informative

Practice question

The two pedigrees show inheritance of an **autosomal dominant trait** (**D** = disease, dominant; **d** = normal, recessive). Numbers in {curly brackets} indicate alleles of a microsatellite repeat polymorphic locus. For each pedigree, state whether the meiosis **in II-1** is <u>informative</u> or <u>uninformative</u>, giving the parental types for II-1 in each case.



Pedigree #1: Informative or uninformative?

Parental types for II-1:

D 12 and d 13

III-1 received d 12 from II-1… we can tell that it is recombinant

Pedigree #2: Informative or uninformative?

Parental types for II-1:

D 9 and d 13

Can't tell whether II-1 gave d 9 (recombinant) or d 13 (parental)

Mapping a gene using molecular markers (cont'd)

Stumbling blocks…

» Which polymorphic loci to test?

» Not all meioses are informative

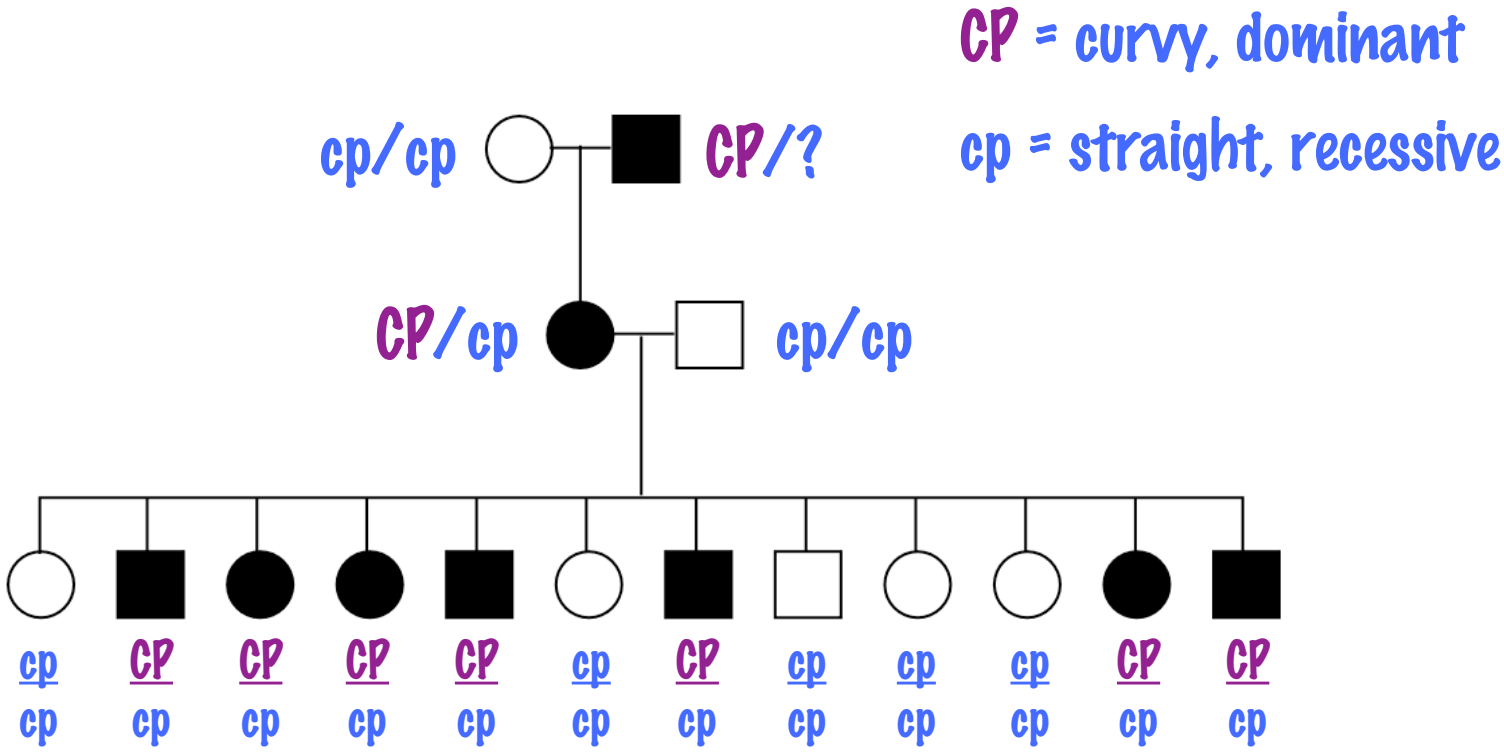
» Pedigrees may be too small to detect linkage with confidence

A solution—

Play the odds: What is more likely to give this pedigree outcome, linkage or non-linkage?

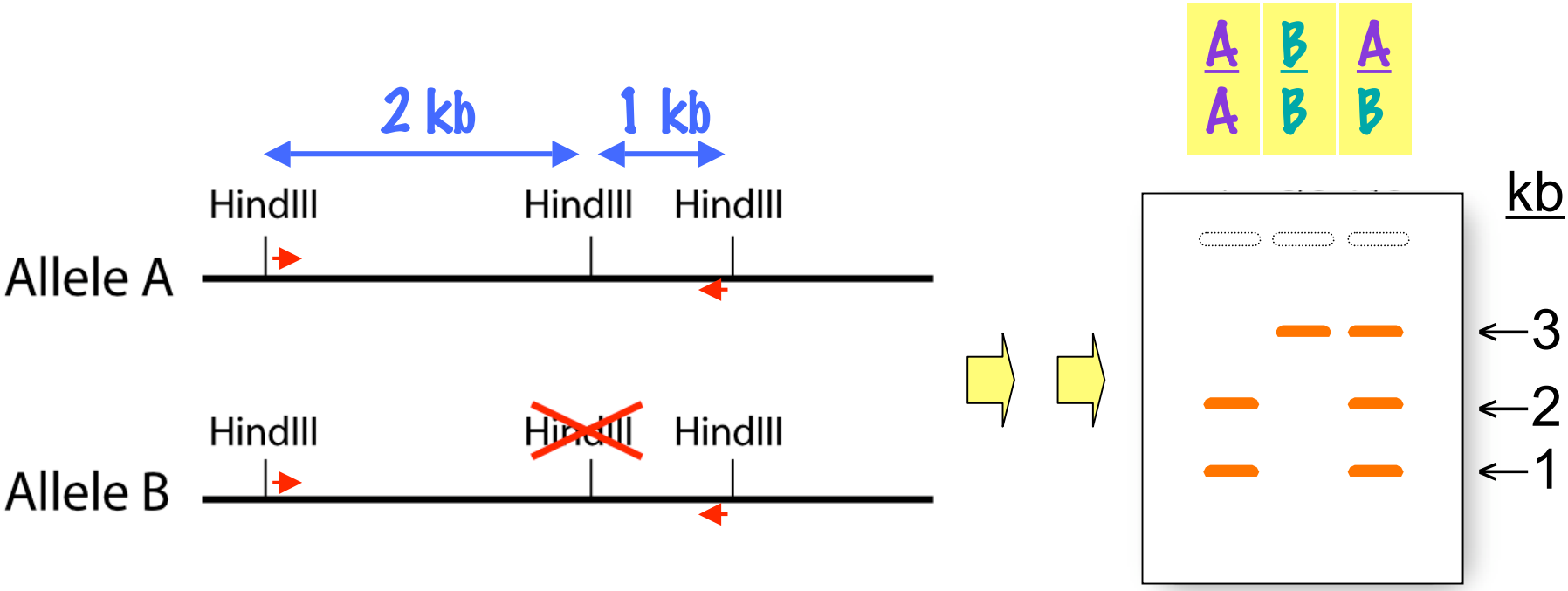
Mapping a gene using molecular markers

A very simple (hypothetical) example… mapping the curvy pinky gene



Mapping a gene using molecular markers (cont'd)

Suppose you know of an RFLP:

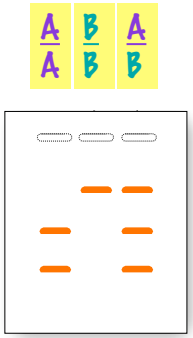


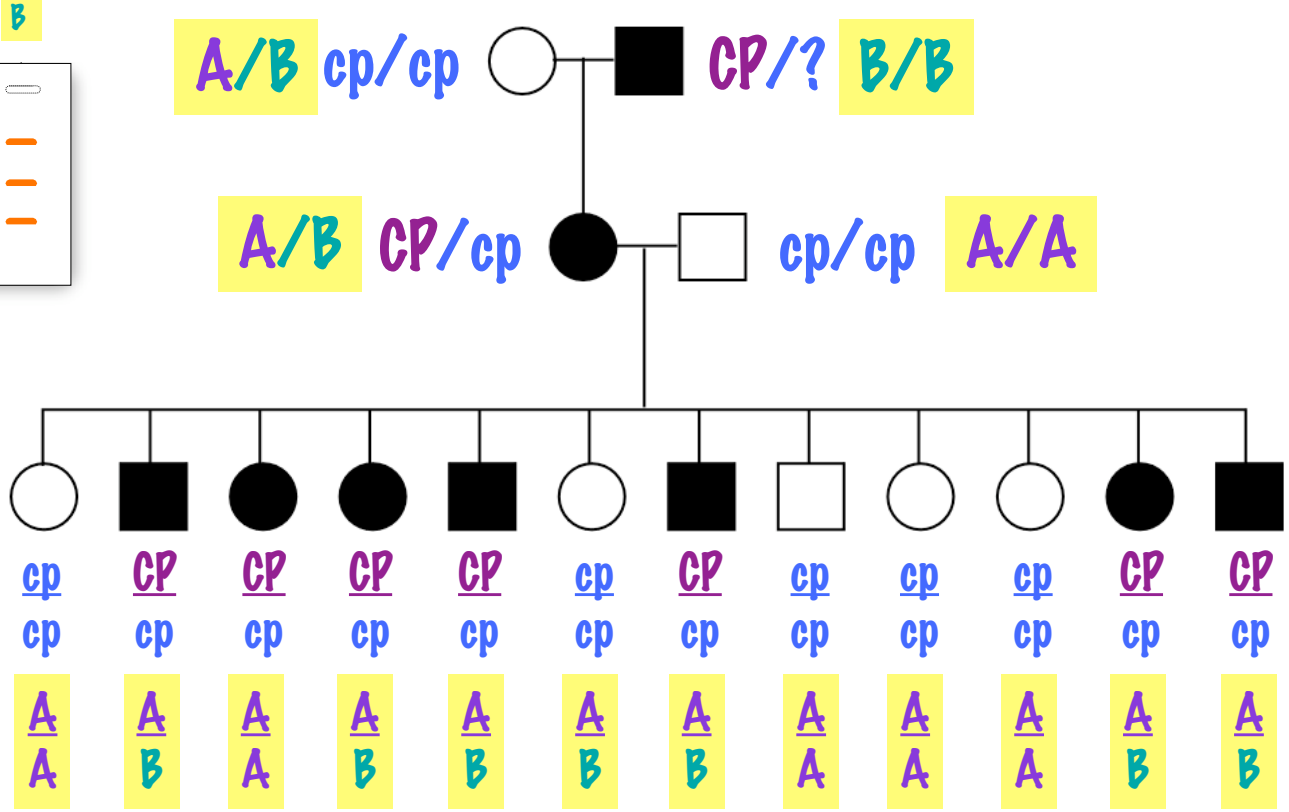
Is the curvy pinky gene linked to this RFLP locus?

Mapping a gene using molecular markers (cont'd)

Genotype all members of the family for the RFLP

Suppose we find that the RFLP genotypes are:





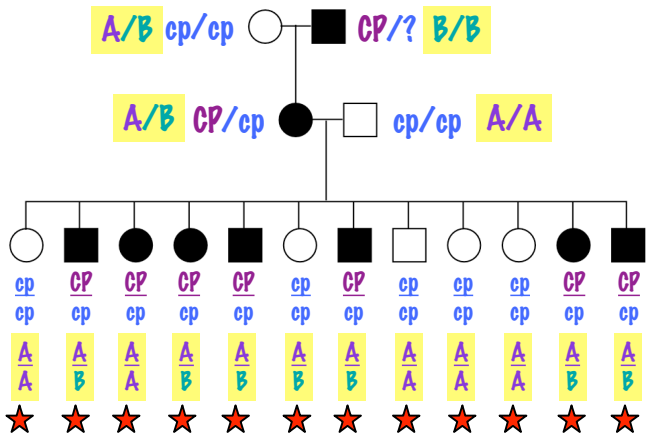
Gametes from II-1...

Parental types?

B CP

A cp

Mapping a gene using molecular markers (cont'd)



Gametes from II-1...

Parental types?

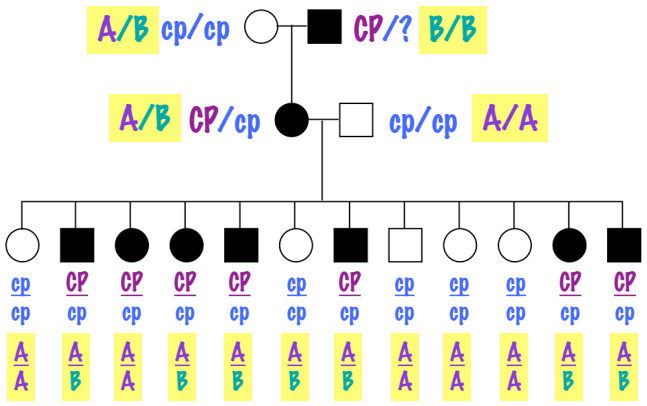
B CP

A cp

eggs \ sperm	A cp	Exp. for I.A.	Obs.
A CP	A/A CP/cp	3	1
A cp	A/A cp/cp	3	4
B CP	A/B CP/cp	3	6
B cp	A/B cp/cp	3	1

Result: Parental = 10/12; non-parental = 2/12. Hmmm...

Mapping a gene using molecular markers (cont'd)



A solution—

Play the odds: What is more likely to give this pedigree outcome, linkage or non-linkage?

this set of parental genotypes giving this set of offspring genotypes

Can predict probability of this outcome for I.A.

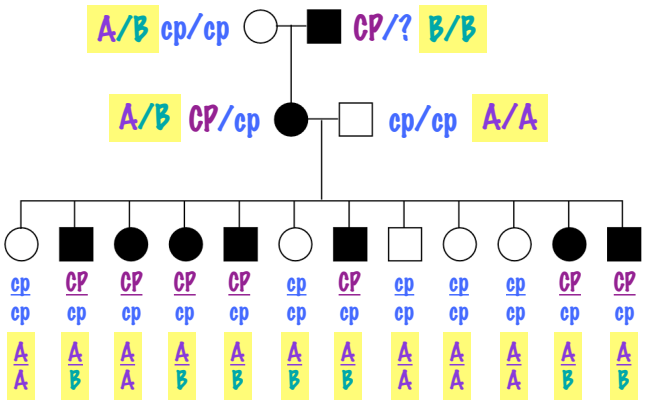
But for predicting outcome if linked… what map distance??

Don't know! So, set up predictions for full range of distances.

Odds of linkage?

A “what-if” exercise…

what are the odds that we will see this pedigree if the two loci are linked at (x) cM?



What are the odds that these parents (II-1 and II-2) would have this set of children (of these genotypes) if the loci were linked at (x) cM?

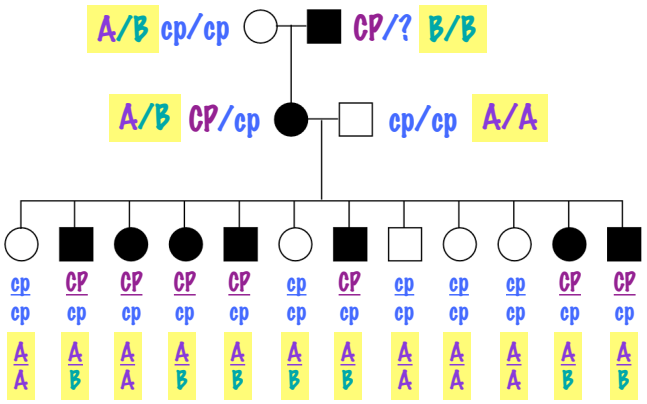
“Odds” ≠ probability!

“Odds” is a comparison: probability if linked vs. probability if unlinked

The odds of linkage

A “what-if” exercise…

what are the odds that we will see this pedigree if the two loci are linked at (x) cM?

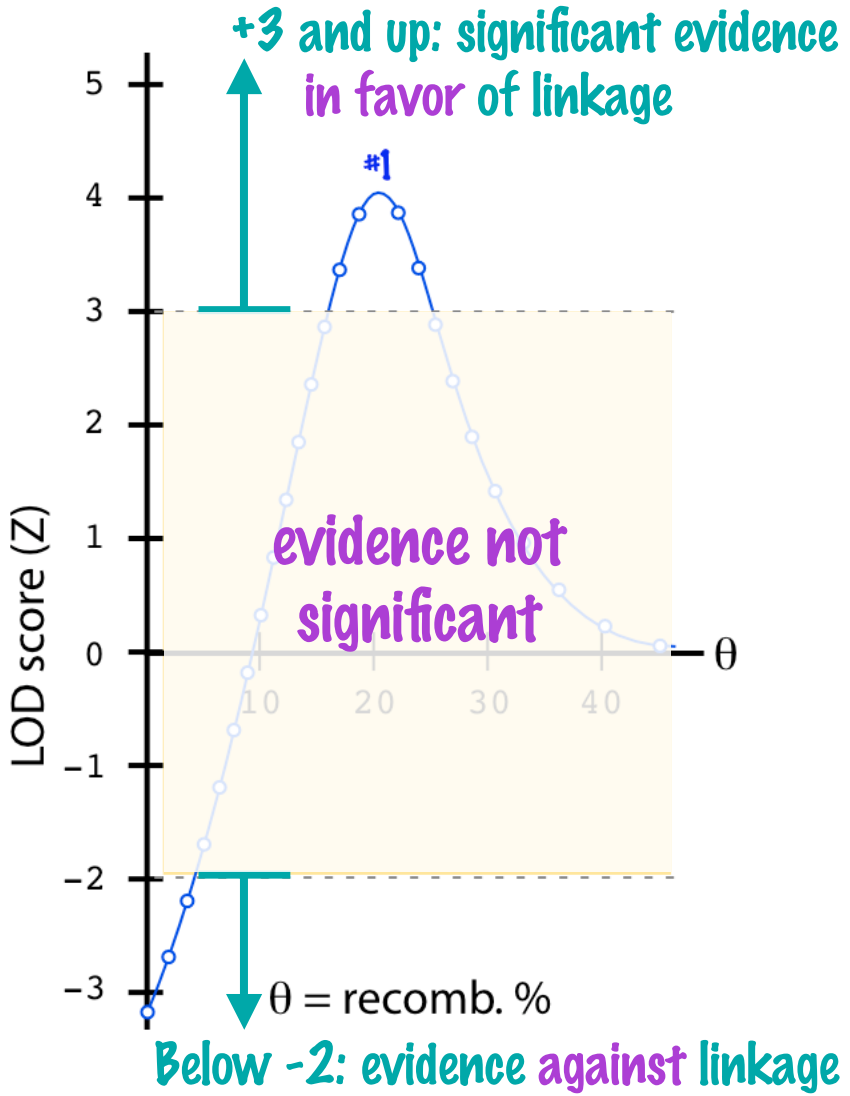


LOD scores

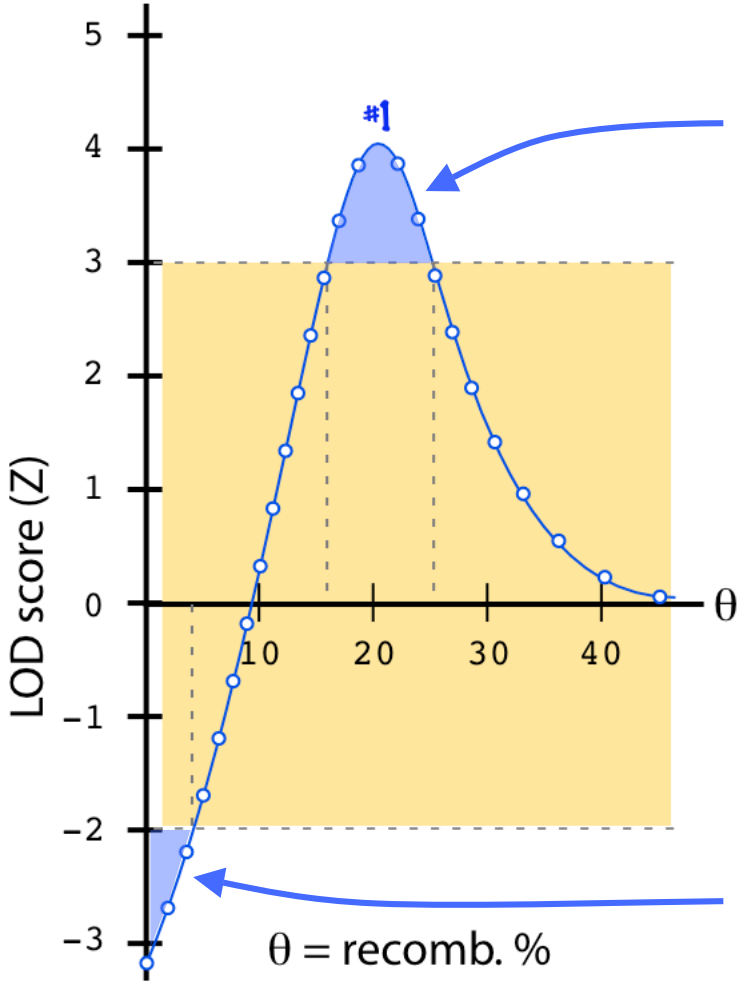
1. Consider the disease and PS #1
2. What are the odds of getting the observed pedigree if the two loci are linked at 0 cM?
   LOD score for ~0 cM
3. What the odds if they are linked at 2 cM?

   LOD score for 2 cM
4. What the odds if they are linked at 4 cM?
   LOD score for 4 cM

   etc., up to 50 cM



Interpreting the LOD scores plot



Consider the disease and PS #1

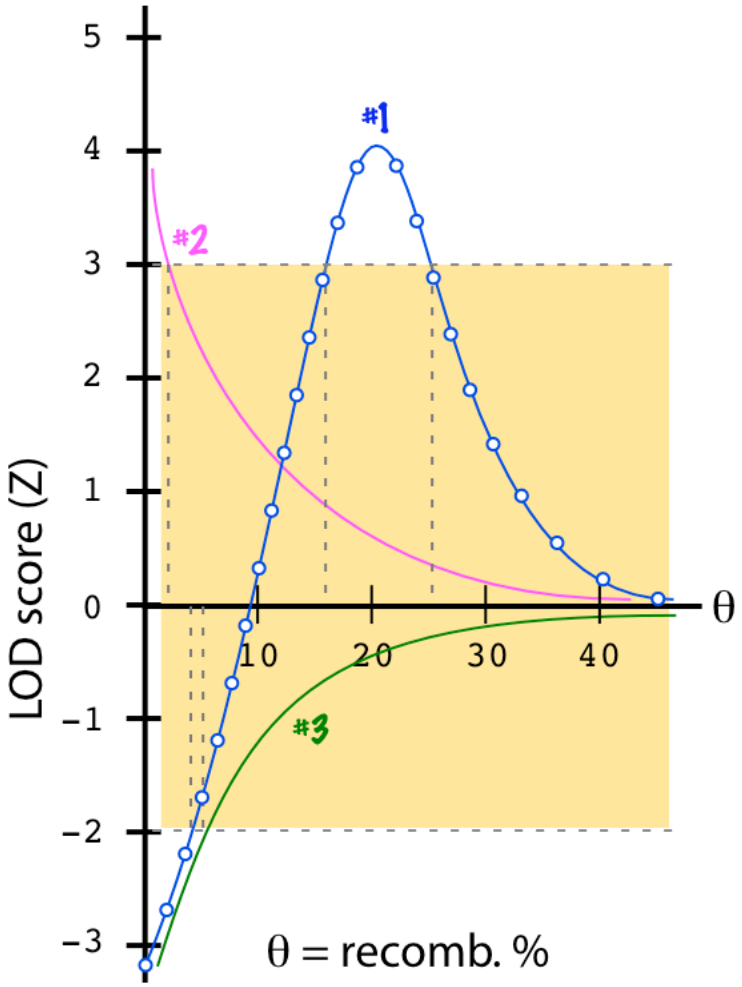
Evidence in favor of linkage:
at map distance of ~17 cM - 25 cM

Max. probability of linkage at ~20 cM

Then repeat with the disease and PS #2…

Evidence against linkage:
at map distance of < 4 cM

Interpreting the LOD scores plot (cont'd)



Disease and PS #1

Evidence in favor of linkage at map distance of ~17 cM - 25 cM

Max. probability of linkage at ~20 cM

Evidence against linkage at map distance of < 4 cM

Disease and PS #2

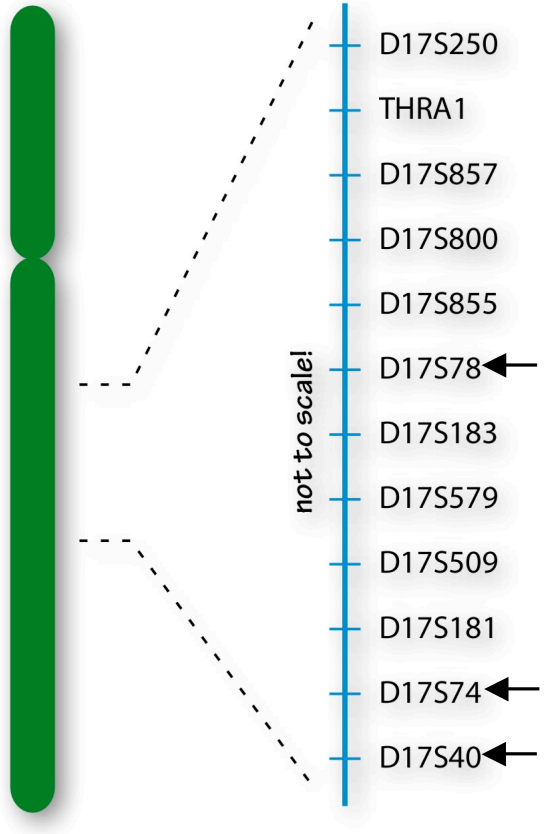
Evidence in favor of linkage at map distance of < 2 cM; max probability at close to 0 cM

Disease and PS #3

Evidence against linkage at < 5 cM

Linkage Mapping of *BRCA1*

Marker	Recombinant frequency				
	0.001	0.10	0.20	0.30	0.40
D17S78	−0.84	−0.65	−0.16	−0.04	+0.00
D17S41	−1.54	−1.12	−0.71	−0.36	−0.14
D17S74	+5.98	+4.83	+3.47	+1.97	+0.65
D17S40	+1.36	+1.01	+0.63	+0.30	+0.07



Positional cloning of the Huntington disease gene

Positional cloning case study—Huntington disease

HD first described in 1872

Affects ~1/10,000 individuals

Disease causes neurodegeneration —

- movement disorder ("chorea")
- lack of coordination
- cognitive changes
- Age of onset ~35-55 years, progresses over ~15-25 years
- Invariably lethal

Why map the gene?

Hope of cloning it if we know where it is

Identify the gene product

Understand its function

Understand the defect in the disease forms

Devise therapy

Risk assessment

Trial and error testing of RFLPs

Nancy Wexler

Random, purified fragments of human genome

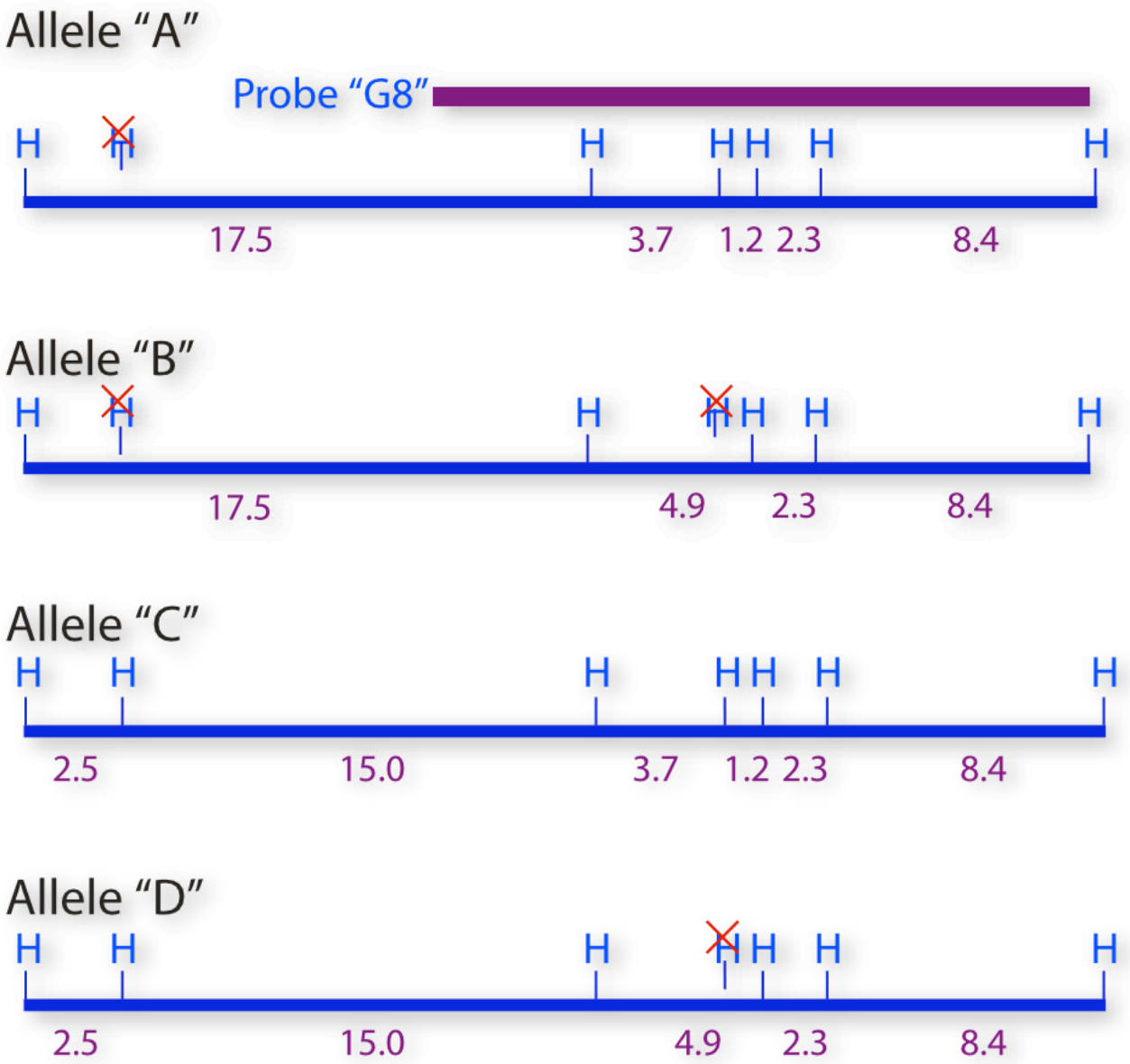
Look for differences in the pattern of bands on the blot => RFLPs

How many probes will need to be tried?

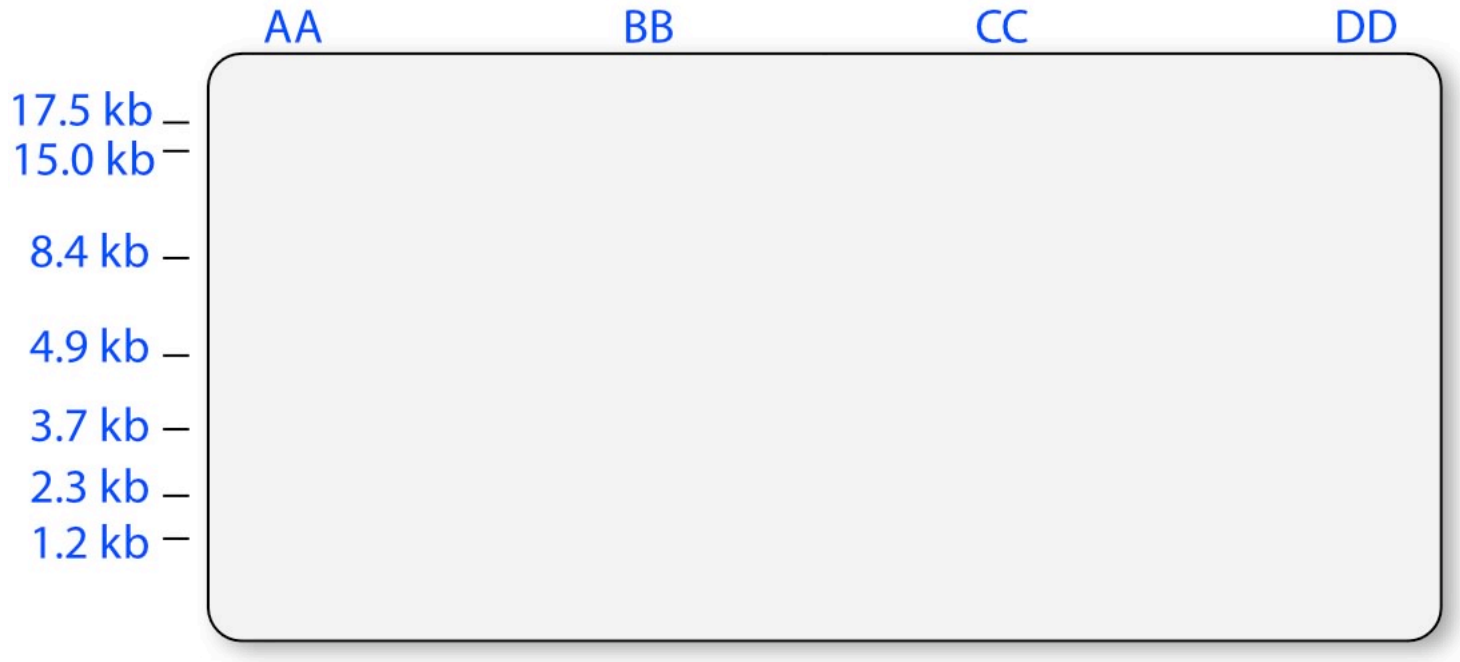
- Human genome ~3,000,000,000 bp
- One RFLP per ~15,000-20,000 bp
- So, need ~1500-2000 probes for 1% coverage!

On their 12th probe... the jackpot!

- linkage of the RFLP to HD!


Allele “A”
Probe “G8”
H H H H H H H
17.5 3.7 1.2 2.3 8.4
Allele “B”
H H H H H H H
17.5 4.9 2.3 8.4
Allele “C”
H H H H H H H
2.5 15.0 3.7 1.2 2.3 8.4
Allele “D”
H H H H H H H
2.5 15.0 4.9 2.3 8.4

What would homozygous genotypes look like on the Southern blot?

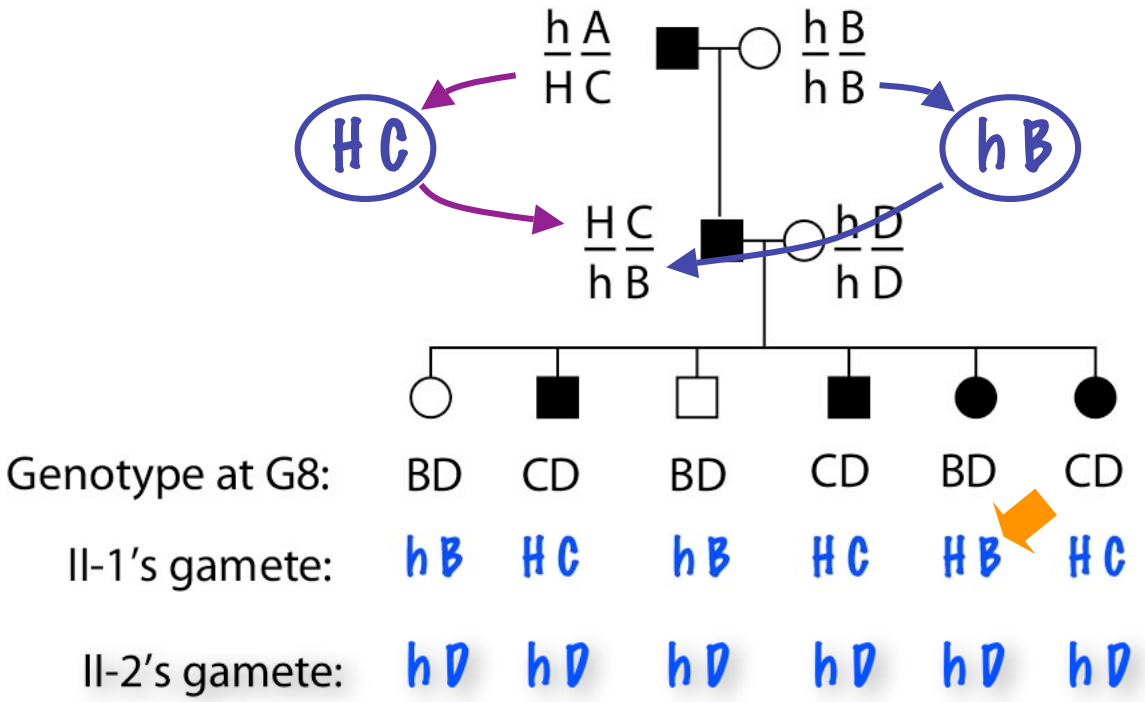


Calculating LOD scores — a hypothetical example

1. Figure out the phase in II-1

   what are the parental types

2. Figure out the gametes produced by II-1

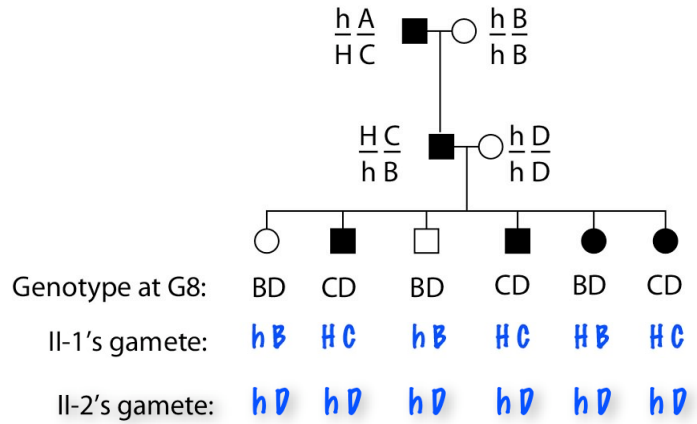


Calculating LOD scores (cont'd)

3. Figure out the probability of the pedigree—

   - if HD and G8 loci are unlinked

   - if they are linked at θ cM



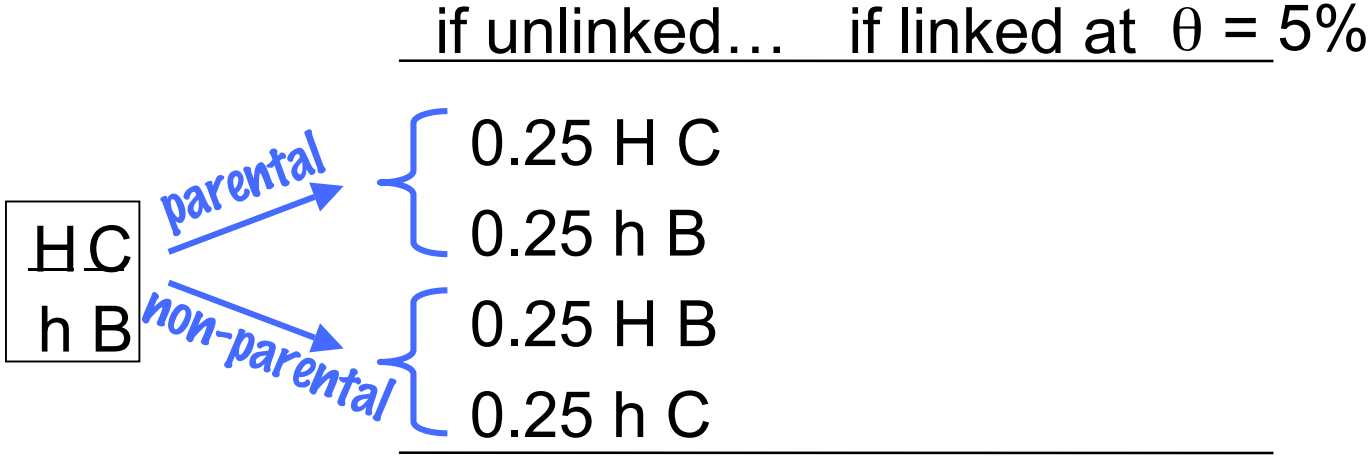
probability of each child's genotype =

(probability of egg) x (probability of sperm) genotypes

overall probability of pedigree =

p(child 1) x p(child 2) x p(child 3)… etc.

Possible gametes from II-1:



Probability of the observed genotype if H/h and G8 loci are…

	unlinked	linked @ θ = 5%
Child #1 h B/h D	0.25 x 1 = 0.25	0.475 x 1 = 0.475
Child #2 H C/h D	0.25 x 1 = 0.25	0.475 x 1 = 0.475
Child #3 h B/h D	0.25 x 1 = 0.25	0.475 x 1 = 0.475
Child #4 H C/h D	0.25 x 1 = 0.25	0.475 x 1 = 0.475
Child #5 H B/h D	0.25 x 1 = 0.25	0.025 x 1 = 0.025
Child #6 H C/h D	0.25 x 1 = 0.25	0.475 x 1 = 0.475

II-1's gametes:

Parental = HC, hB

$$LOD_{\theta=5\%} =$$

$$\log_{10}\left[\frac{\text{probability of observed genotypes if the loci are linked at 5 cM}}{\text{probability of observed genotypes if the loci are unlinked}}\right]$$

$$\log_{10}\left[\frac{0.475^5 \times 0.025}{0.25^6}\right]$$

$$= 0.394$$

Then repeat with $\theta = 10$, $\theta = 15$, etc.

or tighter/looser spacing...!