Bio393: Genetic Analysis **Name:**_____
Problem Set #1
Due on Friday, April 22, 9 AM

Question 1:
You cross AaBBCcddEeFf with AaBbccDdEEFf individuals.

(a) What is the probability of phenotypically aBCDEf individuals?
*aa (1/4) * B- (1) * C- (1/2) * D- (1/2) * E- (1) * ff (1/4) = 1/64*

(b) What is the probability of phenotypically ABCDeF individuals?
*A- (3/4) * B- (1) * C- (1/2) * D- (1/2) * ee (0) * F- (3/4) = 0*

(c) What is the probability of genotypically AaBBccddEeFf individuals?
*Aa (1/2) * BB (1/2) * cc (1/2) * dd (1/2) * Ee (1/2) * Ff (1/2) = 1/64*

Question 2:
On a Friday night late night walk, you discover a strange mouse with a kinked tail. Your love of genetics inspires you to investigate this mutant phenotype.

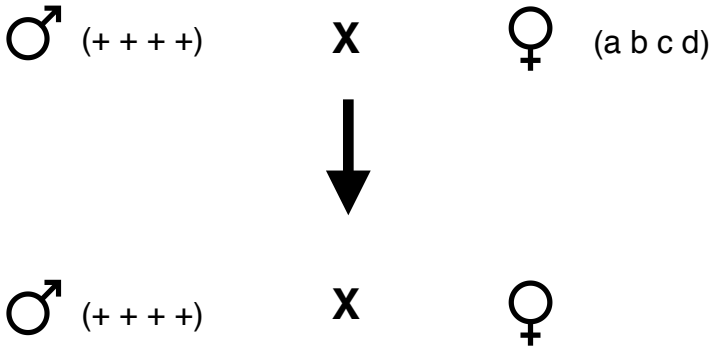
(a) You breed the kinked-tail mouse (a male) with several wild-type females and observe that about half the offspring (both males and females) have kinked tails and half have normal tails. What is the nature of the kinked-tail phenotype?
Autosomal dominant. If it were linked to the X chromosome, then none of the male offspring would have kinked tails.

(b) When two of the kinked-tail offspring from part (a) are crossed, what fraction of the resulting mice would you expect to have kinked tails?
3/4 would have kinked tails.

(c) When you cross kinked-tail offspring from part (a), you find that one third of the resulting kinked-tail males produce no sperm and thus are sterile. The other two thirds of the resulting kinked-tail males (and all of the normal-tail males and all of the females) are fertile. Propose a model to account for these findings.
Two possibilities exist: (1) The kinked-tail mutation causes a dominant kinked-tail phenotype and a recessive male sterility phenotype. (2) The kinked-tail mutation is linked to another mutation that causes recessive male sterility. A cross of heterozygous males would yield 1/3 homozygous kinked-tail mutation or the linked male sterility mutation.

(d) An annoying dorm mate of yours informs you that he has isolated a pure-breeding mouse strain in which males produce no sperm but have normal tails. Also, females are phenotypically normal (fertile with normal tails). You explain to your “friend” that this situation is impossible. Why?
It is impossible to make a pure-breeding strain when the males do not make sperm. Each generation could never give rise to the next generation.

Question #3:
A true-breeding *Drosophila* strain with four different recessive traits (a, b, c, and d) is crossed to the true-breeding wild-type strain. The F1 females that result from this cross are then crossed to wild-type males.



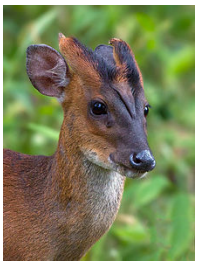
(a) Many flies of both sexes from this second cross are examined and none show the recessive **d** trait. What does this tell you about the chromosome on which the **d** gene resides?
The d gene must be on an autosome. If it were on the X chromosome then 1/2 of all male offspring would have the d phenotype because they would be hemizygous for d.

A total of 200 progeny from the second cross are evaluated for each of the three remaining traits. The 100 females among the progeny all appear as wild-type (*i.e.* none exhibit any of the recessive traits). For the 100 males among the progeny, eight different phenotypic classes are observed. The phenotypes and numbers of each of the phenotypic classes are given below. For simplicity, phenotypes of the three recessive traits are designated **a**, **b**, and **c**, while the corresponding wild-type phenotypes are designated with a "+".

Phenotype	Number
+ + + (females)	100
+ + + (males)	18
a b c (males)	22
a b + (males)	21
+ + c (males)	19
a + c (males)	6
+ b + (males)	4
+ b c (males)	7
a + + (males)	3

(b) Give as much information as you can about the chromosomal positions of the three markers, a, b, and c. Include in your answer any relevant map distances in cM.
The recombination distance between a and b is 6+4+7+3/100 = 20 cM. The recombination distance between a and c is 21+19+7+3/100 = 50 cM. The recombination distance between b and c is 21+19+6+4/100 = 50 cM. Genes a and b are linked, and c is unlinked to both. It is not known whether c is closer to a or b.

Question 4:



The Indian muntjac or barking deer is the mammal with the lowest diploid number of chromosomes, where 2n=6. Please draw out the following:

(a) A mitotic cell in anaphase

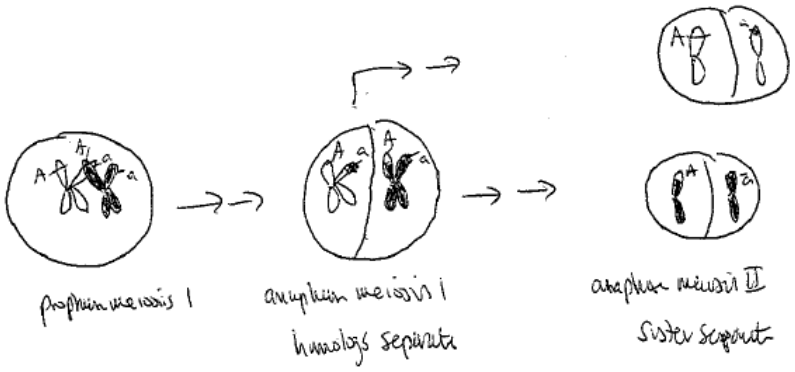
(b) A meiotic cell in telophase of meiosis I

(c) A meiotic cell in anaphase of meiosis II

Question 5:
Consider an individual heterozygous for albinism.

(a) What gamete genotypes would you expect this individual to produce and in what proportions?
The individual is Aa. The gamete genotypes are A, A, a, or a. Each is produced in equal proportions or 1/2 A and 1/2 a.

(b) Diagram how the chromosomes behave during meiosis to explain your answer to (a).
Not all steps are drawn.



Question 6:
The cells shown in the diagram are in various stages of mitosis or meiosis. All the cells come from the same individual.

(a) What is the diploid number of chromosomes in this animal?
Six.

(b) How did you arrive at your answer in part (a)?
The center cell is undergoing mitosis because it is a equatorial division both cells receive six chromosomes. Also, the right cell is undergoing meiosis II when sisters separate from each other in a reductional division. Each gamete made from this division results in a haploid number of chromosomes (three). The left cell is undergoing meiosis I when homologs separate from each other.

(c) Give the names of each stage shown.
Left - meiosis I anaphase
Center - mitosis anaphase
Right - meiosis II anaphase

Question 7:
PhiP and IQ are heterocyclic amines that are mammary gland carcinogens in mice. Both of these chemicals are present in certain food products such as cooked meats. To better understand the biology behind the carcinogenic properties of PhiP and IQ, we would like to identify genes that protect cells from their toxicity.

Wild-type *Saccharomyces cerevisiae* yeast grow at a reduced rate in the presence of 50 mM PhiP but arrest completely in the presence of 100 mM PhiP.

Using yeast, design a screen to isolate mutants that are hypersensitive to PhiP. Be as specific as possible.

Mutagenize wild-type yeast and plate many cells on complete media lacking PhiP. After colonies have grown up, replica plate the cells onto a complete media plate with 50 mM PhiP. Compare the colonies that grow on the complete media plates with and without 50 mM PhiP. Colonies that fail to grow on 50 mM PhiP are hypersensitive to the compound.

Question 8
The roundworm nematode *C. elegans* is a powerful and tractable model to understand how parasitic roundworms become resistant to anthelmintic (anti-nematode) compounds. Design a screen to identify strains with recessive albendazole resistance on chromosome III. Resistant mutants will survive when grown on plates containing albendazole. You have albendazole and normal worm plates for propagating *C. elegans*, the mutagen EMS, the laboratory wild-type strain N2, and triple mutant strain *bli-3; lin-1; unc-32*. The triple mutant strain is true-breeding for mutant alleles that each confer recessive phenotypes. *bli-3* is located on chromosome I and causes a recessive Blistered phenotype. *lin-1* is located on chromosome II and causes a recessive Multivulva phenotype. *unc-32* is on chromosome III and causes a recessive Uncoordinated phenotype. All three mutant phenotypes can be observed in the same mutant worm.

Use EMS to mutagenize +/+ males then cross to bli-3; lin-1; unc-32 hermaphrodites. The cross progeny will not be blistered, multivulva, or uncoordinated because those mutations cause recessive phenotypes. These animals should be grown on normal plates. If the mutation is on the third chromosome, the genotype is:

$$\frac{unc\text{-}32 \quad +}{+ \quad mut}$$

When this hermaphrodite is allowed to cross with itself (self) on albendazole plates. Only homozygous m/m mutants will survive. If the mutation is linked to unc-32, then you will never (or rarely) see uncoordinated animals that are resistant to albendazole. If the mutation is linked to other chromosomes, then you will see 25% of the albendazole resistant progeny are also uncoordinated.

Question 9:
Billy Bob wants to study the function of the yeast *URA5* gene, which encodes an enzyme that catalyzes a step in uracil biosynthesis. To begin his study, he plans to design an experiment to look for mutations in the *URA5* gene. He knows that having non-functional *URA5* gene allows for growth of yeast on medium containing 5-fluoro-orotic acid (5-FOA) (because wild-type cells catalyze a reaction that turns 5-FOA into a toxin that kills the cell). For his screen, he used the following protocol:
1. Mutagenize wild-type yeast.
2. Spread mutagenized cells on 10 plates (Set #1).
3. Let the cells grow into well-separated colonies.
4. Replica plate colonies onto new plates (Set #2) to detect *ura5* mutant strains.

(a) Which type of plate did Billy Bob use for set #1? for set #2? Explain your answer.
He performed a genetic screen for mutants that require uracil to survive. For set #1, he used complete media plates so that all cells (non-mutant and mutant) grow. For set #2, he used complete media lacking uracil.

(b) What phenotype is expected for *ura5* mutant strains?
Mutants in ura5 require uracil to grow. Therefore, they would grow on set #1 plates but fail to grow on set #2 plates.

Three days later, when he looks at the second set of plates, Billy Bob is crestfallen to realize that he sees NO colonies that fit his criterion for potential *ura5* mutant strains.
(c) Explain to him why he might not have found any strains of interest, even if the mutagenesis in Step 1 worked.
Three reasons: (1) He might not have screened enough cells. Mutagenesis is a random process. In a perfect screen, one needs to score enough cells so that every gene in the genome has been mutated. Oftentimes, this level of screening is not possible or practical. (2) Mutants that require uracil for survival might also be inviable when grown on complete media. In other words, these mutants might be lethal regardless of uracil status. (3) Some functions have multiple genes that act redundantly. One would need to mutate two (or more) redundant genes to see a uracil requirement phenotype. Option #1 is the most likely for this phenotype.

Following your thoughtful explanation, Billy Bob decides to try to use selection to find strains with mutations in the *URA5* gene. He once again mutagenizes wild-type cells and then plates cells on a set of 10 plates.
(d) What type of plates did Billy Bob use for his selection and why?
He used plates with complete media and 5-FOA. In this way, wild-type cells will die because they will turn 5-FOA into a toxin that will kill the cell. Mutant cells that require uracil will not process 5-FOA and will survive on those plates.

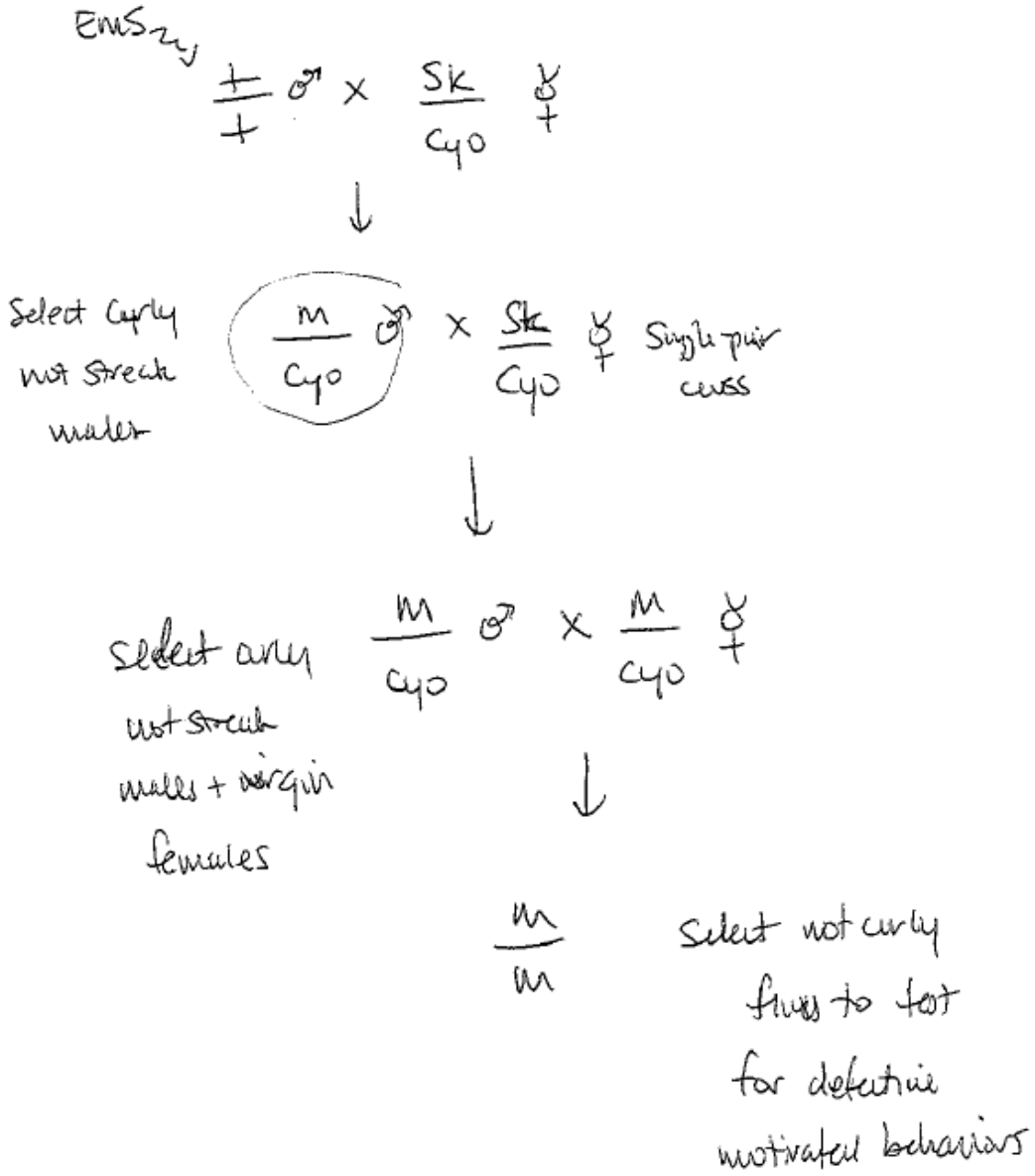
(e) What phenotype does he expect to see for *ura5* mutant strains?
Survival on 5-FOA plates

Billy Bob is ecstatic to find that his selection has worked--he finds strains with the appropriate phenotype. However, his enthusiasm is a bit damped when he realizes that he cannot yet be SURE that these strains have mutations in the *URA5* gene.

(f) Why can't he be sure that these strains have *ura5* mutations?
These strains could have mutations in other ura genes not ura5.

Question 10:
You are interested in motivated behaviors and have experience with the fruit fly *Drosophila*. You design a phenotype assay where you shoot the flies with a laser and observe how quickly they fly away from the heat stimulus.

(a) Draw out the genotypes and crosses for how you would generate flies with mutations on chromosome II to assay in your new system. You have a wild-type stock, a strain with the genotype Sk/Cyo, and a bottle of EMS. Sk causes a dominant streak phenotype and is on chromosome two. CyO is a second chromosome balancer with a dominant curly wing phenotype.



(b) You identify 10 mutant strains in your screen. Four have dominant phenotypes, and six mutants have recessive phenotypes. All six mutants with recessive phenotypes are defective in the same gene. You rudimentarily map the mutant alleles so that you can use different deficiencies (Df) and duplications (Dp) to test the genetic effects of the individual mutant alleles using dosage. Remember that deficiencies have one fewer copy of the wild-type allele of the gene mutated from the screen, and duplications have one more copy of the wild-type allele of the gene mutated from the screen. Please use the following tables to describe how do the mutations affect gene function.

Mutant #1 (m1)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m1/+	2 sec
m1/m1	4 sec
m1/Df	4 sec
m1/Dp	1 sec
+/Df	0.01 sec
+/Dp	0.01 sec

m1 is a dominant negative (antimorph) because its mutant phenotype gets less mutant with wild-type copies of the gene.

Mutant #2 (m2)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m2/+	~10 sec
m2/m2	~10 sec
m2/Df	~10 sec
m2/Dp	0.01 sec
+/Df	~10 sec
+/Dp	0.01 sec

m2 is a haploinsufficient locus. It has a dominant phenotype observed in the deletion heterozygote.

Mutant #3 (m3)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m3/+	6 sec
m3/m3	12 sec
m3/Df	4 sec
m3/Dp	10 sec
+/Df	0.01 sec
+/Dp	4 sec

m3 is a hypermorph causing an increase in wild-type gene function. Its mutant phenotype becomes worse with more wild-type or mutant copies of the locus.

Mutant #4 (m4)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m4/+	5 sec
m4/m4	5 sec
m4/Df	5 sec
m4/Dp	5 sec
+/Df	0.01 sec
+/Dp	0.01 sec

m4 is a neomorph causing an altered gene function. Its mutant phenotype is not modified by extra or fewer wild-type gene copies.

For mutants #5-10, you perform similar tests. Please write out the results as an allelic series (*e.g.* m5>m10.

Mutants #5-10 (m5-m10)	
Genotype	**Phenotype**
Wild-type (+/+)	0.01 sec
m5/+	0.01 sec
m6/+	0.01 sec
m7/+	0.01 sec
m8/+	0.01 sec
m9/+	0.01 sec
m10/+	0.01 sec
m5/m5	~10 sec
m6/m6	~10 sec
m7/m7	4 sec
m8/m8	3 sec
m9/m9	~10 sec
m10/m10	3 sec

Mutants #5-10 (m5-m10)	
Genotype	**Phenotype**
Df/Df	~10 sec
m5/Df	~10 sec
m6/Df	~10 sec
m7/Df	~10 sec
m8/Df	8 sec
m9/Df	~10 sec
m10/Df	~10 sec
m5/m8	8 sec
m6/m8	8 sec
m7/m8	6 sec
m8/m8	3 sec
m9/m8	8 sec
m10/m8	4 sec

All mutants cause a recessive phenotype. m5, m6, and m9 cause a complete loss of gene function because their mutant phenotypes do not get worse when combined with a deficiency. m7, m8, and m9 cause a partial loss of function or they are hypomorphs. m8 is the least mutant because its mutant phenotype retains the most wild-type function when combined with a deficiency. The homozygous m7 phenotype is more severe than the m10 phenotype. The allelic series order is:

m6 = m9 = m5 > m7 > m10 > m8

Question 11:
You isolate ten new mutant yeast strains that are defective in synthesis of leucine, an amino acid. These Leu- mutants (numbered 1-10) were all isolated in a strain of mating type a (MAT a). *S. cerevisiae* yeast are either mating type a or α. As it turns out, your high school classmate, now at the University of Chicago, has independently isolated ten yeast Leu- mutants (numbered 11-20) in a strain of mating type α (MAT α). You and your ex-classmate decide to combine your resources and determine how many different genes are represented by your 20 mutant strains. You cross each of the MAT a strains to each of the MAT α strains. Your experimental observations are shown in the table below, where an empty square indicates that the diploid did not grow on minimal medium and a filled square indicates that the diploid did grow on minimal medium.

a/α	1	2	3	4	5	6	7	8	9	10
11	■	■		■	■		■	■	■	■
12	■	■	■	■			■	■	■	■
13	■	■	■	■	■		■	■	■	
14	■	■	■	■	■		■	■	■	
15	■	■	■	■			■	■	■	■
16			■		■		■		■	■
17	■	■		■	■		■	■	■	■
18	■	■	■	■	■			■		■
19										
20	■	■		■	■		■	■	■	■

(a) What property do mutants 6 and 19 share? *dominant phenotype*

(b) Which mutations do you know to be in the same gene?

[1,2,4,8,16] [3,11,17,20] [5,12,15] [7,9,18] [10,13,14]

(c) Could mutations 6 and 10 be in the same gene? *Yes*

(d) Based on this experiment, what is the minimum number of genes required for leucine synthesis?
Five
(e) Based on this experiment, what is the maximum number of genes required for leucine synthesis?
Seven - five that have a recessive phenotype and two that have a dominant phenotype

Question 12:
One way to isolate nonsense suppressor mutations in tRNA genes is to select for the simultaneous reversion of nonsense mutations in two different genes. This selection works because it is extremely unusual to get back mutations in two different genes at the same time. The yeast *HIS1* and *HIS2* genes are required for histidine synthesis and strains harboring mutations in either gene will not grow unless histidine is provided in the growth medium.

(a) If you wanted to isolate nonsense suppressor mutations, explain why it would be a bad idea to start with a strain that has an amber mutation (TAG) in *HIS1* and an ochre mutation (TAA) in *HIS2*.

You would need nonsense suppressors for both the amber and ochre stop codons. Those two suppressors are encoded by different genes. Therefore, it would be very difficult to identify mutations in both genes in one selection.

Instead of starting with a double mutant, you start with a strain containing an amber mutation in just *HIS1*. After mutagenesis with EMS, you select his+ suppressor mutants by their ability to grow on medium without histidine. In this case, it is necessary to consider the possibility of a back mutation in *HIS1* as well as extragenic suppressor mutations in tRNA genes.

(b) Explain why it would be very unlikely in this case to acquire an intragenic suppressor mutation in *HIS1* and not a revertant.
The amber mutation in HIS1 causes an early stop in the coding sequence. Intragenic suppressors act by eliminating or reducing the effect of the first mutation in the gene. It is difficult to envision how the effects of a truncated protein could be alleviated by changing additional amino acids in the same gene.

Question 13:
(a) Assume that hairy toes are inherited as a recessive trait with 80% penetrance in humans. A couple decide to have a baby but worry about the stigma of hairy toes. This malady affected both of their fathers. What is the probability that their first child will have hairy toes?

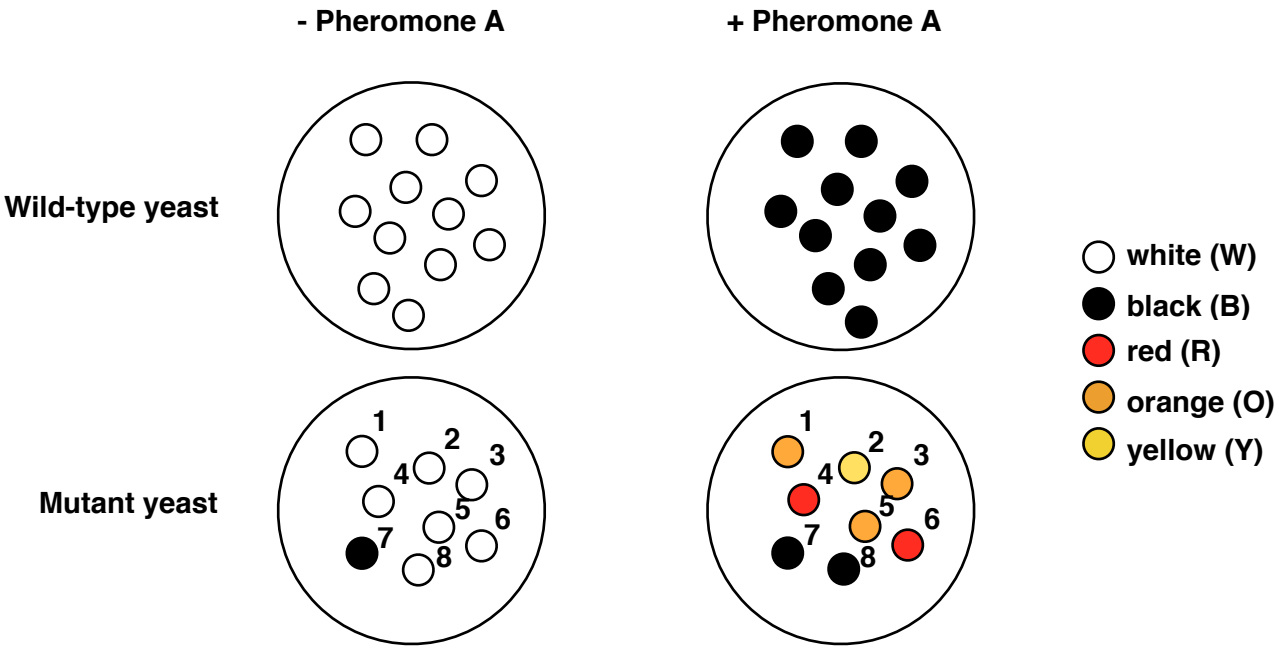
*Because both the man and woman had fathers with hairy toes, they must be carriers for this trait. Therefore, they have a 25% chance that their child with be homozygous for the allele that causes the recessive hairy toe trait. Because the penetrance is 80%, even if the child inherits both hairy toe alleles, he/she might not have hairy toes. 0.25 * 0.80 = 0.2 or 20% chance of having hairy toes.*

(b) After taking a genetics class, the mother decides that the risk of a hairy-toed baby is worth it. She believes that the trait has variable expressivity. How would she be able to tell?

She would see that her hairy-toed baby has more or less severe hairy toes that her father or father-in-law. Of course, she would likely hope that her baby has a less severe hairy toe phenotype.

Question 14:
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?

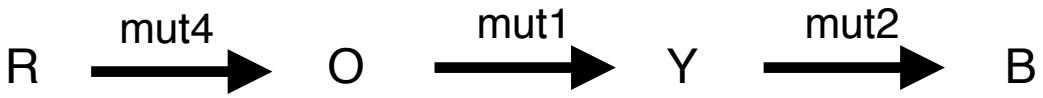
[1,3,5] [2] [4,6]

(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	*orange*
mut2	*yellow*
mut4	*red*
mut1 mut2	*orange*
mut1 mut4	*red*
mut2 mut4	*red*

Question 15:
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.

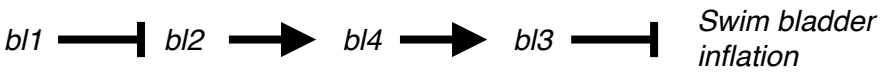
(a) Did you perform a screen or a selection? Describe the logic behind your answer.

In principle, you could perform either, but a selection would be easier. After mutagensis 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.

(d) What type of screen would you perform to isolate mutants to test between the two models proposed in part (c)? Describe the crosses to separate these two possibilities.

For the sake of clarity, let's assume you have a strain of bl1 and that the mutant phenotype is partially sinking for every mutant animal.

A non-complementation screen for bl1 null mutants might differentiate (1) and (2). Null alleles of bl1 would have a full sinker phenotype and would enhance the bl1 sinker phenotype as heterozygotes. If no strong sinker mutants are identified then it might be a redundant pathway because in the F1 all animals will be heterozygous for the enhancer mutant and the bl1-1 mutation. In other words, you might already be looking at the null phenotype of bl1 in the bl1-1 mutation. New alleles can not get more severe.