ANNOUNCEMENTS

- I'VE PUT ALL THE COURSE MATERIALS AT MY OWN FACULTY WEBSITE:

  HTTP://WWW.SJSU.EDU/PEOPLE/RACHAEL.FRENCH/

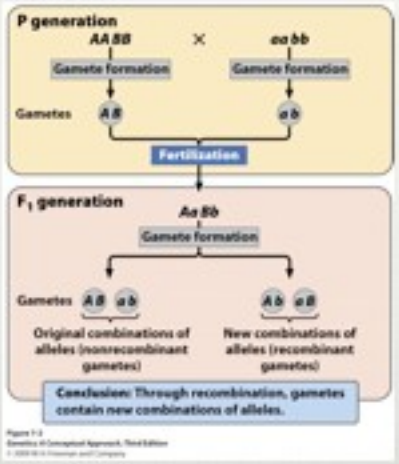
LECTURE 6: LINKAGE AND GENE MAPPING
JULY 13, 2011

1. Linkage (Chapter 7)
   - Recombination
   - Linked Genes
   - Crossing Over Between Linked Genes
     - Recombination Frequency
     - Arrangement of Alleles
   - Inter- vs. Intrachromosomal Recombination
   - Intrachromosomal Recombination Results from Physical Exchange Between Chromosomes
   - Predicting the Outcome of Crosses with Linked Genes
   - Sturtevant and Genetic Maps

LECTURE 6: LINKAGE AND GENE MAPPING
JULY 13, 2011

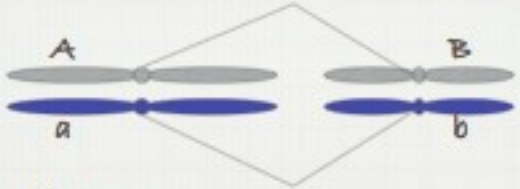
1. Linkage (Chapter 7)
   - Constructing a Genetic Map based on Recombination Frequencies
     - Two-point testcrosses
     - Three-point testcrosses
   - Interference and Coefficient of Coincidence
   - Mapping Human Genes
   - Mapping with Molecular Markers

RECOMBINATION: THE SORTING OF ALLELES INTO NEW COMBINATIONS

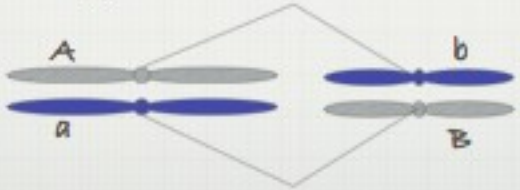


- When one of the F_2 progeny reproduces, the combination of alleles in its gametes may differ from the combinations in the gametes from its parents

RECOMBINATION CAN RESULT FROM INDEPENDENT ASSORTMENT



Is as likely as:



Ab or aB gametes are as likely as AB or ab gametes

RECOMBINATION CAN *ALSO* HAPPEN WHEN GENES ARE LINKED

- Linked genes
  - Are located on the same chromosome
  - Do not assort independently - they tend to be inherited together.

Review:

- Principle of Segregation: Each diploid organism has two alleles at a locus that separate in meiosis, one allele going into each gamete.
- Principle of Independent Assortment: The two alleles at a locus act independently of alleles at other loci.
- Recombination: the sorting of alleles into new combinations.

MENDEL'S TWO PRINCIPLES AND THE CHROMOSOME THEORY OF HEREDITY

- Mendel had no idea of what biological processes resulted in the data underlying his principles of heredity.
- In 1903, Walter Sutton proposed a biological basis for Mendel's principles, called the Chromosome Theory of Heredity (Ch 3)
  - Genes are found on chromosomes

MENDEL'S TWO PRINCIPLES AND THE CHROMOSOME THEORY OF HEREDITY

- To restate Mendel's principles in relation to Chromosome Theory of Heredity:
- The Principle of Segregation
  - A diploid organism has two alleles for a trait, each located at the same locus (position) on two homologous chromosomes.
  - Homologous chromosomes segregate during meiosis, with each gamete receiving one homolog and all of its alleles.

MENDEL'S TWO PRINCIPLES AND THE CHROMOSOME THEORY OF HEREDITY

- The Principle of Independent Assortment

  During meiosis, each pair of homologous chromosomes assorts independently of other homologous pairs.

LINKED GENES

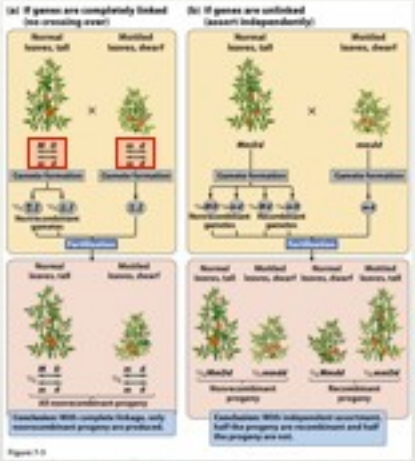
- The number of chromosomes is much smaller than the number of genes
- Therefore some genes must be present on the same chromosomes

  Linked genes:

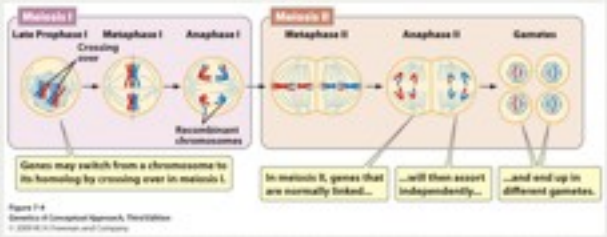
  - Genes that are located close together on the same chromosome.
  - Travel together during meiosis, usually arriving in the same gamete.
  - Should not (usually) assort independently.

- Genes on the same chromosome belong to the same linkage group.

COMPLETE LINKAGE VS. UNLINKED GENES THAT ASSORT INDEPENDENTLY



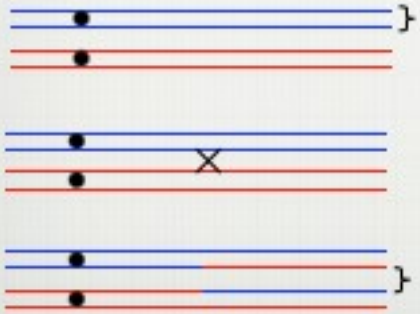
CROSSING OVER CAN PRODUCE RECOMBINATION BETWEEN LINKED GENES



- Genes occasionally switch from one homologous chromosome to another through the process of crossing over.
  - Genes that exhibit crossing over are incompletely linked.
  - Recombination takes place in Prophase I of meiosis.

LINKAGE AND CROSSING OVER:

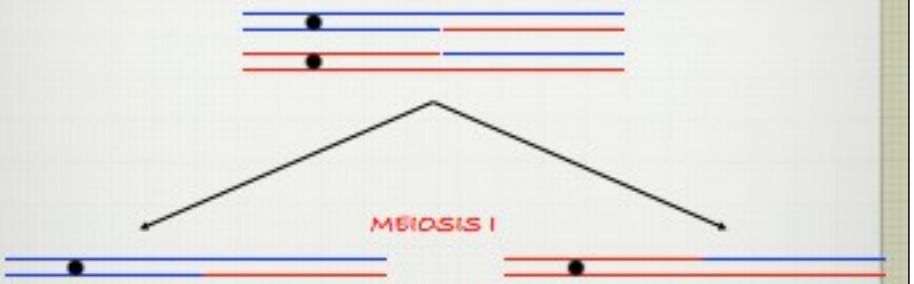
Crossing over involves the breakage and rejoining of homologs



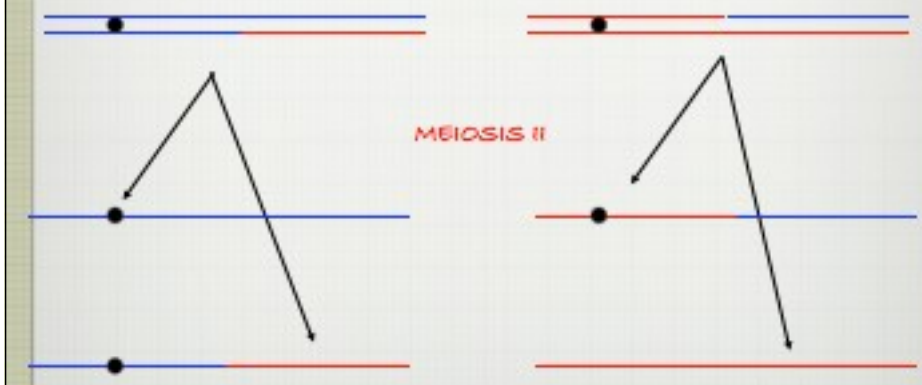
Two sister chromatids

Recombinant chromatids

RESULTS IN MEIOSIS:



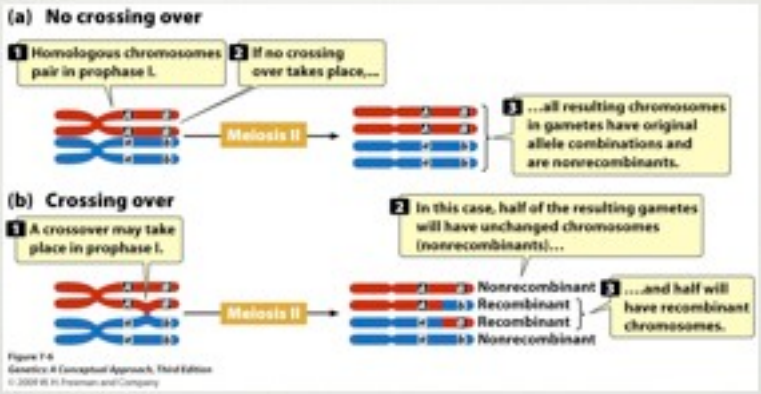
RESULTS IN MEIOSIS:



SO:

- A single recombination event during meiosis results in four types of gametes (for that chromosome):
  - Two parental (non-recombinant chromosomes)
  - Two recombinant chromosomes (reciprocal)

A SINGLE CROSSOVER PRODUCES HALF NONRECOMBINANT GAMETES AND HALF RECOMBINANT GAMETES

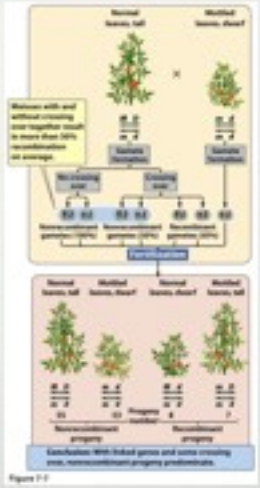


IMPORTANT THINGS TO NOTICE ABOUT CROSSING OVER

- When there is a single crossover during meiosis, half the gametes are recombinants and half are nonrecombinants.
- The total percentage of recombinant gametes is always half the percentage of meioses in which crossing over takes place.
- If crossing over between two genes takes place in every meiosis, only 50% of the resulting gametes will be recombinants.
- The frequency of recombinant gametes is always half the frequency of crossing over
- The maximum proportion of recombinant gametes is 50%.

EXAMPLE OF CROSSING OVER BETWEEN LINKED GENES

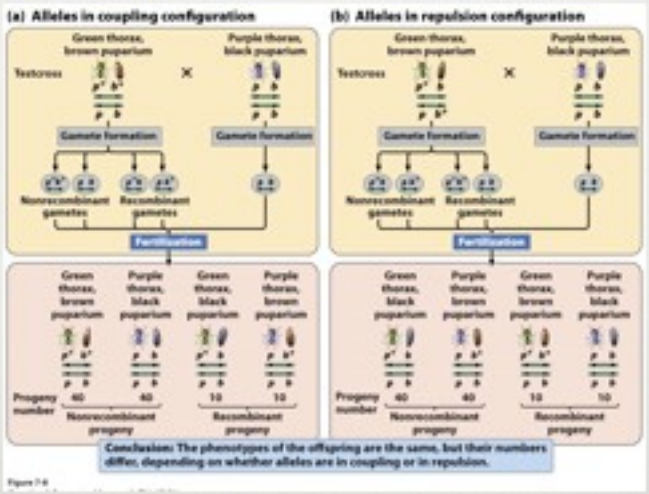
- If the genes were unlinked, we would expect a 1:1:1:1 phenotypic ratio in the progeny.
- When linked genes undergo some crossing over, we observe:
  - mostly nonrecombinant progeny
  - fewer recombinant progeny.
- The genes do not assort independently – they are linked.



CALCULATING RECOMBINATION FREQUENCY

- Recombination Frequency is the percentage of recombinant progeny produced in a cross
- Recombination Frequency = $\frac{\text{Number of recombinant progeny}}{\text{Total number of progeny}} \times 100\%$
- e.g. $\frac{8+7}{55+53+8+7} \times 100\% = 12.2\%$ (or 0.122)

THE ARRANGEMENT OF ALLELES DETERMINES THE OUTCOME OF THE CROSS



TWO TYPES OF RECOMBINATION

Recombination produces new allele combinations in gametes

1. Interchromosomal Recombination
   - Between genes on different chromosomes
   - Arises from independent assortment - random segregation of chromosomes in anaphase I of meiosis
   - Mendel discovered while studying dihybrid crosses
   - Produces 50% nonrecombinant gametes and 50% recombinant gametes

TWO TYPES OF RECOMBINATION

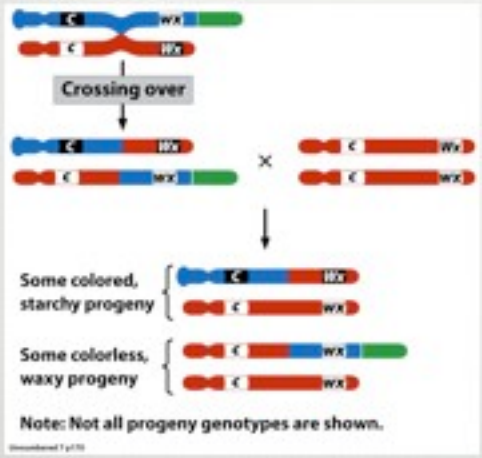
Recombination produces new allele combinations in gametes

2. Intrachromosomal Recombination
   - Between genes located on the same chromosome
   - Arises from crossing over - the physical exchange of DNA in prophase I of meiosis
   - Produces fewer than 50% recombinant gametes.
     - Unless genes are very far apart on the same chromosome
     - In that case they assort independently, as if they were on different chromosomes

INTRACHROMOSOMAL RECOMBINATION RESULTS FROM PHYSICAL EXCHANGE

- McClintock and Creighton discovered a strain of corn that had an abnormal chromosome 9, containing a densely staining knob at one end and a small piece of another chromosome attached to the other end.
  - Realized they could use this knob to visually distinguish the two members of a homologous pair.
  - Studied the inheritance of two traits determined by linked genes on chromosome 9.
    - C = colored kernels, c = colorless kernels
    - Wx = starchy kernels, wx = waxy kernels

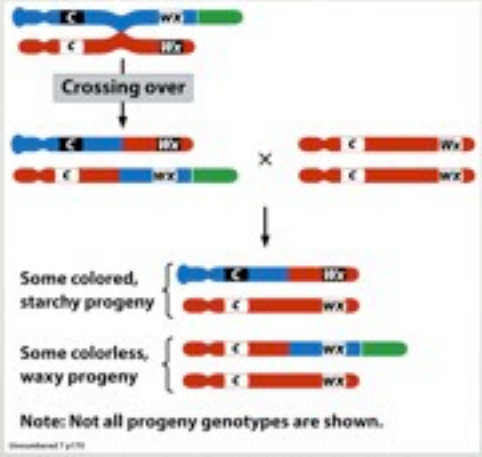
CREIGHTON AND MCCLINTOCK'S EXPERIMENT



Their Prediction:

- If crossing over entails physical exchange between chromosomes, then the colorless, waxy progeny resulting from recombination should have a chromosome with an extra piece but not a knob.

CREIGHTON AND MCCLINTOCK'S EXPERIMENT



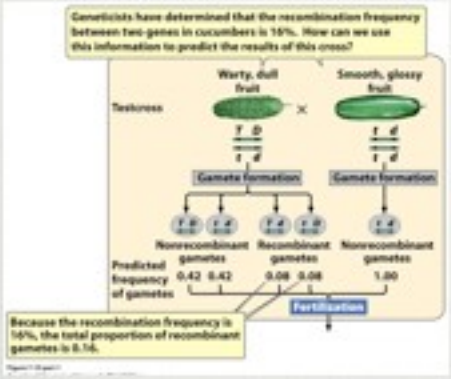
Their Prediction:

- Some colored starchy progeny should possess a knob, but not the extra piece.

IMPLICATIONS OF CREIGHTON AND MCCLINTOCK'S WORK

- Correlated physical exchange of material with genetic exchange of information
  - by using homologous chromosomes that were visually distinguishable.
- First evidence in plants that corresponding segments of genetic material on the chromatids of homologous chromosomes are able to cross over during meiosis.

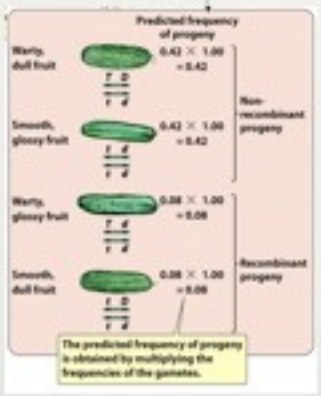
PREDICTING THE OUTCOMES OF CROSSES WITH LINKED GENES



The recombination frequency allows a prediction of the proportions of offspring expected for a cross entailing linked genes

1. Calculate gamete frequency

PREDICTING THE OUTCOMES OF CROSSES WITH LINKED GENES



The recombination frequency allows a prediction of the proportions of offspring expected for a cross entailing linked genes

2 Multiply gamete frequencies (of two parents) to predict frequency of offspring

GENE MAPPING

- An undergraduate researcher figured out how to map genes
  - Thomas Hunt Morgan was a very famous Drosophila geneticist who taught a biology class at Columbia in the early 1900's.
  - Two undergraduates in his class in 1909, sophomore Alfred Henry Sturtevant and freshman Calvin Bridges, asked if they could work in his lab.

GENE MAPPING

- Morgan and his students made some of the first observations that some genes did not segregate randomly, but were inherited together.
  - Morgan hypothesized:
    - These genes were on the same chromosome and therefore traveled together during meiosis.
    - Closely linked genes (rarely shuffled by recombination) lie close together.
    - Loosely linked genes (more frequently shuffled by recombination) lie further apart.

STURTEVANT CONSTRUCTED THE FIRST CHROMOSOME MAP

- Sturtevant hypothesized that variation in the strength of linkage indicated how genes are positioned along a chromosome, providing a way to map genes.
  - Worked out the first genetic map, which was remarkably accurate.

STURTEVANT CONSTRUCTED THE FIRST CHROMOSOME MAP

- Went on to become a geneticist, elucidating:
  - Gene mapping, basic mechanisms of inheritance in Drosophila, cytology, embryology, and evolution

RATES OF RECOMBINATION ARE ROUGHLY PROPORTIONAL TO PHYSICAL DISTANCE

- Hypothesis:
  - Crossover events occur at random along the chromosome
  - Two genes that lie far apart are more likely to undergo a crossover than are two that lie close together
  - Recombination frequencies can provide
    - A way to determine the order of genes along a chromosome
    - An estimate of relative distances between the genes

CONSTRUCTING GENETIC MAPS

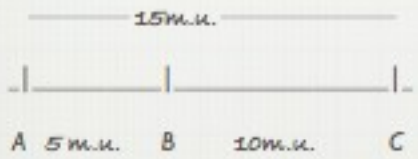
- Genetic maps are chromosome maps calculated by using the genetic phenomenon of recombination
  - Distances are measured in map units (m.u.), also called centiMorgans (cM)
  - 1 m.u. = 1% recombination
- Physical maps are chromosome maps calculated by using physical distances along the chromosome
  - Distances are expressed as numbers of base pairs

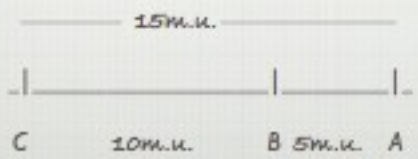
RECOMBINATION MAPPING

- Distances are approximately additive:
  - Distance from gene A to gene B is 5 m.u.
  - Distance from gene B to gene C is 10 m.u.
  - Distance from gene A to gene C is 15 m.u.
  - Then gene B must be located between genes A and C

RECOMBINATION MAPPING

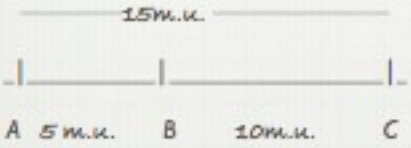
- We can draw two equivalent genetic maps:





QUESTION

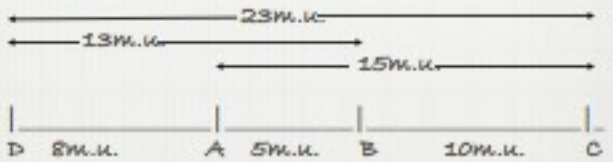
What if we add information about a fourth gene to our genetic map?



- Gene pair — Recombination Frequency (%)

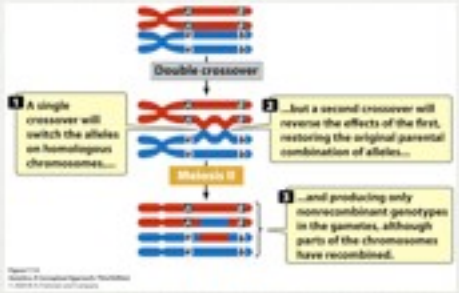
A and D	8
B and D	13
C and D	23

QUESTION



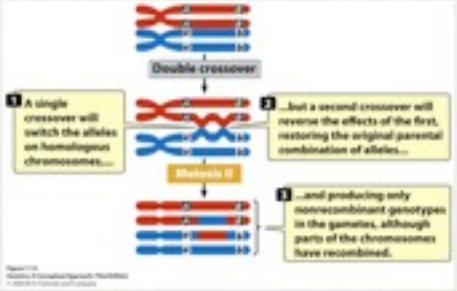
We could also draw this map with from right to left (i.e. C, B, A, D).

CONSTRUCTING RECOMBINATION MAPS



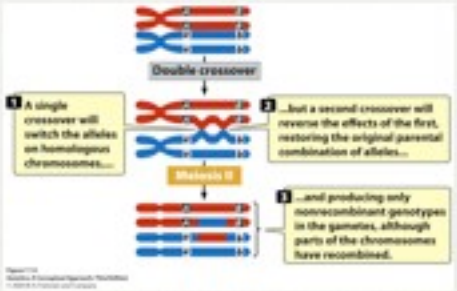
- We cannot distinguish between genes on different chromosomes and genes located far apart on the same chromosome that exhibit 50% recombination.

CONSTRUCTING RECOMBINATION MAPS



- A testcross for two genes that are relatively far apart on the same chromosome tends to underestimate the physical distance, because double crossovers are not detected

CONSTRUCTING RECOMBINATION MAPS



- Double crossovers are less frequent between genes that are close, so genetic maps based on short distances are usually more accurate.

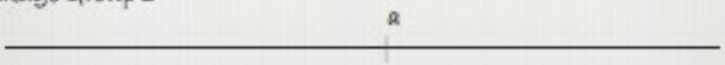
CONSTRUCTING A GENETIC MAP

- Conduct a series of testorosses between pairs of genes
- Determine the recombination frequencies between them
- Two-point testoross (or two-point oross for short): a testoross between two genes

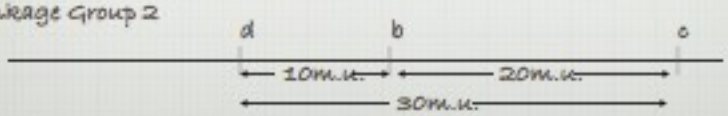
CONSTRUCTING A GENETIC MAP

Gene loci in testoross	Recombination Frequency (%)
a and b	50
a and c	50
a and d	50
b and c	20
b and d	10
c and d	28

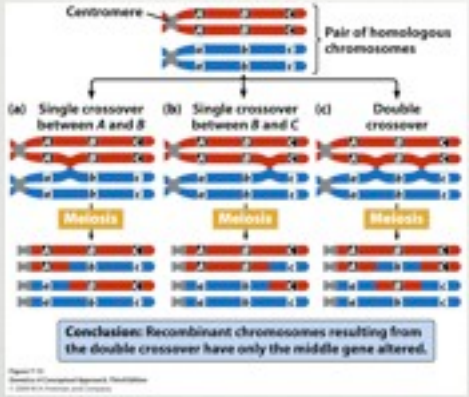
Linkage Group 1



Linkage Group 2

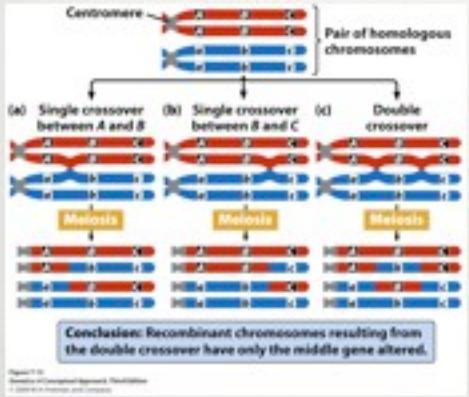


USING A THREE-POINT TESTCROSS TO MAP THREE LINKED GENES



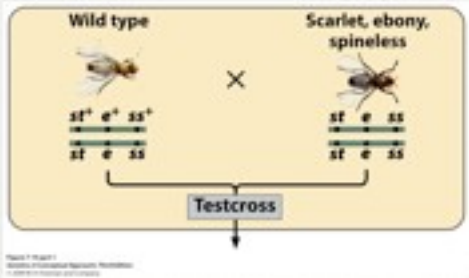
- Numerous two-point crosses are not efficient.
- Three-point testcross, or three-point cross, a testcross for three genes.
  - Establishes the order of genes in a single experiment

USING A THREE-POINT TESTCROSS TO MAP THREE LINKED GENES



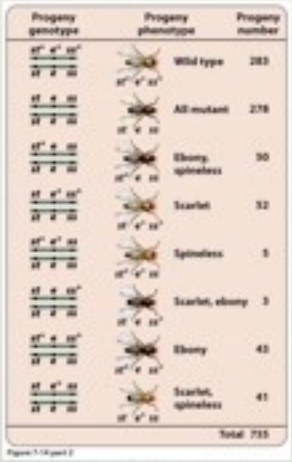
- Three-point testcross, or three-point cross, a testcross for three genes.
  - Some double crossovers can usually be detected, providing more accurate map distances.

MAPPING GENES WITH A THREE-POINT CROSS



- The order of genes is arbitrary until we determine which is the middle gene
- Alleles are in coupling configuration, but can be done with alleles in repulsion.

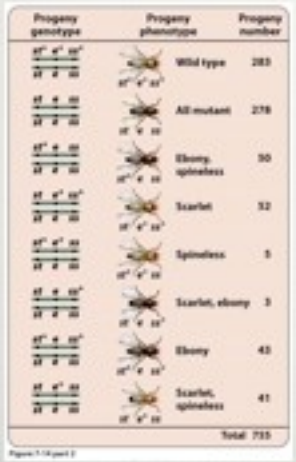
DETERMINING GENE ORDER



Identify the middle locus by examining the double-crossover progeny

1. Determine which progeny are nonrecombinants (parental)
   - 2 most numerous classes
2. Identify the double-crossover progeny
   - 2 least numerous classes - the probability of double crossovers is always lower than single crossovers

DETERMINING GENE ORDER

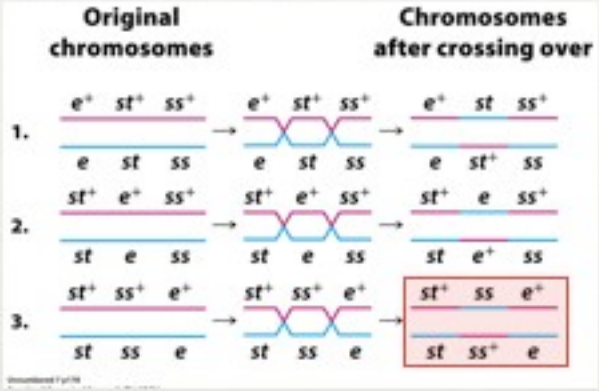


3. To determine which gene is in the middle:

   a) Draw the chromosome of the heterozygous parent with all three possible gene orders

   b) See if a double-crossover produces the combination of genes observed in the double crossover progeny

DETERMINING GENE ORDER



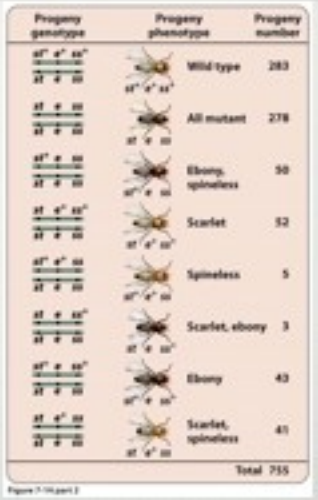
A QUICK SYNTHESIS OF THIS INFORMATION

- Identify the classes with the highest progeny – nonrecombinants.
- Identify the classes with the fewest progeny – double crossovers.

Progeny genotype	Progeny phenotype	Progeny number
	Wild type	283
	All mutant	278
	Ebony, spineless	50
	Scarlet	52
	Spineless	5
	Scarlet, ebony	3
	Ebony	43
	Scarlet, spineless	41
	Total	755

A QUICK SYNTHESIS OF THIS INFORMATION

- In double crossovers, only the middle alleles differ from the nonrecombinants.
- In this case, ss must be in the middle.
- Rewrite the order of the genes for each progeny class to determine where the crossovers took place.



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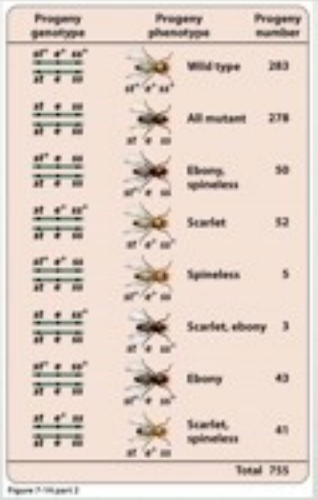
A QUICK SYNTHESIS OF THIS INFORMATION

- The four remaining classes are two different types of single crossovers.
  - Between st and ss
  - Between ss and e



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CALCULATING THE RECOMBINATION FREQUENCIES

- To determine the map distances, calculate the recombination frequencies between each pair of loci.
- Add up all the crossovers (both single and double) that take place between two genes, and divide this number by the total number of progeny from the cross (and multiply by 100%)
- e.g. Crossovers between:

  a) st and ss = $\underline{st^+/ss\ e} + \underline{st/ss^+\ e^+} + \underline{st^+/ss/e^+} + \underline{st/ss^+/e}$

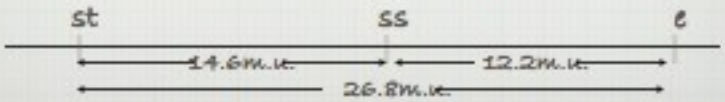
  (Only alleles inherited from the heterozygous parent are listed)

CALCULATING THE RECOMBINATION FREQUENCIES

$$\text{Recombination frequency} = \frac{50 + 52 + 5 + 3}{755} \times 100\% = 14.6\%$$

- ss and e = $st^{+}\ ss^{+}/e + st\ ss/e^{+} + st^{+}/ss/e^{+} + st/ss^{+}/e$

$$\text{Recombination frequency} = \frac{43 + 41 + 5 + 3}{755} \times 100\% = 12/2\%$$



INTERFERENCE AND COEFFICIENT OF COINCIDENCE

- Theoretically, we should be able to calculate the proportion of double-recombinant gametes by using the multiplication rule of probability
- Proportion (probability) of gametes with double crossovers between st and e is equal to:
  - the probability of recombination between st and ss multiplied by the probability of recombination between ss and e, or 0.146 x 0.122 = 0.0178
- Multiply this probability by the total number of progeny gives the expected number of double-crossover progeny, or 0.0178 x 755 = 13.4.

INTERFERENCE AND COEFFICIENT OF COINCIDENCE

- Only 8 crossovers were observed - why?
  - Crossovers aren't independent events - the occurrence of one crossover tends to inhibit additional crossovers in the same region of the chromosome
  - Double-crossovers are thus less frequent than expected.
- The degree to which one crossover interferes with additional crossovers in the same region is termed interference.

CALCULATING INTERFERENCE

1. Determine the coefficient of coincidence, the ratio of observed double crossovers to expected double crossovers.

$$\text{Coefficient of coincidence} = \frac{\text{number of observed double crossovers}}{\text{number of expected double crossovers}}$$

$$= \frac{5 + 3}{0.146 \times 0.122 \times 755} = 0.6$$

We are only observing 60% of the double crossovers that we expected.

observed, because of interference.

CALCULATING INTERFERENCE

2. Interference = 1 - coefficient of coincidence = 1 - 0.6 = 0.4

40% of the double-crossover progeny expected will not be observed, because of interference.

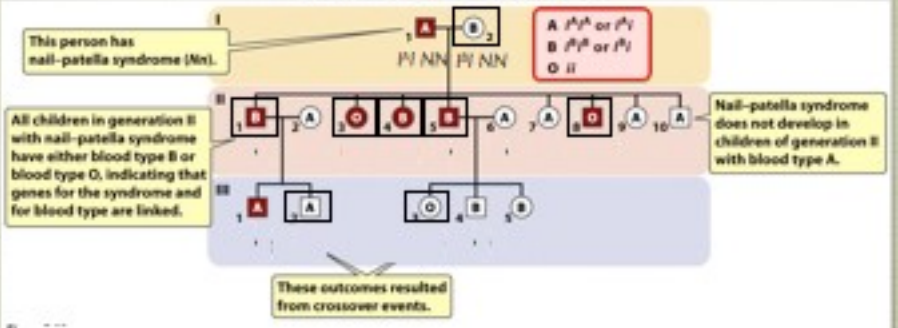
MAPPING HUMAN GENES

- Efforts in mapping human genes hampered by:
  - Inability to perform desired crosses
  - Small number of progeny in most families
  - Restricted to analysis of pedigrees, which are often incomplete and provide limited information
    - use data from several families to test for independent assortment

MAPPING HUMAN GENES

- Nonetheless, many genes have been mapped by using pedigree data to analyze linkage.
  - An early example was linkage between the loci for:
    - Nail-patella syndrome:
      - Autosomal dominant disorder
      - Abnormal fingernails and absent or rudimentary kneecaps
    - ABO blood type:
      - Autosomal locus with multiple alleles

NAIL-PATELLA SYNDROME AND ABO BLOOD TYPES

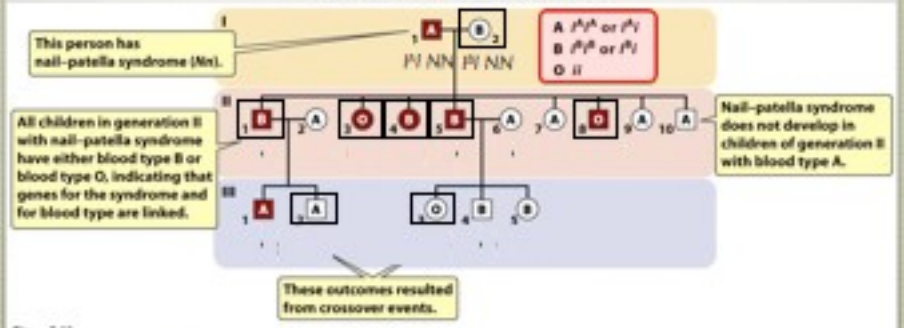


Nail-patella syndrome is rare. So we can assume:

1. People having this trait are...

   heterozygous for the uncommon dominant allele (Nn)

NAIL-PATELLA SYNDROME AND ABO BLOOD TYPES



2. unaffected people are...

homozygous for the wild type allele (nn)

*Remember that the wild type allele is the most common allele within the population.

DETERMINING IF LOCI ARE LINKED

- In the previous pedigree,
  - 13 children from matings in which the genes encoding nail-patella syndrome and ABO blood types segregate
  - 2 are recombinants
  - RF=2/13? Sample size too small to say

DETERMINING IF LOCI ARE LINKED

- Disease-causing alleles are mapped by LOD (logarithm of odds) analysis
  - examines the probability of having linkage at a particular RF compared to the probability of independent assortment.
- A LOD score of 3 indicates linkage with the specified recombination is 1000 times as likely to produce what was observed as independent assortment.

MAPPING WITH MOLECULAR MARKERS

- Gene mapping was originally limited by the availability of genetic markers, variable genes with easily observable phenotypes.
  - e.g. flower color, seed shape, blood types, etc.
- Molecular techniques in the 1980s: examine variations in DNA
  - Almost unlimited number of markers.

MOLECULAR MARKERS

1. Restriction Fragment Length Polymorphisms (RFLPs)
   - Variations in in DNA sequence detected by cutting the DNA with restriction enzymes
2. Microsatellites
   - Variable numbers of short DNA sequences repeated in tandem
3. Single Nucleotide Polymorphisms (SNPs)
   - Individual variations in the DNA nucleotides

MOLECULAR MARKERS

- Gene mapping with molecular markers is done in the same manner as mapping with traditional phenotypic markers
  - Cosegregation of two or more markers is studied
  - Map distances are based on rates of recombination between markers.

FOR NEXT TIME:

- READ CHAPTER 8 (BACTERIAL AND VIRAL GENETICS)