**MGCB 31400**

**AND**

**BIOS 21236**

**Genetic Analysis of Model Organisms**

**Fall 2016**

**Problem Set #2**

**Due Monday, October 24th in class**

**We will not accept late problem sets.**

Please answer each question in the space provided using LEGIBLE writing. If you need additional space, please use the back of the same sheet ONLY. If you would prefer to type up your answers, the assignment with be available on the Chalk site the day it is distributed. Please print SINGLE-SIDED however.

**Please write your full name on the top of each of the 11 pages to assist grading.**

If you have questions or concerns regarding this problem set please email Audrey at aud.will@mac.com

1. In 1915, Bridges used XXY females (females carrying two X chromosomes and a Y chromosome) to show that sex linked genes are present on the X chromosome. [XXY flies are fertile females, XYY flies are fertile males, and XXX flies die.]

He noticed that when females of genotype Xw Xw Y were mated with XBar Y males, there were two classes of “exceptional” progeny, *i.e.,* not expected by Mendelian segregation of markers on a sex chromosome. Specifically, approximately 5% of the male progeny had Bar eyes, while 5% of the female progeny had white eyes.

[Xw represents an X chromosome carrying a recessive allele of the *white* gene. Flies hemizygous or homozygous for *w* mutations have white eyes, instead of the normal red eye. XBar represents an X chromosome with a dominant *Bar* mutation. Flies carrying this chromosome have Bar eyes.]

a) Why are the “exceptional” progeny unusual, when compared to the phenotypes of progeny from a mating between XY males and XX females that carry the same sex-linked markers?

b) Propose an explanation for the presence of these exceptional flies. Don’t worry about the relative percentages of the exceptional flies, just their presence. Your answer should contain a diagram noting the possible segregation patterns of the sex chromosomes of an XXY female during meiosis, and the genotypes, viability, and phenotypic sex of her F1 progeny.

In your diagram, if it is not clear from the genetic markers present on the chromosome, label whether the X and Y chromosomes in the F1 progeny come from the mother (Xm, Ym) or the father (Xp, Yp).

c) If Bar males carrying an extra Y chromosome (i.e., of genotype XBar YY) are mated to Xw Xw females, are there any “exceptional” progeny produced? If so, what are the phenotypes of the exceptional progeny, if not, why not? (A diagram would help in answering the question.)

d) What classes of progeny, if any, from the cross in part C can produce “exceptional” progeny (*i.e.,* not expected by Mendelian segregation of a sex chromosome) in the next generation after mating to individuals hemizygous or homozygous for a different recessive X chromosomal marker (*e.g., v*)? Why?

e) Taken together, how do these genetic and cytological data prove that sex linked genes are carried on the X chromosome?

2. Eric Wieschaus examined the phenotypes of embryos that completely lacked major portions of the genome, e.g., embryos lacking an X chromosome, or lacking both copies of one arm of an autosome. He found that all these embryos developed normally up to the onset of cellularization (about an hour before gastrulation). However, deletion of certain large chromosome regions caused a mutant phenotype during cellularization. Ultimately, Wieschaus showed that only seven genes were required zygotically for the process of cellularization.

a) How can an embryo completely lacking a large component of its genome develop normally for so long??

b) To produce embryos lacking both copies of a chromosome arm he crossed females and males each with a compound autosome; that is to say these flies instead of having two normal chromosomes 2 homologues had a pair of chromosomes, one of which contained two copies of 2L joined by a centromere while the other chromosome contained two copies of 2R in a similar configuration (see diagram).



It turns out that Drosophila has a mechanism that can segregate such compound chromosomes even though they cannot form chiasmata. Assuming that the two compound chromosomes segregate from each other at meiosis 1, what fraction of the embryos would lack all copies of 2L. Show your reasoning.

c) He now wanted to examine embryos lacking only a portion of one arm of the second chromosome. To do so, he crossed females with the compound 2 chromosome to males that were heterozygous for a translocation between chromosome 2 and the Y chromosome.



(In this diagram, the two thin lines represent the two sections of the Y chromosome. Assume that the chromosome with the majority of the Y joined to the distal part of 2L segregates in a manner identical to a wild-type Y chromosome.)

What fraction of embryos lacked the distal region of 2L? Show you reasoning.

3. All stocks of flies are kept in a self-maintaining genotype. For example, crossing males and females of genotype *let*/*TM3*, where *let* is a lethal mutation, and *TM3* is a balancer for the third chromosome only produces flies of the parental genotype.

Female flies of genotype *P[OvoD1]*/*TM3* are sterile because *P[OvoD1]* causes dominant female sterility; however, males of the same genotype are fertile. Thus, it is not obvious how to maintain a self-propagating stock of this mutation.

Conversely, male flies of the genotype *Tub85B*/*TM3* are sterile because the *Tub85B* mutation causes dominant male sterility, however, females of the same genotype are fertile.

Describe how you can create a stock that maintains these two mutations indefinitely. Be specific about the cross you would initially perform, and detail why the stock maintains itself from generation to generation.

4. To further our understanding of the neural circuitries underlying behavior, you transform flies with a P element that contains a wild-type copy of the white gene (w+) and a basal promoter upstream of the lacZ gene. You obtain many hundreds of lines and you assay the expression of lacZ in the brain of adult flies of each line.

In many lines, you do not see any expression of lacZ. However, in one line you discover that lacZ is expressed in a subset of neurons that use dopamine as a neurotransmitter. From physiological studies, you know that dopamine is essential for a specific learning behavior, the association of an environmental stimulus (i.e., an odor) with a noxious stimulus (an electric shock). If flies are exposed to both stimuli concurrently, they learn to associate the odor with the noxious stimulus and will avoid the odor in the future. You are excited about this, as this gives you a potential opportunity to examine how flies learn.

a) What is the most likely explanation for this pattern of lacZ expression in this line of flies?

b) You identify the insertion site of the P element and examine the genomic structure surrounding the P element. Nearby, there is a gene that encodes a novel transcription factor. You are very interested in studying this gene, but there are no mutations in the gene. Propose a series of crosses using the P element that could allow you to obtain a loss-of-function mutation in the gene encoding the transcription factor. (Hint: what happens when a P element excises?)

c) You obtain mutations in the transcription factor and you show that in these mutants, the neurons are not formed and the fly cannot perform associative learning (i.e., coupling the odor to the noxious stimuli). Thus, these neurons are likely to be necessary for associative learning.

However, you now wish to examine whether the activity of these neurons is sufficient to cause flies to avoid the odor even if the noxious stimulus is not present (i.e., is their activity sufficient for associative learning). You obtain a light-activated protein that, after exposure to light, increases the activity of any neuron in which the protein is expressed. You reason that if you express this protein in the same neurons as the transcription factor, you could activate the neurons and cause associative learning in the absence of the noxious stimuli.

How would use P element construct(s) to express the light-activated protein in this specific subset of neurons? What information do you need to have for these construct(s) to be successful? Diagram the structure of any P element that you would use.

5. *Drosophila* genetics as we know it would not be possible without the existence of balancer chromosomes.

a) What are the three main features that define balancer chromosomes? Why is each important to the function of a balancer chromosome?

b) You are researching a particularly interesting recessive mutation in *Drosophila* that you have decided to call *gonadless (gdl)*, which cause homozygous adult females to fail to develop a gonad. However, males carrying this mutation are viable and fertile.

You map the mutation to the X chromosome and put it in trans to an X-chromosome balancer, FM7. The characteristics of an X-chromosome balancer are slightly different from that the balancers for the autosomes. In particular, flies homozygous or hemizygous for FM7 are viable; however, homozygous FM7 females are sterile, while hemizygous FM7 males are fertile. As an aside, why is it necessary that an X chromosome balancer have the above characteristics? (Think about one of the main purposes of balancer chromosomes.)

c) You then cross *gdl*/FM7 female flies to male flies hemizygous for the *gdl* mutation. You leave the F1 male and female progeny together to produce F2 progeny. What is the ratio of fertile to infertile flies in each gender? (Assume that the different fertile genotypes in the F1 mate with equal frequency.)

d) Are the flies arising from the cross described in part B a stable stock (i.e., will they indefinitely maintain the *gdl* mutation given random mating)? Why or why not?

e) You continue to experiment with the *gdl* mutation. Using all of the mapping techniques that are available, you narrow down the location of the mutation to a region that contains three transcription units: A, B and C. How might you determine which of the three genes is altered by the *gdl* mutation without sequencing this region? (There are multiple correct answers).

f) The lab you’re working in is very interested in *gdl* and is interested in identifying as many genes as possible that when mutated cause failure in gonad specification. After working on the mutagenesis screen above for years you’ve isolated the following:

|  |  |
| --- | --- |
| Complementation group: | Number of mutants identified: |
| A | 7 |
| B | 15 |
| C | 2 |
| D | 8 |
| E | 10 |
| F | 5 |

You really want to convince your boss you’ve isolated enough mutants and that you should graduate. Calculate the probability (or write down the equation for the calculation) that you have missed a seventh complementation group. What assumptions are you making in this calculation?

6. One of the most striking aspects of development is its robustness. Yes, there is a small amount of variability between individuals of a species, but largely all of the members of a species share a suite of defining morphological characteristics. Thus, developmental processes result in fixed outcomes despite genetic differences between individuals and different environmental conditions in which each individual is raised. There are a variety of theories as how developmental robustness is achieved, but examples of the molecular mechanisms underlying robustness are very limited. This question deals with one reason that might be so.

Assume that one wished to conduct a forward genetic screen in Drosophila for genes in which recessive, loss of function mutations caused greater variability in a defining organismal character, for example wing size. Note this is not a screen for genes that when mutated cause significant but uniform changes to wing size (e.g., all flies of the genotype have smaller wings); this is a screen for genes that when mutated increase the variability of wing size (e.g., some flies of the genotype have smaller wings, other flies of the genotype have larger wings). For the purpose of the question, assume that the screen does not involve generation of mosaic individuals.

a) Write out the crossing scheme you would use to screen for EMS-induced loss-of-function mutations on one chromosome. Assume you have an appropriate balancer for that chromosome (Bal). In your answer, be sure to:

i) Indicate which generation would you screen.

ii) State how you would determine whether you have identified a recessive mutation that  
 causes wing size variability.

b) Based on your answer to (a) briefly state why such a screen is so difficult to do in practice.