

## Genetics 201 2003 Problem Set 3

Due at start of lecture on Friday Nov. 14

Turn in written answers for problems 1, 2, and 5.

No late problem sets will be accepted.

1). You obtain two lines of *Drosophila*, one having light green eyes and the other having bright orange eyes. (Remember that wild type *Drosophila* have deep red eyes.) When you cross a green female with a orange male, you obtain 251 wild type females and 248 green males in the F1 generation. When you cross F1 males and females, you obtain the following F2 phenotypes: 260 wild type females, 253 green females, 77 wild type males, 179 green males, 183 orange males, and 80 brown-eyed males (brown is a new phenotype).

Explain these results in terms of the number of genes that determine eye color in these crosses, and whether they are on the X or on an autosome. Include any relevant information on linkage and distances.

2). Screening for tumor metastasis in fruit flies?? In a recent paper published in *Science*, two Yale investigators performed a genetic screen in *Drosophila* to identify mutations sufficient to cause noninvasive tumors of the eye disc to invade neighboring tissues. From their screen, they observe that cooperation between oncogenic *Ras<sup>VI2</sup>* expression and inactivation of any one of a number of genes affecting cell polarity leads to metastatic behavior.

a). Inspired by their work, you plan to perform a screen for recessive mutations on chromosome 2 that alone are sufficient to cause tumor metastasis. You may assume that these mutations will not affect viability or fertility. To test for metastatic potential, you plan to use an assay like that used by the Yale group, in which a normally noninvasive tumor is injected into the eye disc, then scored for whether or not it invades surrounding tissues.

Using the metastasis assay, EMS, a wild-type strain, and the strain listed below, write out the crosses you will perform to isolate the desired mutations. Please include the gender and number of flies for each cross and how you will identify the desired progeny at each step.

Strain: *Pm / CyO*

Description: *Pm* is a chromosome 2 marker that has a dominant plum-eyes phenotype.

*CyO* is a chromosome 2 balancer that has a dominant curly-wing phenotype. *CyO/CyO* is homozygous lethal.

b). You are fascinated by one of the mutants found in your screen, as its phenotype causes extreme tumor metastasis. You name this mutant *x<sub>tm</sub>* and decide to map its location on chromosome 2. Your undergrad has isolated *x<sub>tm</sub>* in a strain with two other recessive markers on chromosome 2, bent bristles (*bb*) and heart-shaped wings (*hw*). You perform the following cross and analyze the F2 for their bristle and wing phenotypes and metastatic potential:

P	<i>x<sub>tm</sub> bb hw / x<sub>tm</sub> bb hw</i> males	x	WT females
F1	F1 females	x	<i>x<sub>tm</sub> bb hw / x<sub>tm</sub> bb hw</i> males
F2	analyze the 700 F2 phenotypes:		
	305	wild-type	
	4	no metastasis, normal bristles, heart-wings	
	12	metastasis, normal bristles, heart-wings	
	299	metastasis, bent bristles, heart-wings	
	5	metastasis, bent bristles, normal wings	
	32	no metastasis, bent bristles, heart-wings	
	14	no metastasis, bent bristles, normal wings	
	29	metastasis, normal bristles, normal wings	

Draw a genetic map that shows the order of the *x<sub>tm</sub>*, *bb*, and *hw* loci and the distances between them.

c). To further characterize *x<sub>tm</sub>*, you wish to classify it according to its mutation type (neomorph, hypermorph, etc.). You cross *x<sub>tm</sub>* mutant flies to a panel of different strains with or without the corresponding wild-type region (*x<sub>tm</sub><sup>+</sup>*), and test the eyes of the progeny using your metastasis assay. You observe the following:

<i>x<sub>tm</sub> / x<sub>tm</sub></i>	extreme metastasis
<i>x<sub>tm</sub> / Df (x<sub>tm</sub><sup>+</sup>)</i>	very extreme metastasis
<i>Df (x<sub>tm</sub><sup>+</sup>) / Df (x<sub>tm</sub><sup>+</sup>)</i>	extremely extreme metastasis
<i>x<sub>tm</sub> / x<sub>tm</sub><sup>+</sup></i>	no metastasis

What is the most likely nature of the *x<sub>tm</sub>* mutation, and why?

3a). During your rotation in a *Drosophila* lab, you wind up studying mutations in genes that cause the flies that carry them to have an altered appearance: *ro* = round eyes, *sw* = small wing, *ab* = abnormal abdomen, *br* = bent bristles, *cn* = cinnamon eyes. All of the loci are linked on one arm of chromosome 2.

In order to obtain the location of the *ab*, *br* and *cn* loci relative to each other on chromosome 2, you cross a wild type male to an *ab*<sup>-</sup>, *br*<sup>-</sup>, *cn*<sup>-</sup> female. You then cross F1 females to an *ab*<sup>-</sup>, *br*<sup>-</sup>, *cn*<sup>-</sup> males and count the progeny:

<i>ab</i> <sup>+</sup> , <i>br</i> <sup>+</sup> , <i>cn</i> <sup>+</sup>	327
<i>ab</i> <sup>-</sup> , <i>br</i> <sup>-</sup> , <i>cn</i> <sup>-</sup>	316
<i>ab</i> <sup>+</sup> , <i>br</i> <sup>-</sup> , <i>cn</i> <sup>-</sup>	2
<i>ab</i> <sup>-</sup> , <i>br</i> <sup>+</sup> , <i>cn</i> <sup>+</sup>	2
<i>ab</i> <sup>+</sup> , <i>br</i> <sup>-</sup> , <i>cn</i> <sup>+</sup>	20
<i>ab</i> <sup>-</sup> , <i>br</i> <sup>+</sup> , <i>cn</i> <sup>-</sup>	23
<i>ab</i> <sup>+</sup> , <i>br</i> <sup>+</sup> , <i>cn</i> <sup>-</sup>	32
<i>ab</i> <sup>-</sup> , <i>br</i> <sup>-</sup> , <i>cn</i> <sup>+</sup>	<u>28</u>
	750

From other three point crosses, you determine that *ro* lies midway between *cn* and *ab* and that *sw* is 6.8 map units from *br*, and is farthest from the centromere.

Determine the relative location and map distance between the five loci and show their location relative to the centromere.

b). You decide that you would like to compare your mapping results with those of a collaborating lab that is located near the nuclear plant in Los Alamos, NM. You cross a homozygous wild type female of yours to a mutant male of theirs and then cross the F1 females to your mutant males. Much to your surprise, you obtain the following recombinant progeny classes with approximate frequencies noted:

Rare (25-75 of each class below / 1000 progeny)

*ab*<sup>+</sup>, *br*<sup>+</sup>, *cn*<sup>-</sup>, *ro*<sup>+</sup>, *sw*<sup>+</sup>  
*ab*<sup>-</sup>, *br*<sup>-</sup>, *cn*<sup>+</sup>, *ro*<sup>-</sup>, *sw*<sup>-</sup>  
*ab*<sup>+</sup>, *br*<sup>+</sup>, *cn*<sup>+</sup>, *ro*<sup>+</sup>, *sw*<sup>-</sup>  
*ab*<sup>-</sup>, *br*<sup>-</sup>, *cn*<sup>-</sup>, *ro*<sup>-</sup>, *sw*<sup>+</sup>

Very Rare (1-5 or fewer of each class below / 1000 progeny)

*ab*<sup>+</sup>, *br*<sup>+</sup>, *cn*<sup>-</sup>, *ro*<sup>+</sup>, *sw*<sup>-</sup>  
*ab*<sup>-</sup>, *br*<sup>-</sup>, *cn*<sup>+</sup>, *ro*<sup>-</sup>, *sw*<sup>+</sup>  
*ab*<sup>-</sup>, *br*<sup>+</sup>, *cn*<sup>+</sup>, *ro*<sup>+</sup>, *sw*<sup>+</sup>  
*ab*<sup>+</sup>, *br*<sup>-</sup>, *cn*<sup>-</sup>, *ro*<sup>-</sup>, *sw*<sup>-</sup>

Puzzled by this result, you perform microscopic analysis on meiotic chromosomes of heterozygous F1 females (from the Los Alamos/HMS cross). You find that during prophase I, chromosome 2 polytene chromosomes form strange loop-shaped structures.

-Explain the results obtained from the Los Alamos/HMS cross, including the Very Rare progeny classes.

-In your explanation, include a diagram showing the pairing of chromosome 2 during meiosis in the heterozygous F1 females, showing all relevant loci and the centromere.

-Also, explain why certain classes of recombinant progeny are missing.

(Hint: after drawing your diagram, consider the possibility of recombination and the resulting meiotic products. You can also obtain more background information from Griffiths Ch. 12).

c). Balancer chromosomes are tools used by *Drosophila* geneticists to prevent recombination from occurring on a particular chromosome. *CyO* is a balancer for chromosome 2 that contains multiple inversions, as well as a marker with a dominant wing morphology phenotype (*Cy*, curly wing) and a recessive lethal phenotype. Imagine that you have a version of the *CyO* balancer (*CyO-1*) that has an inversion spanning the entire region of chromosome 2 you are studying in your flies. The *CyO-1* balancer expresses the *Cy* marker described above (dominant curly wing, recessive lethal phenotypes), but the rest of the chromosome is wild type.

State what classes of progeny would be obtained in the following cross:

$$CyO-1 (ab+, br+, cn+) / ab- br- cn- \times CyO-1 (ab+, br+, cn+) / ab- br- cn-$$

Briefly explain why each potential class of progeny is present or absent.

4). As a new member of a *Drosophila* lab, you are looking into a variety of different projects that involve the early patterning of the *Drosophila* embryo. In a variety of F3 screens, your lab has successfully isolated many recessive lethal mutations that affect embryo polarity. However, you have a suspicion that these screens have failed to isolate many important genes that are involved in embryonic patterning. Specifically, you are interested in maternal effect genes.

a). Why would the previous screens that were done in your lab not isolate mutations in maternal effect genes that produced recessive phenotypes?

b). Given the following set of stocks, design a labor-efficient screen that would isolate recessive maternal effect mutations (on chromosome 2) that cause lethal embryonic patterning defects. Remember that maternal effect mutations do not affect the ability of the mothers to form morphologically normal eggs or to lay eggs.

Strains available:

**Genotype**

*cn bw / cn bw*

**Description**

*cn*: recessive eye-color marker on chromosome 2, confers cinnabar color

*bw*: recessive eye-color marker on chromosome 2; confers brown eyes

Flies homozygous for both *cn* and *bw* have white eyes  
Strain is otherwise wild type.

*cn bw / CyO.123*

*CyO.123*: Chromosome 2 balancer that carries the following markers:

- Dominant curly-wing marker, recessive lethal mutation

- *cn* eye color marker

- Dominant temperature sensitive lethal mutation (embryos with mutation fail to develop at temperature of 29°C or above; high temperature does not affect adult flies).

*Fs(2)D / CyO.123*

*Fs(2)D*: chromosome 2 containing dominant female sterile mutation that causes females not to lay eggs; no other relevant markers and is otherwise wild type

In your outline, include numbers and sexes of progeny used for each cross, as well as the genotype and phenotype of the relevant progeny and parents. Be sure to specify how you will identify the progeny you want. Also, describe how you would screen for the desired maternal effect phenotype in your mutants and indicate how you would establish the mutations in permanent stocks. (see hints on following page)

Hint 1: Single F2 progeny do not have to be isolated for crosses. Instead, you can make use of the special characteristics of the chromosomes listed above to save yourself some work.

Hint 2: The homozygous F3 females do not need to be segregated away from their siblings. Think about Hint 1 and what progeny you will get from that set of crosses, and then think about how you will screen your mutant females for the maternal effect phenotype.

5). You have started a rotation in a *Drosophila* development lab that studies leg development. Excited about the expertise in genetics you've gained in Genetics 201, your rotation supervisor asks you to design a screen using P-element mutagenesis to identify recessive zygotic mutations on chromosome 2 that confer an extra pair of legs (an 8-legged fly!) As your lab is an established *Drosophila* lab, you have many lab stocks available to you.

Strain Genotype	Description
$w^+$	Wild-type flies (red eyes)
$w$	Flies homozygous (or hemizygous) mutant at white ( $w$ ) locus, a recessive marker on the X chromosome that causes white eyes
$w; +/+ ; Tny P[w^+]^5 / Tny P[w^+]^5$	These flies carry five copies of a modified P-element containing the $w^+$ allele on chromosome 3. <i>Tny</i> marker is a dominant marker on chromosome 3 that produces tiny flies; nonlethal.
$w; Tft \square 2-3/CyO; +/+$	<i>Tft</i> is a dominant marker on chromosome 2 that results in tufted bristles. $\square 2-3$ is a stable source of transposase, also on chromosome 2. <i>CyO</i> is a 2 <sup>nd</sup> chromosome balancer which carries a recessive lethal mutation and has a dominant curly-winged phenotype
$w; Gla / CyO; +/+$	<i>Gla</i> is a dominant 2 <sup>nd</sup> chromosome marker that causes glassed-over eyes (distinguishable from eye color).

a) Describe a P-element screen to identify new zygotic mutations on chromosome 2 that cause the recessive, nonlethal phenotype of extra leg formation. You may use any of the lab stocks listed above. Be sure to note gender and number of flies used where relevant. Also, indicate the phenotypes you will use to identify the desired class of progeny at each step.

(To be labor-efficient, your screen design ideally should allow you to distinguish between the following: flies in which P-elements have mobilized and hopped to a new location; and flies in which the P-element has not mobilized).

b) Surprisingly, your screen seems to have worked and you've identified 15 different mutations that cause the recessive phenotype of 2 extra legs. You call them *bileg* mutants (*bil1*, *bil2*, etc). *Bileg* mutant flies are fertile and viable.

You wish to determine whether any of your *bileg* mutants represent mutations in a previously identified gene, *exlg* (extra leg). The *exlg* gene is located on chromosome 2; *exlg/exlg* mutants have an eight-legged phenotype identical to that of your *bileg* mutants.

-Describe a genetic test you could perform to determine whether or not your *bileg* mutations are in the *exlg* gene. You have available a stock of homozygous *exlg* flies, plus homozygous stocks of each *bileg* mutant. Please include genotypes and how you would interpret potential results. What possible exceptions might you encounter?

c) You find that *bil4* is a new, recessive loss-of-function mutation of the *exlg* gene. This gene has not been previously cloned; now, with the P-element insertion, you should readily be able to do so. Giddy about your results, your rotation supervisor wants you to extend your rotation by a few weeks and clone *bil4*.

-How would you take advantage of the P-element insertion to clone and map *bil4*? (Note: You may assume the modified P-element used in this problem,  $P[w^+]$ , has the same features as the one described in lecture).