Bio393: Genetic Analysis   Name:_____
Problem Set #1
Due on Friday, April 7, 3 PM, Cook 3125

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.

♂ (+ + + +)   **X**   ♀ (a b c d)

⬇

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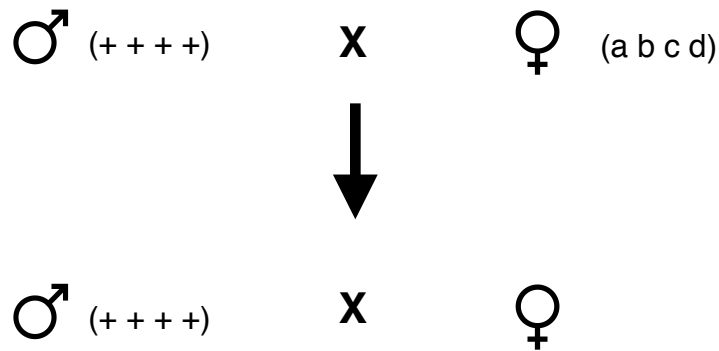
(a) Many flies of both sexes from this second cross are examined and none show the recessive **d** trait. What does this tell you about the chromosome on which the **d** gene resides?
The d gene must be on an autosome. If it were on the X chromosome then 1/2 of all male offspring would have the d phenotype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

A cross between two yellow mutant mice gives rise to the following offspring: two thirds yellow and one third agouti mice. Draw out the cross and make a hypothesis for what could give rise to this phenotypic ratio.

	Y	y
Y	YY (x)	Yy
y	Yy	yy

Y = dominant; yellow
y = recessive; agouti

The 2:1 ratio can arise if one of the genotypes is lethal.

Mice that have inherited the Y allele will be yellow because the allele acts in a dominant manner. However, mice homozygous (YY) for the yellow allele are not viable because they are recessive lethal. As a result, only heterozygous yellow mice (Yy) survive. yy mice are agouti. Thus, there will be two Yy (yellow) for every one agouti (yy).
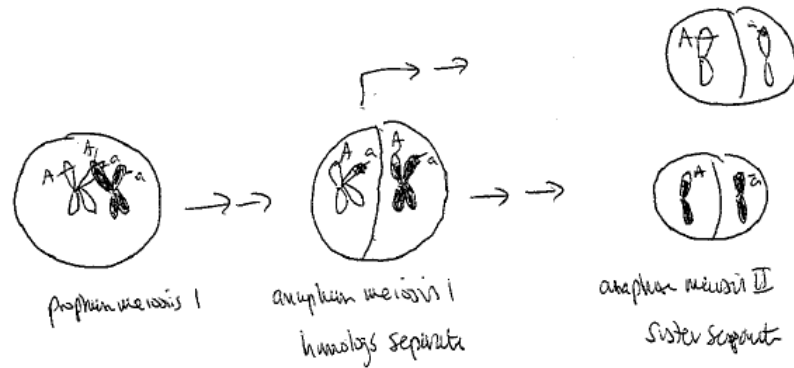
Question 5:
Consider an individual heterozygous for albinism.

(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.

prophase meiosis I   anaphase meiosis I   anaphase meiosis II
                          homologs separate   Sister separate

Question 6:
You perform a selection for mutant *Arabidopsis* plants that can grow in the presence of high salt. You get four mutants (m1-m4). To determine how many genes are mutated, you perform complementation tests and get the following results when plants are grown on high salt.

	m1	m2	m3	m4
m1	Alive	Dead	Alive	Dead
m2	Dead	Alive	Dead	Alive
m3	Alive	Dead	Alive	Dead
m4	Dead	Alive	Dead	Alive

(a) You were lucky that your results were interpretable. What should you have done first?

Check whether the mutant phenotypes are dominant or recessive. If the phenotype is dominant, then you might be confused about a failure to complement.

(b) How many genes are there?

2

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 *Caenorhabditis 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:

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

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?
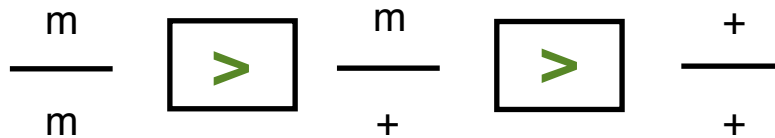*These strains could have mutations in other ura genes not ura5.*

Question 10:

For the following questions, write the phenotypic relationships that would show the mutation effect in the boxes.

Use > or < symbols to denote when mutant phenotypes will be worse or better (or = equal).

(a) Hypermorph (increase in wild-type function)

$$\frac{m}{m} \quad \boxed{>} \quad \frac{m}{+} \quad \boxed{>} \quad \frac{+}{+}$$

(b) Neomorph (altered function)

$$\frac{m}{m} \quad \boxed{= \text{ or } >} \quad \frac{m}{+} \quad \boxed{>} \quad \frac{+}{+}$$
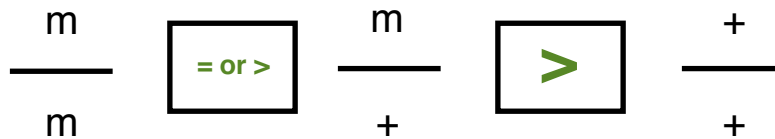
(c) Hypomorph (partial loss of gene function)

$$\frac{m}{m} \quad \boxed{>} \quad \frac{m}{+} \quad \boxed{=} \quad \frac{+}{+}$$
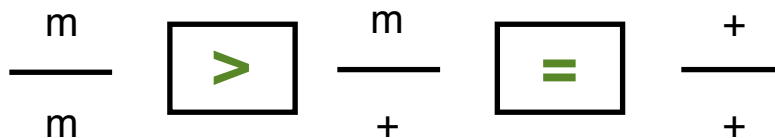
Question 11:
You are interested in motivated behaviors and have experience with the fruit fly *Drosophila*. You design a phenotype assay where you shoot the flies with a laser and observe how quickly they fly away from the heat stimulus.

(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.

$$EMS \searrow$$

$$\frac{\pm}{+} \, \male \quad \times \quad \frac{Sk}{Cyo} \, \female$$

$$\downarrow$$

Select Curly not Streak males
$$\boxed{\frac{M}{Cyo}} \, \male \quad \times \quad \frac{Sk}{Cyo} \, \female \quad \text{Single pair cross}$$

$$\downarrow$$

Select any not streak males + virgin females
$$\frac{M}{Cyo} \, \male \quad \times \quad \frac{M}{Cyo} \, \female$$

$$\downarrow$$

$$\frac{M}{M} \quad \text{Select not curly flies to test for defective motivated behaviors}$$
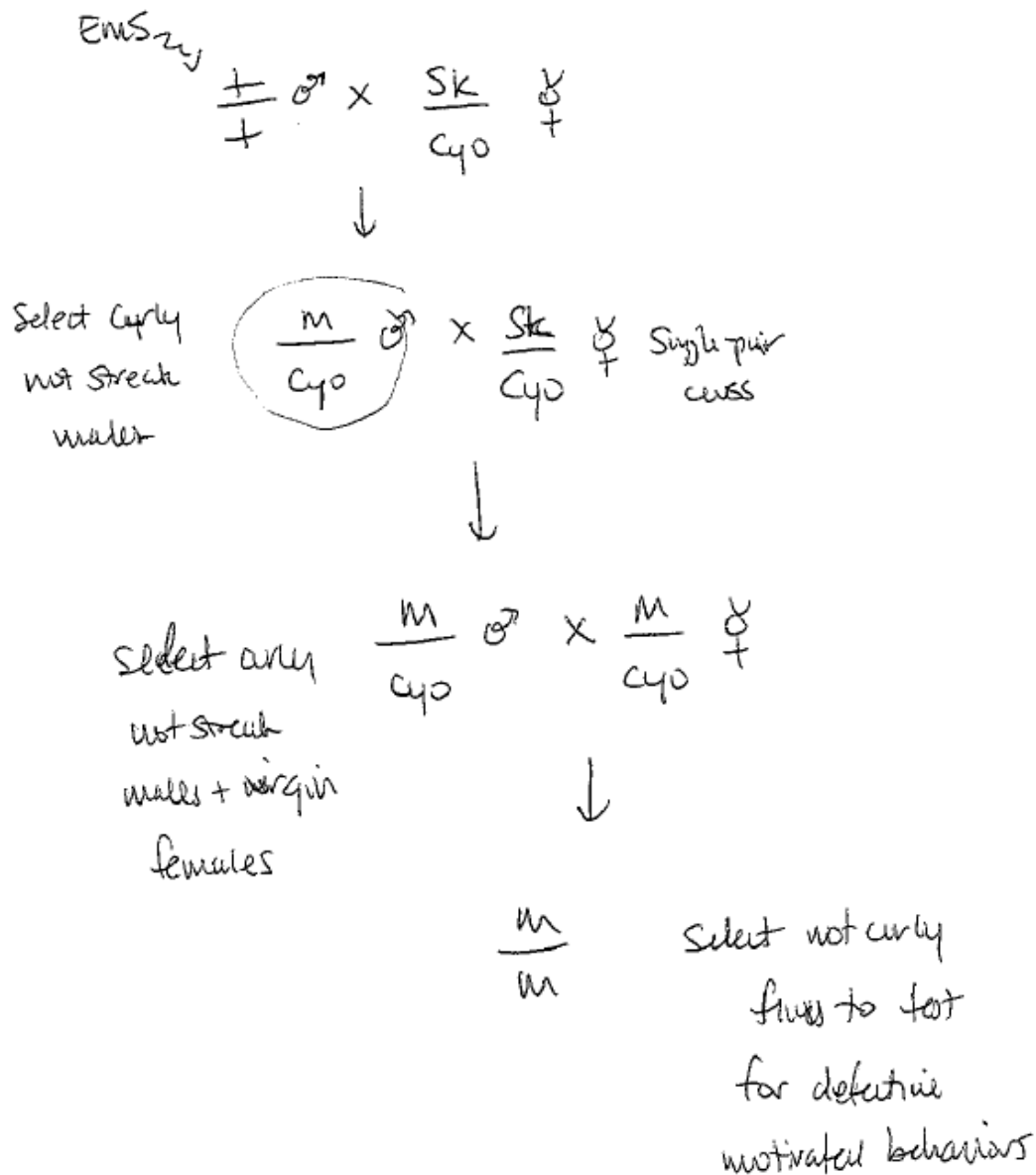
(b) You identify 10 mutant strains in your screen. Four have dominant phenotypes, and six mutants have recessive phenotypes. All six mutants with recessive phenotypes are defective in the same gene. You rudimentarily map the mutant alleles so that you can use different deficiencies (Df) and duplications (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 12:
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 <u>did not grow</u> on minimal medium and a filled square indicates that the diploid <u>did grow</u> 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