Genomic Maps and Linkage Analysis

- Genomic maps
  - Linkage maps
  - Physical maps
Question: Find the closest Thai food restaurant in the University District
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How can the principles of genetic linkage be applied to constructing linkage map?

What's a linkage map?
As an undergraduate student in Morgan’s lab, Sturtevant created the first genetic maps.
Constructing a Linkage Map

Alfred Sturtevant’s major insight

If crossovers occur 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.
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...

Colorless 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:  
\[ + + \]
\[ a \quad c \]

Parent 2:  
\[ a \quad c \]
\[ a \quad c \]

+ = wild type, dominant
a = aggressive
c = cranky

Map:
\[ a \quad 3 \text{ cM} \quad c \]

Count 10,000 progeny
Predicting Progeny From a Known Map

Predict the progeny phenotypes and numbers from this cross:

Parent 1:  

\[
\begin{array}{c}
+a \\
a \\
+ \\
\end{array}
\]

\[
\begin{array}{c}
a \\
+ \\
c \\
+ \\
\end{array}
\]

\(\text{+= wild type, dominant}\)

Parent 2:  

\[
\begin{array}{c}
a \\
+ \\
c \\
+ \\
\end{array}
\]

\[
\begin{array}{c}
+ \\
a \\
c \\
a \\
+ \\
\end{array}
\]

Map:  

\[
\begin{array}{c}
a \\
7 \text{ cM} \\
c \\
a \\
3 \text{ cM} \\
c \\
\end{array}
\]

Count 10,000 progeny

Recombinant types =

\[
\begin{array}{c}
+ c \\
a + \\
\end{array}
\]

\(150 \text{ each}\)

Predicted recombinant products in (a-c) =

\(3\% = 0.03 \times 10000 = 300\)

Parental types =

\(++ \text{ and } a \ c = 4850 \text{ each}\)
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 = $B\text{ }e$

Recombinant gametes = $B\ E\text{ and }b\ e$, 20% total = 10% each

Parental type gametes = $B\ e\text{ and }b\ E$, 80% total = 40% each
**Practice Question**

| Progeny phenotypes: | BE 0.51 | Be 0.24 | bE 0.24 | be 0.01 |

<table>
<thead>
<tr>
<th><strong>parental</strong></th>
<th>0.4 Be 0.4 bE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be/Be 0.16</td>
<td>bE/Be 0.16</td>
</tr>
<tr>
<td>Be/bE 0.16</td>
<td>bE/bE 0.16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>non-parental</strong></th>
<th>0.1 BE 0.1 be</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be/BE 0.04</td>
<td>bE/BE 0.04</td>
</tr>
<tr>
<td>Be/be 0.04</td>
<td>bE/be 0.04</td>
</tr>
</tbody>
</table>

**gametes and frequencies**

<table>
<thead>
<tr>
<th>0.4 Be</th>
<th>0.4 bE</th>
<th>0.1 BE</th>
<th>0.1 be</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be/Be 0.16</td>
<td>bE/Be 0.16</td>
<td>BE/Be 0.04</td>
<td>be/Be 0.04</td>
</tr>
<tr>
<td>Be/bE 0.16</td>
<td>bE/bE 0.16</td>
<td>BE/bE 0.04</td>
<td>be/bE 0.04</td>
</tr>
<tr>
<td>Be/BE 0.04</td>
<td>bE/BE 0.04</td>
<td>BE/BE 0.01</td>
<td>be/BE 0.01</td>
</tr>
<tr>
<td>Be/be 0.04</td>
<td>bE/be 0.04</td>
<td>BE/be 0.01</td>
<td>be/be 0.01</td>
</tr>
</tbody>
</table>
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?

- 1 linkage group, or 2? Just 1!

- Loci = plural (the location in the chromosome that is occupied by alleles of a gene)
- Locus = singular

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

→ gene order and map distance
Physical Maps

- A map of the locations of identifiable landmarks in the genome
  - many types of “landmarks” used
### Types of Physical Maps

<table>
<thead>
<tr>
<th>Resolution</th>
<th>Cytogenetic (Chromosome) Map</th>
<th>cDNA Map</th>
<th>Radiation Hybrid Map</th>
<th>Contig Map</th>
<th>Restriction Map</th>
<th>Sequence Map</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Based on distinctive banding patterns observed in stained chromosomes</td>
<td>Locations of expressed DNA along the genome</td>
<td>Order of DNA markers (STS) that uniquely occur in the genome</td>
<td>Order of overlapping DNA fragments spanning the genome</td>
<td>Describes the order and distance between DNA restriction enzyme sites</td>
<td>The complete DNA sequence of a genome</td>
</tr>
</tbody>
</table>
Physical Maps

- A map of the locations of identifiable landmarks 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: distance measured in base pairs
Integrated Genetic and Physical Maps

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

geneic map (recombination) units = cM
physical map (DNA) units = bp
Integrated Genetic and Physical Map of the Human X-Chromosome
Variation in Recombination Rates

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

my names for the alleles

5’..TCTGATC..3’
3’..AGAACTAG..5’

or

5’..TCTCAGATC..3’
3’..AGAGCTAG..5’

or

alleles at this locus
(may not be coding for anything!)
DNA Marker Genotypes

Do a DNA test for DM1

Suppose you detect DM1\textsuperscript{T} and DM1\textsuperscript{C}

conclude?

Conclude:

One homologue had DM1\textsuperscript{T} allele, one homologue had DM1\textsuperscript{C} allele...

this fly is heterozygous for this DNA marker
How do we test for linkage in general?
What kind of a cross do we set up?

heterozygote \times \text{ homozygous (recessive)}
Testing for linkage

Step 1. Generate the heterozygous flies.

true-breeding red-eye
DNA test: $\text{DM1}^T$ only detected
Genotype: $\frac{\text{pr}^+}{\text{pr}^+} \quad \frac{\text{DM1}^T}{\text{DM1}^T}$

true-breeding purple-eye
DNA test: $\text{DM1}^C$ only detected
Genotype: $\frac{\text{pr}}{\text{pr}} \quad \frac{\text{DM1}^C}{\text{DM1}^C}$

$\frac{\text{pr}^+}{\text{pr}^+} \quad \frac{\text{DM1}^T}{\text{DM1}^T}$ \times \frac{\text{pr}}{\text{pr}} \quad \frac{\text{DM1}^C}{\text{DM1}^C}$

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

$\frac{\text{pr}^+}{\text{pr}^+} \quad \frac{\text{DM1}^C}{\text{DM1}^T}$ and $\frac{\text{pr}}{\text{pr}} \quad \frac{\text{DM1}^C}{\text{DM1}^C}$
Step 2. Do a testcross.

\[
\begin{align*}
\text{pr}^+ & \quad \text{DM}1^c \\
\text{pr} & \quad \text{DM}1^r \\
\times & \\
\text{pr} & \quad \text{DM}1^c \\
\text{pr} & \quad \text{DM}1^c
\end{align*}
\]

Step 3. Score the progeny—

- For each progeny fly: what eye color?
- which allele(s) at DM1?
Sample results...

<table>
<thead>
<tr>
<th>Gamete?</th>
<th>P/NP?</th>
<th>Phenotype:</th>
<th># of Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>red, DM1&lt;sup&gt;T&lt;/sup&gt; &amp; DM1&lt;sup&gt;C&lt;/sup&gt;</td>
<td>322</td>
</tr>
<tr>
<td></td>
<td></td>
<td>purple, DM1&lt;sup&gt;C&lt;/sup&gt; &amp; DM1&lt;sup&gt;C&lt;/sup&gt;</td>
<td>318</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red, DM1&lt;sup&gt;T&lt;/sup&gt; &amp; DM1&lt;sup&gt;C&lt;/sup&gt;</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>purple, DM1&lt;sup&gt;T&lt;/sup&gt; &amp; DM1&lt;sup&gt;C&lt;/sup&gt;</td>
<td>82</td>
</tr>
</tbody>
</table>

Note: The image contains a diagram showing the cross of two genotypes, pr<sup>+</sup> pr<sup>DM1<sup>T</sup></sup> x pr<sup>pr</sup> DM1<sup>C</sup> DM1<sup>C</sup> x pr<sup>pr</sup> DM1<sup>C</sup> DM1<sup>C</sup>. The progeny are red, DM1<sup>T</sup> & DM1<sup>C</sup> (322), purple, DM1<sup>C</sup> & DM1<sup>C</sup> (318), red, DM1<sup>T</sup> & DM1<sup>C</sup> (78), and purple, DM1<sup>T</sup> & DM1<sup>C</sup> (82).
Sample results…

<table>
<thead>
<tr>
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<th># of progeny</th>
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</thead>
<tbody>
<tr>
<td>pr(^{+}) DM(^{1})(^{T})</td>
<td>P</td>
<td>red, DM(^{1})(^{T}) &amp; DM(^{1})(^{C})</td>
<td>322</td>
</tr>
<tr>
<td>pr DM(^{1})(^{C})</td>
<td>P</td>
<td>purple, DM(^{1})(^{C}) &amp; DM(^{1})(^{C})</td>
<td>318</td>
</tr>
<tr>
<td>pr(^{+}) DM(^{1})(^{C})</td>
<td>NP</td>
<td>do later</td>
<td></td>
</tr>
<tr>
<td>pr DM(^{1})(^{T})</td>
<td>NP</td>
<td>purple, DM(^{1})(^{T}) &amp; DM(^{1})(^{C})</td>
<td>78</td>
</tr>
<tr>
<td>pr DM(^{1})(^{T})</td>
<td>NP</td>
<td>purple, DM(^{1})(^{T}) &amp; DM(^{1})(^{C})</td>
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<th>P/NP?</th>
<th>phenotype:</th>
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<tbody>
<tr>
<td>pr&lt;sup&gt;+&lt;/sup&gt; DM&lt;sub&gt;T&lt;/sub&gt;</td>
<td>P</td>
<td>pr&lt;sup&gt;+&lt;/sup&gt; DM&lt;sub&gt;T&lt;/sub&gt; &amp; DM&lt;sub&gt;C&lt;/sub&gt;</td>
<td>322</td>
</tr>
<tr>
<td>pr DM&lt;sub&gt;C&lt;/sub&gt;</td>
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</table>
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 I

\( I^A \): enzyme that adds ‘A’ sugar

\( I^B \): enzyme that adds ‘B’ sugar

\( i \): defective enzyme… no sugar added

\( I^A i \) and \( I^A I^A \): only ‘A’ sugar added \( \rightarrow \) blood type A

\( I^B i \) and \( I^B I^B \): only ‘B’ sugar added \( \rightarrow \) blood type B

\( I^A I^B \): both sugars added \( \rightarrow \) blood type AB

\( ii \): no sugar added \( \rightarrow \) blood type O

\( I^A \) and \( I^B \) 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 I

I^A: enzyme that adds ‘A’ sugar

I^B: enzyme that adds ‘B’ sugar

i: defective enzyme... no sugar added

H gene - enzyme that synthesizes the H substance

RBC

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