

LECTURE 15

Introduction to Linkage

- A. To this point we have considered segregation and assortment of gene pairs that are located on different, non-homologous chromosomes, *viz.*, segregation/assortment from the dihybrid **A/a; B/b** is expected to yield the following gametes

$$\frac{1}{4} AB \quad \frac{1}{4} Ab \quad \frac{1}{4} aB \quad \frac{1}{4} ab$$

1. The dihybrid when testcrossed (x **a/a; b/b**) is expected to yield a 1:1:1:1 phenotypic ratio
 2. This will be true if genes in cross are unlinked [or linked but separated by > 50% recombination]
 3. Note that the testcross essentially “samples” the gametes produced and in their relative frequency
- B. By definition, “linkage” is where two (or more) non-homologous genes are physically situated on the same (homologous) chromosome
1. The number of linkage groups is expected to equal the number of non-homologous chromosomes (the number of chromosomes in the haploid complement)
 2. Phases or configurations
 - a) Consider a dihybrid: **AB/ab** → cis or coupling phase
Ab/aB → trans or repulsion phase
 - b) Then consider situation where two genes are linked and where no crossing over (physical exchange) occurs between the two genes:

If cis or coupling, **AB/ab** → gametes are $\frac{1}{2} AB$ and $\frac{1}{2} ab$

If trans of repulsion, **Ab/aB** → gametes are $\frac{1}{2} Ab$ and $\frac{1}{2} aB$

- (i) if either dihybrid had been testcrossed (x **ab/ab**), only parental combinations would have been observed (and in a 1:1 ratio); the conclusion from this experiment would be that genes A & B are linked
- (ii) recall that testcrossing a dihybrid when the genes were unlinked (A/a; B/b) yielded a 1:1:1:1 ratio of four phenotypes, yielding the conclusion that the genes were unlinked
- (iii) alternatives (i) and (ii) span the range of “linkage data”, from a 1:1 testcross ratio (genes are completely linked), to a 1:1:1:1 ratio (genes are unlinked)

C. Now consider crossing over – physical exchange between homologous pair of chromosomes

1. In the dihybrid AB/ab

a) If there is no crossing over between genes A & B, gametes are

AB and ab → parental types

b) If there is crossing over between genes A & B, gametes are

Ab and aB → recombinant types

c) Note that which is parental and which is recombinant reverses if the dihybrid is in the trans (repulsion) configuration, i.e., in the dihybrid Ab/aB

Ab and aB → parental types

AB and ab → recombinant types

2. Crossing over occurs during the pachynema stage of Prophase I (Meiosis I) when the chromosomes are at the so-called “four-strand” or “tetrad” stage, i.e., the chromosomes have been replicated but segregation/assortment have yet to occur

a) Consider sister versus non-sister chromatids and sister versus non-sister chromosomes

(i) Crossing over can occur between both sister and non-sister chromatids; both types of exchange can have genetic consequence but only exchanges between non-sister chromatids involve exchanges between chromatin from different lines of descent (i.e., maternal and paternal) and represent genetic recombination

b) Because crossing over occurs at the four-strand stage, products of crossing over must be considered from that perspective; consider products from a single tetrad in a dihybrid AB/ab where a single exchange occurs between genes A & B

(i) Expected consequences from a single tetrad are

$\frac{1}{4}$ AB $\frac{1}{4}$ Ab $\frac{1}{4}$ aB $\frac{1}{4}$ ab (1:1:1:1 in a testcross)

(ii) Note that on average there is usually one crossover per tetrad

(iii) Thus, from one tetrad with a single crossover, recover 50% parentals & 50% recombinants, and that this is the same ratio as expected from a testcross of a dihybrid where the genes A & B are *unlinked*!

(iv) What would happen if the genes were linked but the dihybrid was in the trans (repulsion) configuration?

D. Frequency of crossing over:

1. Between any two (linked) genes, crossing over is a chance event; given that on average only one crossover occurs per tetrad, one would not necessarily expect a crossover to occur between any two genes in any given tetrad
 - a) If crossing over occurs randomly along a chromosome, the chance that a crossover will occur between any two genes should be a function of the distance between the genes, i.e., the further apart are any two genes, the more likely a crossover will occur between them, and conversely, the closer are any two genes, the less likely a crossover will occur between them
 - b) By definition:

% crossing over = the proportion of tetrads that have an exchange between two genes

% recombination = the proportion of recombinants
 - c) Consider a sample of five tetrads:
 - (i) at 100% crossing over, there is (a maximum) of 50% recombination, and the frequency of gametes observed in a dihybrid will be the same as independent assortment despite the fact that the genes are linked
 - (ii) note that the range between 50% recombination and 0% recombination indicates genes are linked; note also that percentage of parentals will always be greater than percentage of recombinants unless the genes are unlinked or separated by more than 50% recombination

E. Linkage mapping:

1. Because the probability of a crossover occurring between any two genes is primarily a function of chromosomal distance, one should be able to utilize crossover occurrences as a means to estimate “distance” between genes, and hence construct a “map”

By definition, 1% recombination = 1 map unit (centiMorgan)

2. Two-point (factor) mapping:

Example: two sex-linked genes in *Drosophila*:

<p>♀♀ sn⁺/-; wild-type sn/sn: singed bristles</p>	<p>♀♀ w⁺/-; wild-type w/w; white eyes</p>
<p>♂♂ sn⁺/Y; wild-type sn/Y: singed bristles</p>	<p>♂♂ w⁺/Y; wild-type w/Y; white eyes</p>

- a) Testcross a dihybrid \rightarrow ♀ ($sn^+, sn^- / w^+, w$) \times ♂ $sn w/Y$,
and score F_1 ♂♂(sons): results are given below

<u>Phenotype</u>	<u>Number</u>	<u>Genotype</u>
singed	184	$sn w^+/Y$
wild-type	64	$sn^+ w^+/Y$
singed, white	66	$sn w/Y$
white	<u>186</u>	$sn^+ w/Y$
	500	

- b) Given these data, one can accomplish the following:

- (i) determine the allelic configuration in the dihybrid (tester) ♀
 - (ii) determine the % recombination (map distance) between the two genes
- c) The data indicate linkage (i.e., the four genotypes are clearly not in a 1:1:1:1 ratio), and given that parentals must be in excess (relative to recombinants), the allelic configuration in dihybrid can be deduced straightforwardly

$sn w^+$ and $sn^+ w$ are in excess: they must be parentals, and hence stem from tetrads where there was no exchange between the two genes, i.e., the dihybrid ♀ was in the *trans* configuration

ergo, the dihybrid ♀ was $sn w^+$ / $sn^+ w$

- d) The map distance between the two genes is the % recombination between them

% recombination = # recombinants / total

$$= 64 + 66 / 500 = 26\% \quad \text{map} = \frac{sn}{\quad} \frac{26 \text{ mu}}{-} \frac{w}{\quad}$$

F. Difficulties with two-factor crosses

1. Do not specify order, i.e., must make a series of crosses for positioning
2. Can underestimate “true” crossover distance
 - a) Odd-numbered crossovers will be scored as single crossovers, and even-numbered crossovers will be scored as no crossovers (parentals)
 - b) Because “map distance” is a function of *detected* crossovers, the “true” distance can be underestimated; the error will increase with increasing physical distance between any two genes