Bio393: Genetic Analysis **Name:**_____
Problem Set #3
Due on Friday, May 15, 2 PM

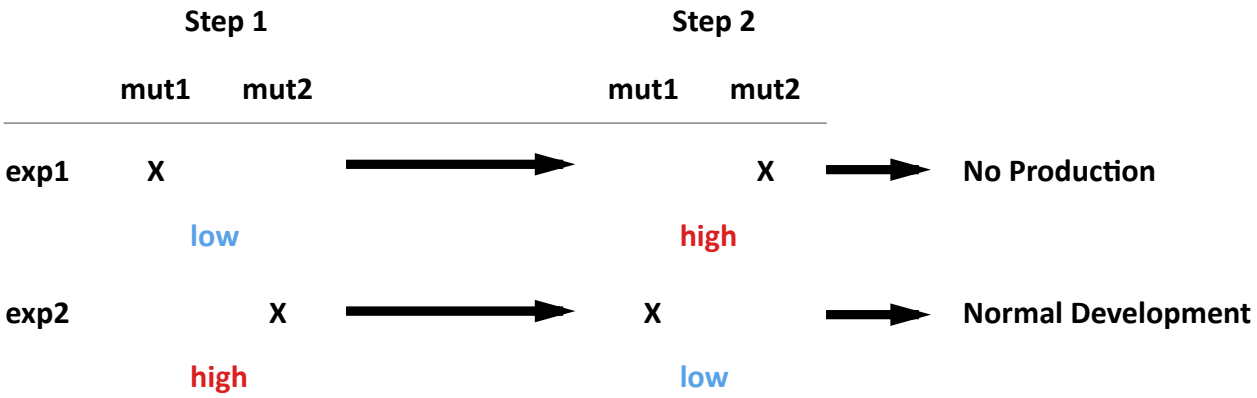
Question 1:
A graduate student working on a plant decides to genetically dissect the process of flowering. She knows that flowering is normally signalled to occur by long daylight hours in this species. When plants of this species undergoing flowering are examined, four stages (I to IV) in the process can be morphologically defined. The student decides to do a screen to understand the genetic basis of flowering. She gets 15 mutants with recessive phenotypes that fail to make flowers. Three mutants accumulate at stage I, five mutants accumulate at stage II, and the remaining seven mutants appear to undergo stages I and II normally, but the tissues in the wild type that normally become flowers instead develop into leaves.

Then, the student does all pairwise complementation tests between mutants with similar phenotypes. She finds that the three mutants that accumulate at stage I identify two complementation groups (genes 1 and 2). One of the mutants (mut1) has a cold-sensitive phenotype. This mutant has a mutant phenotype at or below the screen temperature. The other complementation group is made up of two mutations, one of which is temperature-sensitive (mut2). This mutant has a mutant phenotype at or above the screen temperature. The student realizes that she can use these mutations to ask about the order of function of the products of these two genes by reciprocal temperature shifts and do the following experiments.

Using mut1 mut2 double mutants, she grows the double mutant at low temperature until just past the point when step I of the process has occurred in wild-type controls and then shift the individuals to a high temperature to complete development. She finds that all of the plants fail to produce flowers and that the precursor cells accumulate at stage I.

Using mut1 mut2 double mutants, she grows the double mutant at high temperature until just past the point when step I of the process has occurred in wild-type controls and then shift the individuals to a low temperature to complete development. She finds that all of the plants develop normally.

(a) What do these results tell you about the relationship between the steps controlled by genes 1 and 2?



mut1 is required for development during stage I
mut2 is required for development during stage II

The graduate student also identified a mutant that had flowers in locations where the plant normally grows leaves. This mutant was named “showy”. When crossed to the wild-type, all of the offspring have the “showy” phenotype.

(b) What is most likely explanation for the showy phenotype?

The mutation causes a dominant phenotype that converts leaves to flowers.

(c) Describe two genetic tests that she could do to bolster your suggestion with respect to the nature of the showy phenotype.

1.) If the mutant resulting in the showy phenotype causes a dominant phenotype by constitutively activating the flowering pathway through gain-of-function, then cis-dominant suppression should remove the showy phenotype.

2.) If the mutant resulting in the showy phenotype causes a dominant phenotype by haploinsufficiency, then a deficiency of that locus should causes a similar phenotype.

You can use x-ray mutagenesis to separate these possibilities. If the locus is haploinsufficient, then the showy phenotype will not be suppresses (in cis) by x-ray generated deficiencies. Whereas, if the mutation is causing a gain of function, then removing the locus (in cis) by deficiency will suppress the mutant phenotype.

Reasoning that she might be able to order the functions of genes 2, 4, and 5 using the “showy” phenotype to student constructs the following:

Plants that are homozygous for the mutation in gene 4 and heterozygous for “showy”. These plants have the “showy” phenotype.

Plants that are homozygous for the mutation in gene 5 and heterozygous for “showy”. These plants have the phenotype from a mutated gene 5.

(d) What do these results tell you about the order of function of genes 4 and 5?

mut4(m/m) + showy(m/+) —> Showy

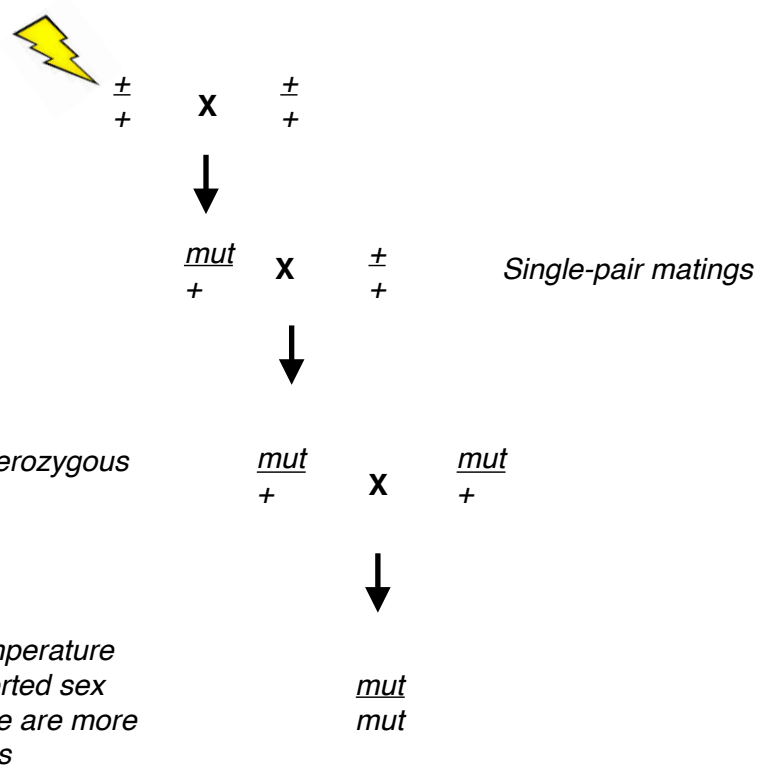
mut5(m/m) + showy(m/+) —> mut5

Given that showy is dominant, the mut4 showy cross suggests that mut4 is upstream of showy. We observe the opposite case in the mut5 showy plants. In this case, the dominant activity of showy is prevented from proceeding by mut5.

mut4 —> Showy —> mut5

Question 2:
You are a beginning faculty member and have decided to genetically dissect the process of sex determination in the Mediterranean fruit fly (medfly) *Ceratitis capitata*, reasoning that such knowledge might allow development of control procedures for this invasive crop pest. Also, it might bring you much needed funding and respect.
Fortunately, you met a researcher who already has sex determination mutants but has not studied them. You decide to collaborate.

(a) In case the collaboration goes sour, describe how you would isolate a heat-sensitive mutant that when homozygous causes males to develop as females? This mutation would have no effect on females. Also, remember there are no balancers in medflies.



You collaborator has three mutants (fem, del1, and del2) that all causes males to develop as females. You would like to know if they have mutations in the same gene. fem is temperature-sensitive; del1 is cold-sensitive; and del2 has a recessive phenotype that is not cold or temperature-sensitive.

(b) If del1 and del2 have mutations in the same gene and fem complements both del1 and del2, describe the conditions under which you would do the complementation tests and the results that would be expected.

The del1 and del2 complementation test is straightforward. Make the heterozygote, raise the offspring at cold temperature, and look for sex ratio distortion.

Once you have established that del2 is deficient in the same function as del1, then you can use the recessive phenotype that is not cold-sensitive. Make the heterozygote between fem and del2, raise the offspring at high temperature, and look for sex ratio distortion.

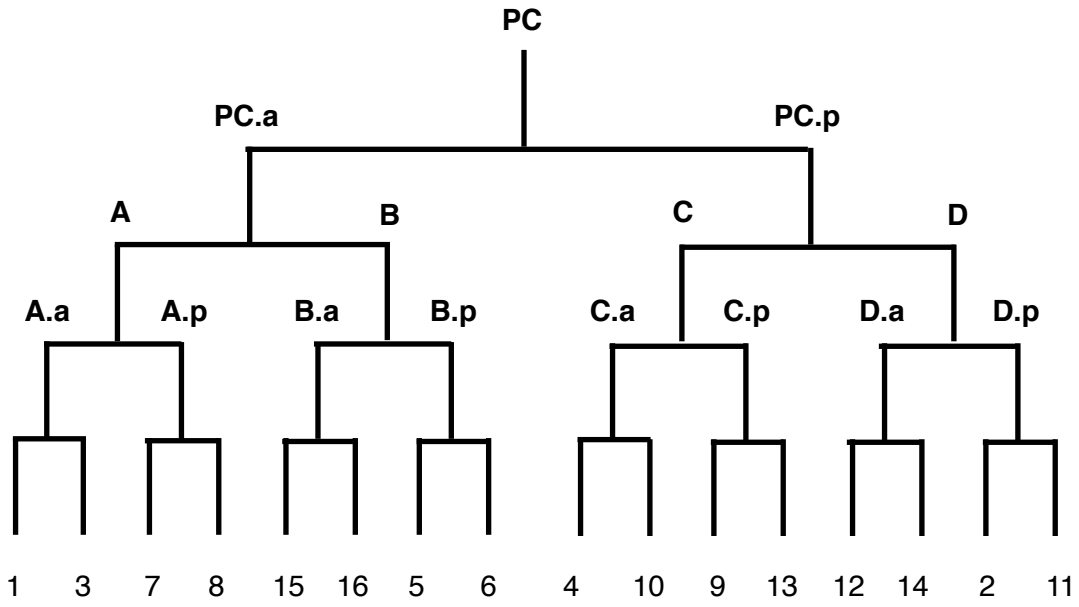
Another mutant called Ix has a dominant phenotype that causes males to develop as intersexes (a mix of male and female).

(c) Using the mutagen EMS, how can you determine if the mutated locus is haploinsufficient or a gain-of-function?

There are at least two ways:

1. *Haploinsufficiency is much more common than gain-of-function. From a standard mutagenesis screen, you should get the Ix dominant phenotype at loss-of-function rates.*
2. *You can perform a cis-dominant suppressor screen for loss-of-function mutations in Ix. If the mutant phenotype is not suppressible by a linked mutation, then it is likely haploinsufficient.*

Question 3:
You are studying a relative of *C. elegans* named *C. horvitzii*. Just like Bob Horvitz and John Sulstan, you want to generate a lineage map of the organism from the zygote the the adult animal. Below is the lineage you have constructed so far.



You need to place cells 1-16 on the lineage. Using ablation to generate the following data:

Ablated cell	Cells that are present
PC.a	2, 4, and 9-14
A	2, 4, 5, 6, and 9-16
A.a	2, and 4-16
B.p	1,2,3,4,7,8, and 9-16
D	1,3,4,5,6,7,8,9,10,13,15,and 16
D.p	1,3,4,5,6,7,8,9,10, and 12-16
C.p	1-8, 10, 11, 12, 14-16

Not Present

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PC.a	x		x		x	x	x	x							x	x
A	x		x				x	x								
A.a	x		x													
B.p					x	x										
D		x									x	x		x		
D.p		x									x					
C.p									x				x			

You have several lineage defective mutants that fail to form certain cells and their direct descendants. Using these mutants, you attempt to discern which cells are needed for the development of particular components of the adult body.

In mutants for lineages A, B, C, and D.a, you always see the tail formed correctly. Mutants for lineage D never have a proper tail.

In mutants for lineages A.p, B, and PC.p, you always see a proper vulva. However, mutants for lineages A and PC.a never have a proper vulva.

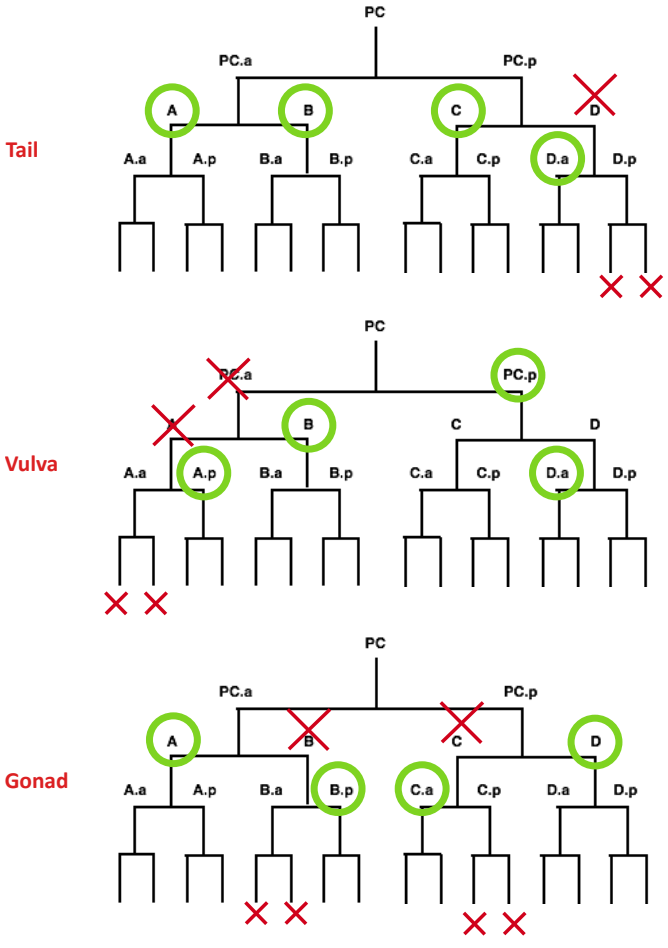
In mutants for lineages A, B.p, C.a, and D, you always see proper formation of the gonad. However, mutants for lineages B and C do not have gonads.

(b) Which cells are responsible for tail, vulva, and gonad formation?

Tail = Dp

Vulva = A.a

Gonad = B.a + C.p



Question 4:
You are interested in the development of a set of cells that make the opening of the secretory duct to the intestine of *Drosophila*. Fourteen cells are arranged in two rings with seven cells in each ring. The cells of the first ring are numbered 1-7 and the second ring 8-14. To investigate the developmental origin of these cells, you induce mitotic recombination in flies heterozygous for a somatic cell marker that you can score. The time at which you induce mitotic recombination is prior to when all 14 cells are formed. When you score for clones homozygous for your somatic cell marker in a large number of flies, you observe the following numbers and patterns of clones:

Cells expressing somatic marker	Number of cases
1,3,5,10	7
8,14	10
2,7,8,9,13,14	4
1,3,4,5,6,10,11,12	4
4,6,11,12	6
8,9,13,14	7
9,13	12
1,10	11
4,11	13
6,12	12
3,5	12
2,7	14
All	2

Say as much as you can about the developmental relationships of the cells that makeup this duct.

It appears that seven precursor cells; each of which give rise to two of the 14 intestinal cells. If all of the cells came from one common precursor, then all or none of the cells would have been labeled. The probability of any one precursor cell being marked is the same as observed from the most abundant two labeled cell classes. These classes also tell us that the precursor cell for each of the cells in the two final rings can contribute cells to either ring. For example, [2,7] came from mitotic recombination in a precursor that contributed to the first ring, but [4,11] came from mitotic recombination in a precursor that contributed to both the first and second rings.