

1. (5 pts) Our atmosphere is 21% O₂, yet some animals encounter environments with much lower O₂ levels. These animals can survive such low O₂ conditions (called hypoxia) because they respond to low O₂ by using a signaling pathway to express hypoxia survival genes (many of which are glycolysis enzymes). You decide to use *C. elegans* and a genetic screen to identify genes that comprise this signaling pathway. Wild-type *C. elegans* can survive in hypoxia (1% O₂). You use EMS in a clonal screen to look for mutants that, while healthy at 21% O₂, have trouble surviving at 1% O₂. You identify 5 independent mutations that give rise to a hypoxia sensitive phenotype: *od1*, *od2*, *od3*, *od4*, and *od5*. You perform a non-complementation test between different mutant alleles. You also compare each allele heterozygous against wild type (+). You test each transheterozygous F1 hermaphrodite for survival at 1% O₂. The data are in the noncomplementation chart below. "Live" indicates survival at 1% O₂. "Dead" indicates that less than 10% of the animals survive at 1% O₂.

		Allele From Hermaphrodite Parent					
		+	<i>od1</i>	<i>od2</i>	<i>od3</i>	<i>od4</i>	<i>od5</i>
Allele From Male Parent	+	Live	Dead	Live	Live	Live	Live
	<i>od1</i>		Dead	Dead	Dead	Dead	Dead
	<i>od2</i>			Dead	Live	Dead	Live
	<i>od3</i>				Dead	Live	Dead
	<i>od4</i>					Dead	Live
	<i>od5</i>						Dead

For each mutation, indicate whether you think it gives a dominant or a recessive phenotype.

od1 _____ dominant (1 pt)

od2 _____ recessive (1 pt)

od3 _____ recessive (1 pt)

od4 _____ recessive (1 pt)

od5 _____ recessive (1 pt)

2. (4 pts) Assign the alleles into non-complementation groups. Are there any alleles that you cannot assign into a group? Which one(s)? Why not?

Group A: *od2* and *od4* (1 pt)

Group B: *od3* and *od5* (1 pt)

od1 cannot be assigned to a group (1 pt).

Reason: it's dominant, and therefore will fail to complement everything (1 pt).

3. (3 pts) Based on the non-complementation data, do you believe that your screen was saturated? Discuss why or why not.

Not Saturated (1 pt)

Assuming a standard distribution, 99% saturation usually results in an average of 4 or 5 mutations (alleles) per gene (1 pt).

Based on our complementation data, we are only getting about 2 alleles per gene (1 pt).

4. (6 pts) You begin to map some of your mutations. You find that one of the mutations, *od2*, falls within a region that is uncovered by a deficiency, called *odDf22*. You cross your mutation to *odD22* and analyze the resulting phenotype, quantifying exactly the fraction of worms that die at 1% O₂. The data is shown in the graph below.

Genotype	Percent Worms That Survive 1% O ₂
+ / +	99%
<i>od2</i> / +	99%
<i>od2</i> / <i>od2</i>	5%
<i>od2</i> / <i>odDf22</i>	1%
+ / <i>odDf22</i>	99%

Is *od2* a loss of function or a gain of function allele? Discuss whether you think *od2* behaves like an amorph, hypomorph, hypermorph, antimorph, or neomorph? Is the gene mutated by *od2* haploinsufficient? Be sure to support your answers with a discussion of the data.

Loss of Function (1 pt).

Reason: the mutation is recessive and *od2* homozygotes are similar in phenotype to *od2* hemizygotes (1 pt).

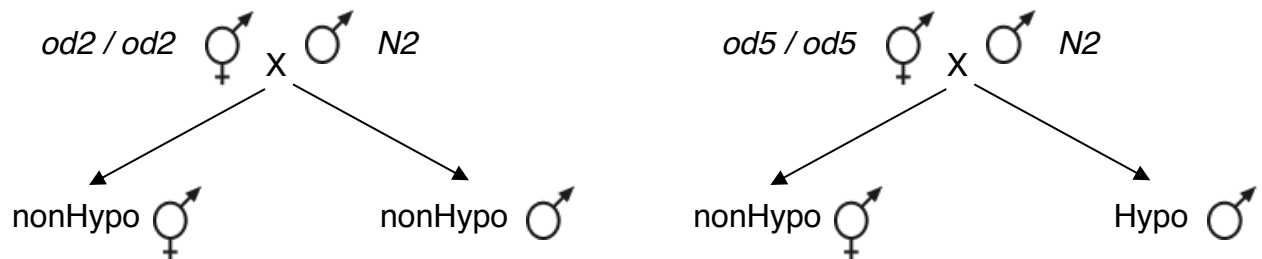
Hypomorph (1 pt).

Reason: recessive loss of function can be either amorph (null) or hypomorph (partial loss of function). The *od2/Df* has a stronger phenotype than that of *od2* homozygotes, suggesting that the *od2* mutation has some remaining gene activity relative to the Df. Thus, *od2* cannot be a null (1 pt).

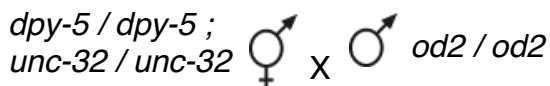
Not haploinsufficient (1 pt).

Reason: Haploinsufficient loci give dominant phenotypes. For example, the +/Df would give a phenotype were it haploinsufficient (1 pt).

5. (4 pts) You do several crosses to try to map *od2* and *od5*. First, you cross mutant hermaphrodites to N2 (wild-type) males and score the cross progeny for the hypoxia-sensitive (Hypo) phenotype (i.e., death at 1% O₂, the phenotype you used to identify these mutants in the first place).



Next, you cross either *od2* or *od5* mutant males into a double mutant for *dpy-5*; *unc-32*. Note that *dpy-5* is on chromosome I, whereas *unc-32* is on chromosome III. You single the F1 cross progeny hermaphrodites and score the different phenotypic classes of hermaphrodite F2 (you look at about 100 F2 each). Those classes are indicated below, each class with its own number.



nonHypo nonDpy nonUnc

1. nonHypo nonDpy nonUnc
2. Hypo nonDpy nonUnc
3. nonHypo Dpy nonUnc
4. nonHypo nonDpy Unc
5. nonHypo Dpy Unc (rare)
6. Hypo nonDpy Unc (rare)



nonHypo nonDpy nonUnc

1. nonHypo nonDpy nonUnc
2. Hypo nonDpy nonUnc
3. nonHypo Dpy nonUnc
4. nonHypo nonDpy Unc
5. Hypo Dpy nonUnc (rare)
6. nonHypo Dpy Unc (rare)
7. Hypo nonDpy Unc (rare)
8. Hypo Dpy Unc (very rare)

On which chromosome do you think *od2* is located? Explain your answer.

I (1 pt)

Reason: You can never recover *dpy-5 od2* double mutants – they must be linked (1 pt)

On which chromosome do you think *od5* is located? Explain your answer.

X (1 pt)

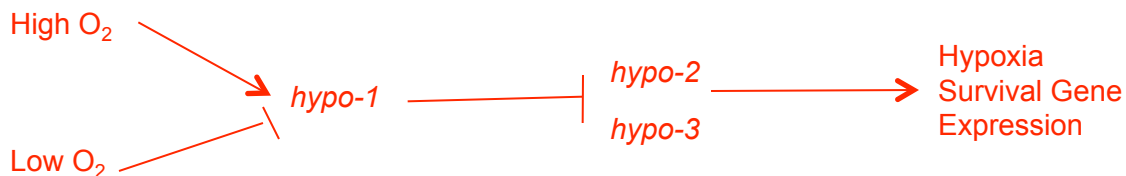
Reason: When crossed to N2 males, the male progeny, but not the hermaphrodites, show the mutant phenotype – a hallmark of X linkage (1 pt)

6. (6 pts) Whereas wild-type worms grow at 1% O₂, they die at 0.1% O₂. You believe that your mutants have trouble expressing the hypoxia survival genes, even under conditions of low O₂. You hypothesize that there might be genes that mutate to give the opposite phenotype from that of your Hypoxia-Sensitive mutants. That is, you screen for mutants that are Hypoxia Resistant – they grow even at 0.1% O₂, presumably because they overexpress the hypoxia survival genes. You identify one such Hypoxia Resistant mutant: *od6*. Linkage analysis places it in a different gene from those mutated in your first screen. You next decide to name the genes mutated by your alleles. You name the gene mutated by *od6* as *hypo-1*. You name the gene mutated by *od2* as *hypo-2*. You name the gene mutated by *od5* as *hypo-3*. You generate double mutants to perform epistasis analysis. In the table below, all genotypes are homozygous for the indicated allele.

Genotype	Phenotype
Wild Type	Normal O ₂ Sensitivity
<i>hypo-1(od6)</i>	Hypoxia Resistant
<i>hypo-2(od2)</i>	Hypoxia Sensitive
<i>hypo-3(od5)</i>	Hypoxia Sensitive
<i>hypo-1(od6); hypo-2(od2)</i>	Hypoxia Sensitive
<i>hypo-1(od6); hypo-3(od5)</i>	Hypoxia Sensitive

Place the three genes into a linear signaling pathway. Be sure to indicate the final outcome of the pathway: upregulation of the hypoxia survival genes. Indicate positive genetic interactions between the wild-type gene products with an arrow. Indicate negative genetic interactions between the wild-type gene products with a T-bar. Be sure to indicate the position of O₂ in the pathway.

Be sure to indicate if there are any genes that you cannot order in the pathway. Indicate which ones, and explain why you cannot order them.



Cannot order *hypo-2* and *hypo-3* (1 pt).

Reason: they have the same phenotype (1 pt).

Correct order of *hypo-1* versus *hypo-2* and *hypo-3* (1 pt).

An indication of the role of oxygen in the pathway (1 pt)

Correct sign between *hypo-2/3* and hypoxia survival gene expression outcome (1 pt)

Correct sign between *hypo-1* and *hypo-2/3* (1 pt)

7. (6 pts) You map the *hypo-2* gene to chromosome I. You use two recessive markers on chromosome I, *dpy-5* and *lin-10*, to do additional mapping. You cross *hypo-2* mutant males to *dpy-5 lin-10* double mutant hermaphrodites. You isolate the cross progeny and allow them to self fertilize. From the F2, you single obvious recombinants (14 Dpy nonLin animals and 11 Lin nonDpy animals) and allow them to self fertilize. You find that 2 of the 11 Lin nonDpy animals, when selfed, give rise to 1/4 Lin Hypo progeny. You also find that 11 of the 14 Dpy nonLin animals, when selfed, give rise to 1/4 Dpy Hypo progeny. You know that *dpy-5* is located at position 0 on chromosome I, whereas *lin-10* is located at position +2.5. What is the map position of *hypo-2* (based on your data)?

	<i>dpy-5</i>	<i>hypo-2</i>	<i>lin-10</i>
	----- ----- -----		
Dpy nonLin:	11		3
Lin nonDpy:	9		2
Total:	20		5

25 total recombinants between *dpy-5* and *lin-10*. 20 of those happened between *dpy-5* and *hypo-2*. Thus, the fraction of distance between *dpy-5* and *hypo-2* relative to the distance between *dpy-5* and *lin-10* is 20/25 or 0.8.

The actual map distance in cM between *dpy-5* and *lin-10* is 2.5 cM. The *hypo-2* gene is 0.8 of this distance to the right of the *dpy-5* locus. $0.8 \times 2.5 = 2.0$ cM to the right of *dpy-5*. Since *dpy-5* is at position 0 on the chromosome, *hypo-2* must be around +2.0.

2 pts for calculating the correct positions of the recombinants.

2 pts for figuring out the ratio of the interval.

2 pts for figuring out the exact position on the map.

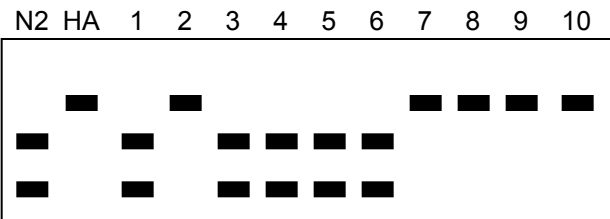
8. (2 pts) You eventually generate a homozygous triple mutant strain: *dpy-5 hypo-2 lin-10*. You cross this strain to males from the Hawaiian strain (HA). How can you identify cross progeny from self progeny in this cross?

Cross progeny will be nonDpy nonHypo and nonLin (2 pts).

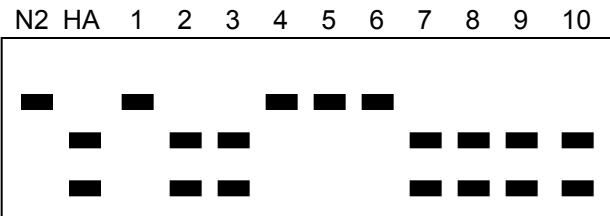
You self the cross progeny and obtain 4 Dpy nonHypo nonLin (#1-#4), 2 Dpy Hypo nonLin (#5 & #6), 2 nonDpy nonHypo Lin (#7 & #8), and 2 nonDpy Hypo Lin (#9 & #10) recombinants. You self these F2 and obtain a strain that is homozygous for the recombinant chromosome for each of these recombinant F2. You have PCR primers for 4 SNPs between *dpy-5* and *lin-10*. You do 4 separate PCR reactions for each recombinant (plus the parental strains as controls). You digest the PCR products with enzymes that distinguish Bristol (N2) from HA. The gel for each SNP is shown below, along with the map of the SNPs. The numbers 1-10 indicate the recombinants. Use this data in for the questions on the next page.



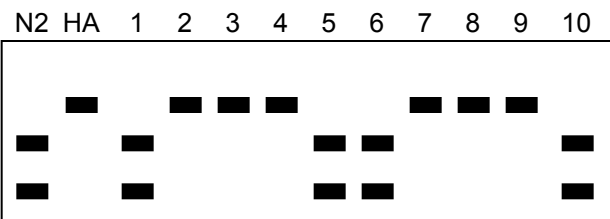
PCR and digest for SNP1



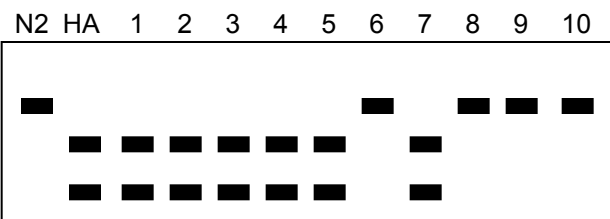
PCR and digest for SNP2



PCR and digest for SNP3



PCR and digest for SNP4



9. (10 pts) For each recombinant in the table below, indicate between which two markers (i.e., the SNPs as well as the genes *dpy-5* and *lin-10*) the recombination event occurred.

Rec.	Phenotype	Location of Recombination?
1	Dpy	Between SNP3 & SNP4
2	Dpy	Between <i>dpy-5</i> & SNP1
3	Dpy	Between SNP1 & SNP2
4	Dpy	Between SNP2 & SNP3
5	Dpy Hypo	Between SNP3 & SNP4
6	Dpy Hypo	Between SNP4 & <i>lin-10</i>
7	Lin	Between SNP4 & <i>lin-10</i>
8	Lin	Between SNP3 & SNP4
9	Lin Hypo	Between SNP3 & SNP4
10	Lin Hypo	Between SNP2 & SNP3

10. (4 pts) Between which markers is *hypo-2* located? Between SNP3 & SNP4

Genetics 502, Spring 2009,

The *C. elegans* Exam

NAME _____

PROBLEM	Possible Pts.	Points Received
1	5	
2	4	
3	3	
4	6	
5	4	
6	6	
7	6	
8	2	
9	10	
10	4	
TOTAL	50	