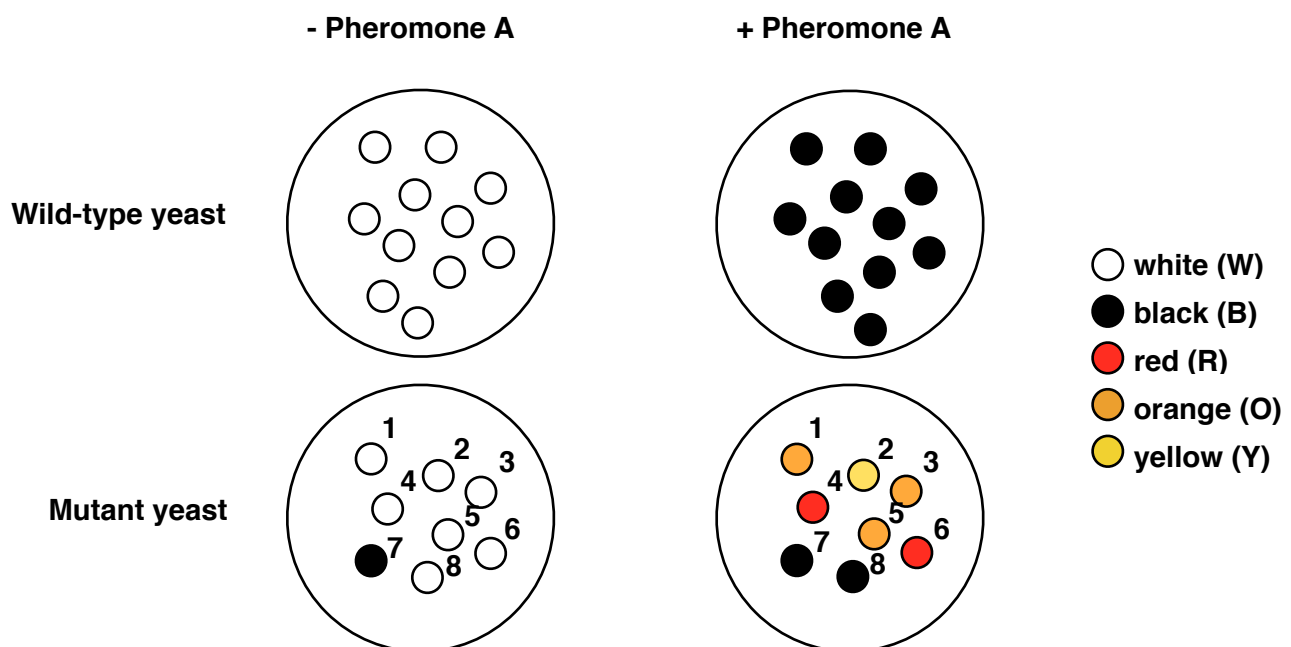


Question 1:

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 bacteria 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:

	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?

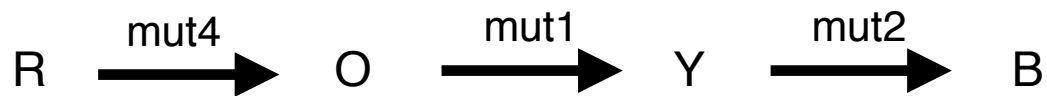
[1,3,5] [2] [4,6]

(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	Black
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.

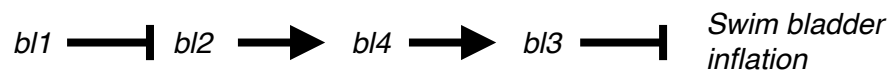
(a) Did you perform a screen or a selection? Describe the logic behind your answer.

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:

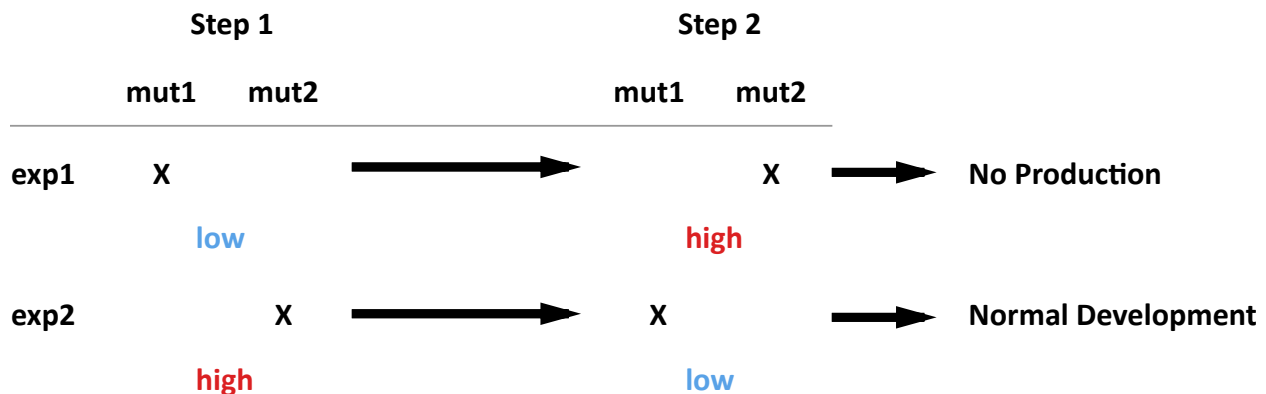
A graduate student working on a plant decides to genetically dissect the process of flowering. She knows that flowering is promoted by long daylight hours in this species. When she looks at plants of this species that are flowering, four stages (I to IV) in the process can be morphologically defined. 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 cause 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 suppressed (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, the 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

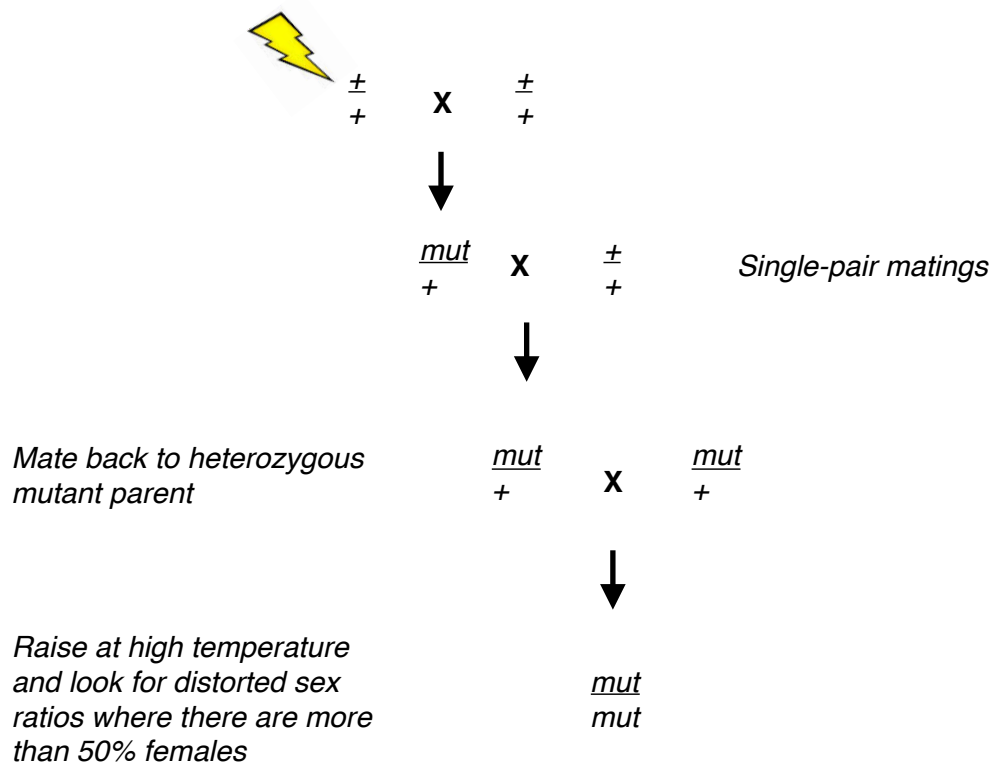
Given that showy is dominant, the mut4 showy cross suggests that mut4 is upstream of showy. We observe the opposite case in the mut5 showy plants. In this case, the dominant activity of showy is prevented from proceeding by mut5.

mut4 → Showy → mut5

Question 4:

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 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:

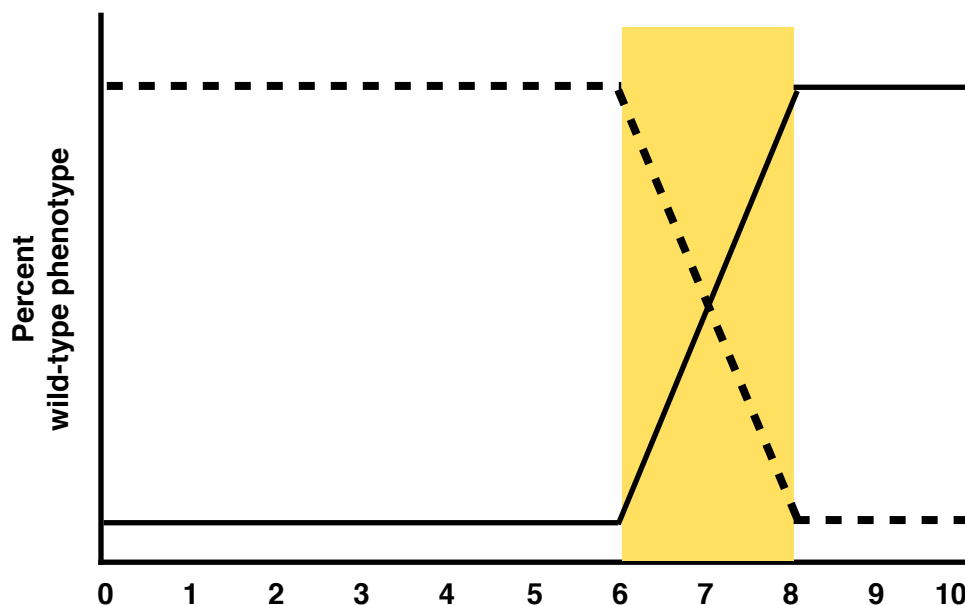
Draw a cross to differentiate between maternal-effect inheritance and cytoplasmic inheritance? Hint: think about multiple generations.

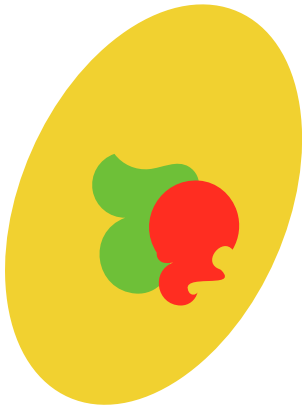
With traits that show cytoplasmic inheritance, every offspring from an affected mother will be affected. The trait will be passed in each generation from mother to child.

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:

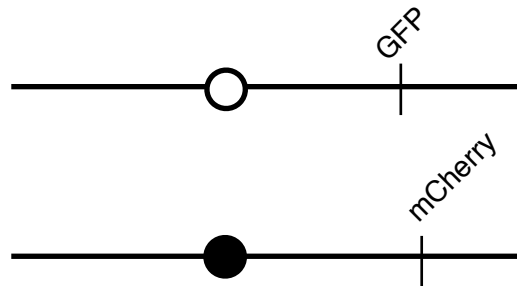
Using a temperature-sensitive allele, you perform upshift and downshift experiments over the course of ten hours with a shift every hour. After the ten hours is complete, you measure the penetrance of the mutant phenotype. You find that the temperature-sensitive period is between six and eight hours.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





Question 7:

In *Drosophila*, you can generate twin spots using cell-specific markers. In the example below, red ommatidia are homozygous for the mCherry gene, green ommatidia are homozygous for the GFP gene, and yellow ommatidia are heterozygous for mCherry and GFP. Draw out the diploid homologous chromosomes with centromeres demarcated as open and closed circles and locations of the GFP and mCherry insertions that would lead to this mitotic recombination result.



Question 8:

A developmental geneticist at the University of Toronto identified four different promoters that drive expression of any gene in different parts of an isopod. She sends you the promoter sequences for expression in carapace, legs, antennae, and the whole animal. She also helps you to make transgenic isopods. You drive expression of the wild-type *red* gene, which when mutated makes the red color phenotype, using all four promoters.

Describe the experiment (strains, promoters, etc.) that will determine where the function of the *red* gene is required in the animal using these reagents and any mutant or wild-type strains. Assume that the *red* mutant phenotype is recessive.

You want to rescue the red mutant phenotype so you will add expression constructs to red mutant isopods by transgenesis. If the gene functions in a particular cell type, then the red mutant phenotype will be rescued and the isopods will be gray.

If the red gene acts autonomously within the carapace, then you would expect that carapace promoter and whole animal promoter would drive red gene expression and rescue the red mutant phenotype. The leg and antennae promoters would not rescue the red mutant phenotype and the isopods will remain red.