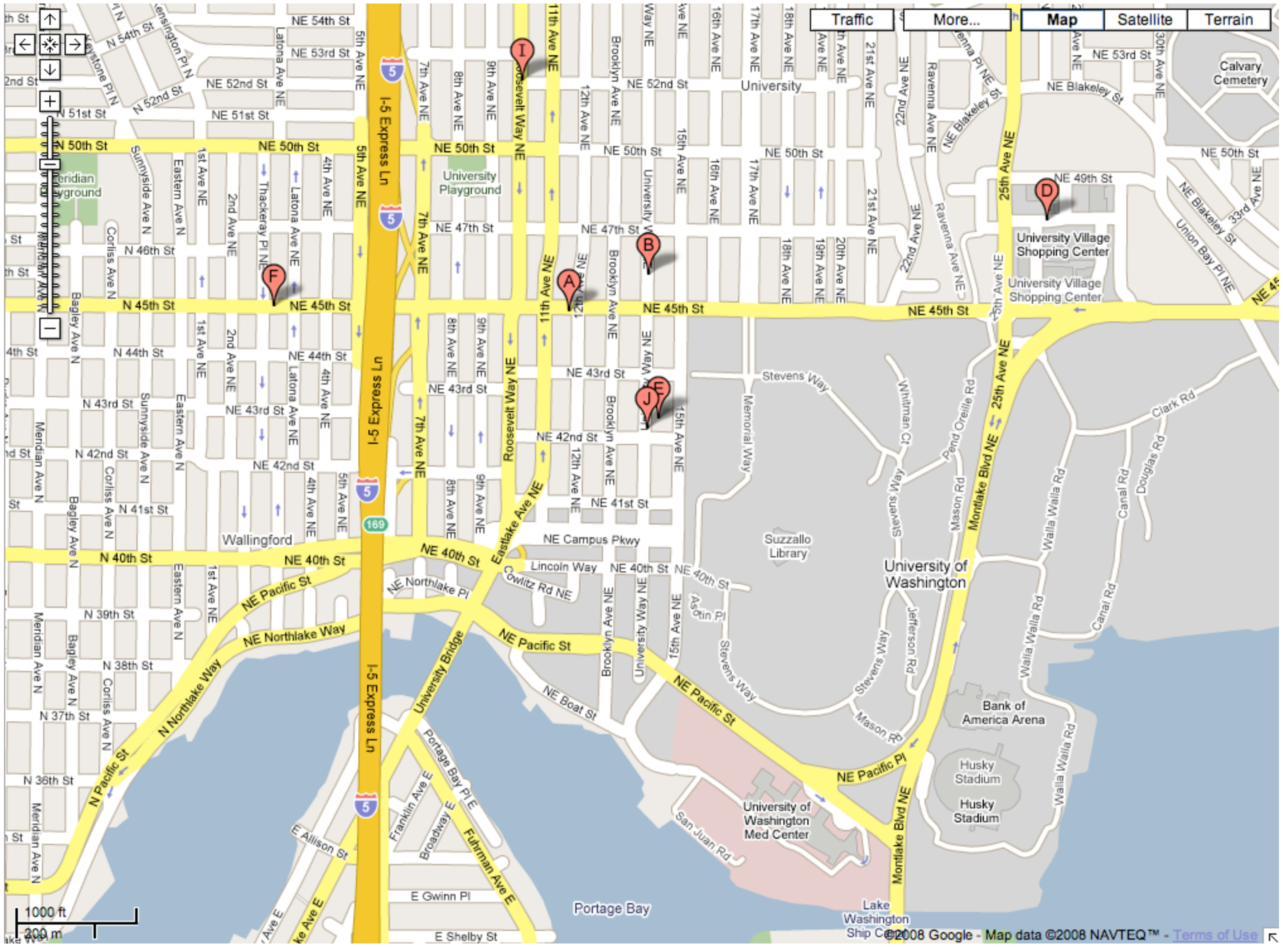
Genome 371, 1 and 5 Feb 2010, Lecture 7 and 8

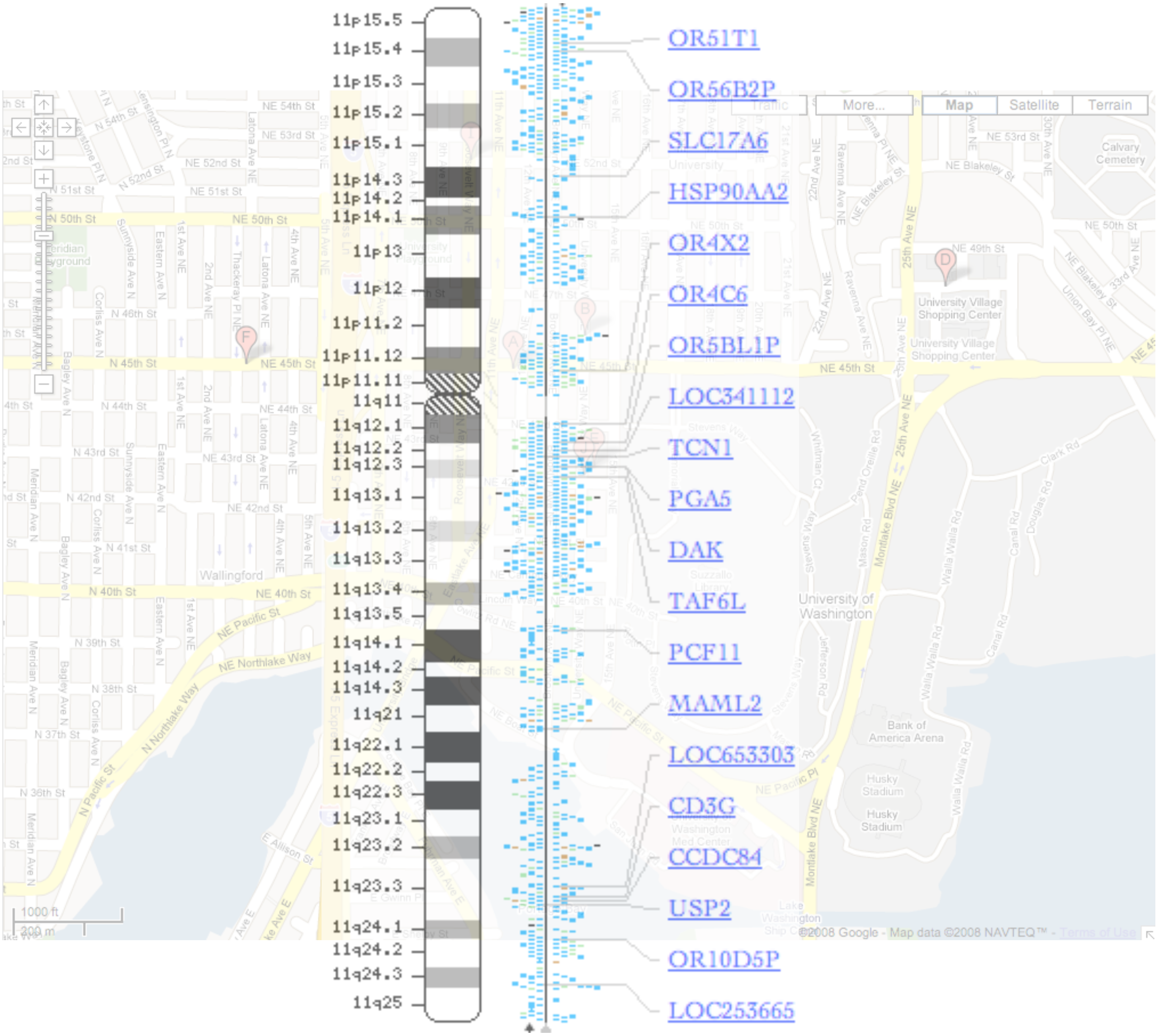
Genomic Maps and Linkage Analysis

- Genomic maps
  - Linkage maps
  - Physical maps

Question: Find the closest Thai food restaurant in the University District



Question: Find the closest Thai food restaurant in the University District



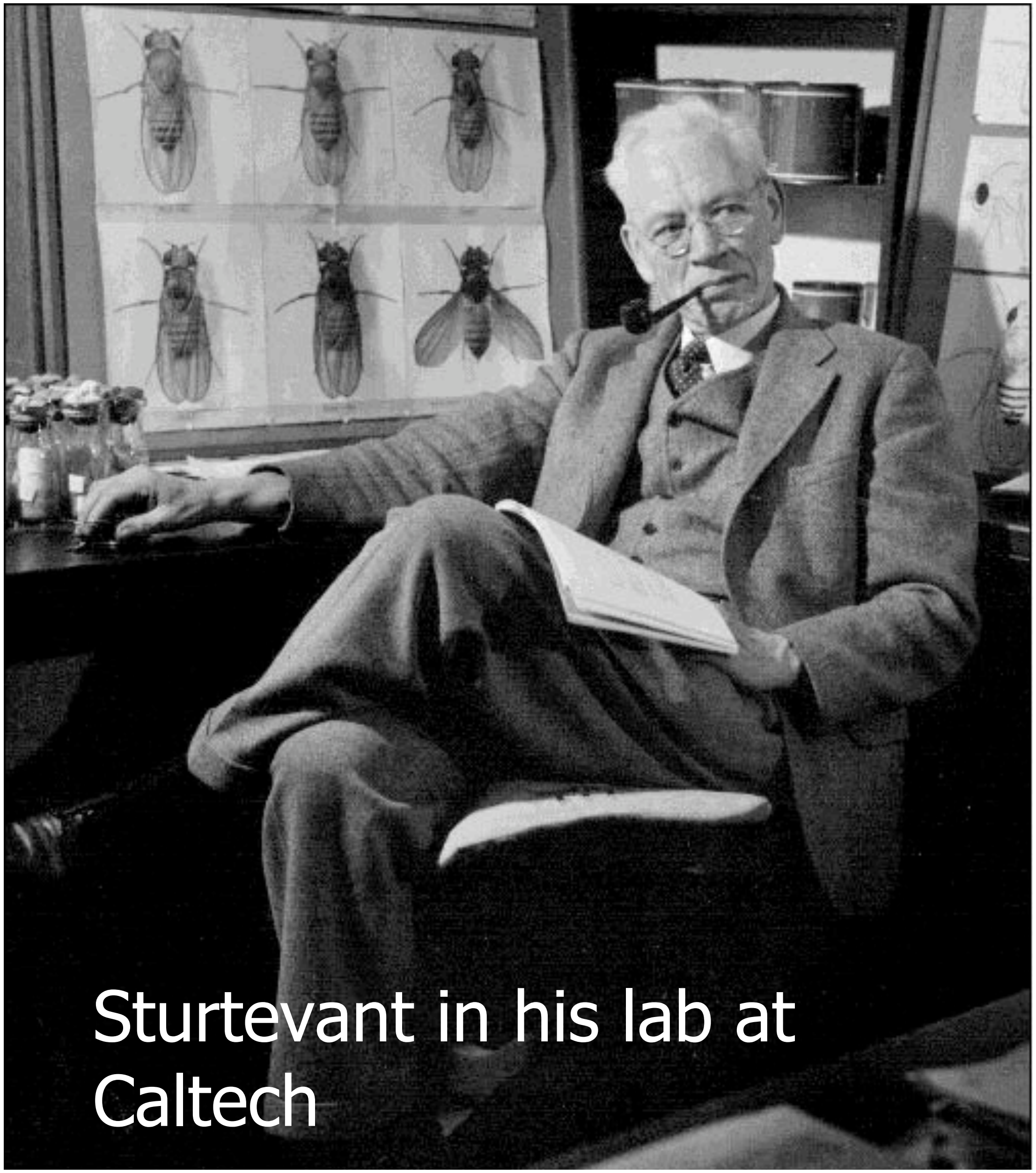
How can the principles of genetic linkage be applied to constructing linkage map?

What's a linkage map?

A Humbling Digression…

Alfred Sturtevant

As an undergraduate student in Morgan's lab, Sturtevant created the first genetic maps

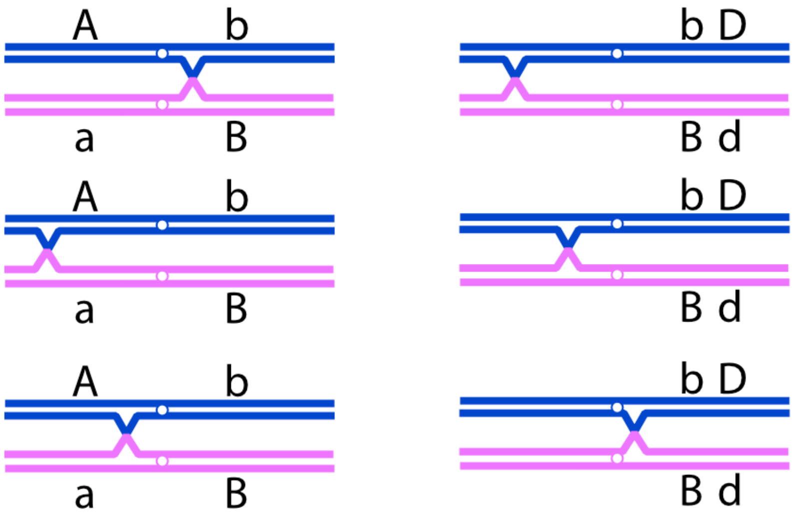


Sturtevant in his lab at Caltech

Constructing a Linkage Map

Alfred Sturtevant's major insight

If crossovers occurs at random:



Probability of crossover between two genes is proportional to the distance between them

Crossover between A and B much more likely than between B and D

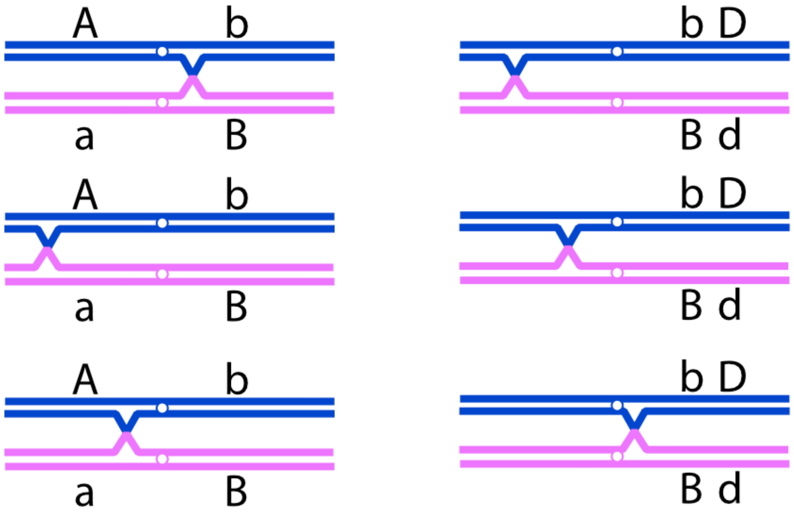
Constructing a Linkage Map (cont'd)

So…

Do testcross

Measure recombinant frequency…

= indicator of map distance between the genes!



Map distance = # of recombinant products ÷ total # of products

- 1 map unit = 1 **centiMorgan (cM)** = 1% of meiotic products being recombinant
- Recombination frequency in adjacent intervals is additive

Recomb. freq. (A•D) = (A•B) + (B•D) …up to a point

Homework Practice Problem

In corn…

Colored kernels (C) is dominant over colorless (c)
Plump kernels (S) is dominant over shrunken (s)
Starchy kernels (W) is dominant over waxy (w).

A trihybrid (Cc Ss Ww) plant is testcrossed and the following progeny are obtained:

2708 Colorless, plump, waxy
2538 Colored, shrunken, starchy
626 Colorless, plump, starchy
601 Colored, shrunken, waxy
116 Colorless, shrunken, starchy
113 Colored, plump, waxy
4 Colored, plump, starchy
2 Colorless, shrunken, waxy

Determine linkage (including map distance) for the genes, and the phase in this cross.

Practice Problem

Where do we begin?

Determine genotypes of offspring

Identify parental types

Calculate map distance between pairs of loci

Determine map order

Summary

* Crossing-over creates new combinations of traits

* Two Parental types in ≈ frequencies
Two Recombinant types ≈ frequencies

* If genes are linked,
Parental types > recombinant types

* The frequency of recombinant types
indicates the distance between linked genes

What is the Maximum Recombination Frequency Between Two Loci?

50% To convince yourself… think about independent assortment

Unlinked Loci: $r = 0.50$

Linked Loci: $r < 0.50$

Loci can **appear** to be unlinked because:

- They are on separate chromosomes
- They are so far apart on the same chromosome that they assort independently

Predicting Progeny From a Known Map

Predict the progeny phenotypes and numbers from this cross:

Parent 1: $\dfrac{+\quad +}{a\quad c}$

Parent 2: $\dfrac{a\quad c}{a\quad c}$

\+ = wild type, dominant

a = aggressive

c = cranky

Map:

Count 10,000 progeny

Predicting Progeny From a Known Map

Predict the progeny phenotypes and numbers from this cross:

Parent 1: $\dfrac{+\quad +}{a\quad c}$

Parent 2: $\dfrac{a\quad c}{a\quad c}$

+ = wild type, dominant

Map: a 3 cM c

Count 10,000 progeny

Recombinant types = + c, a + } 150 each

Predicted recombinant products in (a-c) = 3% = 0.03 x 10000 = 300

Parental types = + + and a c = 4850 each

Practice Question

Brown seed pods (**B**) in a plant species is is dominant to green (**b**), and elongated pods (**E**) is dominant over squished (**e**).

(a) A fully heterozygous plant has the dominant alleles linked in trans (i.e., dominant alleles **not** on the same homologue) at a map distance of **20 cM**. What will be the genotypes of gametes produced by this plant, and in what frequencies (or percentages)?

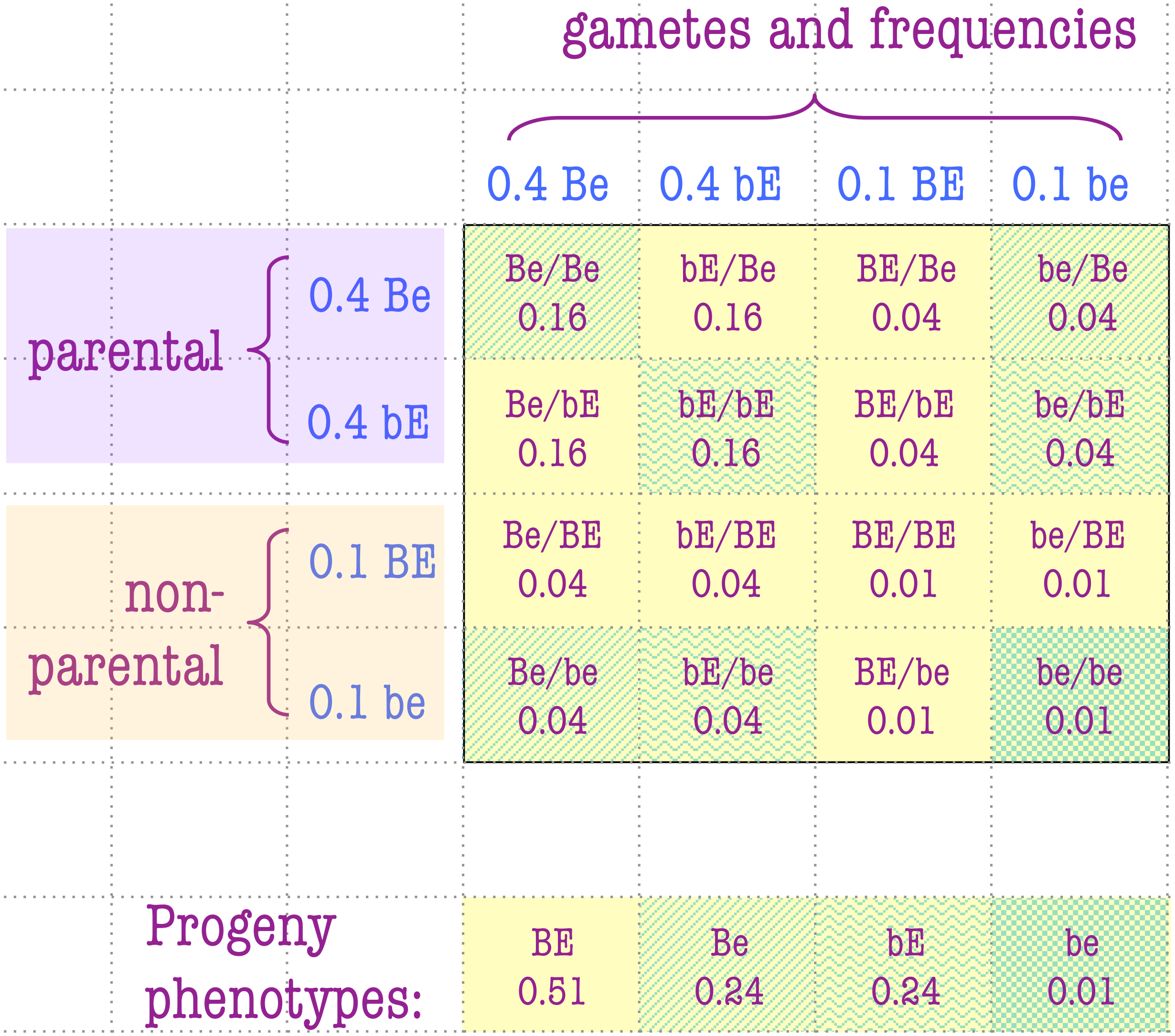
(b) If this plant is **self-pollinated**, what progeny phenotypes will you expect to see, and in what frequencies? Use a Punnett square to illustrate your answer.

Heterozygote genotype = $\frac{B \qquad e}{b \qquad E}$

Recombinant gametes = B E and b e, 20% total = 10% each

Parental type gametes = B e and b E, 80% total = 40% each

Practice Question



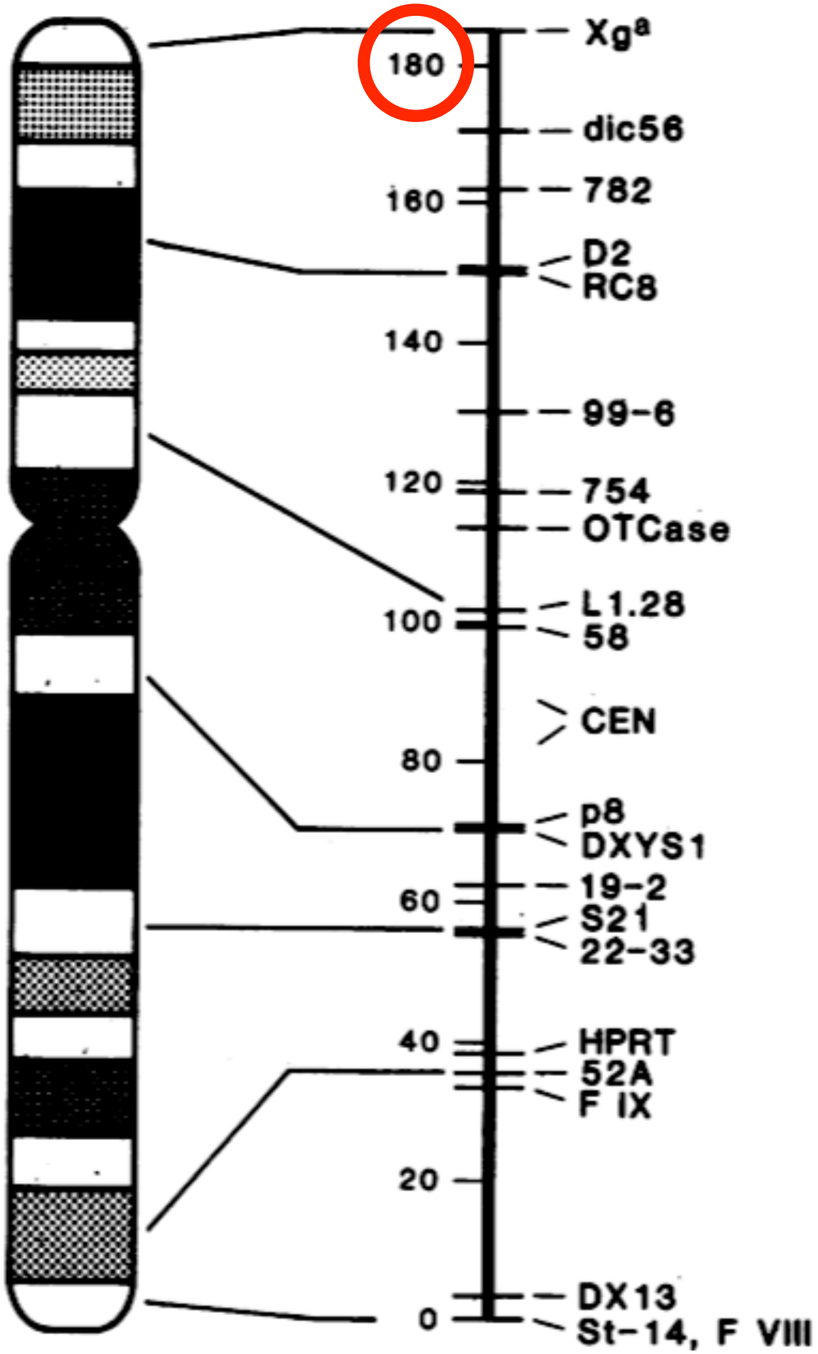
gametes and frequencies

		0.4 Be	0.4 bE	0.1 BE	0.1 be
parental	0.4 Be	Be/Be 0.16	bE/Be 0.16	BE/Be 0.04	be/Be 0.04
	0.4 bE	Be/bE 0.16	bE/bE 0.16	BE/bE 0.04	be/bE 0.04
non-parental	0.1 BE	Be/BE 0.04	bE/BE 0.04	BE/BE 0.01	be/BE 0.01
	0.1 be	Be/be 0.04	bE/be 0.04	BE/be 0.01	be/be 0.01

Progeny phenotypes:	BE 0.51	Be 0.24	bE 0.24	be 0.01

A Genetic Map Is:

A map of the locations of *polymorphic markers* where order and distance is determined by *recombination frequency*

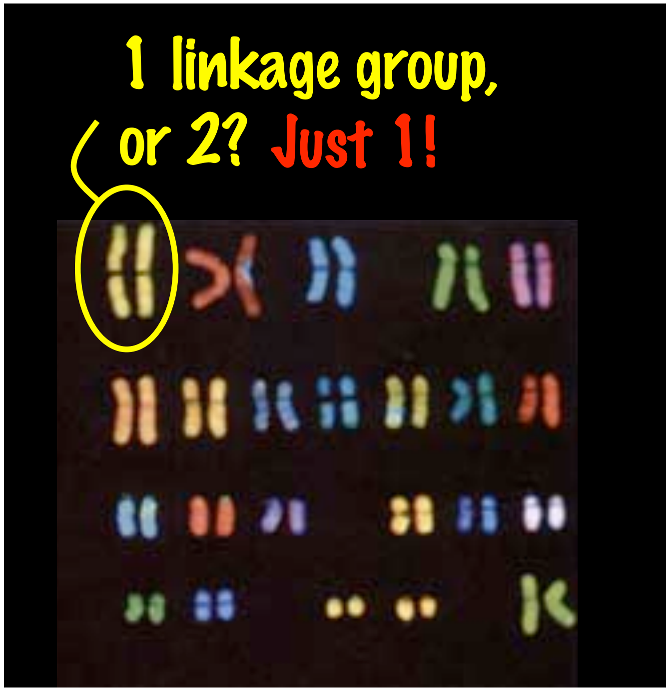


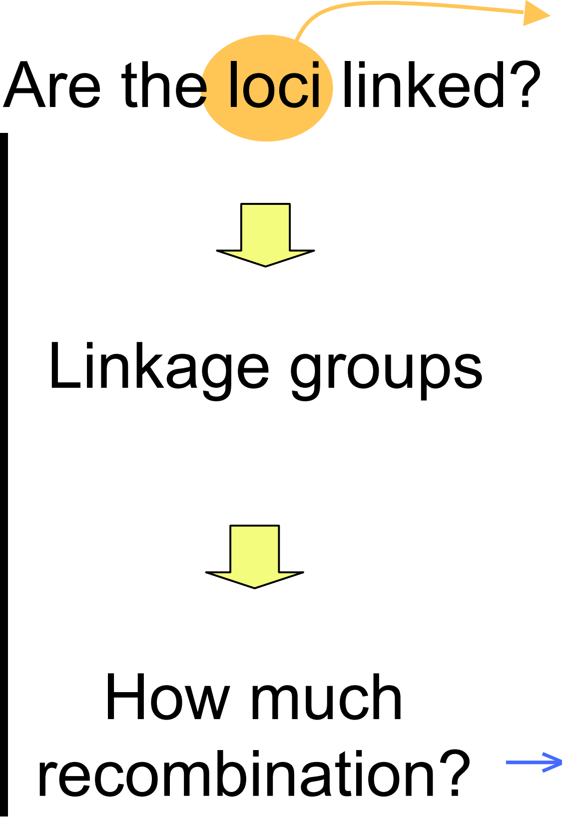
Human X-chromosome map…

180 cM?

What the?

Linkage Groups





Are the loci linked?

Linkage groups

How much recombination? → **gene order and map distance**

loci=plural
locus=singular
the location in the chromosome that is occupied by alleles of a gene

Linkage group = group of loci (genes) known to be associated by linkage

Physical Maps

- **A map of the locations of identifiable <u>landmarks</u> in the genome**
  - many types of "landmarks" used

Types of Physical Maps

Resolution

Low

Cytogenetic (Chromosome) Map - Based on distinctive banding patterns observed in stained chromosomes

cDNA Map - Locations of expressed DNA along the genome

Radiation Hybrid Map - Order of DNA markers (STS) that uniquely occur in the genome

Contig Map - Order of overlapping DNA fragments spanning the genome

Restriction Map - Describes the order and distance between DNA restriction enzyme sites

Sequence Map - The complete DNA sequence of a genome

High

Physical Maps

- A map of the locations of identifiable <u>landmarks</u> in the genome
    - many types of "landmarks" used

- Highest resolution physical map of a genome is its complete DNA sequence

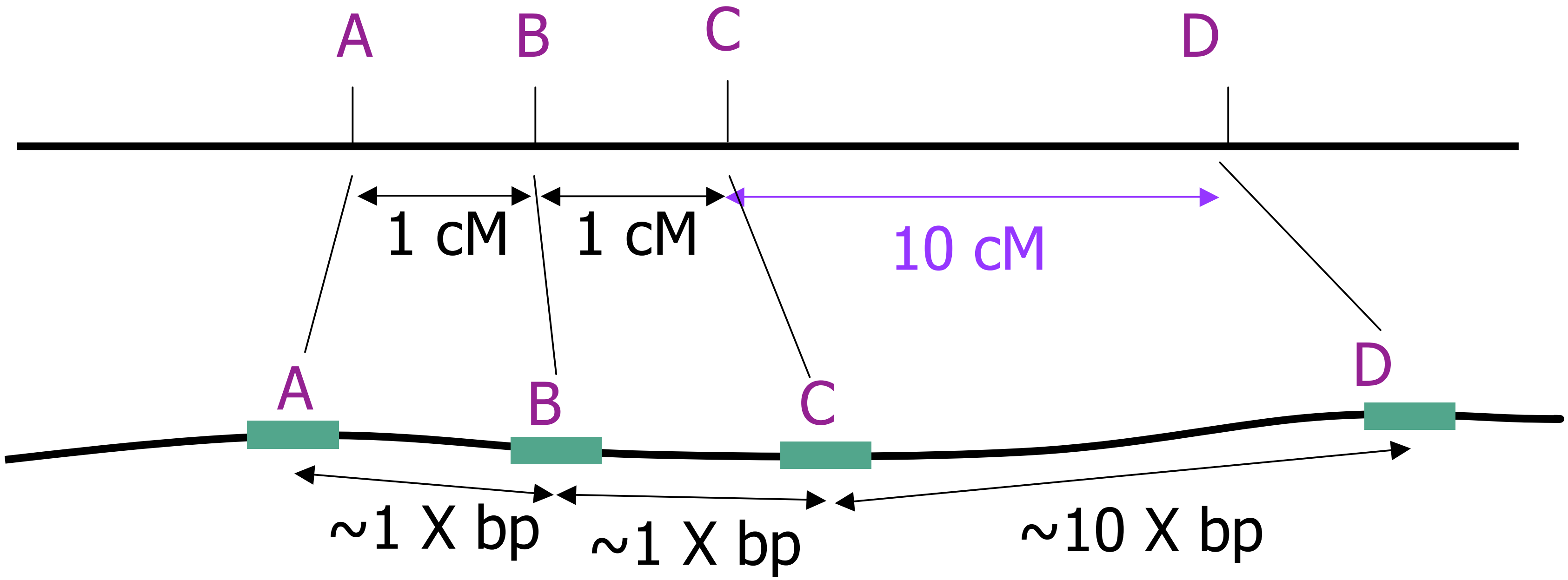
- Primary distinction between genetic and physical map is the units of distance:

Genetic map: recombination distance

Physical map: distanced measured in base pairs

Integrated Genetic and Physical Maps

genetic map (recombination) units = cM



A B C D

1 cM 1 cM 10 cM

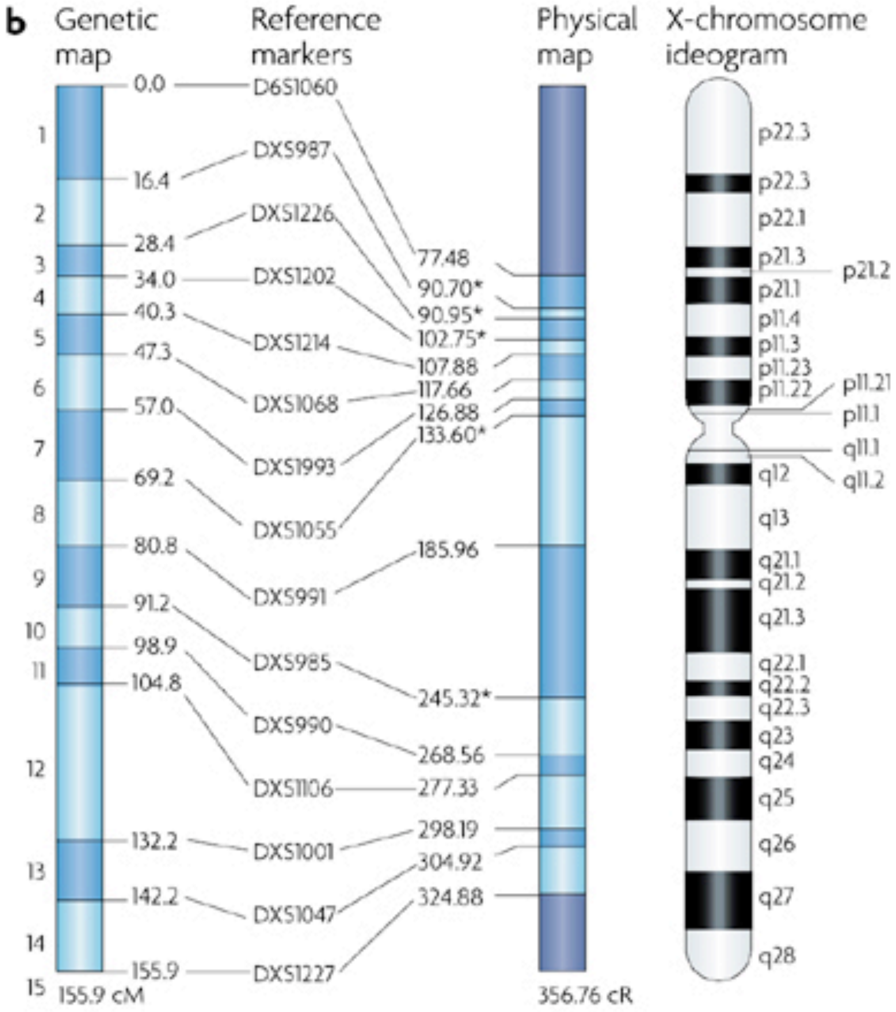
A B C D

~1 X bp ~1 X bp ~10 X bp

physical map (DNA) units = bp

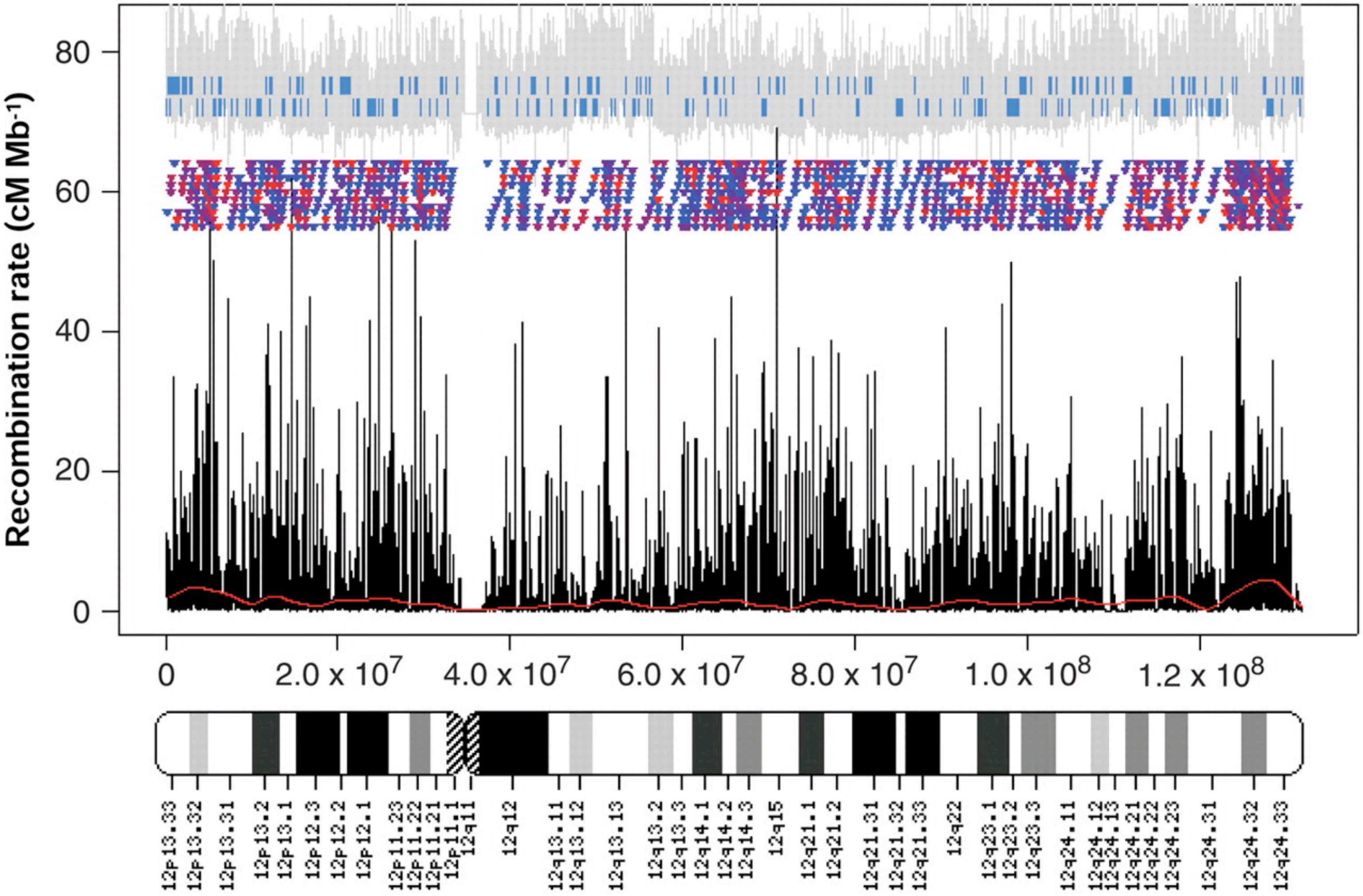
- Order conserved between genetic and physical maps
- Distance separating loci in genetic and physical maps is proportional

Integrated Genetic and Physical Map of the Human X-Chromosome



Beyond the Basics

Variation in Recombination Rates



Myers et al (2005) 310:321

Linkage Analysis With Molecular Markers

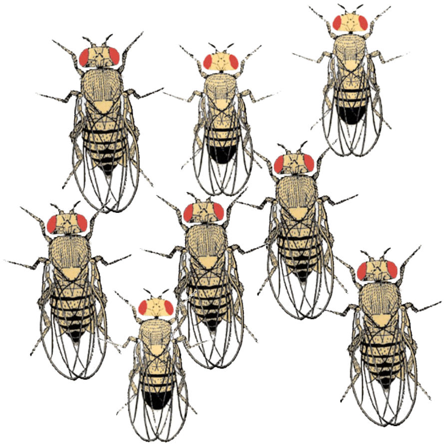
DNA Polymorphisms Are Genomic Landmarks

"Mile Markers" throughout the genome

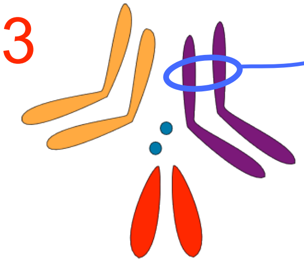
We don't know where the gene for our trait of interest lies, but…

if we can show that our trait is linked to a DNA polymorphism... we'd know roughly where the gene is located!

DNA Polymorphisms… An Example



Sequence
chromosome
#3



my names for
the alleles

5’..TCTTGATC..3’
3’..AGAACTAG..5’ $\mathrm{DM1}^{T}$

or

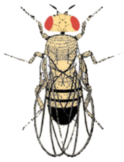
5’..TCTCGATC..3’
3’..AGAGCTAG..5’ $\mathrm{DM1}^{C}$

or

alleles at this locus

(may not be coding for anything!)

DNA Marker Genotypes

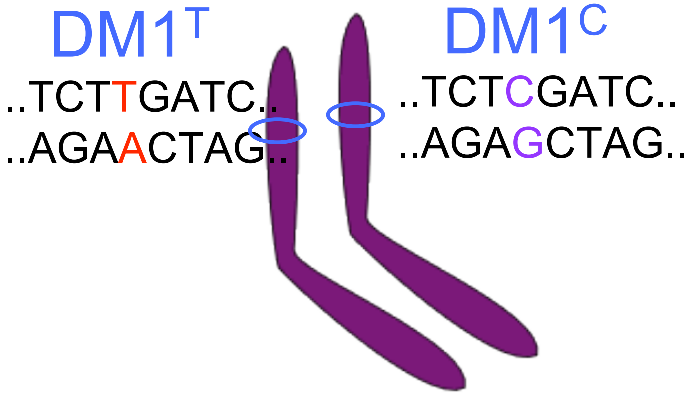


→ Do a DNA test for DM1 → Suppose you detect DM1^T and DM1^C → conclude?

Conclude:

One homologue had DM1^T allele, one homologue had DM1^C allele...

this fly is heterozygous for this DNA marker



DM1^T

..TCTTGATC..
..AGAACTAG..

DM1^C

..TCTCGATC..
..AGAGCTAG..

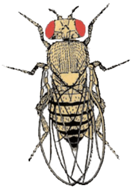
How do we test for linkage in general?

What kind of a cross do we set up?

heterozygote x homozygous (recessive)

Testing for linkage

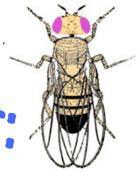
Step 1. Generate the heterozygous flies.



true-breeding red-eye

DNA test:
$DM1^T$ only detected

true-breeding purple-eye

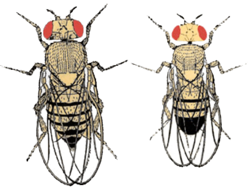


DNA test:
$DM1^C$ only detected

Genotype: $\frac{pr^+}{pr^+}\ \frac{DM1^T}{DM1^T}$ x $\frac{pr}{pr}\ \frac{DM1^C}{DM1^C}$

$\downarrow$

$\frac{pr^+}{pr}\ \frac{DM1^C}{DM1^T}$



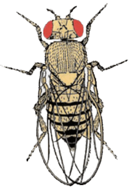
heterozygote!

When the heterozygote makes gametes… what would you consider the parental types among these gametes?

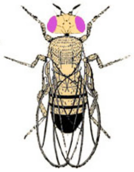
$\boxed{pr^+\ DM1^T}$ and $\boxed{pr\ DM1^C}$

Testing for linkage (cont'd)

Step 2. Do a testcross.



$$\frac{pr^+}{pr} \quad \frac{DM1^C}{DM1^T} \qquad x \qquad \frac{pr}{pr} \quad \frac{DM1^C}{DM1^C}$$

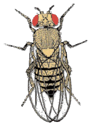


Step 3. Score the progeny—

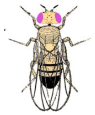
For each progeny fly: what eye color?

which allele(s) at DM1?

Sample results…



$\frac{pr^+}{pr}$ $\frac{DM1^C}{DM1^T}$ x $\frac{pr}{pr}$ $\frac{DM1^C}{DM1^C}$

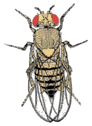


gamete?	P/NP?

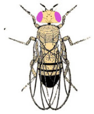
	phenotype:	# of progeny
	red, $DM1^T$ & $DM1^C$	322
do later	purple, $DM1^C$ & $DM1^C$	318
	red, $DM1^C$ & $DM1^C$	78
	purple, $DM1^T$ & $DM1^C$	82

progeny genotype?

Sample results…

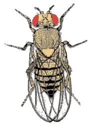


$\frac{pr^+}{pr}$ $\frac{DM1^C}{DM1^T}$ x $\frac{pr}{pr}$ $\frac{DM1^C}{DM1^C}$

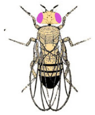


gamete?	P/NP?	progeny genotype?	phenotype:	# of progeny
pr^+ $DM1^T$	P	do later	red, $DM1^T$ & $DM1^C$	322
pr $DM1^C$	P		purple, $DM1^C$ & $DM1^C$	318
pr^+ $DM1^C$	NP		red, $DM1^C$ & $DM1^C$	78
pr $DM1^T$	NP		purple, $DM1^T$ & $DM1^C$	82

Sample results…



$\frac{pr^+}{pr}$ $\frac{DM1^C}{DM1^T}$ x $\frac{pr}{pr}$ $\frac{DM1^C}{DM1^C}$



gamete?	P/NP?		phenotype:	# of progeny
pr^+ $DM1^T$	P	$\frac{pr^+}{pr}$ $\frac{DM1^T}{DM1^C}$	red, $DM1^T$ & $DM1^C$	322
pr $DM1^C$	P	$\frac{pr}{pr}$ $\frac{DM1^C}{DM1^C}$	purple, $DM1^C$ & $DM1^C$	318
pr^+ $DM1^C$	NP	$\frac{pr^+}{pr}$ $\frac{DM1^C}{DM1^C}$	red, $DM1^C$ & $DM1^C$	78
pr $DM1^T$	NP	$\frac{pr}{pr}$ $\frac{DM1^T}{DM1^C}$	purple, $DM1^T$ & $DM1^C$	82

↑

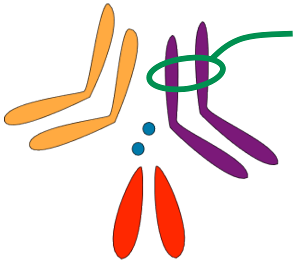
progeny
genotype?

Testing for linkage (cont'd)

Step 4. Interpret the results.

Conclusion? The eye color gene is linked to the DM1 locus

$$\text{Map distance} = \frac{78 + 82}{322 + 318 + 78 + 82} = 20 \text{ cM}$$



pr locus is somewhere near here!

Summary

Genes can be mapped relative to each other based on linkage

Genes can also be mapped relative to known DNA positions (“DNA markers” or polymorphic sites) along chromosomes

…and thus these DNA markers serve as landmarks to establish the physical locations of genes in the genome

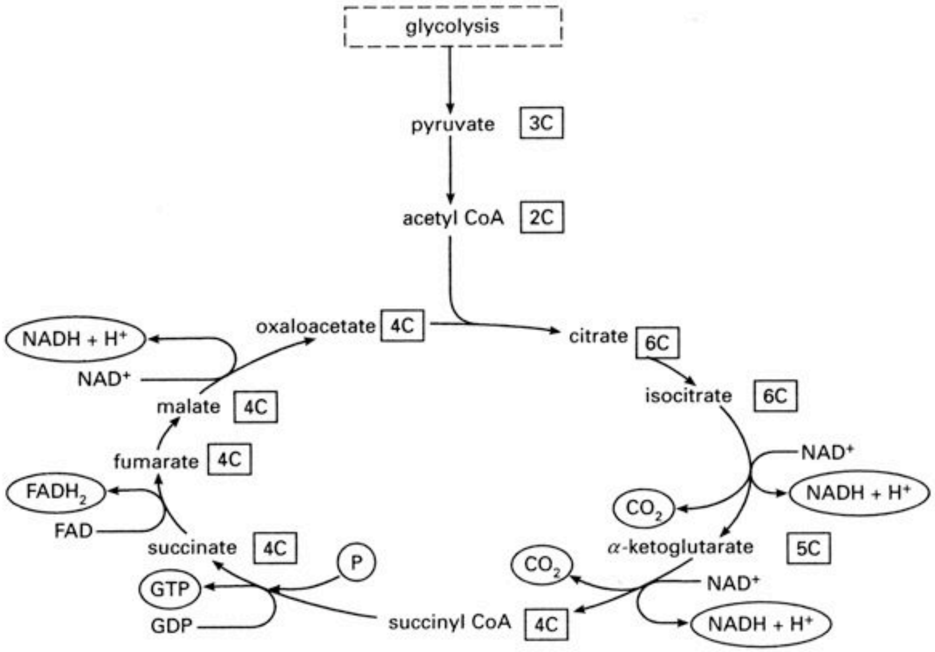
What's the advantage of using DNA markers?

There are LOTS of them, throughout the genome!

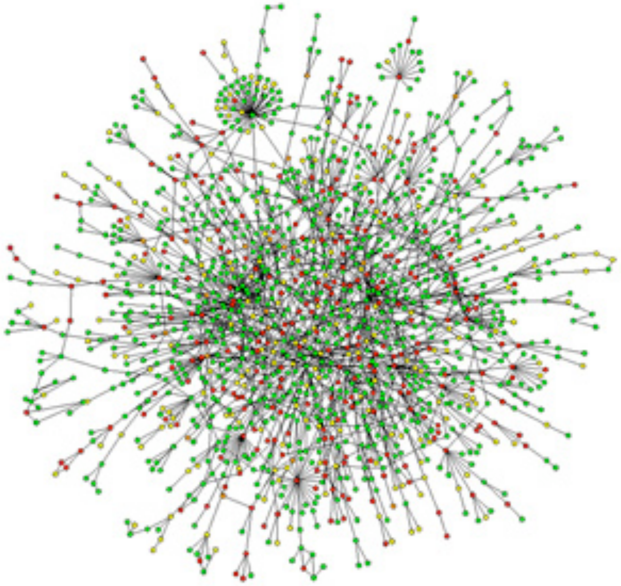
Genetic Interactions

- Genetic Interactions
  - Epistasis

Genes Do Not Act In Isolation



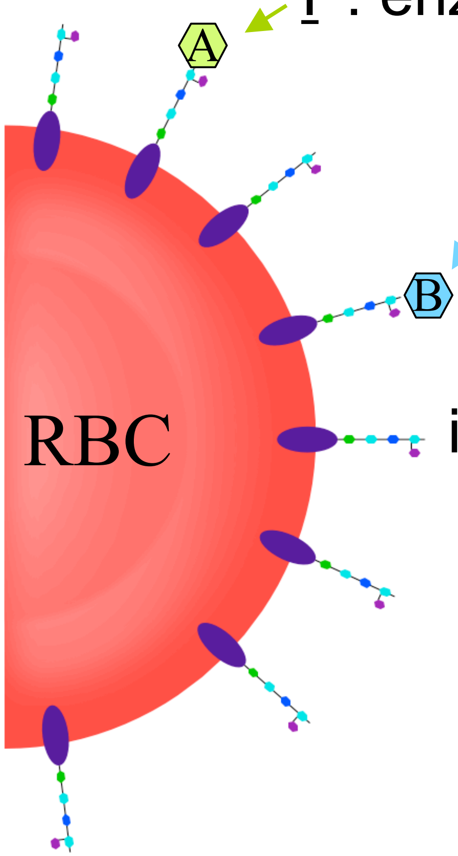
Metabolic pathway



Protein-Protein Network

A Digression into Human Blood Groups…

ABO blood type: determined by alleles of gene $\underline{I}$



$\underline{I}^A$: enzyme that adds ‘A’ sugar

$\underline{I}^B$: enzyme that adds ‘B’ sugar

i: defective enzyme… no sugar added

$\underline{I}^A$i and $\underline{I}^A\underline{I}^A$: only ‘A’ sugar added → blood type A

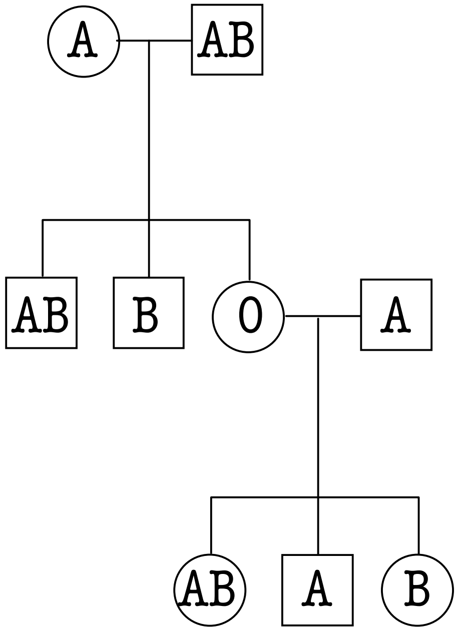
$\underline{I}^B$i and $\underline{I}^B\underline{I}^B$: only ‘B’ sugar added → blood type B

$\underline{I}^A\underline{I}^B$: both sugars added → blood type AB

ii : no sugar added → blood type O

$\underline{I}^A$ and $\underline{I}^B$ are co-dominant; both dominant to i

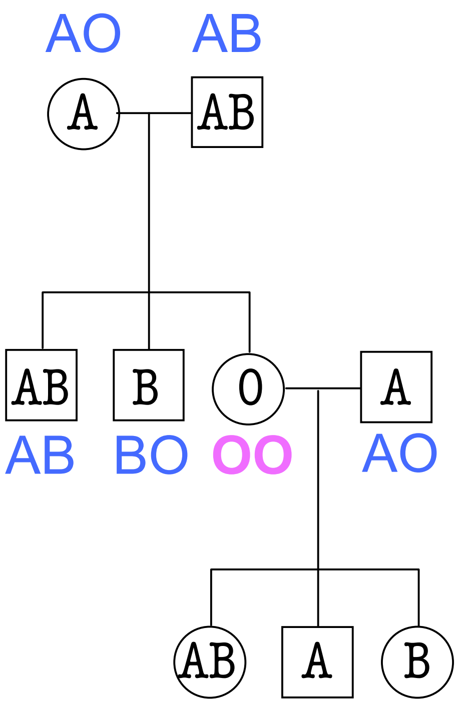
Consider the Following Pedigree



Blood types of the individuals in the pedigree are marked.

What do you find unusual in this pedigree?

Consider the Following Pedigree

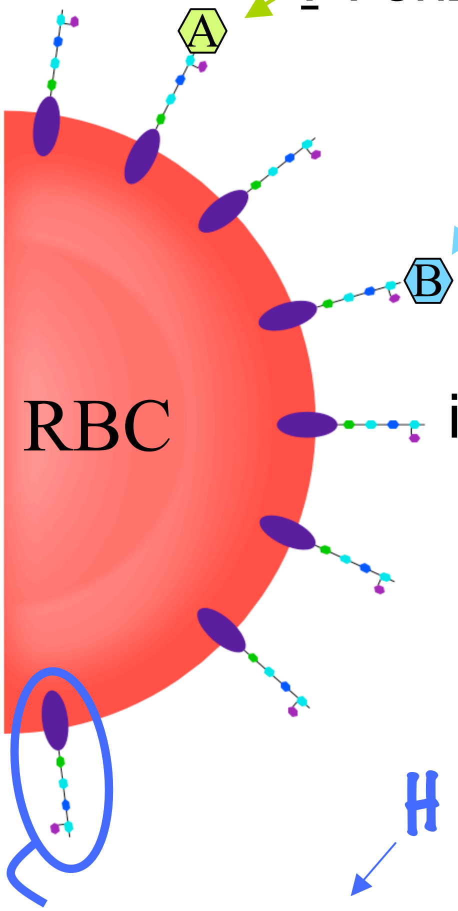


Blood types of the individuals in the pedigree are marked.

What do you find unusual in this pedigree?

ABO Blood Groups… Some Extra Information

ABO blood type: determined by alleles of gene $\underline{I}$



$\underline{I}^A$: enzyme that adds ‘A’ sugar

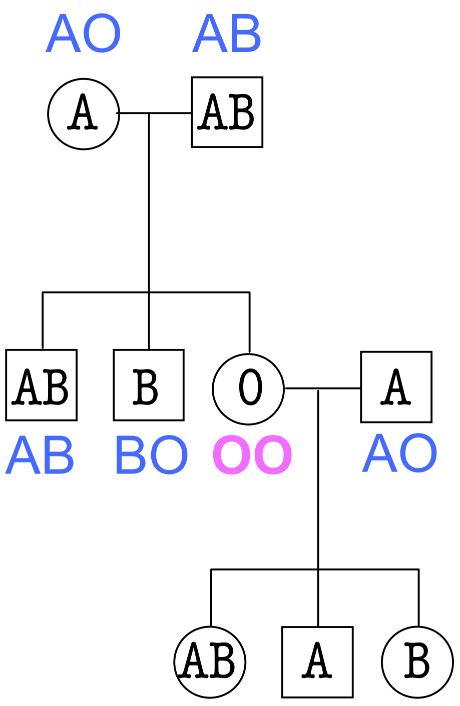
$\underline{I}^B$: enzyme that adds ‘B’ sugar

i: defective enzyme… no sugar added

H gene - enzyme that synthesizes the H substance

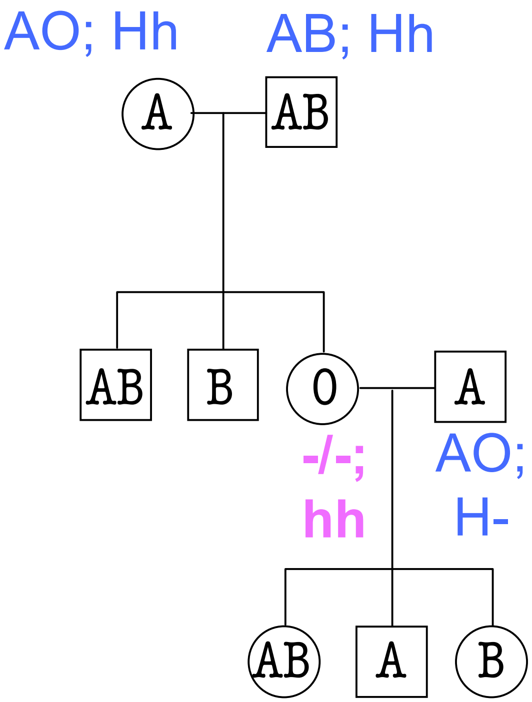
substance H

Back to the Pedigree



Given what you now know about the H gene, how would you explain this pedigree?

Back to the Pedigree



Given what you now know about the H gene, how would you explain this pedigree?

$$\xrightarrow{\text{H gene}} \text{substance H} \xrightarrow{\underline{\text{I}}\text{ gene}} \text{A, B, AB, O blood groups}$$

Epistasis

The Bombay Phenotype is an example of ***epistasis***

The effects of one gene modify the effects of a second gene

Genes H and I gene exhibit epistasis

The alleles that are masking the effect are called epistatic alleles

The alleles whose effect is being masked are called the hypostatic alleles

Epistasis is a form of ***gene interaction*** - the action of two or more genes in contributing to a phenotype

Summary

Epistasis describes the interaction of two (or more) genes

May lead to modified dihybrid ratios

Useful in inferring and ordering steps in a pathway