**Bio393: Genetic Analysis** **Name:**_____
**Midterm**

**Question 1 (6 points):**
White-nose syndrome is devastating insect-eating bat populations across the United States. It is caused by the fungus, *Pseudogymnoascus destructans*, and spreads during hibernation. In effort to control this pathogen, you grow it in the lab and want to identify compounds to kill it. Standard anti-fungal compounds work well, but some strains of the fungus survive treatments. Propose a hypothesis to explain how a mutation in a gene could cause drug resistance.

*Loss or reduction-of-function mutations in genes for the targets of the anti-fungal compounds could affect the efficacy of the drug. Likewise, mutations in genes that encode drug import pathways would prevent drug import and make the cells resistant.*

*Gain-of-function mutations in genes that encode drug export pathways would remove compounds and promote drug resistance. Likewise, gain-of-function mutations in genes that encode drug break-down pathways would degrade compounds faster and promote resistance.*

*There are other explanations.*

**Question 2 (8 points):**
You would like to identify a loss-of-function mutation in every one of the approximately 20,000 genes in *C. elegans.* You perform an EMS-mutagenesis experiment and isolate 2,000 mutant strains. After whole-genome sequencing, you find that 2,000 genes are devoid of predicted loss-of-function mutations and approximately 500 of the 2,000 genes are not mutated at all.

Provide explanations for (1) why you fail to recover loss-of-function mutations in every one of the 20,000 genes and (2) why you fail to recover any mutations in 500 genes.

*Mutagenesis causes approximately one mutation per 1,000 genes. Each strain should harbor 20 mutations. In 2,000 strains, approximately 40,000 genes should be mutated, if every strain was a mutant strain. In other words, each gene should be mutated twice if mutagenesis were completely random. Given statistical sampling, it is possible that not every gene will have a mutation. However, 2,000 genes without predicted loss-of-function mutations is too high a number for statistical sampling issues. (1) It is likely that you fail to recover loss-of-function mutations in every one of the 20,000 genes because complete loss of many of these genes is lethal. (2) For those 500 genes, lack of any mutation is unlikely because silent (synonymous) mutations should be found. It is likely that these genes were missed because of sampling.*

**Question 3 (6 points):**
Blood type in humans is a codominant trait. A man with blood type A whose mother was blood type O has children with a woman with blood type B whose father was blood type O. What blood type(s) could the children inherit? Remember that two copies of the null allele result in blood type O.

(a) O only
(b) A, B, and O
(c) AB, A, B, and O
(d) AB only

**Question 4 (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 5 (8 points):**
Remember that 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 6 (6 points):**
You have three true-breeding strains of gerbils – all of which express the recessive white fur trait. You cross line1 to line2 and get gerbils with brown fur. Then, you cross line1 to line3 and get gerbils with white fur. Last, you cross line2 to line3 and get gerbils with brown fur. What is your interpretation of these results?

(a) The genes mutated in line1 and line2 are different
(b) The genes mutated in line1 and line3 are different
(c) The genes mutated in line2 and line3 are different
(d) Both (a) and (b) are true
(e) Both (a) and (c) are true

**Question 7 (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 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 it 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.*

**Question 8 (4 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)** How can you differentiate revertants (or back suppressors) from extragenic suppressors?

*Extragenic suppressors would be unlinked from the original mutant allele. So, crosses to a wild-type strain will generate mutants again if the suppressor is extragenic. Or, you could cross to the mutant allele. 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 mutation, it is possible that a second-site mutation in the same gene could suppress the effect of the original null mutation.*

**(c)** What could you do to determine if the suppressor was a revertant or an intragenic suppressor?

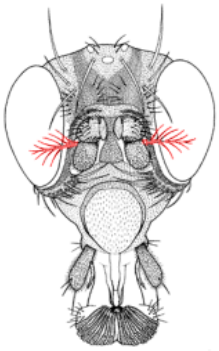
*It is difficult! They are both linked, so crosses to separate the intragenic suppressor from the revertant would likely not separate them unless it was a huge gene and/or you collected many individuals. The easiest way is to sequence the gene and look for a second-site or not.*

**(d)** One of the suppressor mutations also suppresses mutant alleles of different genes. How might that suppressor act?

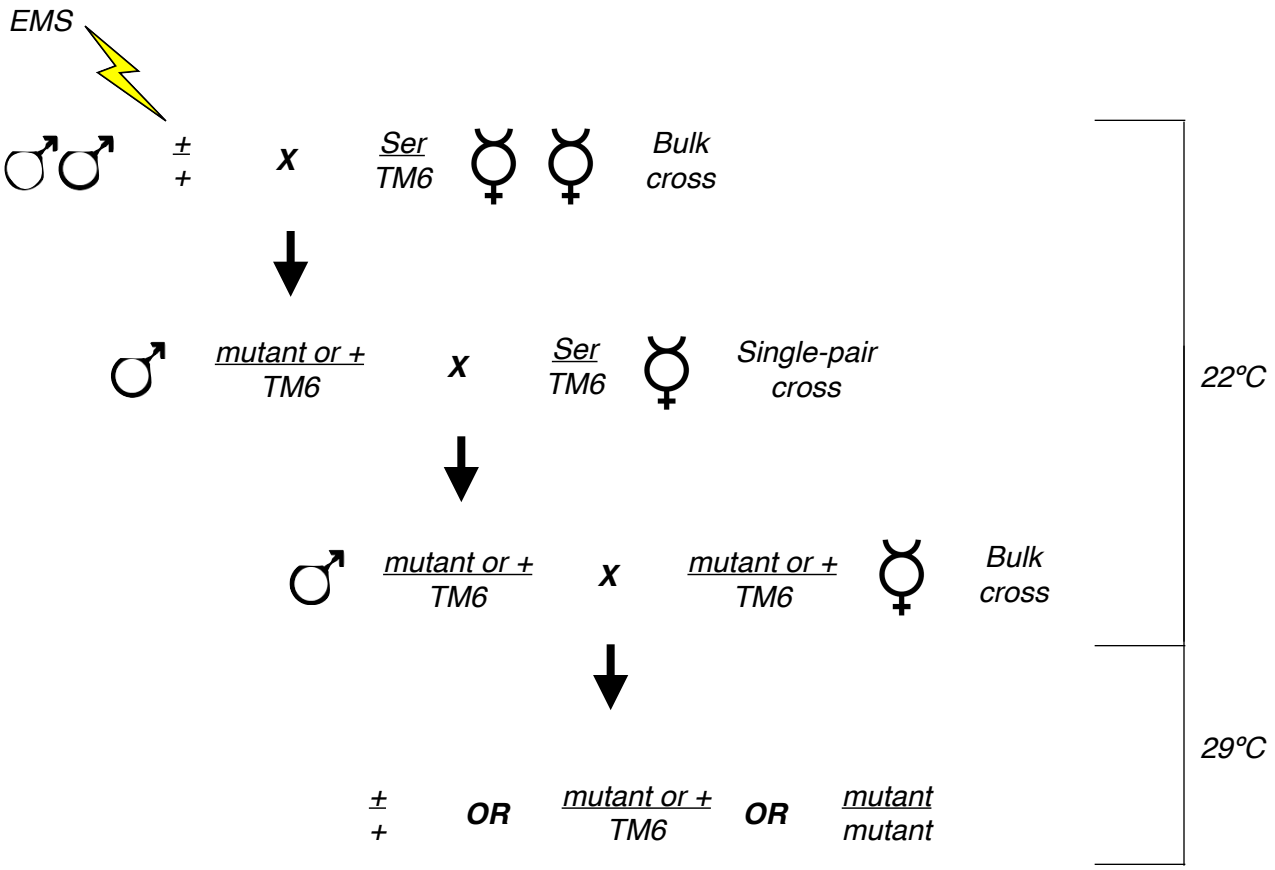
*It is an informational suppressor, so it allele-specific but gene non-specific.*

**Question 9:**

*Drosophila* "hear" sounds by feeling air vibrations with their arista or polarized hair structures between their eyes (red in image below). You are interested in the sensory programs underlying the recognition of air vibrations by the arista. Because few mutants affecting arista development exist, you design a genetic screen to identify them. Your focus is on chromosome 3, specifically on temperature-sensitive alleles. Using these alleles, you can sensitize arista development for future genetic studies. You have wild-type flies, a balancer stock Ser/TM6 [Antp], and two incubators (one at 22ºC and one at 29ºC). TM6 is marked with an *Antennapedia* allele [Antp] that causes a dominant transformation of antennae into legs, so you can easily see the presence of the balancer. Ser causes a dominant serrated wing phenotype.



**(a, 20 points)** Design a genetic screen to identify mutations that cause a temperature-sensitive recessive arista mutant phenotype. Be sure to specify bulk or single-pair crosses along with the rearing temperature for the flies.




*Homozygous mutants can be selected by lack of Antennepedia. When raised at 29ºC, mutants in temperature-sensitive aristaless alleles will not form arista.*

You identify six temperature-sensitive alleles. To determine how many genes could be mutated, you set up complementation crosses.
**(b, 6 points)** Draw out a cross for *aristaless-1* and *aristaless-2* below.

♂ $\frac{\textit{aristaless-1}\ +}{\textit{aristaless-1}\ +}$ **x** $\frac{+\ \textit{aristaless-2}}{+\ \textit{aristaless-2}}$ ♀

↓

$\frac{\textit{aristaless-1} \qquad +}{+ \qquad \textit{aristaless-2}}$

You grow the cross progeny from the complementation test at the restrictive temperature and score for arista. The data are below.

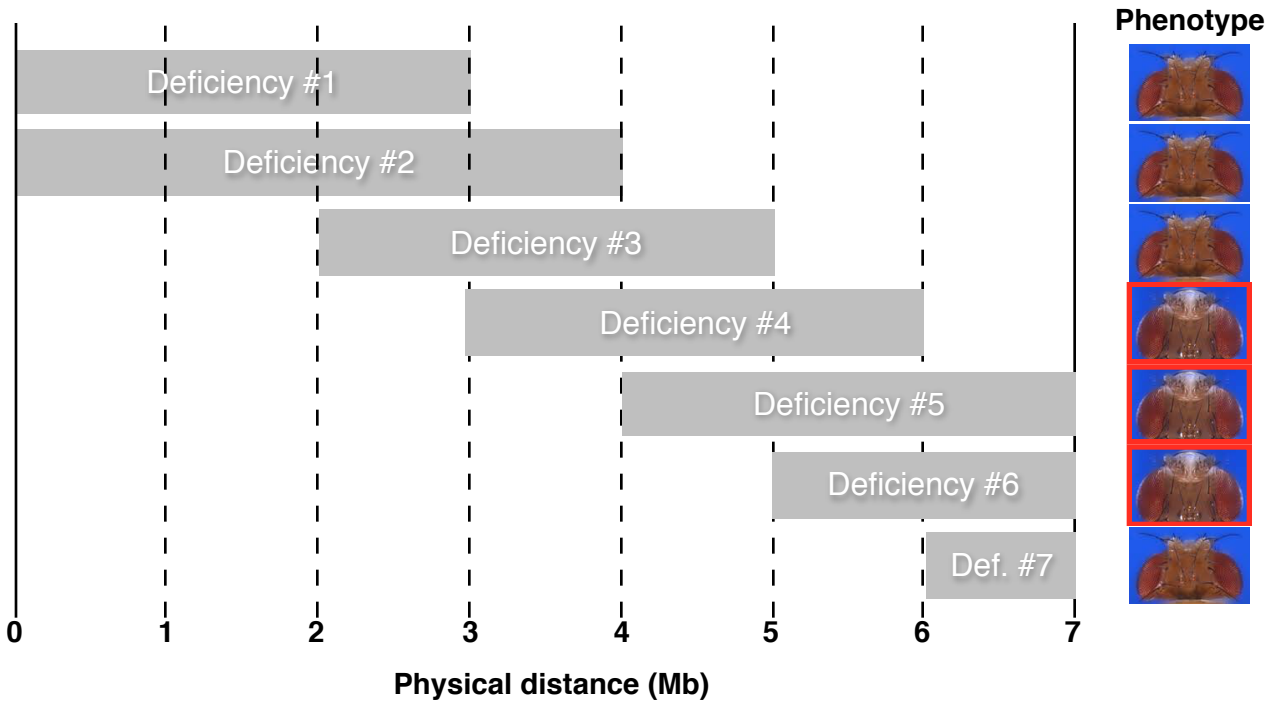
 Wild-type  Aristaless mutant

| | **Wild-type** | ***aristaless-1*** | ***aristaless-2*** | ***aristaless-3*** | ***aristaless-4*** | ***aristaless-5*** | ***aristaless-6*** |
|---|---|---|---|---|---|---|---|
| **Wild-type** |  |  |  |  |  |  |  |
| ***aristaless-1*** | |  |  |  |  |  |  |
| ***aristaless-2*** | | |  |  |  |  |  |
| ***aristaless-3*** | | | |  |  |  |  |
| ***aristaless-4*** | | | | |  |  |  |
| ***aristaless-5*** | | | | | |  |  |
| ***aristaless-6*** | | | | | | |  |

**(c, 6 points)** Write out the complementation groups and any ambiguities.

*[1,3,5,6] and [4]*
*aristaless-2 causes a dominant phenotype so we can not assign it to a complementation group.*

You choose to map the location of the *aristaless-6* mutation using a collection of deficiencies that lack large parts of chromosome 3. Some but not all of these deficiencies remove or delete many genes including the gene mutated in *aristaless-6*. You cross *aristaless-6* mutant flies to each of the deficiencies and look at the aristaless phenotype in the heterozygous flies. The extents of the deficiencies and the aristaless phenotype data from the heterozygotes are below.




**(d, 8 points)** Where is the approximate physical location of the gene mutated in *aristaless-6* and how did you come to that conclusion?

*The gene is located between 5 and 6 Mb on chromosome III. Because deficiencies 4, 5, and 6 fail to complement the recessive aristaless phenotype of the aristaless-6 mutant, they must have the wild-type allele deleted. The other deficiencies complement the aristaless phenotype of the aristaless-6 mutant so they do not have the wild-type allele deleted.*

**(e, 4 points)** In each deficiency heterozygote with the aristaless phenotype, the phenotype is more severe than the homozygous mutants. What is the nature of the *aristaless-6* mutation?

*The phenotype of the heterozygote with the deficiency is more severe than the homozygous mutant phenotype. That genetic dosage result tells us that the aristaless-6 mutation is a hypomorph or a partial loss-of-function mutation.*

**(f, 8 points)** The *Drosophila* community has a very large resource of promoters that drive expression of your favorite gene in any tissue within the fly. Luckily for you, four promoters that express genes in tissues of interest exist: eye, leg, arista, and the whole body. You clone the gene mutated in the *aristaless-6* mutants and are interested in where in the fly the normal gene is required for function. Please describe the four transgenic strains you would like to produce and the predicted experimental outcome for each if the gene is required in the arista.

*You would like to express a wild-type copy of the gene mutated in the aristaless-6 mutant. With the four promoters, you can drive expression in four different tissues in the aristaless-6 mutant flies. In any tissue where the gene acts, you will see rescue of the artistaless-6 mutant phenotype.*

| ***Promoter*** | ***Rescue?*** |
|---|---|
| *eye* | *No* |
| *leg* | *No* |
| *arista* | *Yes* |
| *whole body* | *Yes* |