Chromosome Mapping by Recombination

Genes on the same chromosome are said to be linked.

Crossing over: the physical exchange of homologous chromosome segments
- A given crossover generates two reciprocal recombinant products, so reciprocal recombinant classes usually occur equally frequently.

Meiotic recombination: any process during meiosis that generates a haploid product with new combinations of the alleles carried by the haploid genotypes that united to form the dihybrid meiocyte; it includes both the processes of
  - independent assortment (for unlinked genes)
  - crossing over (for linked genes)

A linkage map is also known as a genetic map. It can be measured in map units, recombinant frequencies, or centimorgans (1 mu = RF of 1% = 1 cM).

Given a known genetic distance (i.e. in mu’s), you can make predictions about the frequency of progeny that you will observe in different classes. Given the frequency of recombination between linked genes, you can calculate the genetic distance between them.

Q 4.4: The cross E/E.F/F x e/e.f/f is made, and the F₁ is then backcrossed to the recessive parent. The progeny genotypes are inferred from the phenotypes. The progeny genotypes, written as the gametic contributions of the heterozygous parent, are in the following proportions:

<table>
<thead>
<tr>
<th></th>
<th>2/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.F</td>
<td></td>
</tr>
<tr>
<td>E.f</td>
<td>1/6</td>
</tr>
<tr>
<td>e.F</td>
<td>1/6</td>
</tr>
<tr>
<td>e/f</td>
<td>2/6</td>
</tr>
</tbody>
</table>

Explain these results.

A: The cross is E/e.F/f x e/e.f/f. If independent assortment exists, the progeny should be in a 1:1:1:1 ratio, which is not observed. Therefore, there is linkage. E f and e F are recombinants equaling one-third of the progeny, the recombination frequency is 33.3%, and the two genes are 33.3 mu apart.


A: If the genes were unlinked, they should assort independently and the four progeny classes should be present in roughly equal proportions. This is clearly not the case. The A/a.B/b and a/a.b/b classes (the parentals) are much more common than the A/a.b/b and a/a.B/b classes (the recombinants). The two genes are on the same chromosome. Their recombination frequency is 10%, therefore they are 10 mu apart.
Three-point test crosses allow you to evaluate the linkage between three genes in one cross.

* When calculating genetic distances using three point crosses, you must count each rarest class twice when you determine recombination frequencies because of double crossovers.

**Q:** Two purebreeding strains of mice are crossed to produce a mouse heterozygous for dominant and recessive alleles of three linked genes (A vs. a, B vs. b, E vs. e). A series of F₁ triply heterozygous mice are testcrossed and the resulting progeny were:

```
ABE  8
ABe  342
AbE  100
Abe  62
aBE  49
aBe  94
abE  317
abe  14
```

**a.** What are the genotypes of the purebred parents of an F₁ triple heterozygote?

**b.** What is the map order of the three genes?

**c.** What are the distances between them?

**A:**

**a.** The most common classes indicate the parental genotypes. In this situation, ABe and abE are the most frequent classes, therefore they are the parental genotypes.

**b.** The rarest classes indicate the double recombinant, which tells you which gene is in the middle. In this situation, ABE and abe are the rarest classes, therefore “e” must be in the middle, so the map order is A-E-B.

**c.**

<table>
<thead>
<tr>
<th>Class</th>
<th>Frequency</th>
<th>Recombination between</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A and B</td>
</tr>
<tr>
<td>ABe</td>
<td>342</td>
<td></td>
</tr>
<tr>
<td>abE</td>
<td>317</td>
<td></td>
</tr>
<tr>
<td>ABE</td>
<td>8</td>
<td>(2)(8)=16</td>
</tr>
<tr>
<td>abe</td>
<td>14</td>
<td>(2)(14)=28</td>
</tr>
<tr>
<td>aBe</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>AbE</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Abe</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>aBE</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>349</td>
<td>216</td>
</tr>
</tbody>
</table>

\[A \quad 21.6 \quad E \quad 13.3 \quad B\]
Interference: crossovers inhibit each other; double recombinant classes can be used to determine the extent of this interference

- If crossovers in two regions are independent, the product rule will accurately predict the frequency of double recombinants; if there are fewer double recombinants than expected, it suggests some kind of interference

- Coefficient of coincidence \( c_{oc} = \frac{\text{observed frequency of double recombinants}}{\text{expected frequency of double recombinants}} \)

- Interference, \( I = 1 - c_{oc} \)
**DNA**

Experimental evidence: the foundation of scientific knowledge

**The question:** What is the genetic material?  
**The answer:** DNA.

How do we know this?

Frederick Griffith (1928): injected different strains of bacteria into mice  
$S$: smooth, kill mice  
$R$: rough, do not kill mice  
$S_{\text{heat-killed}}$: dead, do not kill mice  
$S_{\text{heat-killed}} + R$: rough cells are transformed into smooth cells and can kill mice by extracts from $S$ cells

Conclusion: some sort of hereditary material in the dead $S$ strain was transformed into the live $R$ strain, and turned it into an $S$ strain

Avery, MacLeod and McCarty (1944): injected mixtures of $R$ cells and $S$ cell extracts in which different components had been depleted to determine which component of the extract transformed $R$ cells into $S$  
Depleting the extracts of DNA prevented transformation, therefore DNA is the transforming agent, the hereditary material

Conclusion: DNA is the hereditary material
Hershey and Chase (1952): grew some phage on $^{32}$P and some on $^{35}$S, infected different bacterial cultures with each, then sheared the empty phage carcasses off the bacterial cells and pelleted them; the hereditary material carried by the phage will be found in the bacteria

$^{32}$P: labels DNA
$^{35}$S: labels protein

Conclusion: DNA is the hereditary material

Q 7.24: A student in Griffith’s lab found three cell samples marked “A,” “B,” and “C.” Not knowing what each sample contained, the student decided to try injecting these samples into some of her mice, both singly and in combinations, to see if she could determine what each sample contained. She observed the responses of the infected mice after an incubation period and recovered blood samples from each test group to look for the possible presence of infecting cells. She recorded her observations in the table below. Assuming that each sample contained only one thing in a pure form, what do you think were in samples “A,” “B,” and “C”?

<table>
<thead>
<tr>
<th>Sample injected</th>
<th>Response of mice</th>
<th>Type of cells recovered from mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Dead</td>
<td>Live S cells</td>
</tr>
<tr>
<td>B</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>None</td>
<td>Live R cells</td>
</tr>
<tr>
<td>A+B</td>
<td>Dead</td>
<td>Live S cells</td>
</tr>
<tr>
<td>A+C</td>
<td>Dead</td>
<td>Live R and live S cells</td>
</tr>
<tr>
<td>B+C</td>
<td>Dead</td>
<td>Live S cells</td>
</tr>
<tr>
<td>A+B+C</td>
<td>Dead</td>
<td>Live S cells</td>
</tr>
</tbody>
</table>

A: “A” must be live S cells because it kills mice when injected and S cells are recovered from the mice. “B” must be DNA from S cells as it transforms sample C when coinjected. “C” must be live R cells because it kills mice when injected and S cells are recovered from the mice.
The question: What is the structure of DNA?
The answer: A double helix with strands running antiparallel and bases that pair, A-T and G-C.

How do we know this?

Erwin Chargaff
After studying the base composition of the DNA from many different organisms, developed Chargaff’s Rules
1. The total amount of pyrimidine nucleotides (T+C) always equals the total amount of purine nucleotides (A+G)
2. The amount of T always equals the amount of A, and the amount of C always equals the amount of G, but A+T does not always equal G+C

Q 7.16: If thymine makes up 15% of the bases in a specific DNA molecule, what percentage of the bases is cytosine?
A: 35%

Q 7.28: If you extract the DNA of the coliphage φX174, you will find that its composition is 25% A, 33% T, 24% G, and 18% C. Does this composition make sense in regard to Chargaff’s rules? How would you interpret this result? How might such a phage replicate its DNA?
A: Chargaff’s rules are that A=T and G=C. Because this is not observed, the most likely interpretation is that the DNA is single-stranded. The phage would first have to synthesize a complementary strand before it could begin to make multiple copies of itself.

Rosalind Franklin
Got an X-ray diffraction pattern that yielded crucial information about the structure of DNA
• long
• thin
• has two similar parts that are parallel to each other and run along the length of the molecule
• helical

Watson and Crick put it all together (1953)
DNA is composed of two linear strands of DNA that twist around each other to form a double helix; the key structure is two nucleotide strands side-by-side twisted into a double helix and held together by hydrogen bonds between the bases; the components are
• sugar-phosphate backbone: alternating deoxyribose and phosphate groups connected by phosphodiester bonds; the way the sugars and phosphates bond requires each strand to have a 5’-3’ polarity
• nitrogenous bases: attached to the deoxyribose of the backbone, hydrogen bonds between bases from either strand hold them together and, because
the paired strands are antiparallel, cause the molecule to assume a double helical conformation

* DNA bases are dATP, dTTP, dCTP, and dGTP (d stands for deoxy). RNA bases are ATP, UTP, CTP, and GTP.
The question: How does DNA replicate?
The answer: Semiconservatively, and with the help of a lot of different proteins.

How do we know this?

Meselson and Stahl (1958)
1. Grow bacteria for many generations on heavy nitrogen
2. Switch the bacteria to regular nitrogen
3. Collect the bacteria after 1 or 2 generations and isolate the DNA
4. Using cesium chloride gradient centrifugation, separate DNA containing regular nitrogen from DNA containing heavy nitrogen

Conclusion: DNA replication is semiconservative, meaning that, after replication, each double-stranded molecule is composed of one parental strand and one newly synthesized strand

Q: What would the samples have looked like if DNA replication were conservative?
The replication fork:

Proteins required for DNA replication:
- Primase: makes an RNA primer; needed because DNA polymerase can only attach nucleotides to the 3’ OH of an already existing oligonucleotide, it cannot polymerize starting from a single base
- Helicase: unwinds DNA
- DNA polymerase: adds nucleotides
- Topoisomerase: causes nicks to prevent supercoiling
- Ligase: stitches together Okazaki fragments
- Single strand binding proteins: keep DNA strands apart
- Telomerase: special enzyme for finishing off DNA replication at telomeres, contains a small RNA molecule that acts as a template for synthesis of the repetitive DNA found at the ends of linear chromosomes