Bio393: Genetic Analysis **Name:**_____
Midterm (10 pages, 120 points)

Question 1 (12 points):
Alfred Sturtevant kept careful track of horse coat colors on his farm when he was growing up. He observed that a black mare crossed to a chestnut stallion produced all bay offspring. Mating these bay offspring gave rise to offspring of four different coat colors: black, bay, chestnut, and liver. Crossing liver offspring back to the black mare gave all black offspring. Crossing liver offspring back to the chestnut stallion gave all chestnut offspring. Explain how coat color is being inherited in horses and what the genotypes of each color are.

Coat color is controlled by two different genes (A and B). The black mare is AAbb and the chestnut stallion is aaBB. The bay offspring are AaBb. When you cross two bay offspring, you get all four phenotypic classes.

Black offspring are A-bb.
Chestnut offspring are aaB-.
Bay offspring are A-B-.
Liver offspring are aabb.

Question 2 (6 points):
Using linkage mapping, you determined the genetic distance between A and B is 150 cM. What is the minimum number of intermediate markers between A and B that you would need to make this estimate possible? Please explain your reasoning.

You need at least three intermediate markers to estimate the recombination distance between A and B. Because 50 cM is the maximum recombination distance (it looks like independent assortment), three markers gives an average distance of 37.5 cM if they are all equally spaced between A and B. With two (or fewer) markers, the distance between any two markers would be 50% or appear to be independently assorting.

Question 3 (6 points each):
Over the past summer, you mentored a high school student in the lab. He worked hard but did not take any notes or keep a lab notebook. You had him look for suppressors of a mutant phenotype caused by a point mutation resulting in a null phenotype (not necessarily a stop codon mutation).

(a) Propose a cross to differentiate revertants (or back suppressors) from extragenic suppressors and how the outcomes of the cross would show either option.

Extragenic suppressors are often unlinked from the original mutant allele.

So, crosses to a wild-type strain will generate heterozygotes for the suppressor and the original mutant allele. After crossing these heterozygotes, you would get mutant individuals in the next generation because one-sixteenth will be mutant without the suppressor allele.

Or, you could cross to the mutant allele. In the next generation, revertants would still express the wild-type phenotype whereas suppressors with a recessive phenotype will look mutant.

(b) Is it possible that he isolated an intragenic suppressor? Explain why or why not.

Yes. Because this allele is not necessarily a stop codon (nonsense) mutation, it is possible that a second-site mutation in the same gene could suppress the effect of the original null mutation.

Question 4 (6 points):
Please give two reasons why it is difficult to identify the mutation causing the mutant phenotype of interest just from sequencing the whole genome of a wild-type and a mutant organism.

(1) Mutations (whether natural or induced) are all over genomes. Sequencing will find random mutations throughout the genome that are different between any two strains.
(2) Depending on how diverse the species is, much of the genome of the wild-type and mutant strains could be heterozygous and difficult to find the causal mutations.
(3) Different species have loss of genomic DNA in the somatic cells versus the germline (chromosome diminution). The loss of chromosome regions could be different in different individuals, so sequencing might miss common genomic regions.
(4) Some mutant phenotypes are caused by more than one mutation. It would be difficult to find both of them in a search through a large number of mutations.

Question 5 (18 points):

On a recent hike in the woods, you start digging through some rotting logs (highly recommended). You see some terrestrial isopods of the species *Porcellio scaber*, including an entire log populated by isopods that are bright purple. You begin to dream of Norwestern-marketed isopod pets for every new Wildcat. Oh the profits! You collect a bunch of individuals along with some wild-type looking (grayish) individuals from another log.

Back in the lab, you cross purple by purple isopods. You only get purple isopods. You also cross gray by gray isopods, and you only get gray isopods. Feeling confident that you have pure-breeding strains, you cross a purple isopod with a gray isopod. All of the F1 individuals are gray. You then mate these gray F1 individuals back with the purple parent (a test cross). After a lot of work, you are excited to count 1000 offspring. The color phenotype data are below.

Phenotype	Number
Gray	513
Purple	482
Red	3
Blue	2

(a, 12 points) Describe a genetic model for how the four color phenotypes are controlled and the relationships between the gene(s).

The dominant phenotype is gray. One model is that purple is the loss of two different genes red and blue. Single mutations in red make a red recessive isopod phenotype, and single mutations in blue make a blue recessive isopod phenotype. From the described test cross, you identify single red and blue mutants. Given that only five out of 1000 were identified, the two genes are very close. They are 0.5 cM apart.

You identify a rare white isopod in a culture of your gray true-breeding strain. You generate a true-breeding white strain of isopods. When you cross white and purple isopods, you get gray isopods again. You allow these F1 isopods to interbreed. In the next generation, you see gray, purple, and white isopods again (along with rare red and blue isopods) at a 9:3:4 ratio. When you cross these purple isopods, you get white isopods about two-thirds of the time.

(b, 6 points) Assuming that white mutants have an absence of red and blue pigments, what can you say about the relationship of the white gene and the previous color gene(s)?

The white gene confers a recessive white phenotype and is epistatic to the two color genes red and purple.

In first cross, the white isopod is homozygous for the white mutation and for both the wild-type red and blue genes. It was isolated in the gray true-breeding stock. The purple isopod is mutant for both the red and blue genes but wild-type at the white gene locus. This cross generates heterozygotes at all three loci.

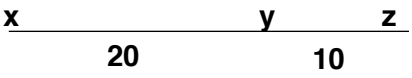
In the second cross, the heterozygotes interbreed. Because the white gene is epistatic to both red and blue, one quarter of the offspring are homozygous for the white mutant allele and are white. Of the remaining non-white offspring, one quarter of them are purple.

You get white isopods about two-thirds of the time when mating the purple isopods because two thirds of the offspring are heterozygous for the white mutation.

Question 6 (18 points):
Your poli sci roommate does not believe all this mumbo jumbo about genetics. He says that there is no way for you to predict the phenotypes of offspring from the following cross.

$$\frac{x-\ y-\ z-}{+\ +\ +} \times \frac{x-\ y-\ z-}{x-\ y-\ z-}$$

You look up on wikipedia that the x, y, and z genes are all linked. Also, x is 20 map units from y, and y is 10 map units from z. The gene order is x, y, z. Fill out the table below for numbers of offspring with each phenotype (Total offspring = 1000).



Phenotype	Number of offspring
x y z	***360***
+ + +	***360***
x y +	***40***
+ + z	***40***
x + +	***90***
+ y z	***90***
x + z	***10***
+ y +	***10***

Recombination between x and y should occur with a frequency of 0.2 and between y and z with a frequency of 0.1.

*However, recombination will also occur in both intervals in double recombinants with a frequency of 0.2 * 0.1 = 0.02*

Therefore, if we account for double recombinants:

freq(xy+) = 0.2 - 0.02 = 0.18
freq(+yz) = 0.1 - 0.02 = 0.08

Multiply 1000 by expected freq. and divide by 2 to account for both classes:

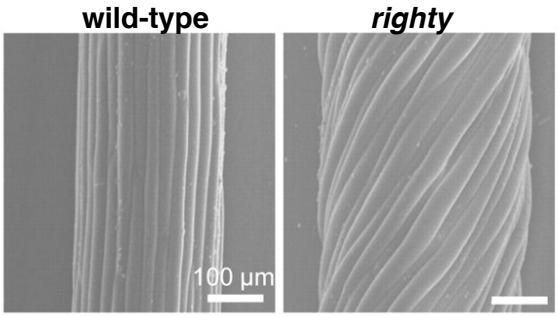
*n(xy) = (1000 * 0.18) / 2 = 90*
*n(yz) = (1000 * 0.08) / 2 = 40*

n(double recombinants) =
*1000 * 0.02 = 20.*

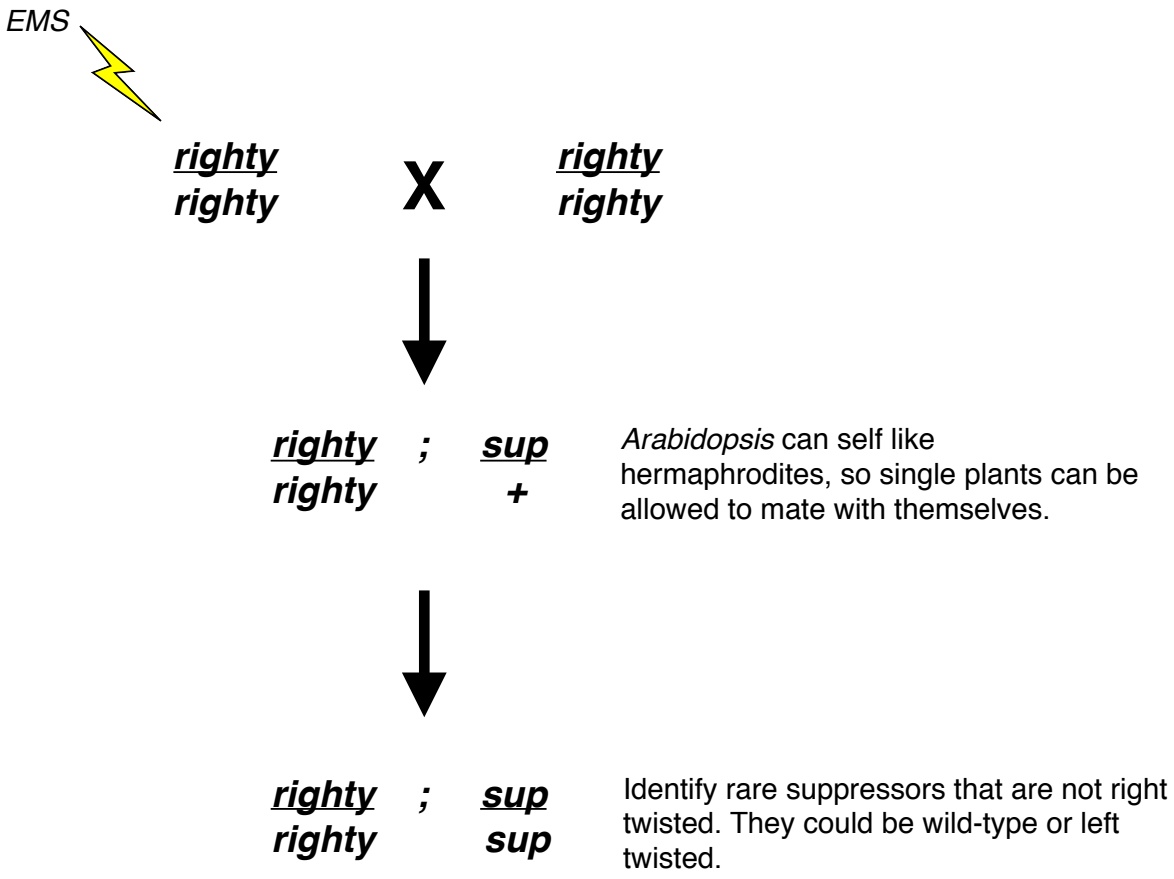
Finally, we can calculate the number of parentals:

*Probability of not having a recombinant between x and y (0.8), and probability of not having a recombinant between y and z (0.9). Therefore, the probability of both is 0.8*0.9=0.72 or 720/1000*

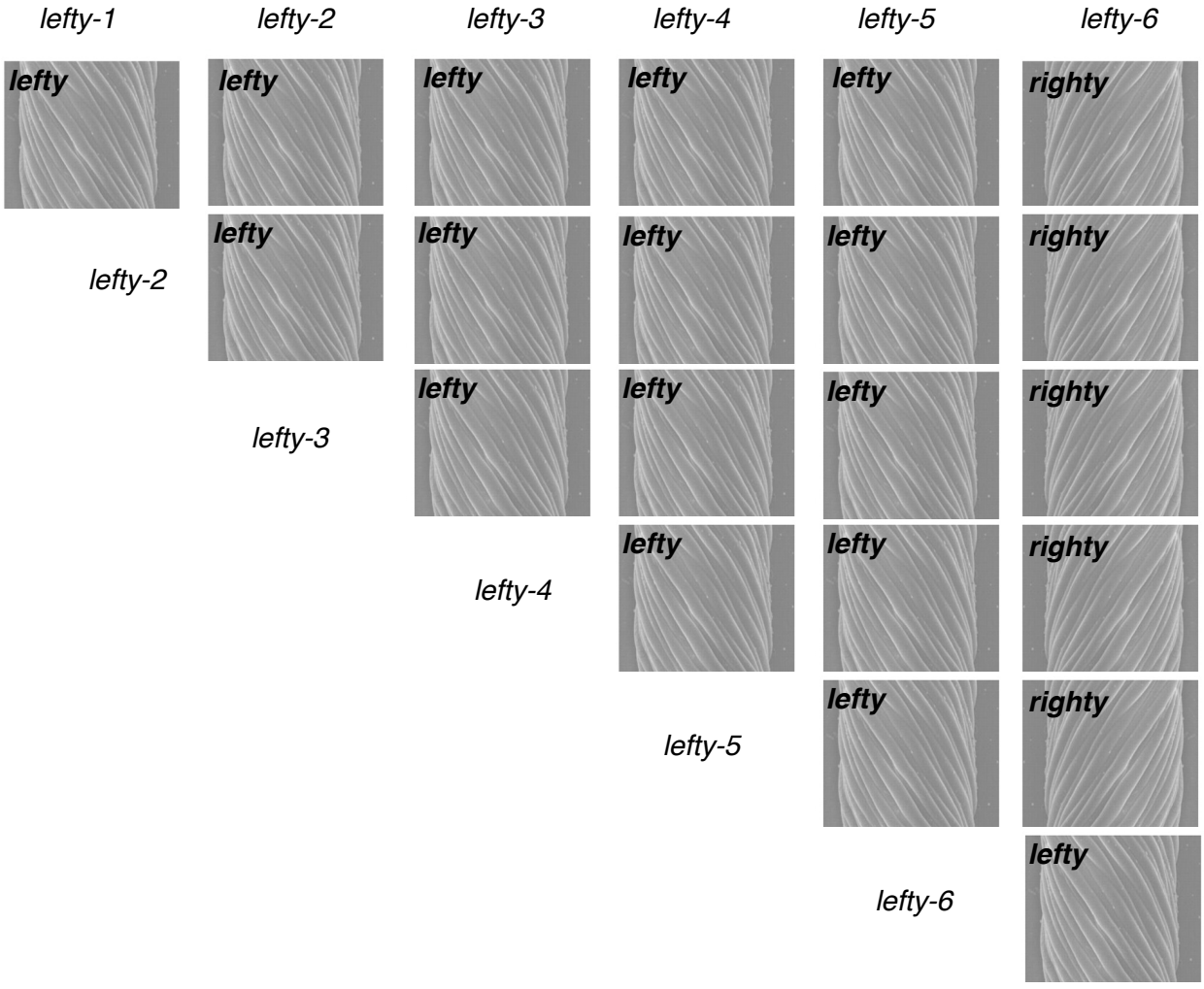
Question 7 (36 points):
The mustard weed *Arabidopsis thaliana* is a powerful model to understand many plant traits. You are interested in the development of the axial organ or stem because you want to genetically engineer taller plants. In wild-type plants, the axial organ grows with little twisting and the flowers are radially symmetric. The post-doc you work with gave you a mutant strain that has a right-handed helical twist (*righty*).



(a, 12 points) Write out a genetic screen to identify suppressor mutants (*sup*) of *righty*.



(b, 6 points) You are incredibly successful in your screen and identify six independent suppressor mutants. Interestingly, all of them have a recessive left axial twist phenotype (*lefty* mutants). Describe the number of genes and which mutants share a common perturbation of function.



Two genes were identified from the screen. lefty-1, lefty-2, lefty-3, lefty-4, lefty-5 all fail to complement each other. lefty-6 complements all the other lefty mutants, so it is the sole mutant in that gene.

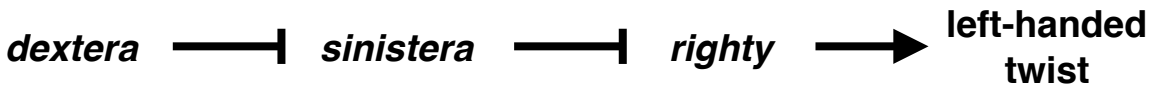
(c, 4 points): Please describe what a mutant with a dominant left axial twist look like in the crosses in part (b).

It would have a lefty phenotype when crossed to any mutant strain. The complementation test with this allele would be uninformative.

(d, 4 points): Your PI tells you to keep screening because you have not found many of the genes for control of axial organ outgrowth orientation. Given the data you have so far, why would she say that statement?

You found five mutations in one gene and only one mutation in the other gene. If the screen were saturated for the mutagen you used, then you would expect that you would have more mutations in the gene with only one mutation.

(e, 10 points) A collaborator sends two other mutants to you so that you can build a genetic pathway for control of axial organ twist. Using your original *righty* mutant and the two new mutants, right-handed twist (*dextera*) and left-handed twist (*sinistera*), along with the genetic relationships shown below, fill out the single and double mutant phenotypes that would give you this pathway. Describe the reasoning behind your double mutant phenotypes after the table.



Genotype	Phenotype
righty	**Right-handed twist**
dextera	**Right-handed twist**
sinistera	**Left-handed twist**
dextera; sinistera	**Left-handed twist**
dextera; righty	**Right-handed twist**
sinistera; righty	**Right-handed twist**

In the model described in this answer, the double mutant phenotype is the phenotype of the gene that acts most downstream in the pathway.

Question 8 (4 points each):
With respect to a typical genetic screen, please answer the following questions:

(a) What types of mutations are **most** common and why?

Reduction or loss-of-function mutations are most common because it is easier to break something than to make it work better. Alternatively, the most common mutation would be nucleotide changes that do not affect any coding sequences.

(b) What type of phenotype is **least** common and why?

Dominant phenotypes are least common mutant phenotype because they are often caused by more rare events like gain-of-function. Alternatively, the most common phenotype would be the wild-type phenotype. Obtaining mutants is a rare event.

(c) What phenotype is **most** often observed and why?

Sterility or lethality is mutant phenotype most often observed because a large number of genes function in offspring production or viability. Alternatively, the most common phenotype would be the wild-type phenotype. Obtaining mutants is a rare event.

Please fill out the post-midterm survey at bio393.andersenlab.org