Bio393: Genetic Analysis
Midterm Key

Question 1 (9 points):
You are interested in optimizing corn ear size in *Zea mays* so you perform a screen for larger ears. A rare mutant is identified with large ears. Upon backcrossing and selfing to re-isolate the mutant phenotype, you find that only 1/16 of the offspring have large ears of corn.

(a, 3 points): Propose a model for why only 1/16 of the offspring have larger ears.

*The large ear corn phenotype could be caused by two alleles in unlinked genes that confer a recessive phenotype. We only observe large ear corn plants 1/16th of the time because we have a 1/4 *1/4 chance of homozygosing the two unlinked alleles.*

Unfortunately, the larger ears are heavier and make the corn stalks bend and break more easily. Your friend has a strain with more stout stalks that could stabilize the heavier ears. His strain has normal size ears. You cross his strain with your strain and then self the resulting cross progeny. Only two plants out of 128 have big ears and more stout stalks.

(b, 6 points) Write out the cross and propose a model for the phenotypic ratio that you observe.

Two plants out of 128 is a ratio of 1/64, which suggests three unlinked alleles that confer recessive phenotypes.

Let's say that our two loci from part (a) are called ear1 and ear2. Only when both unlinked mutant alleles are homozygous do we see a big ear corn phenotype.

Then, let's say the strain from our friend has the mutant stout allele that makes thicker corn stalks.]

Our cross is:

ear1; ear2; stout+ X ear1+; ear2+; stout

ear1; ear2; stout+
ear1+ ear2+ stout

1/64 are homozygous for ear1; ear2; stout

Question 2 (5 points):
Using linkage mapping, you determined the genetic distance between A and B is 100 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 two intermediate markers to estimate the recombination distance between A and B. Because 50 cM is the maximum recombination distance (it looks like independent assortment), two markers gives an average distance of 33 cM if they each are equally spaced between A and B. With one marker, the distance between any two markers would be 50% or appear to be independently assorting.

Question 3 (6 points):
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).

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.

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 three-sixteenths will be mutant without the suppressor allele being homozygous.

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.

Question 4 (14 points):
Your 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 30 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 the numbers of offspring with each phenotype (Total offspring = 1000).

Phenotype	Number of offspring
x y z	**315**
+ + +	**315**
x y +	**35**
+ + z	**35**
x + +	**135**
+ y z	**135**
x + z	**15**
+ y +	**15**

Recombination between x and y should occur with a frequency of 0.3 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 0.3 * 0.1 = 0.03*

Therefore, if we account for double recombinants:

freq(xy+) = 0.3 - 0.03 = 0.27
freq(+yz) = 0.1 - 0.03 = 0.07

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

*n(xy) = (1000 * 0.27) / 2 = 135*
*n(yz) = (1000 * 0.07) / 2 = 35*

n(double recombinants) =
*1000 * 0.03 = 30.*

Finally, we can calculate the number of parentals:

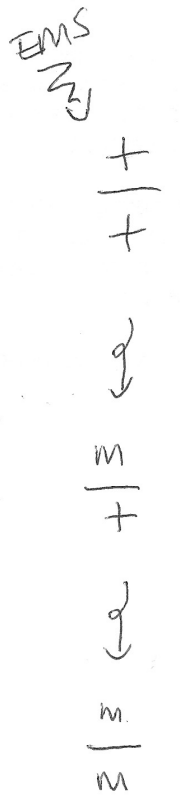
*Probability of not having a recombinant between x and y (0.7), and probability of not having a recombinant between y and z (0.9). Therefore, the probability of both is 0.7*0.9=0.63 or 630/1000*

Question 5 (36 points):
RNA interference (RNAi) is a conserved endogenous process found in most organisms. You would like to understand the genes that control this amazing process, so you turn to the obligate intracellular parasite *Toxoplasma gondii* because it readily and strongly induces RNAi. You have a variety of constructs that induce RNAi to cause 100% penetrant mutant phenotypes. One such example is the gene *let-1* that causes 100% lethality in the wild-type strain when inhibited by RNAi.

(a, 10 points) Describe and write out a selection that will allow you to identify mutations in genes that promote RNAi. *T. gondii* does not have balancer chromosomes, can self cross, and (for our purposes) is diploid. You can use a mutagen.

Mutations in genes that promote RNAi would be RNAi deficient or RNAi resistant. We have the let-1 RNAi construct that causes 100% lethality in the wild-type strain. This situation sets up a system where we can use selection to identity a large number of RNAi deficient mutants using a lethal RNAi like let-1. If we RNAi let-1, then all animals will die except for mutants that fail to do RNAi because they have mutations in genes that promote RNAi.

Perform the following mutagenesis and in the last generation raise all of the offspring after exposure to let-1 RNAi. All heterozygotes and wild-type individuals in that generation will die. Only RNAi deficient mutants will survive.



(b, 6 points) You would also like to identify mutations in genes that inhibit RNAi. Your collaborator sends you two RNAi constructs. One construct when applied to wild-type cells causes a weakly penetrant coloration defect where ~5% of the cells are less opaque than the wild type, and the other causes a weakly penetrant disorganized movement defect where ~10% of the cells move weirdly. Both mutant phenotypes do not affect the ability of the *T. gondii* cells to grow or reproduce. Using these two RNAi constructs, describe and write out a screen to identify negative regulators of RNAi.

You can use the same mutagenesis protocol and crosses as (a), but you will expose the last generation of animals to both of the new RNAi constructs instead of let-1. We want genes that normally inhibit RNAi, so mutants in those genes will be RNAi sensitive. We can identify them in the last generation because they will have increased levels of the opaque mutant phenotype AND increased levels of weird cell movement.

(c, 2 points) Why do you need to use two RNAi constructs in the screen in part (b)?

We need to two RNAi constructs because if we use just one we might identify mutants that enhance just the opaqueness OR the weird cell movement RNAi phenotypes and not generally inhibit RNAi.

(d, 8 points) From your selection in part (a), you identify two complementation groups. Describe the cross, genotypes, and phenotypic results for two mutations that complement each other (mut1 and mut2) and then two mutations that fail to complement each other (mut1 and mut3).

We will cross the two mutants to get a heterozygote for both.

If mut1 and mut2 complement, then we would get

mut1 mut2+
mut1+ mut2

These individuals would not be mutant because they are deficient in different functions. Therefore, they would die in the presence of let-1 RNAi.

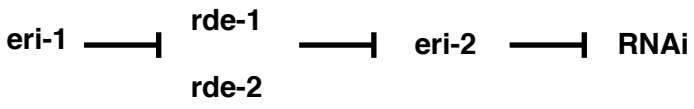
If mut1 and mut3 fail to complement, then we would get

mut1
mut3

These individuals would be mutant because they are deficient in the same function. Therefore, they would survive in the presence of let-1 RNAi.

(e, 12 points) You have two mutants in separate genes that promote RNAi (*rde-1* and *rde-2*; rde = RNAi defective) and two mutants in separate genes that inhibit RNAi (*eri-1* and *eri-2*; eri = enhanced RNAi). Using an RNAi construct with intermediate penetrance, you can measure enhancement or suppression of RNAi by these different mutant genes. You obtain the following results from single and double mutants. Please draw out a linear pathway for RNAi responsiveness in *T. gondii*. Note any ambiguities.

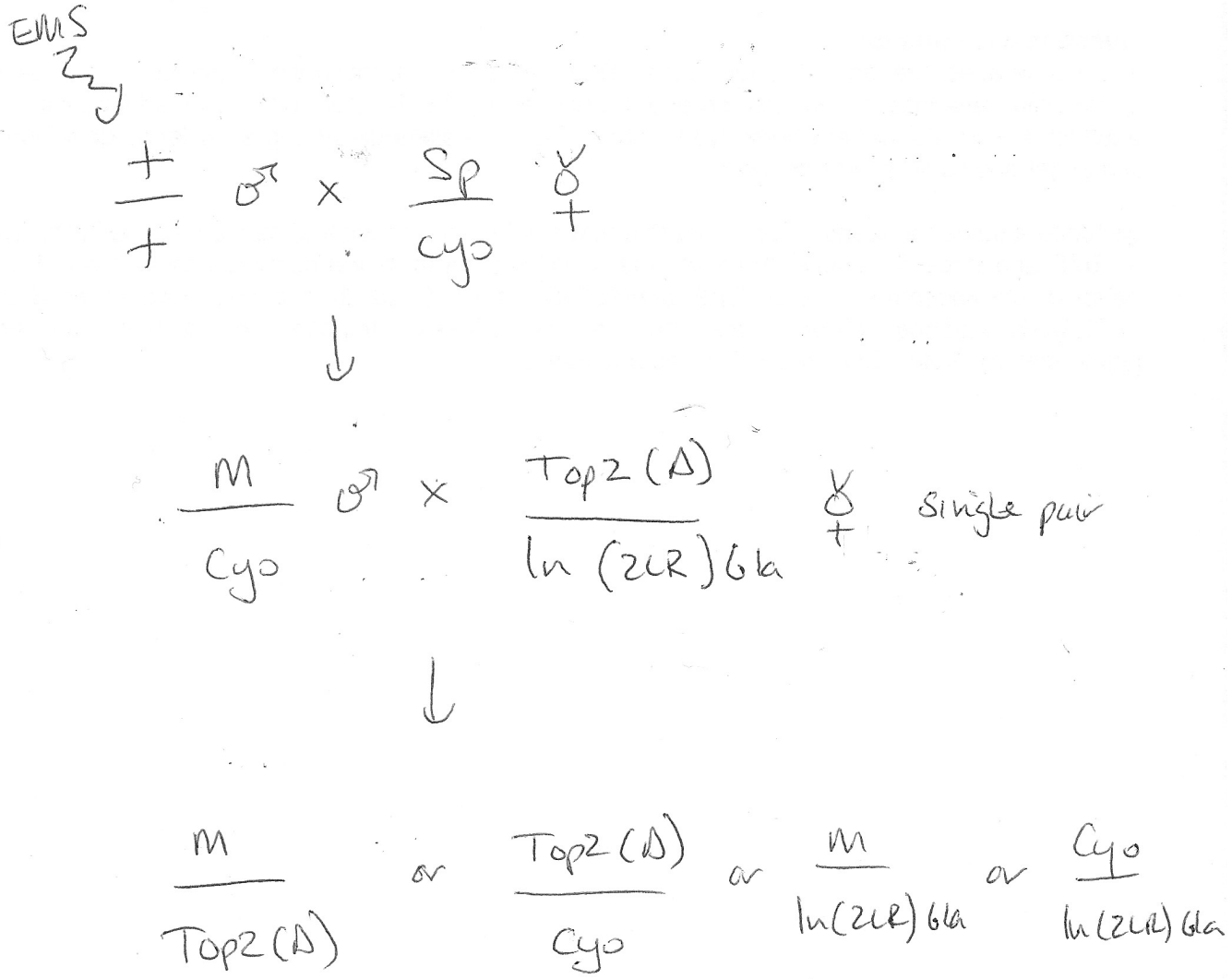
Genotype	Percent mutant after RNAi
+	50
rde-1	0
rde-2	0
eri-1	100
eri-2	100
rde-1; eri-1	0
rde-2; eri-1	0
rde-1; eri-2	100
rde-2; eri-2	100



We do not know the order of rde-1 and rde-2 because they have the same mutant phenotype.

Question 6 (15 points):
Topoisomerases are essential for proper DNA replication, transcription, and chromosome segregation. In *Drosophila melanogaster*, topoisomerase II (encoded by the *Top2* gene) is required for proper development to adulthood. Animals that are homozygous for a *Top2* null allele die during early larval development. You would like to get additional alleles of *Top2*.

Describe a screen to identify *Top2* but not mutations in other genes that cause larval lethality. You have a bottle of EMS and three fly stocks: the wild type, *Sp/Cyo* (Sternal plural dominant mutant with the Curly of Oster balancer chromosome), and a *Top2(Δ)/In(2LR)Gla* stock (*Top2(Δ)* is a null allele of *Top2* balanced by the *In(2LR)Gla* balancer chromosome that causes recessive lethality and a dominant increase in eye pigmentation). Note, *Cyo/In(2LR)Gla* flies are viable.



In the last generation, you will see if you get any non-Cyo (non-curly winged), non-In(2LR)Gla (non-eye pigmentation) individuals. If you do not and you see larval lethality, then those mutants must have failed to complement top2(Δ).

Question 7 (13 pts):
You are interested in understanding the genetics of congenital heart defects, so you perform an ENU-mutant screen in mice. After lots of work and heavy mutagenesis, you identify a mutant with a recessive heart defect and generate a pure-breeding stock. You notice that this stock also has a patchy hair loss phenotype, and your classmate (a self-purported mouse expert) points out that the stock is more aggressive than the parental stock.

You decide to cross wild-type parental males to your mutant stock. All of the resulting male offspring from that cross are patchy, but the mice have normal hearts and are less aggressive. After crossing siblings from that cross, you can identify males and females with heart defects and aggressiveness (but no patchiness) and use them to establish a stock that lacks patchy but has heart defects and aggressiveness.

(a, 6 points) Write out the cross and genotypes along with a brief explanation of why you performed this cross.

Let's say that the different mutant phenotypes are in three different loci (for the sake of writing the cross); h for heart defects, p for patchy hair, and a for aggressiveness. We are performing this cross to outcross what could be unlinked (and unrelated) mutations.

h p a mutants are crossed to h+ p+ a+ wild-type individuals to get heterozygotes. In that generation, you see patchiness in 100% of male offspring, indicating that the mutation that confers patchiness is on the X chromosome.

h a p X h+ a+ p+

h a p males AND *h a p females*
h+ a+ Y *h+ a+ p+*

Once you cross siblings, you get rid of patchiness (again) showing that it is unlinked and unrelated.

(b, 7 points) Provide a brief explanation for whether (or not) the same gene could be causing all three abnormal traits (heart defects, patchiness, and aggressiveness).

Mutations in the same gene could be causing both heart defects and aggressiveness (or they could be very closely linked and we haven't found a recombinant yet). The mutation that causes patchiness is definitely in a different gene because it is on a different chromosome (X).

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