

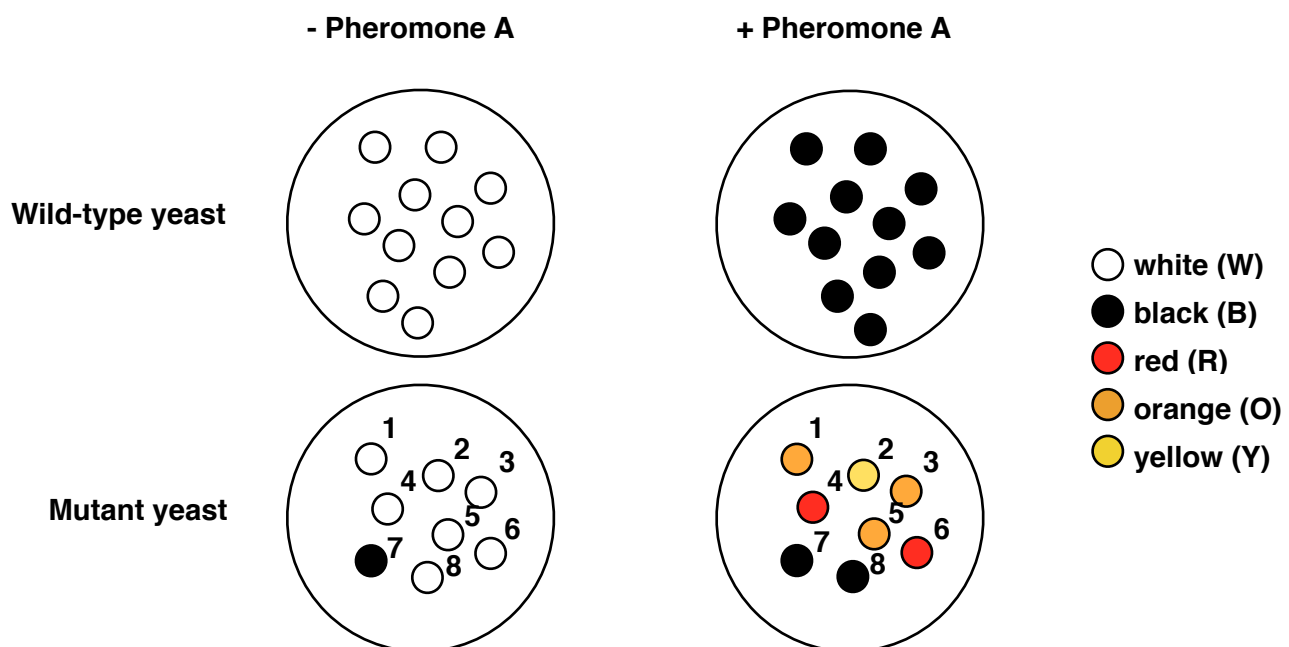
Bio393: Biomedical Genetics
Problem Set #3
Due on Friday, April 26, 5 PM

Name: _____

Question 1:

Your lab studies how yeast respond to different chemical signals (pheromones). You work with a newly isolated haploid strain. When you grow colonies of this yeast on a petri plate, the colonies are white. If you grow the yeast on a petri plate with Pheromone A, the colonies are black!

You want to understand how this response works and decide to perform a mutant screen. You mutagenize the yeast and plate all of the resulting mutants on petri dishes with rich media but no Pheromone A. You then replica plate the mutants onto petri dishes with rich media plus Pheromone A. Below are some of your results:



	WT	mut1	mut2	mut3	mut4	mut5	mut6	mut7	mut8
no pheromone	W	W	W	W	W	W	W	B	W
pheromone A	B	O	Y	O	R	O	R	B	B

(a) You know that you mutagenized 1000 cells, but when you plated the mutants onto rich media without Pheromone A, only 800 colonies grew. Why did 20% of the cells die?

Mutagenesis induces random mutations throughout the genome. Those cells likely had mutations in essential genes and could not grow.

(b) Of the colonies that grew, you isolated seven mutants (labeled 1-7 above). Why is colony 8 not interesting to you?

Colony 8 has the wild-type phenotype. We are only interested in mutants.

You perform complementation tests among the eight isolated mutants and grow the diploid strains in the presence of pheromone A. You get the following results. All crosses with mut7 are black even on plates with no pheromone.

	WT	mut1	mut2	mut3	mut4	mut5	mut6	mut7	mut8
WT	B	B	B	B	B	B	B	B	B
mut1		O	B	O	B	O	B	B	B
mut2			Y	B	B	B	B	B	B
mut3				O	B	O	B	B	B
mut4					R	B	R	B	B
mut5						O	B	B	B
mut6							R	B	B
mut7								B	B
mut8									B

(c) Can you tell which of these mutations confers a dominant phenotype?

Mutant 7 has a dominant black pigment phenotype.

(d) How many genes are involved in the black pigment biosynthetic pathway?

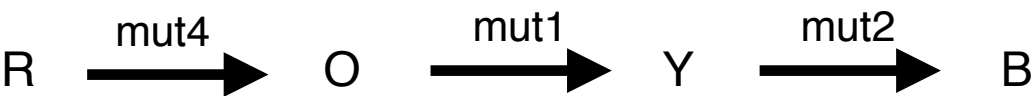
[1,3,5] [2] [4,6]

(e) You decide to use your mutants to order the genes in the black pigment biosynthetic pathway. You do not know the intermediates involved, but you can tell when a particular intermediate builds up because of the color of the colony. In order to build the pathway, you need to make double mutants. Using two representative mutations in different genes, show how you would build a double mutant strain. For this exercise, assume that each gene is unlinked from each other and from the mating locus.

You have to build the strains without knowing the genetic interactions and then confirm the presence of the two mutants using complementation.

For example, let's build the mut1 mut2 double mutant. Cross mut1 haploid cells to mut2 haploid cells to get heterozygotes for both. In the spores generated from meiosis of the diploid cell, some tetrads (or groups of four spores) will have four different genotypic classes (tetratypes for aficionados). The four different genotypic classes will be observed because one spore will have the wild-type phenotype and the other three will be two single mutants one double mutant. Because you do not know which one is a single or a double mutant, you grow up cultures of each mutant spore to get a population of haploids. Mate each of these haploid mutants to both mut1 and mut2 single mutants. Double mutants should fail to complement the mutant phenotype of both mut1 and mut2.

(f) You construct double mutant cells, where each mutation is in a different gene (e.g. mut1 mut2 is mutated for both the mut1 and mut2 genes). You then grow each of your double mutants in the presence of Pheromone A. Given the pathway below, fill in the chart for the single and double mutant phenotypes in the presence of Pheromone A.



	+ Pheromone A
WT	<i>Black</i>
mut1	<i>Orange</i>
mut2	<i>Yellow</i>
mut4	<i>Red</i>
mut1 mut2	<i>Orange</i>
mut1 mut4	<i>Red</i>
mut2 mut4	<i>Red</i>

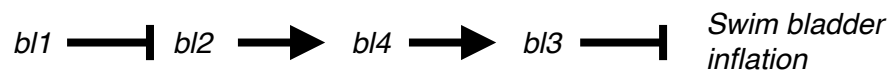
Question 2:
 Ever since childhood, you have often wondered how do fish control their depth in the water. You know that the swim bladder can inflate or deflate to move the fish up or down in the water, but how is the swim bladder made? After Bio393, you join a zebrafish lab to do a mutant hunt for swim bladder defective mutants. You identify mutant fish that are either floaters or sinkers (bladder (bl) mutants). Floaters have defective swim bladders that are constitutively inflated, while sinkers have swim bladders that are constitutively deflated.

(a) Did you perform a screen or a selection? Describe the logic behind your answer.
In principle, you could perform either, but a selection would be easier. After mutagenesis and crossing to generate homozygous mutant animals, you can collect mutants that are found at the top of the tank (floaters) and those mutants that are always at the bottom of the tank (sinkers). You do not have to go through all of the other wild-type animals that are swimming up and down in the water.

(b) The mutant fish fall (or maybe sink - ha!) into three complementation groups (bl1 through bl3) with one additional mutant that has a dominant hypermorphic phenotype (bl4). You want to figure out the swim bladder regulatory pathway, so you make double mutants to measure genetic interactions. The phenotypes are below:

Genotype	Phenotype
bl1	partially sinks
bl2	floats
bl3	floats
bl4	sinks
bl1 bl2	floats
bl1 bl3	floats
bl1 bl4	sinks
bl2 bl3	floats
bl2 bl4	sinks
bl3 bl4	floats

Draw out the gene regulatory pathway for swim bladder inflation.



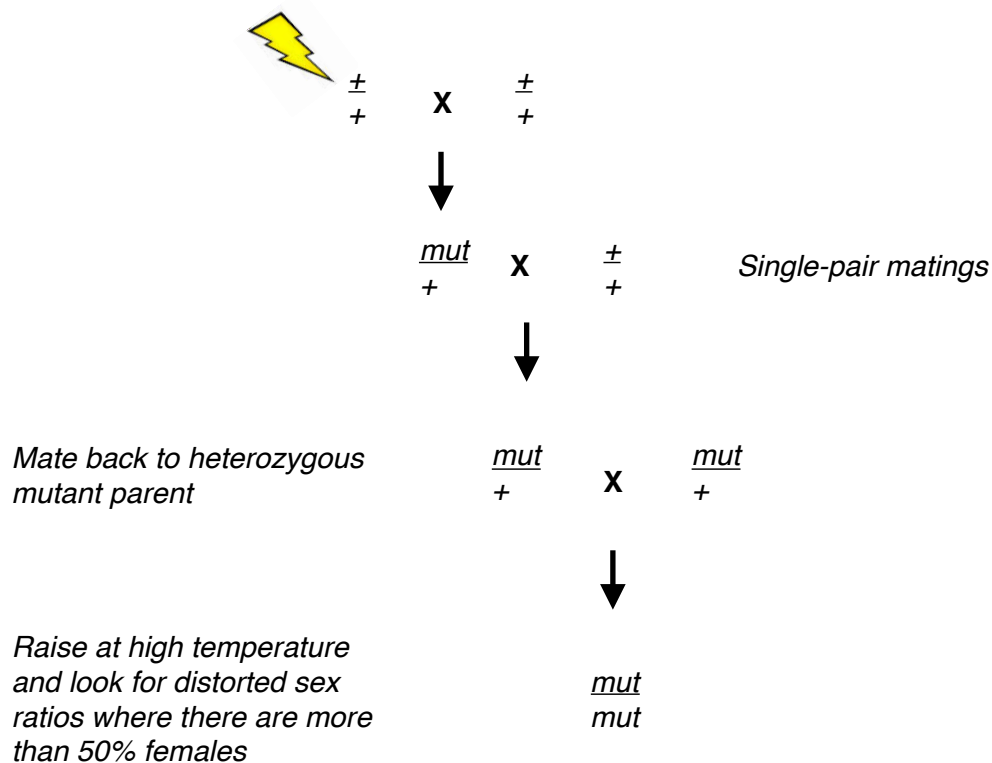
(c) The bl1 mutant only partially sinks. Propose two explanations for this mutant phenotype.

- (1) *The bl1 mutant could be a hypomorph.*
- (2) *The bl1 mutant could be redundant with some other gene.*
- (3) *The bl1 mutant phenotype could have variable expressivity. Because I didn't say that it was a strain, you don't know. If the bl1 mutant stock had some partial sinkers and some complete sinkers then it could be variable expressivity.*
- (4) *Formally, I didn't say that whether we are looking at a population of mutants or a single mutant individual. If it was a population, then incomplete penetrance could be an explanation.*

Question 3:

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.



Your 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 lx 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 lx dominant phenotype at loss-of-function rates.*
- 2. You can perform a cis-dominant suppressor screen for loss-of-function mutations in lx. If the mutant phenotype is not suppressible by a linked mutation, then it is likely haploinsufficient.*

Question 5:

Draw a cross to differentiate between maternal-effect inheritance and cytoplasmic inheritance? Hint: think about multiple generations.

With traits that show cytoplasmic inheritance, every offspring from an affected mother will be affected. The trait will be passed in each generation from mother to child.

With traits that show maternal-effect inheritance, the offspring's phenotype is dependent on the maternal genotype. Some mothers might not have the alleles that confer the dominant or recessive mutant phenotype so the offspring will be unaffected.

Question 6:

Using a temperature-sensitive allele, you perform upshift and downshift experiments over the course of ten hours with a shift every hour. After the ten hours is complete, you measure the penetrance of the mutant phenotype. You find that the temperature-sensitive period is between six and eight hours. Please draw the upshift (solid line) and downshift (dotted line) on the graph below.

