

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

Name: _____

Question 1:

You cross AaBBCcddEeFf with AaBbccDdEEFf individuals.

- (a) What is the probability of phenotypically aBCDEf individuals?
- (b) What is the probability of phenotypically ABCDeF individuals?
- (c) What is the probability of genotypically AaBBccddEeFf individuals?

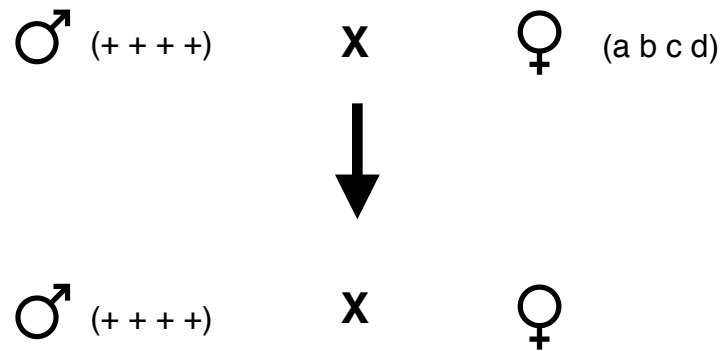
Question 2:

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?
- (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?
- (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.
- (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?

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?

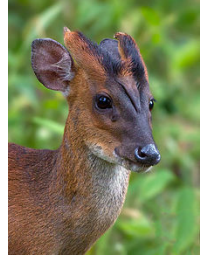
A total of 200 progeny from the second cross are evaluated for each of the three remaining traits. The 100 females among the progeny all appear as wild-type (*i.e.* none exhibit any of the recessive traits). For the 100 males among the progeny, eight different phenotypic classes are observed. The phenotypes 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 “+”.

<u>Phenotype</u>	<u>Number</u>
+ + + (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.

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:

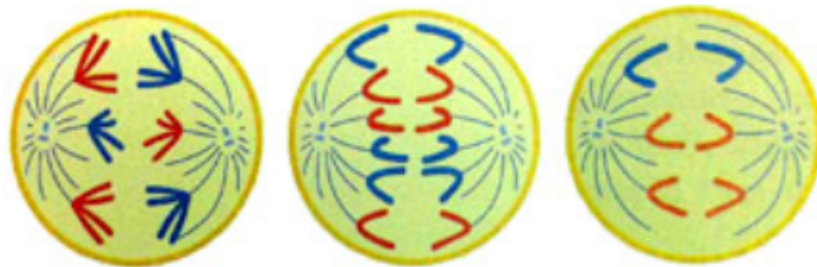
Consider an individual heterozygous for albinism.

(a) What gamete genotypes would you expect this individual to produce and in what proportions?

(b) Diagram how the chromosomes behave during meiosis to explain your answer to (a).

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 animals?

(b) How did you arrive at your answer in part (a)?

(c) Give the names of each stage shown.

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.

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 that are resistant to albendazole and have mutant alleles 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.

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 that affect its function. 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 wildtype yeast with appropriate treatment.
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.

(b) What phenotype is expected for *ura5* mutant strains?

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 criteria 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.

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?

(e) What phenotype does he expect to see for *ura5* mutant strains?

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?

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 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 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	0.1 sec
+/Dp	0.1 sec

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

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	0.1 sec
+/Dp	4 sec

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

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

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?
- (b) Which mutations do you know to be in the same gene?
- (c) Could mutations 6 and 10 be in the same gene?
- (d) Based on this experiment, what is the minimum number of genes required for leucine synthesis?
- (e) Based on this experiment, what is the maximum number of genes required for leucine synthesis?

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*.

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⁺ revertants 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*.

Question 13:

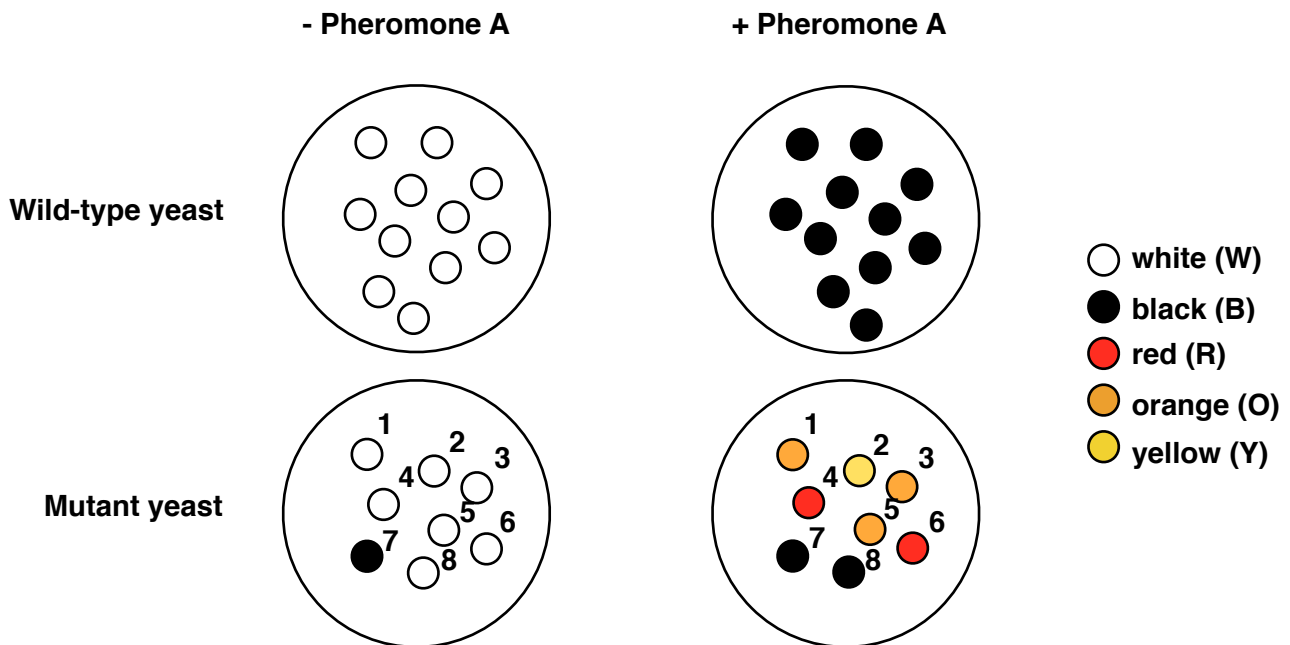
(a) Assume that hairy toes are inherited as a recessive trait with 80% penetrance in humans. A couple decide to have a baby but worry about the stigma of hairy toes. This malady affected both of their fathers. What is the probability that their first child will have hairy toes?

(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?

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?

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

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:

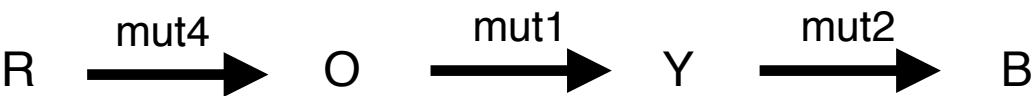
	WT	mut1	mut2	mut3	mut4	mut5	mut6	mut7	mut8
WT	B	B	B	B	B	B	B	B	B
mut1		O	B	O	B	O	B	B	B
mut2			Y	B	B	B	B	B	B
mut3				O	B	O	B	B	B
mut4					R	B	R	B	B
mut5						O	B	B	B
mut6							R	B	B
mut7								B	B
mut8									B

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

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

(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.

(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	
mut2	
mut4	
mut1 mut2	
mut1 mut4	
mut2 mut4	

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.

(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.

(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.