Chapter 9
Gene Interactions

Figure 9-1
The coat colour on this juvenile horse is called Bay Roan Tobiano. Bay is the brown base coat colour; Roan is the mixture of white hairs with the base coat, making a 'foggy' colour; and Tobiano is the white patches. The genes causing the Roan and Tobiano coat colours, respectfully, are found on the same chromosome and are linked. Knowing this, we can predict which coat colour genes are from which parents, and how those genes will be inherited in this horse's offspring.

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As we learned in Chapter 3, Mendel reported that the pairs of loci he observed segregated independently of each other; for example, the segregation of seed color alleles was independent from the segregation of alleles for seed shape. This observation was the basis for his Second Law (Independent Assortment), and contributed greatly to our understanding of heredity as single units. However, further research showed that Mendel's Second Law did not apply to every pair of genes that could be studied. In fact, we now know that alleles of loci that are located close together on the same chromosome tend to be inherited together. This phenomenon is called **linkage**, and is a major exception to Mendel's **Second Law of Independent Assortment.** Researchers use linkage to determine the location of genes along chromosomes in a process called genetic mapping. The concept of gene linkage is important to the natural processes of heredity and evolution, as well as to our genetic manipulation of crops and livestock.

A Genetic Nomenclature & Symbols

Nomenclature and symbols have been covered in previous chapters. This will be a brief review to recover the topics.

A **gene** is a hereditary unit that occupies a specific position (**locus**) within the genome or chromosome and has one or more specific effects upon the phenotype of the organism and can mutate into various forms (**alleles**). A **genotype** is the specific allelic composition of a cell or organism. Normally only the genes under consideration are listed in a genotype and the alleles at the remaining gene loci are considered to be wild type. A **phenotype** is the detectable outward manifestation of a specific genotype. In describing a phenotype usually only the characteristics under consideration are listed while the remaining characters are assumed to be wild type (normal).

A.1 Gene names and symbols

Usually, gene names are unique and their corresponding symbols are unique letters or combinations of letters. So, for example, the "*vermillion*" gene in Drosophila is represented by the letter "*v*", while "*vg*" is the symbol for the "*vestigial*" gene and "*vvl*" is the symbol for the "*ventral veins lacking*" gene locus. Note however that the same letter symbols may represent a different gene in another organism. Gene symbols and gene names are typically shown in *italics* text. In lectures we may not always use italics for gene names and symbols.

The normal, or wild type, form of a gene is usually symbolized by superscript plus sign, "+". *e.g.* " a^+ ", " b^+ ", *etc*. or it is sometimes abbreviated to just "+". A forward slash is occasionally used to indicate that the two symbols are alleles of the same gene, but on homologous chromosomes (see **Figure 9-2**).

A typical mutant form of the gene, of which there can be many, can be symbolized by a superscript minus sign, "-". *e.g.* " a^- ", " b^- ", *etc*, or sometimes abbreviated to just "*a*", "*b*", *etc* (no superscript). Therefore if the genotype of a diploid organism is given as a^+/a^-, it means there is a wild type allele and mutant allele of the "*a*" gene at the "*a*" locus. This may also be abbreviated to *+/a*.

In some species of diploids, the dominant allele is typically designated with the upper case letter(s), while the recessive allele is given the lower case letter(s). For example, in Mendel's peas the dominant Rough allele is "*R*", while the recessive smooth allele is "*r*".

B Recombination

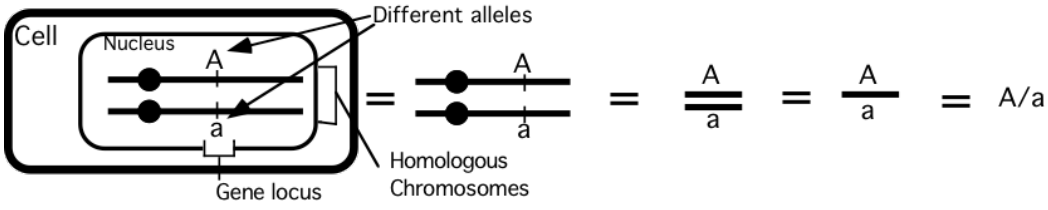

Figure 9-2

A diagram of how chromosomes, loci and alleles look in the cell, and how we depict them written.

(Original-J.Locke- CC BY-NC 3.0)

The process of meiosis leading to a separation of chromosomes, and crossing over is necessary for the understanding of this chapter. Refer to Chapter 7 for a review of these concepts.

The term "recombination" is used in several different contexts in genetics. In reference to heredity, **recombination** is defined as a process that results in gametes with combinations of alleles that were not present in the gametes from the parental generation (**Figure 9-3**). Recombination is important because it contributes to the genetic variation that may be observed between individuals within a population and that may be acted upon by selection for evolution.

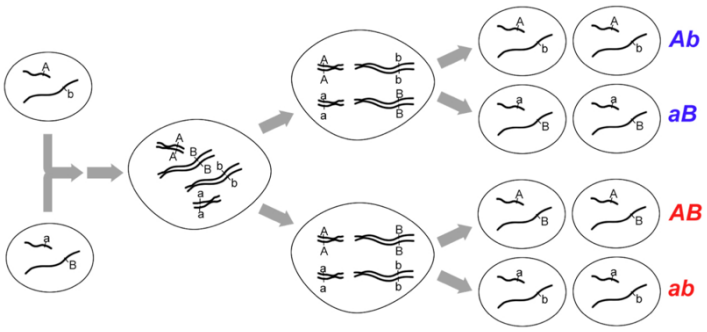

Figure 9-3

When two loci are on non-homologous chromosomes, their alleles will segregate in combinations identical to those present in the parental gametes (*A;b, a;B*), and in recombinant genotypes (*A;B, a;b*) that are different from the parental gametes.

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B.1 Inter- and intrachromosomal recombination

Interchromosomal recombination occurs either through **independent assortment** of alleles whose loci are on different chromosomes (Chapter 3). **Intrachromosomal recombination** occurs through **crossovers** between loci on the same chromosomes. It is important to remember that in both of these cases, recombination is a process that occurs during meiosis (mitotic recombination may also occur in some species, but it is relatively rare).

As an example of interchromosomal recombination, consider loci on two different chromosomes as shown in Figure 9-3.

We know that if these loci are on different chromosomes there is no physical connection between them, so they are **unlinked** and will segregate independently as did Mendel's traits. The segregation depends on the rel-

ative orientation of each pair of chromosomes at metaphase. Since the orientation is random and independent of other chromosomes, each of the arrangements (and their meiotic products) is equally possible for two unlinked loci as shown in Figure 9-3 on page 80.

Intrachromosomal recombination occurs through crossovers. Crossovers occur during prophase I of meiosis, when pairs of homologous chromosomes have aligned with each other in a process called **synapsis**. Crossing over begins with the breakage of DNA of a pair of non-sister chromatids. The breaks occur at corresponding positions on two non-sister chromatids, and then the ends of non-sister chromatids are connected to each other resulting in a reciprocal exchange of double-stranded DNA. Generally every pair of chromosomes has at least one crossover during meiosis, but often multiple crossovers occur in each chromatid during prophase I.

Because interchromosomal recombination happens through independent assortment, genes in this situation are always unlinked. Intrachromosomal recombination has instances of linked genes, and so they will be the focus of this chapter.

B.2 Inheriting Parental and Recombinant Gametes

If we consider only two loci and the products of meiosis results in recombination, then the meiotic products (gametes) are said to have a **recombinant genotype**. On the other hand, if no recombination occurs between the two loci during meiosis, then the products retain their original combinations and are said to have a non-recombinant, or **parental genotype.** The ability to properly identify parental and recombinant gametes is essential to apply recombination to experimental examples.

To properly identify recombinant and parental gametes, you need to know the genotype of the parents (the P generation) of the individual in question. This is most easily demonstrated in a dihybrid. If, for two genes, one parent has the genotype *AABB,* they can only produce one type of gamete: *AB* (**Figure 9-4** left). *Note: the absence of slashes and semicolons indicate that we don't know whether the genes are linked or not.* The same is true for the other parent. If it is *aabb*, then it can also only produce one type of gamete: *ab*

This is an encore presentation of the online exercise for writing out linked and unlinked genes.

http://tinyurl.com/oog-linked

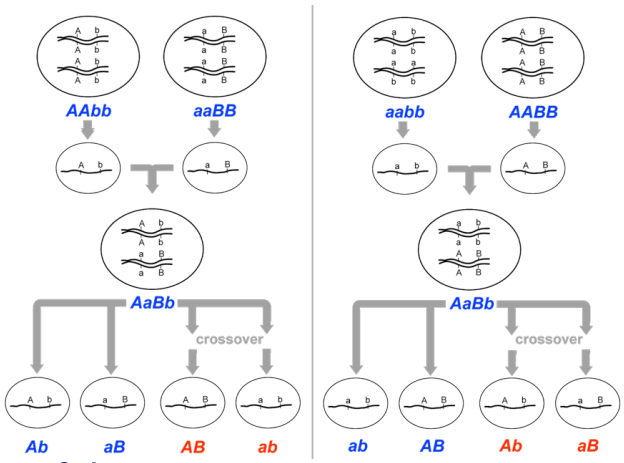

Figure 9-4

The genotype of gametes can be inferred unambiguously if the gametes are produced by homozygotes. However, recombination frequencies can only be measured among the progeny of heterozygotes (i.e. dihybrids). Note that the dihybrid on the left contains a different configuration of alleles (*Ab/aB*) than the dihybrid on the right (*AB/ab*) due to differences in the genotypes of their respective parents. Therefore, different gametes are defined as recombinant and parental among the progeny of the two dihybrids. In the cross at left, the recombinant gametes will be genotype *AB* and *ab*, and in the cross on the right, the recombinant gametes will be *Ab* and *aB*.

(Figure 9-4 right). When those two gametes combine, they create an individual (F_1) that has a genotype written as *AaBb*.

Only the F_1 dihybrid individual produces four different gametes: *AB*, *ab*, *Ab* and *aB*. The **parental gametes** are those that the F_1 obtained from its parents, in this case *AB* and *ab*. *Ab* and *aB* are **recombinant gametes** and are evidence of a recombination event happening, resulting in a different combination of alleles (Figure 9-4).

This idea also applies for independently assorting genes (which would be *A/a*; *B/b*). The frequency of each type of gamete from the dihybrid would be equal. Each would account for about ¼ each of the whole set of gametes. If the genes are linked (*ie.* on the same chromosome), the recombinants will be much less frequent than the parental arrangement.

For the above example, the P generation has one parent homozygous for both dominant alleles, and the other homozygous for both recessive alleles. It is very important to note that this will not always be the case. In some instances one parent will be homozygous with one gene dominant and the other gene recessive (*AAbb*) and the other parent will be the opposite (*aaBB*). This

situation will change which is the parental and recombinant gametes (compare left and right in Figure 9-4).

B.3 Coupling and repulsion configuration

When looking at an organism that is heterozygous at two loci, just by looking at them you cannot tell how the mutant and wild type alleles are arranged. Both mutant alleles could be on one homologous chromosome, and both wild type alleles could be on the other (e.g. *ab/a⁺b⁺)*. This is known as a **coupling** (or cis) **configuration** (See **Figure 9-5**) When one wild type allele and one mutant allele are on one homologous chromosome, and the opposite is on the other, this is known as a **repulsion** (or trans) **configuration** (e.g. *a⁺b/ab⁺*). The way to determine the orientation is to look at the parents (or P generation) of that cross if you know the genotypes of them. If the parents are homozygous in both genes, and one shows both dominant phenotypes and the other shows both recessive phenotypes, then you know that the individual you are looking at is in coupling configuration. If one parent has one dominant and one recessive phenotype, and the other has the opposite, then you know the individual is in repulsion configuration.

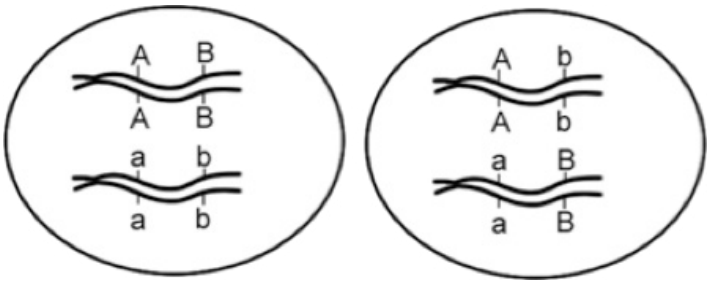

Figure 9-5
Alleles in coupling configuration (*AB/ab*,left) or repulsion configuration (*Ab/aB* right).
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B.4 Recombination frequency

Recombination frequency represents distance between genes (**d**). It is a calculation to define the number of parental and recombinant gametes. The equation is as follows:

$$d = \frac{\#\ \text{Recombinant gametes}}{(\text{Total}\ \#\ \text{gametes})}\ X\ 100\%$$

The total number of gametes is, of course, the sum of both parental and recombinant gametes.

Through identifying and defining parental and recombinant gametes, you can calculate ***d*** and from there decide the degree of linkage.

Based upon the equation and independent assortment, you can see that the recombination frequency cannot be higher than 50%. The unit for this distance is more formally known as a centiMorgan (cM - note the capital M!). If alleles are assorting independently, there will a random distribution of the alleles in the progeny, and so 50% will be recombinant gametes and 50% will be parental gametes, making ***d*** approximately 50 cM. If a gene is linked you will see a higher percentage of parental gametes, making ***d*** < 50 cM. You will never see more recombinant gametes than parental, and so in no situation will recombination frequency be higher than 50 cM, except slightly with regards to standard experimental error. If you calculate a recombination frequency higher than 50 cM, you need to make sure you accurately defined parental and recombinant gametes.

C Unlinked Genes and Complete and Partial Linkage

When comparing any two genes, they can be varying distances apart. Their ***d*** allows us to categorize them into the degree of linkage. The amount of linkage can be placed on a sliding scale.

Table 9-2 shows generally how we categorize the degree linkage using recombination frequency. Because ***d*** is based upon experimental results that will have some experimental error, these should be treated as guidelines and not hard rules in determining the distance between genes.

C.1 Unlinked genes

Unlinked genes are genes that appear to segregate and show independent assortment. There will be a ran-

Table 9-1
The linkage description is listed corresponding to its recombination frequency. Note: values between 0.30 and 0.50 may be partially linked, or many not be linked at all. It is often difficult to distinguish between these two possibilities because of experimental error.

Linkage Description	Distance
Unlinked	~50 cM (~50%)
Partial linkage	< 30 cM or 30%
Complete linkage	0.00 cM or 0%
You messed up!	>> 50 cM

dom and even distribution of gamete types, and a ***d*** of 50 cM is the expectation. This situation is describes two instances, either the genes are on completely different chromosomes, or they are far enough apart on a single chromosome that the crossovers are so numerous so as to randomly assort the alleles (Figure 9-3 on page 80). Either way, because the alleles are assorting independently you should see an equal number of recombinant and parental gametes, so ***d*** will be ~50 cM. Note, because of real life variability this value can be anywhere from ~40 cM to ~60 cM.

C.2 Complete Linkage

Having considered unlinked loci, let us turn to the opposite situation, in which two loci are so close together on a chromosome that the parental combinations of alleles always segregate together (**Figure 9-6**). This is because during meiosis they are so close that there are no crossover events between the two loci and the alleles at the two loci are physically attached on the same chromatid and so they always segregate together into the same gamete. In this case, no recombinants will be present following meiosis, and the recombination frequency will be 0.00. This is **complete** (or **absolute**) **linkage** and is rare, as the loci must be so close together that crossovers are never detected between them.

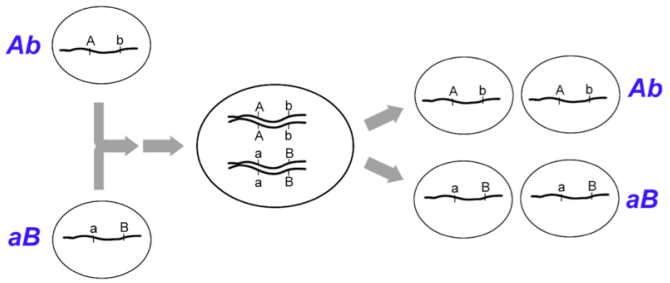

Figure 9-6

If two loci are completely linked, their alleles will segregate in combinations identical to those present in the parental gametes (*Ab*, *aB*). No recombinants will be observed.

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C.3 Partial Linkage

It is also possible to obtain recombination frequencies between 0% and 50%, which is a situation we call **incomplete** (or **partial**) **linkage**. Incomplete linkage occurs when two loci are located on the same chromosome but the loci are far enough apart so that crossovers occur between them during some, but not all, meioses (**Figure 9-7**). Genes that are on the same chromosome are said to be **syntenic** regardless of whether they are completely or incompletely linked or unlinked. All

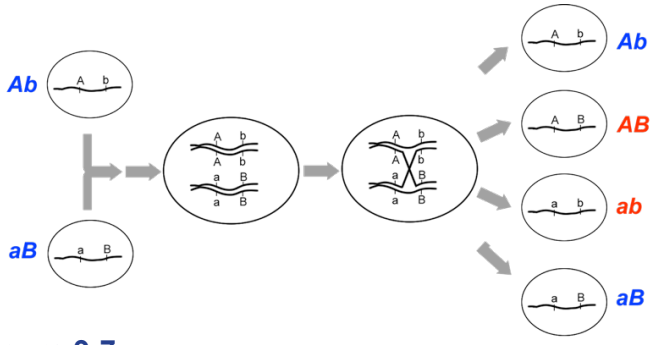

Figure 9-7

A crossover between two linked loci can generate recombinant genotypes (*AB*, *ab*), from the chromatids involved in the crossover. Remember that multiple, independent meioses occur in each organism, so this particular pattern of recombination will not be observed among all the meioses from this individual.

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linked genes are syntenic, but not all syntenic genes are linked (they may be so far apart that the proportion of gametes resemble independent assortment).

Because the location of crossovers is essentially random along the chromosome, the greater the distance between two loci, the more likely a crossover will occur between them. Furthermore, loci that are on the same chromosome, but are sufficiently far apart from each other, will on average have multiple crossovers between them and they will behave as though they are completely unlinked. A recombination frequency of 50% (***d*** = 50 cM) is therefore the maximum recombination frequency that can be observed, and is indicative of loci that are either on separate chromosomes, or are located very far apart on the same chromosome (*ie* syntenic, but not linked!)

D Experimentally Determining Recombination Frequency

Let us now consider a complete experiment in which our objective is to measure recombination frequency (**Figure 9-8**). We need at least two alleles for each of two genes, and we must know which combinations of alleles were present in the parental gametes. The simplest way to do this

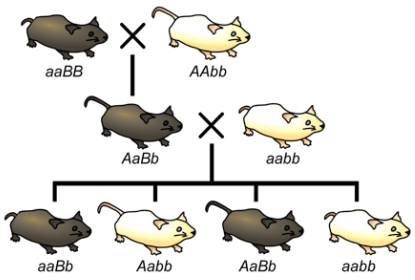

Figure 9-8

An experiment to measure recombination frequency between two loci. The loci affect coat color (B/b) and tail length (A/a).

(Wikipedia Mod by Deyholos-CC BY-NC 3.0)

is to start with pure-breeding lines that have contrasting alleles at two loci. For example, we could cross short-tailed mice, brown mice (*aaBB*) with long-tailed, white mice (*AAbb*) (Figure 8, P cross). Based on the genotypes of the parents, we know that the parental gametes will be *aB* or *Ab* (but not *ab* or *AB*), and all of the progeny will be dihybrids, *AaBb*. We do not know at this point whether the two loci are on different chromosomes, or whether they are on the same chromosome, and if so, how close together they are. That is why the genotype in this paragraph does not contain slashes or semicolons.

The recombination events that may be detected will occur during meiosis in the dihybrid individual. If the loci are completely or partially linked, then prior to meiosis, alleles *aB* will be located on one chromosome, and alleles *Ab* will be on the other chromosome. These are the parental gametes based on our knowledge of the genotypes of the gametes that produced the dihybrid. Thus, recombinant gametes produced by the dihybrid will have the genotypes *ab* or *AB*.

Now that we have identified the parental and recombinant gametes, how do we determine the genotype of the gametes produced by the dihybrid individual? The most practical method is to use a testcross (Figure 8 shows F_1 crossed with a tester), in other words to mate *AaBb* to an individual that has only recessive alleles at both loci (*aabb*). This will give a different phenotype in the second generation for each of the four possible combinations of alleles in the gametes of the dihybrid (**Figure 9-9**).

We can then infer unambiguously the genotype of the gametes produced by the dihybrid individual, and therefore calculate the recombination frequency between these two loci. For example, if only two phenotypic classes were observed in the F_2 (i.e. short tails and brown fur (*aaBb*), and white fur with long tails (*Aabb*))

♂ \ ♀	AB	Ab	aB	ab
ab	Aa Bb	Aa bb	aa Bb	aa bb
phenotype	Long Brown	Long White	Short Brown	Short White
recombinant or parental	R	P	P	R

Figure 9-9

An experiment to measure recombination frequency between two loci. The loci affect coat color (*B/b*) and tail length (*A/a*).
(Wikipedia-Modified Deyholos-CC BY-NC 3.0)

we would know that the only gametes produced following meiosis of the dihybrid individual were of the parental type: *aB* and *Ab*, and the recombination frequency would therefore be 0%. Alternatively, we may observe multiple classes of phenotypes in the F_2 in ratios such as shown in **Table 9-2**. Given the data in this table, the calculation of recombination frequency is straightforward:

$$d = \frac{\text{\# recombinant offspring}}{\text{Total offspring}} \times 100$$

$$d = \frac{13+17}{48+42+13+17} \times 100\ cM$$

$$= 25\ cM$$

Because the recombination frequency is below 30%, we can say that the tail length gene and the fur colour gene are partially linked.

Note: The use of linkage and recombination frequency, will be extended to Genetic Mapping in the next chapter.

Table 9-2

An example of quantitative data that may be observed in a genetic mapping experiment involving two loci. The data correspond to the F_2 generation in the cross shown in Figure 9-8 on page 83.

tail phenotype	fur phenotype	number of progeny	gamete from dihybrid	genotype of F_2 from test cross	(P)arental or (R)ecombinant
short	brown	48	*aB*	*aaBb*	P
long	white	42	*Ab*	*Aabb*	P
short	white	13	*ab*	*aabb*	R
long	brown	17	*AB*	*AaBb*	R

Summary:

- Recombination is defined as any process that results in gametes with combinations of alleles that were not present in the gametes of a previous generation.
- The recombination frequency between any two loci depends on their relative chromosomal locations.
- Unlinked loci show a maximum 50% recombination frequency (a distance of 50 cM).
- Loci that are close together on a chromosome are linked and tend to segregate with the same combinations of alleles that were present in their parents.
- Crossovers are a normal part of most meioses, and allow for recombination between linked loci.
- Measuring recombination frequency is easiest when starting with pure-breeding lines with two alleles for each locus, and with suitable lines for test crossing.

Key terms:

allele
complete (absolute) linkage
coupling (cis) configuration
crossover
distance
    (d = #recomb/#total*100)
gene
genotype
incomplete (partial) linkage
independent assortment
interchromosomal recombination
intrachromosomal recombination
linkage
locus
parental genotype (and gametes)
phenotype
recombinant genotype (and gametes)
recombination
recombination frequency
    (see *distance*)
repulsion (trans) configuration
Second Law of
    Independent Assortment
synapsis
syntenic
unlinked

Study Questions:

1. Compare recombination and crossover. How are these similar? How are they different?
2. Explain why it usually necessary to start with pure-breeding lines when measuring genetic linkage by the methods presented in this chapter.
3. If you knew that a locus that affected earlobe shape was tightly linked to a locus that affected susceptibility to cardiovascular disease human, under what circumstances would this information be clinically useful?
4. In a previous chapter, we said a 9:3:3:1 phenotypic ratio was expected among the progeny of a dihybrid cross, in absence of gene interaction.
    a) What does this ratio assume about the linkage between the two loci in the dihybrid cross?
    b) What ratio would be expected if the loci were completely linked? Be sure to consider every possible configuration of alleles in the dihybrids.
5. Given a dihybrid with the genotype *CcEe*:
    a) If the alleles are in coupling (cis) configuration, what will be the genotypes of the parental and recombinant progeny from a test cross?
    b) If the alleles are in repulsion (trans) configuration, what will be the genotypes of the parental and recombinant progeny from a test cross?
6. In this question, white flowers (w) are recessive to purple flowers (W), and yellow seeds (y) are recessive to green seeds (Y). If a green-seeded, purple-flowered dihybrid is testcrossed, and half of the progeny have yellow seeds:
    a) What can you conclude about linkage between these loci?
    b) What do you need to know about the progeny in this case?

7. If the progeny of the cross *aaBB* x *AAbb* is testcrossed, and the following genotypes are observed among the progeny of the testcross, what is the frequency of recombination between these loci?

   a) *AaBb* 135
   b) *Aabb* 430
   c) *aaBb* 390
   d) *aabb* 120

8. Draw the cell in metaphase I of the cell cycle in a case where crossover did not occur in prophase I, and in a case where it did.

9. What is meant by the sentence "All linked genes are syntenic, but not all syntenic genes are linked."?