Note: please find the handouts in this year's handout list if the links do not work.

UN2005/UN2401 '18 Lecture #23

Handouts: 23A - Linkage vs Independent Assortment
23B – Independent Assortment, AND Genes, Enzymes & Pathways.
23C - How to measure RF's in diploids. This handout is online only – no paper copies.
23D – Recombination & Linkage

These notes are relatively long because some of the sections are for reference or fyi. Also, recombination is covered twice, from different angles. We will not get to Population Genetics and Evolution, so that material is posted separately. You are not responsible for that material, but it is well worth reading (after finals?).

I. Pedigrees

See Handout 22B, and Notes of Lecture 22, VIII, esp. VIII-B.

II. Reshuffling at Meiosis

More details in Topic VII of Lecture #21.

A. Why discuss this now? To follow inheritance of more than one gene requires knowledge of details of meiosis (which differ from mitosis), in particular events in first div of meiosis. Lecture #21 has all the details; here are the main points:

B. Prophase I -- Big Differences from Mitosis -- duration & crossing over. Time when genetic recombination (reshuffling of genes) occurs.

C. Metaphase I & Anaphase I -- Important differences from Meiosis

1. Chromosomes line up in pairs at Metaphase I

2. Homologs, not sisters, separate at Anaphase I. Therefore independent Assortment (reshuffling of chromosomes) occurs during meiosis I.

D. Products of human meiosis -- Reminder (see Sadava fig. 42.4 (43.3) or Becker fig. 25-8 (20-9))

1. In females: You get one large (haploid) egg per meiosis.

2. In males: When male germ cells go through meiosis, 4 sperm are formed.

E. Mitosis vs Meiosis Overall: Steps of meiosis are diagrammed in detail in parallel to the steps of mitosis on handout 21A.

To review Meiosis, and compare to Mitosis, fill in the table in Lecture 21, and do or finish problems 8-1, 8-2 (parts A to E), 8-3, & 8-8 (parts A-D & G). .

III. Crosses with Multiple genes (on separate chromosomes) -- Genotypes

A. Consider a dihybrid cross (for example) AABB X aabb. What will the offspring (F_1) be?

1. Terminology:

a. Monohybrid cross = AA X aa. It gives an F_1 that is hybrid for 1 gene (Aa).

b. Dihybrid cross = AABB X aabb or AAbb X aaBB. It gives an F_1 that is hybrid for 2 genes (AaBb), and so on.

2. Procedure: Same procedure as with monohybrid crosses (such as AA X aa) -- figure out the parents, then the gametes, and mix the gametes to get zygotes.

3. Results: In this case (AABB X aabb), first parent must produce AB gametes and second parent ab gametes, so all zygotes must be AaBb = F_1. (If you think gametes of first parent could be AA or BB, you should convince yourself why you are wrong. Put the genes on homologous chromosomes, go through meiosis, and see why only AB gametes are possible.)

B. What will gametes of AaBb be? See handout 23A (right panel).

Suppose you want to cross the F_1's (AaBb) from above to get the F_2. How do you do it? Repeat standard procedure as in previous cases. Figure out gametes, then zygotes. So let's figure out the gametes of AaBb. What do you expect? See handout 23A or Becker fig. 25-14 (20-14).

Let's start with the simplest case: The two genes in question are on separate chromosomes. (See right panel of Handout 23A, or Becker fig. 25-14 (20-14) or Sadava fig. 12.5 (12.7). Other cases are considered later.

- **Location of genes on chromosomes:** Assume the two genes in question are on two, different, non-homologous autosomes. In other words, alleles of gene alpha (A & a) are on one pair of autosomes/homologs; alleles of gene beta (B & b) are on a different pair of autosomes/homologs.
- **Line up at meiosis:** Each pair of homologs lines up independently at meiosis (meta. I), so in this case there are two possible lineups at metaphase I of meiosis -- all "straight" chromosomes on top of metaphase plate and all "wiggly" below, or one straight and one wiggly on each side.
- **Results of each individual meiosis:** The results of the two lineups are shown on the handout -- the 4 products of meiosis can be two each of AB and ab, ***or*** two each of Ab and aB.
- **Results of many meioses:** Will produce many gametes which will be a mixture of all 4 kinds of gametes in equal proportions. When you get all 4 kinds in ratios of 1:1:1:1 this is called independent assortment.
- **Genetic Consequences:** In this case, the A's (A and a) were inherited independently of the B's (B and b). So allele A (or allele a) is just as likely to go with allele b or allele B. This follows from the line up at meiosis, as shown on handout, and explained above.

C. What will the zygotes be for the case of independent assortment? See Handout 23B.

1. Using a Square: Can make a 4 X 4 Punnett square with gametes (AB Ab, aB, ab) on each side and fill it all in. (See Sadava fig. 12.4 (12.6) for an example.) This method is tedious, subject to error, and unwieldy when crosses involve more than 2 genes. In order to use this method to get the gametes and do the square, we have to assume that the two genes assort (are distributed) independently. But if this is so, there is an easier way to figure out the offspring -- the branch method.

2. Using the Branch/probability method: This is another way to figure out what zygotes are expected, and in what proportions. This method works for any number of genes as long as they assort independently. This is the "branch" method (shown on your handout; it is just the application of elementary probability).

a. Figure out what would happen if you did the cross with one gene (here Aa X Aa). Use a small square if you don't remember. In this case results will be three genotypes, AA, Aa, aa in ratios of 1:2:1.

b. Figure out what would happen if you did the cross with the other gene(s), Bb X Bb.

c. Put the results together.

(1*) Number of possible different genotypes*: Since the alleles of the alpha gene and the alleles of the beta gene are distributed independently, any expected alpha genotype can go with any expected beta genotype. This gives the following possibilities:

AA with BB, Bb or bb; Aa with BB, Bb or bb; aa with BB, Bb, or bb. (This is most easily followed using a branching diagram as shown on the handout.) This shows that 9 different genotypes are expected; the square gives the same result.

(2) Proportions of different genotypes: Figure out the chance of each alpha combination (AA, Aa, or aa) and of each beta combination; chance of any particular combination (say aaBb) is the product of the individual chances (= 1/4 X 1/2 for aaBb).

The general rule is: if the chances of any two (alternative) outcomes are known, the chance of both happening at the same time is their product .

d. Important: Don't panic if probability is not your strong point. All the probability you need to handle genetics (at the level of this course) is as follows:

- The chance of this AND that both happening = chance of this TIMES chance of that.
- The chance of this OR that happening = chance of this PLUS chance of that.
- To summarize for simple cases: AND = multiply; OR = add*. See Sadava fig. 12.6 (12.8).

*Note: the "or means add" rule only works if the two alternatives are mutually exclusive. For example, you can't be both AA and Aa at the same time, so chance of being one or the other is the sum of the two chances. If the alternatives can both occur, the rule is different. (Famous example: Suppose there is a chance of 50% that it will snow today, and a 50% chance tomorrow. What is the chance it will snow either today or tomorrow? The answer is ***not*** 100%!)

e. How this works for crosses with many genes. Suppose you have a cross involving more than two genes, such as AaBBccDd X aaBbCCDd. If all genes assort independently, you can figure out the results for each gene (Aa X aa; BB X Bb etc.) and multiply the probabilities to figure out the chances of getting some combination such as AaBbCcDd. In this case, that would be 1/2 X 1/2 X 1 X 1/2.

To review independent assortment, try problem 10-1 (A-C).

IV. Crosses with Multiple genes -- Phenotypes *(See Handout 23B)*

A. Common Features to all cases on 23B

- Complete dominance of A over a, and B over b
- Independent inheritance of genes alpha (alleles A & a) and beta (alleles B & b); 9 genotypes as above.
- A → one polypeptide;
  B → different polypeptide.

B. Simplest Case: One gene -- One Trait. Two genes & two traits overall, but each gene controls only one trait or characteristic.

1. How many phenos? In this case, there are 4 different phenotypes corresponding to A_B_; A_bb;
aaB_ and aabb. A_B_ gets both the alpha job and the beta job done, aabb does neither and so on.

2. How many of each pheno? In this case, you get an F2 that is 9:3:3:1 in terms of phenotype. How is this figured out?

a. For gene alpha: In this case, you need to know what proportion are A_ (= what is chance of getting A_)?

A_ = AA plus Aa.
If chances of AA and Aa are known, chance of either one (of A_) = sum of the two chances.
From Aa X Aa, 1/4 should be AA and 1/2 Aa
How many A_ ? Chance of A_ = 1/4 + 1/2 = 3/4
How many aa? 1/4 expected from Aa X Aa. Alternatively, we know 3/4 are A_ and everyone else is aa.

b. For gene beta: By the same reasoning, 3/4 should be B_ and 1/4 bb.

c. For both genes:

What proportion should be A_B_? That's chance of A_ X chance of B_ = 3/4 X 3/4 = 9/16.
What proportion A_bb? 3/4 X 1/4 = 3/16.
Using this reasoning, the proportions of the 4 phenotypes should be 9/16: 3/16:3/16:1/16.
This gives the famous ratio of 9:3:3:1.
To say it another way,
9/16 should be able to do both jobs
3/16 able to do the beta job or alpha job (but not the other)
1/16 should be able to do neither.

To review the genotypes and phenotypes expected in cases like this (independent assortment, and one gene per trait) try 10-1 part D, 10-2, 10-3 & 10-5.

B. Pleiotropy -- more traits than genes: One gene can affect Two (or many) Traits.

Suppose one gene → 1 peptide. This one peptide (not to mention different peptide variants generated by alternative splicing or modifications) can have multiple direct or indirect effects. This is called pleiotropy. In this case, one gene affects several traits. (For an example, see Waardenburg syndrome, described in problem 10-18.)

C. More genes than traits: Two (or more) genes can affect One Trait. See handout 23B (top).

Both genes/peptides can affect the same trait, for example by affecting the same or different steps in a single pathway. The following cases will be discussed in detail.

1. Two genes control a single step. Left case on Handout 23B.

Suppose 2 different peptides → 1 enzyme (with two subunits). Enzyme catalyzes, say, X → Z.

Then an absence of either gene product will cause a block in the same step (X to Z). Therefore, aa B_, A_bb and aabb will have the same phenotype - - in all these cases, X to Z will be blocked, so X will not be broken down and/or Z will not be made.

If you do the cross AaBb X AaBb, what is the expected ratio of normal to mutant phenotypes?

If X is colorless and Z is colored, say black, then phenotypic ratios will be 9:7 black to colorless.

In this case, normal is often called Z+ or X+, since it is able to make Z and/or break down X, while mutant is called Z- or X- since it is unable to make Z and/or breakdown X. Using this terminology, phenotypic ratios will be 9:7 Z+: Z-.

2. What if the 2 genes affect different steps in the same pathway? Top right case on Handout

a. Z+ vs Z-. (Inactive gene A or inactive gene B gives the same phenotype -- inability to catalyze synthesis of Z).

This time suppose the 2 peptides (products of genes A and B) catalyze **different** steps in the **same** pathway. For example, suppose

- enzyme A, product of gene A, catalyzes X → Y
- enzyme B, product of gene B, catalyzes Y → Z. (See picture on handout.)

Phenotype: If you define normal phenotype as ability to make Z, then defect in gene/enzyme A or gene/enzyme B produces the same effect = a block in the pathway. (Although a different intermediate will build up in each case.)

Ratios: If you are only interested in ability to make Z, a cross of AaBb X AaBb gives normal: mutant phenotype in a ratio of 9:7. (Lack of product of gene A has same phenotype as lack of product of gene B, & so ratios are same as in case discussed above).

What if Z is colored? If both X and Y are colorless (but Z is pigmented), then ratios will be 9:7 of pigmented (normal) to colorless (albino).

b. Epistasis. (Inactive gene A gives a different phenotype than inactive gene B) -- bottom right case on Handout 23B. Note that gene/enzyme relations are the same as in previous case, but phenotypes are different.

Phenotype: Suppose X and Y serve different functions (other than to make Z) and/or X, Y and Z are different colors (as indicated below & on handout). Then a block in X →Y looks/acts differently than a block in Y → Z. For example, in this case (as on handout)

- aaB_ or aabb is white/colorless A_bb is brown A_B_ is black.

Ratios: In this case, if you define phenotypes by color, a cross of AaBb X AaBb → offspring with three phenotypes in ratios of 9 (black): 3 (brown) :4 (white).

This type of situation is known as **epistasis** -- effects of gene A (when it is aa in this case) override (are epistatic to) the effects of gene B. In other words, if genotype is aa, it doesn't matter what the state of the B's are -- aa__ is always white. The gene controlling the first step is said to be epistatic to the gene controlling the second step.

For a similar case, with a picture, see Sadava fig. 12.11 (12.13).

c. Epistasis vs Dominance. Epistasis is sometimes confused with dominance, as both cases refer to how the effects of one allele or gene overrides the effects of another. However they are quite different.

(1). *Dominance* refers to the effects of one allele overriding the effects of another allele (of the same gene). For example, A is dominant to a.

(2). Epistasis refers to the effects of one gene overriding the effects of another gene. For example, gene A (really the aa genotype) is epistatic to gene B.

(3). What interacts? In both cases it is the gene products, the proteins made by the genes, that interact, NOT the genes or alleles themselves.

Note: The term 'epistasis' is often used in a more general sense to refer to cases where the effects of one gene influence (but don't necessarily override) the effects of another. In other words 'any time two different genes contribute to a single phenotype and their effects are not merely additive, those genes are said to be epistatic.' For a very thorough discussion of epistasis (beyond the scope of this course), and the source of this quote, see http://www.nature.com/scitable/topicpage/epistasis-gene-interaction-and-phenotype-effects-460

3. General Case.

- For each pathway you can think of, there will be a characteristic number of phenotypes and ratios of phenotypes (From AaBb X AaBb).
- From the pathway, you can usually predict the pattern of inheritance.
- From the pattern of inheritance, you can often deduce the pathway.
- Cases above assume genes are autosomal, and there is complete dominance. If genes are on the X (sex-linked), or there is incomplete or co-dominance, that will affect the ratios and numbers of phenotypes as well -- but the underlying principles are the same.

See problem set 10 for examples and to test your understanding. To get practice in matching up patterns of inheritance and the underlying metabolic pathways, try 10-4, 10-15 & 10R-5.

Note on Terminology: In the 3 examples on Handout 23B, the genes are called gene A and gene B, and the alleles are A, a, B & b. It is probably a better practice to call the genes and the alleles by different names to avoid confusion. For example, in the cases discussed here, the genes are called alpha and beta to keep them straight from the alleles A and B.

V. Linkage and crossing over -- Suppose gene alpha (alleles A and a) & gene Beta (alleles B and b) are on the same chromosome. What are the genetic consequences? (In all cases above, the two genes were on separate chromosomes.)

A. What gametes do you get? *(Left Panel of Handout 23A*)

Consider two genes, alpha and beta, that are on the same pair of homologs. In any particular meiosis, we assume there are only two possibilities regarding crossing over (recombination) -- either no crossover occurs in the region between between the 2 genes (arrow (1) on handout, left panel) or there is one crossover in the region between the 2 genes (arrow (2) on handout, left panel); also shown in picture below.

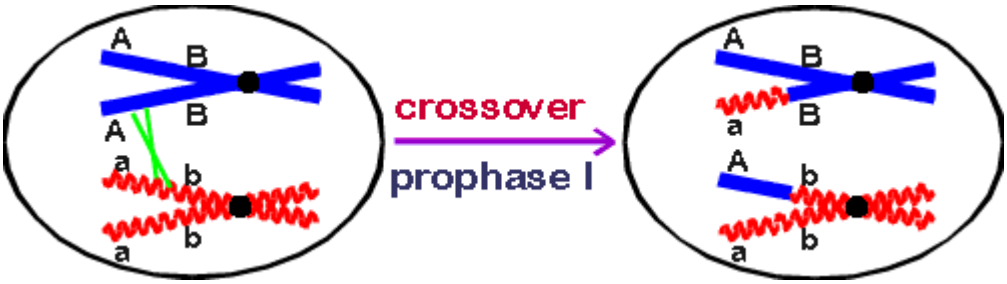
Note: We are ignoring the possibilities of multiple crossovers between the two genes for now.

1. If there is no crossover between genes alpha and beta, what gametes will you get? Only 2 kinds, AB and ab, in equal proportions. (This is assuming that you start with AB on one homolog and ab on the other.)

2. What is 'one crossover event?' A single crossover involves two double stranded DNA molecules. **Both molecules are cut and rejoined (at equivalent spots), so you get two new combinations or recombinant DNA molecules. (We are deliberately ignoring the molecular details of the cut and rejoin event. See a genetics text if you are interested.) We assume most meiotic crossovers are between non-sister chromatids.

3. What if one crossover occurs between the two genes? In that case, the equivalent parts of two homologous (not sister) chromatids are exchanged (see handout) switching an A and an a allele. What gametes will you get this time? The result will be 4 different gametes, two that are AB and ab (the parental combination), and two that are Ab and aB (called recombinants). See Sadava fig. 12.16 (12.18) or Becker fig. 25-16 (20-16).

**Note that crossing over occurs after DNA replication, so there are 2 chromatids per chromosome, and 4 chromatids total. (This is sometimes called the '4 strand stage' where 'strand' = a chromatid or double stranded DNA molecule, NOT a single DNA strand.) Note that only 2 of the 4 chromatids are involved in any one crossover event, but that 4 chromatids are present per cell.



B. Terminology:

1. Parents. If you have a double heterozygote, there are 2 possibilities: you can start with (1) AB/ab or (2) Ab/aB. Letters before slash = alleles on one chromosome (from one parent); alleles after slash = alleles on other homologous chromosome (from other parent). This way of writing the genotype of a diploid is used because it is easy to type. Although it is the standard way of writing genotypes, it is often easier to understand what is happening if you draw out the two homologs and write the alleles in the corresponding positions (loci) on each homolog. Here is an example:

(1) AB/ab		(2) Ab/aB
====A========B====	one chromatid*	====A========b====
====a========b====	other chromatid	====a========B====

***Important:** ====A========B==== or ====A========b==== represents a double stranded DNA molecule = one chromatid with two alleles (A & B in the first case; A and b in the other). However, the ds DNA is sometimes written ------A-------B----- or A B for convenience. since it represents one chromatid. When we write ------A-------B----- (or A B) it represents a ds DNA molecule, NOT one strand of a double stranded DNA.

2. Products of Meiosis.

a. Possibilities: Products of meiosis (gametes or spores) can be classified as parental or recombinant

b. Definitions:

(1). Parental = has alleles that were on one homolog in the doubly heterozygous (dihybrid) parent = combination of alleles you started with (before meiosis).

(2). Recombinant = has a new combination of alleles on one homolog. (How many copies of each homolog per product of meiosis?)

Diploid Parent	Parental Gametes/Spores	Recombinant Gametes/Spores
AB/ab (1 above)	AB and ab	Ab and aB.
Ab/aB (2 above)	Ab and aB	ab and AB

c. Recombination is reciprocal. Every recombination event generates two recombinant chromatids and two recombinant products of meiosis. Therefore, if you look at many gametes or spores from many meioses, the numbers of the two types of recombinants should be equal.

3. How do you get parentals and recombinants from an individual Meiosis? (Handout 23A)

a. How do you get parentals? If the two genes of interest, in this case genes alpha and beta, are relatively close together, most meioses will occur without a crossover between the genes, and most gametes will be the parental type. (In this case, most meioses follow arrow (1) on the left panel of 23A.)

b. How do you get recombinants?

(1). If the two genes of interest are relatively close to each other, we can ignore multiple crossovers -- we can assume there is only 0 or 1 crossover per meiosis between the two genes, as shown on the left panel of handout 23A

(a). There will be 2 recombinant type gametes from each meiosis with a single crossover. (These are the meioses that follow arrow (2).) There will also be two parental gametes if there is only one crossover.

(b). The number of meioses that follow arrow (2) on 23A will increase as the distance between alpha and beta increases. Therefore the number of recombinant gametes will increase.

(2). If the two genes of interest are relatively far apart, then multiple crossovers between them can occur in a single meiosis. This is not shown on handout 23A.

c. Multiple crossovers -- what are the consequences?

(1). Are all crossovers counted? If the two genes of interest are relatively far apart on the same chromosome, then multiple crossovers between them can occur in a single meiosis. However, in that case, not all crossover events may be detected, as some crossovers may return the genes to the original, parental, combination. In this case, the number of ***detectable*** recombinants will not be strictly proportional to the actual amount of recombination.

(2). How many chromatids involved? Each individual crossover event involves two nonsister chromatids. However, If multiple crossover events occur, then more than 2 chromatids may be involved -- the same two chromatids may not be involved in each separate event. Therefore, in a single tetrad (4 products of a single meiosis) all 4 chromatids may be recombinant. However, if you look at many meiotic products from many meioses, not more than ½ of the products can be recombinant. This is explained below.

(***3). Genes on the same chromosome can act unlinked.*** If the genes of interest are far enough apart, so that many multiple crossovers occur between them, then the two genes can be inherited as if they were on separate chromosomes – that is, the numbers of parentals and recombinants will be equal.

To review the expected gametes and terminology, see problem 10-6.

4. Measurement of Linkage

a. Definition of Linkage:

- Genes are said to be linked if there are more parental gametes than recombinant ones. In other words, if A is more likely to go with B or with b, then the genes are linked.
- Linkage is said to be zero when the number of recombinants and parentals is equal. This means A is equally likely to go with B or b.

b. How do you assess linkage?

- Linkage is determined by measuring the amount of recombination.
- The amount of recombination is assessed by examining the products of <u>many</u> meioses, either gametes or spores.
- RF = recombination frequency = % of haploid meiotic products (gametes or spores) that are recombinant
- Higher RF (more recombination) means less linkage, and vice versa.

c. Calculation of RF

RF = % of haploid meiotic products that are recombinant
= (# recombinants/# total meiotic products) X 100
= frequency of recombinants that are recovered

To calculate RF, you need to classify products of meiosis, not zygotes, as recombinant or parental. In cases where gametes cannot be examined directly, the genotypes of the gametes (parentals or recombinants) are inferred from the phenotypes of the zygotes. You do not have to know how to do this; if you are curious, or want to know how to do it, see 23C -- How to do RF's with Diploids. (On line only.)

d. Why use RF? RF is calculated by examining the products of many meioses, not one. RF is used as an indication of the actual incidence of crossing over because we seldom examine the results of a single meiosis. Instead, we look at the total results from many meioses.

e. RF is proportional to distance within the proper range

- What is the 'proper range?' When there is either zero or one crossover per meiosis (in the interval being checked). In this case, what looks like a parental is parental (no crossovers) and what looks like a recombinant is recombinant (result of a single crossover). In this range, RF and distance are strictly proportional.

- In the proper range: 1% RF = 1 unit of distance = 1 centiMorgan (cM) = one map unit.

- What is outside the proper range? When there are multiple crossovers per meiosis (in the interval being checked). In this case, you 'lose' some recombinants -- multiple crossovers can put the genes back into the parental combination, and what looks like a parental may be a multiple recombinant. In this range, RF does not reflect the real incidence of crossing over, and RF and distance are not proportional. The RF will be an underestimate of distance. (How to correct for multiple crossovers is covered in Genetics courses, not here.)

- Practical consequences: RF is usually considered to be proportional to distance for values of RF up to 20-25%. Values above that are generally not accurate because of multiple crossovers. Lower values are more accurate than higher ones, because the possibility of multiple crossovers (in any given interval) decreases with distance.

f. Uses & Limits of RF: (See graph and table on handout 23D)

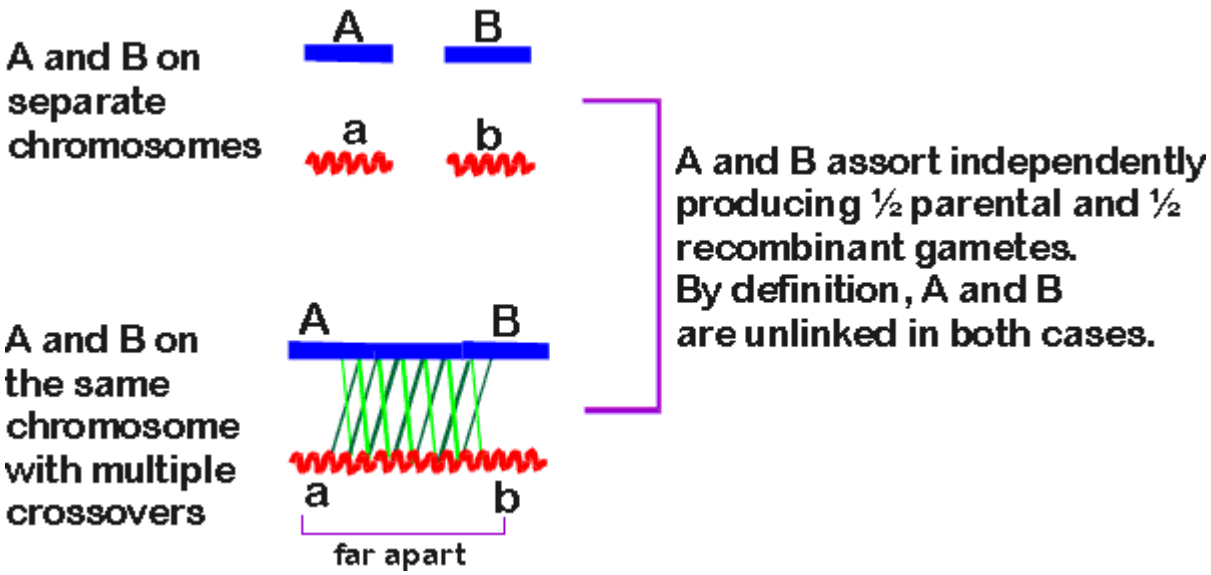
(1). Min RF = 0.

(2). Proportionality: If the two genes are relatively close, the number of recombinants and the RF will be proportional to distance. RF will increase linearly as genes get further apart.

(3). Lack of Proportionality: As distance between the genes increases, the amount of recombination increases, but the RF, which depends on the number of ***detectable*** recombinants from a large number of meioses, will not increase proportionally because of multiple crossovers. See note at ** and above.

(3). Max RF = 50%. Genes on the same chromosome can act unlinked: If the genes of interest are far enough apart, so that multiple crossovers occur between them, the two genes can be inherited as if they were on separate chromosomes (independent assortment). In other words, if you look at many meiotic products from many meioses, no more than ½ of the products will be recombinant (RF = 50%).**

**Another way to see this: Suppose there are multiple crossovers between the genes. An odd number of crossover events will produce a recombinant; an even number of crossovers will switch it back, and produce a parental combination. If there are many crossovers, the number of even crossovers should be about equal to the number of odd crossovers, so the number of parental and recombinant combinations should be about equal. In other words, A is just as likely to end up connected to B or to b.



C. What is the significance of all this? Why does linkage and crossing over matter?

1. Biological significance.

a. Reshuffling. Crossing over allows more combos by reshuffling existing alleles and/or mutations. Without crossing over, the same alleles would remain together on each chromosome (homolog) indefinitely. Allowing more combos provides more raw material for natural selection/evolution.

b. Repair. Crossing over also allows repair -- if a and b are defective alleles, crossing over allows you to get a chromosome with both A and B from Ab plus aB.

c. Distribution -- Crossing over may promote proper pairing of homologs. This helps ensure a proper lineup at homologs at metaphase I, and proper distribution at anaphase I to daughter cells.

Note: In eukaryotes, significant crossing over between homologs occurs only at prophase I of meiosis. It does not occur in somatic cells during in mitosis. Crossing over in meiosis only affects the next generation, not the generation in which it occurs. If crossing over occurs in the germ cells of a multi-cellular organism, the gametes of the organism are changed, but the somatic cells of the organism are unaffected. *(In advanced courses and some laboratories, scientists may consider the effects of crossing over during mitosis in somatic cells, but this is very rare and it does not affect the next generation, so we are ignoring it.)*

2. Historical Significance -- Recombination frequencies were used to estimate distances and make genetic maps. These results indicated genes were arranged in a linear order ('linear' here means 'unbranched' -- the 'linear' chromosomes could be joined into a circle, but it was still unbranched).

3. Current Laboratory and/or medical significance. These days, genetic maps are often used to infer linkage (or to provide ‘positional information) and not the other way around. Maps and recombination frequencies are used to figure out which genes (or 'markers') are close together, and tend to be inherited together. This allows presymptomatic predictions of who will get certain diseases, and the cloning of genes responsible for diseases (when the protein made is unknown). An example is explained briefly below.

VI. An Example of the use of Linkage -- How the linkage of the HD gene was used.

A. Without a marker (linked gene or polymorphism) -- Who will get HD?

1. The Problem. *HD is a dominant disease.* Symptoms of HD don't develop until late adulthood (usually). How to tell who will get the disease?

2. Background: HD is a Case where the disease allele encodes an altered protein (exact function unknown). More details on HD are in notes of Lecture 22 – p.15. The disease causes degeneration of nervous system. (H allele causes disease; h is the normal allele that does NOT cause disease.) What gene or protein involved was unknown until recently, when gene isolated. For more background, see the 'First Mention' column of the *NY Times*, from 12/8/09. The column describes the first time HD was discussed in the newspaper. For more recent developments, see the Wikipedia article on huntingtin (the protein encoded in the HD gene.)

3. Prediction (pre-linkage). Suppose there is an affected parent and normal parent. They have a kid. What is chance kid will have HD? (You figure it out. Is affected parent likely to be Hh or HH?) Without any DNA testing, kid will have to wait 40-50 yrs to find out if s/he has disease or not, well after deciding whether or not to have kids. This is not optimal!

B. With a marker -- Who will get HD?

1. The Idea. Suppose you have a parent who is A H/a h, and gene alpha and the gene responsible for HD are closely linked. Then if a kid inherits allele A, s/he will probably get allele 'H' but if kid inherits the 'a' allele s/he will probably get the 'h' allele. So you can infer which allele of HD the kid got from which allele of the linked gene (A or a) was inherited.

2. What's a marker? In the case described above, the alpha gene acts as a 'marker' of which chromosome the kid inherited. In the case of HD, and many similar diseases the marker was really not a gene but a variable section of the DNA containing a polymorphism. (Actually an RFLP – see handout 18B and below.)

3. Polymorphisms. There are regions of the DNA that are very variable. These regions are said to be polymorphic because they come in multiple forms (with different base sequences). These highly variable regions often occur in sections of the DNA that do not affect phenotype -- in spacers, introns etc. These various forms or polymorphisms are important (even though they have no effect in phenotype) because the variations can be used as the basis of identifications, as previously explained. These differences can also be used as "genetic markers" that can be followed through crosses. You can follow the multiple forms of the DNA sequence through a pedigree just as you would alleles A vs a or B vs b.

4. Haplotypes. A polymorphic section of the DNA comes in multiple versions that are distinguished by their differences in the number and/or lengths of fragments generated by PCR, restriction endonuclease digestion, etc. Each version is often called a haplotype. A haplotype is similar to an allele in that it is one alternate version of a DNA locus. The term "allele" is usually used to refer to a section of the DNA that has a known function; differences in alleles are reflected in differences in phenotype. The term "haplotype" is usually used to refer to a section of the DNA that has no known function (or whose function is irrelevant). Different haplotypes are distinguished by their differences in DNA sequence. The "phenotype" is the genotype = the state of the DNA. Just as different alleles of the same gene can be be followed through crosses, so can different haplotypes.

5. Some details for HD (For more information see HD-details.html or HD-details.pdf.)

a. The polymorphism. The gene that causes HD (when defective) is linked to a polymorphic region with two RFLPs (variable restriction sites) as shown on handout 18B. When the region is cut up with restriction enzymes, there are 4 possible haplotypes (3 are shown on handout 18B). For historical reasons, the 4 haplotypes are known as A, B, C & D. Problem 10-17 shows a pedigree for a family in which both HD and the RFLP's are segregating, that is, being passed from generation to generation. (Note that the problem refers to the marker region with the RFLP as the A/B/C/D gene.) For a diagram of the DNA and more details, see HD-details.html.

b. Role of the RFLP or polymorphic region. A linked polymorphism can be used as a "marker" to indicate which chromosome the child has inherited from the affected parent.

c. An example: Suppose normal parent is AA and affected parent is AB and H, and you know (from family history) it's A H/B h (H allele on same chromosome as A). If kid is AB, will s/he be likely to get HD? If kid is AB, and there was no cross over, kid got chromosome from affected parent with B & h and kid will be okay. If kid is AA, kid will get H (if there was no crossover). Note that you need a family history to know whether affected parent is A H/ B h or A h/ B H.

d. Additional Details

(1). Use of test. A test like the one described above (using linkage between a polymorphic region and the HD gene) was used for predicting inheritance of HD until the gene itself was isolated. (Now you can test for the sequence of the gene itself.) Many other similar procedures have since been developed for predicting inheritance of other genetic diseases.

(2). Phase. Does HD (or whatever disease we are talking about) always go with same particular haplotype of the A/B/C/D region (or whatever the linked marker gene is)? No. In any particular family, HD can go with any version of the A/B/C/D region. It depends which haplotype (A, B, C or D) was on the chromosome at the time the original mutation of h → H occurred.

(3). Effects of crossing over. The closer the linked marker (such as A/B/C/D) is to the disease gene, the more accurate the predictions can be, because the lower the chance of crossing over between the "marker" and the actual **disease gene.**

C. Positional Cloning. Problems of phase and crossing over (see above) can be eliminated by identifying the gene itself. How to do that? The HD gene and many other disease genes have been located by their linkage to a polymorphism. The genes were cloned on the basis of this "positional information" -- their position relative to some variable site. If you want to know the details (FYI), see HD-details.html.

VII. Mapping & Wrap up of Linkage and Crossing over -- Review & Summary

Most of this is review and will not be covered in class. Please read it to be sure you didn't miss anything.

A. Mapping -- How do you measure and use RF?

1. Do the Cross: Cross two double homozygotes to get a heterozygote, and then get heterozygote to go through meiosis and tally products of meiosis.

If you do AAbb X aaBB, heterozygote will be Ab/aB. Gametes will be AB, ab Ab, and aB.
If you do AABB X aabb, heterozygote will be AB/ab. Gametes will be same, but what counts as a 'recombinant' will be different.
If you cross two mutants with mistakes at different points in the DNA, cross will be like this:

Parent 1	X	Parent 2	---------->	Heterozygote
----x----------		----------x----		----x----------
----x----------		----------x----		----------x----

What will gametes be this time?
In all these cases, it is important to keep track of what alleles or mutations are on one homolog and what is on the other (in the parents).
Remember that ---------- represents a double stranded DNA molecule.

2. Calculate RF. Once heterozygote goes through meiosis, classify haploid products of meiosis as parental or recombinant and calculate RF using formula as above.

a. If products of meiosis are spores -- in this case, haploids can be grown by mitosis and their phenotype (& genotype) directly classified as parental or recombinant.

b. If products of meiosis are gametes -- in this case, determining genotypes of products of meiosis cannot be done directly, and you have to look at the diploid organisms that are formed from the gametes. To keep life simple, you use a test cross -- one parent is homozygous recessive for all genes in question. From the phenotypes of the diploid zygotes/organisms you infer the genotypes of the gametes (from the heterozygous parent). This is discussed in detail below and on 23C -- How to do RF's with Diploids..

3. How you make a simple map.

a. The principle: RF is proportional to distance, up to a point, as explained above. Therefore, within the proper range (see below), map distances are additive, just like regular distances.
b. Units: 1% RF corresponds to 1 map unit (in the proper range). One map unit is also known as one centiMorgan or 1 cM.
c. Procedure -- An example: Suppose you want to order genes A, B and C, and you do the appropriate crosses. For example:

AB/ab --(meiosis)--> 14% Ab and aB
Bc/bC --(meiosis)--> 4% BC and bc

Then:
RF between gene A and gene B is 14% and distance is 14 mu or cM.
RF between gene B and gene C is 4% and distance between them is 4 mu or cM.

Where is gene C? Data put gene C 4 cM from B, but C could be on side nearer to A or away from A. (Draw both possibilities.) How do you tell which case it is?
You need to measure the RF between A and C. It will be 10 or 18%, depending on whether order is A-C-B or A-B-C.
For a typical map, see Sadava text p. 258 (248); for a worked out example try fig. 12.22 (in 9th ed only).

4. Why maps are not completely additive. **Short answer:** Because you don't count the product of a double (or quadruple) crossover as a recombinant. Details:

a. How crossovers are counted. Double crossovers and no crossovers both → parental allele combinations in the gametes and are counted as "parentals," so RF's don't really count # switches, but approximate it -- RF's really measure the # of ***detectable*** recombinant combos in products of meiosis. (See legend to graph.)

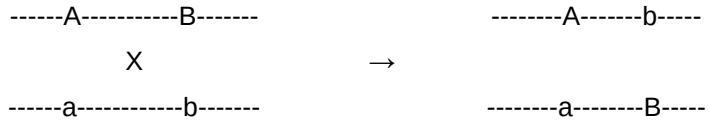
b. When you can ignore multiple crossovers. If you stick to low values of RF and distance, in the linear part of the curve, then you can ignore multiple crossovers, since they are rare. In that case, RF and distance are proportional. For most purposes, values under 25% are considered ok. If you use larger values, you have to correct for multiple crossovers. (How to do so will not be discussed here; it will be covered in genetics courses.)

To go over how to relate RF and map distance, try problem 10-11, parts A-D.

To review complementation vs crossing over and get practice making a map, try problem 11-14. (Note that in this problem, crossing over is occurring between mutations -- locations on the DNA as shown in example above -- as vs. between genes A and B.)

B. When and how does crossing over occur? Some details to review and/or notice. This is for reference & will not be covered in class. However, you are expected to know the information in this section.

We sometimes draw crossing over as if there were two single chromosomes (one chromatid per chromosome) involved like so:



But sometimes we draw crossing over as if occurred when each chromosome is already doubled, and there are 2 chromatids per chromosome. (See pictures below.) Which is it? (Remember that in all these pictures, both above and below, each line represents a double stranded DNA molecule.)

1. Each single crossover event involves one pair of chromatids, but crossing over occurs at a stage (prophase I of meiosis) when there are 4 homologous chromatids, 2 per chromosome.

2. Crossing over happens only at meiosis (pro. I), not mitosis. Only affects next generation, not the generation in which it occurs. If crossing over occurs in the germ cells of a multi-cellular organism, the gametes of the organism are changed, but the somatic cells of the organism are unaffected.

3. Crossing over requires at least two things

a. Enzymes for pairing, cutting, and rejoining of DNA. Some of the enzymes involved are probably the same ones as for repair of damaged DNA. These are NOT restriction enzymes. The enzymes of restriction/modification and the enzymes of recombination are different.

b. Homologous DNA sequences.

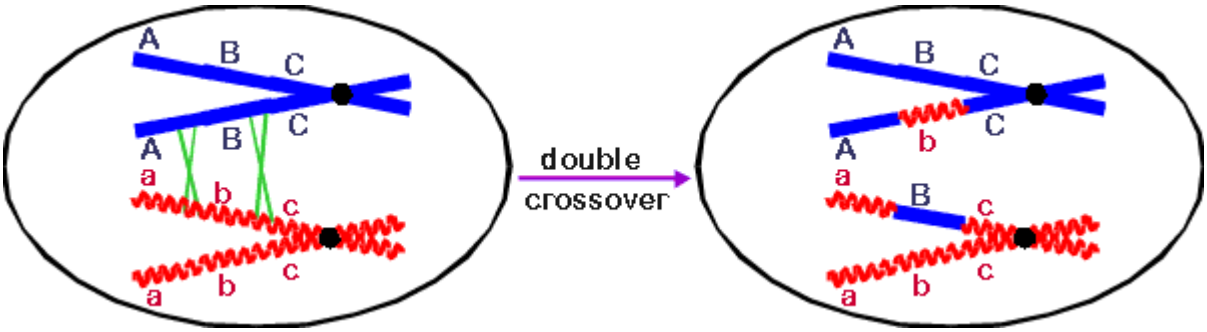
4. Why does it occur only in meiosis? Some of the necessary enzymes for pairing, cutting and rejoining are active only in germ cells at pro. I. (Homologous DNA's are present in all 2N cells. However, homologous chromosomes are paired at Prophase I of meiosis, but not at prophase of mitosis.)

5. Each crossover involves an actual cut and rejoining between two molecules. This has some important consequences.

a. Crossing over is Reciprocal. Every time there is a cut and rejoin that produces, say, Ab, a reciprocal aB is also produced.

b. A double crossover involves 2 separate events. A double crossover requires two separate cut and rejoining events. In the case shown below, both events involve the same pair of chromatids. If ABC and abc crossover to give AbC and aBc, that's a double crossover -- it takes two cut and rejoining events and is rarer than either one alone.

This has practical consequences. It means that you can usually tell double recombinants from single recombinants, even if you don't know the gene order, by seeing which types of recombinants occur less frequently. This often helps to establish gene order. For example, in this case, since the genes are in order of A-B-C, then ABc and abC recombinants should be more common than AbC and aBc. If the order of genes were A-C-B, then AbC -- really ACb -- would be more common than ABc --really AcB -- and so on.)



Note: If crossing over occurs between a circular DNA and a linear fragment, as in bacterial transformation, integration of genes from the fragment also requires a double crossover -- two separate cut and rejoining events. As explained above, double cross over events in a small interval are rare, but with bacteria and their viruses, you can select for the rare individuals who are the products of rare events.

To review crossing over, map units and the effects of double crossovers, see problem 10-8. (Note this problem is about a haploid organism with an unfamiliar life cycle. Be sure you know the answer to part A before continuing.)

C. How do you count recombinants in a diploid organism? (FYI) See handout 23C -- How to do RF's with Diploids (on line). Remember, this is FYI!!

1. The problem: You can't look directly at gametes -- you need to look zygotes (or at diploid organisms that develop from zygotes by mitosis). Then you have to infer the genotype of the gametes from the phenotype of the zygotes.

2. The solution: To make the results easier to analyze, you usually make one parent doubly heterozygous and one doubly homozygous recessive. (See note at ** below.) In other words, you do a test cross and analyze zygotes. (Test cross = Any cross where one parent is homozygous recessive for all genes under consideration.) Advantages of a this particular set up:

a. You don't have to worry about crossing over in the homozygous parent. If it occurs, it has no effect on the gametes.

b. Homozygous parent can contribute only recessive alleles.

(1). Any dominant allele in the zygote came from the heterozygous parent. Therefore you can deduce genotype of zygote from phenotype of the zygote.

(2).You can easily classify each gamete from the heterozygous parent as parental or recombinant. You can figure out genotype of gamete from genotype of the zygote.

3. Example: Suppose you do the cross Ab/aB X aabb, and you get A_B_ pheno offspring. Then

a. What goes in the blanks? Has to be a and b.

b. How was the zygote formed -- from recombinant or parental gamete from Ab/aB? Must be AB gamete from double heterozygote parent met ab from homozygous recessive parent. (Is this a recombinant or a parental??)

4. More info: Details of how to measure the RF in a diploid are explained on Handout 23C = How to measure RF's with diploids. For an example of such a cross, see Sadava figs 12-15 & 12-17 (12.17 & 12.19). These are both about the same cross. For an example of how you use similar results to make a map, see fig. 12.22 (9th ed).

**Note: If you did a cross with fruit flies in intro lab, you may have set it up differently because there is no crossing over in male flies.

You do not have to know how to measure RF in diploids. If you want to try it anyway, see problems 10-10, 10-11E, and 10-12 to 10-14. (There are additional problems on this in 10 and 10R.) If you have the 10th ed. of Sadava, see Working with Data, p. 248. Note that all editions of Sadava have genetics problems within, or at the end of, the genetics chapter.

Population Genetics and Evolution will be introduced if we have time. No problems on this topic will be assigned. The material is posted online for anyone who is interested. You are not responsible for this material, but it is highly recommended.