**Additional Problem Set**

1. The *C. elegans* gene *her-1* is defined by both dominant and recessive mutations. The dominant mutations partially transform XX animals into males, while recessive mutations transform X0 animals into hermaphrodites.

a) Animals that are hemizygous for a deficiency (Df/+) that removes the *her-1* gene are normal: XX animals are hermaphrodites and X0 animals are male. What does this tell you about the dominant *her-1* mutations described above?

**The dominant mutations are not haploinsufficient. They are gain-of-function mutations.**

b) Certain *her-1* recessive alleles transform some of the X0 animals into hermaphrodites, but also produce X0 animals that are intersexual, possessing both male and hermaphroditic characteristics. When X0 animals that are homozygous for these mutations (*her-1*/*her-1*) are compared to X0 animals that are hemizygous for these mutations (*her-1*/*Df*), the hemizygous X0 animals have fewer intersexual and more hermaphrodite individuals than *her-1*/*her-1* mutants. What can you say about these *her-1* alleles?

**The *her-1* mutations that produce intersexual animals are hypomorphic.**

c) Recessive loss-of-function mutations in the *C. elegans* gene *tra-2* cause X0 and XX animals to adopt a male fate. The HER-1 protein is a secreted ligand that binds to the TRA-2 receptor to inhibit its ability to signal. Describe the epistatic interactions between *her-1* and *tra-2* mutations that are consistent with this model.

***her-1(lf); tra-2(lf)* XX animals develop as males.**

d) You isolate a suppressor of one of the *tra-2* recessive mutations. This suppressor mutation suppresses only one of the seven different *tra-2* mutations tested. What type of suppressor could this be and why?

**It is either an informational suppressor or an interaction suppressor because it is allele specific.**

e) In an additional test, you find that the suppressor mutation can also suppress a loss-of-function *unc-54* allele, which causes animals to be

paralyzed. Does this information affect how you think about this suppressor?

**It is an informational suppressor since it is allele specific, gene nonspecific.**

f) During *C. elegans* development, the zygote divides to produce a AB blast cell, which generates the nervous system, and a P1 blast cell, which generates the muscle cells. Both the nervous system and muscles are sexually dimorphic in *C. elegans*. In genetic mosaic experiments, you produce animals that are mutant for *her-1(lf)* in AB and wild type for *her-1* in P1. Mosaic XO animals of this type are male in all tissues. You also produce animals that are mutant for *her-1* in P1 and wild type for *her-1* in AB. Mosaic XO animals of this type are also male for all tissues. Provide an explanation for these results.

***her-1* functions nonautonomously. The production of *her-1* in either the progeny of the AB or P1 cell is sufficient to specify the sexual fate of all cells in the animal.**

2. The three deficiencies *Df1, Df2* and *Df3* remove genes on the *C. elegans* fourth chromosome. In mapping experiments you cross males hemizygous for these three deficiencies (*Df*/+) to hermaphrodites that are doubly mutant for recessive mutations in *dpy-5*, which is on chromosome I, and one of four mutations on chromosome IV: *ced-3, ham-1, unc-30* and *unc-31*. The phenotypes of the progeny of these crosses are shown in the below.

| | ♀ parent | | | |
|---|---|---|---|---|
| ♂ parent | *dpy-5; ced-3* | *dpy-5; ham-1* | *dpy-5; unc-30* | *dpy-5; unc-31* |
| *Df1*/+ | + | - | - | + |
| *Df2*/+ | + | + | - | - |
| *Df3*/+ | - | + | + | + |

"+" indicates complementation for the *ced-3, ham-1, unc-30* or *unc-31* mutations; "-" indicates failure to complement

The *dpy-5* mutation causes a Dumpy phenotype; the *ham-1* mutation causes a cell differentiation defect; the *unc-30* and *unc-31* mutations cause an uncoordinated phenotype

a) In these crosses, why are all of the hermaphrodites made homozygous for the *dpy-5* mutation?

**To distinguish self (*dpy-5*/*dpy-5)* from cross (*dpy-5*/+*)* progeny.**

b) What are the phenotypic classes and sexes of the progeny produced when *Df1* / + ♂ are crossed with *dpy-5, unc-31* ♀?

**Dpy, Unc ♀**

**Wild-type (or NonDpy, NonUnc) ♂ and ♀.**

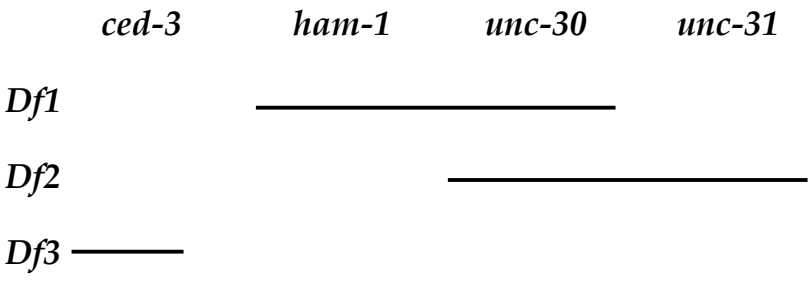
c) What are the phenotypes of the progeny produced when *Df1* / + ♂ are crossed with *dpy-5, unc-30* ♀?

**Dpy, Unc ♀**

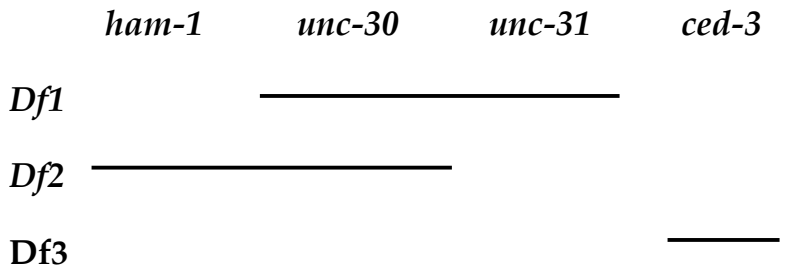
**Unc (or NonDpy, Unc) ♂ and ♀**

**Wild-type (or NonDpy, NonUnc) ♂ and ♀**

d) Draw a map showing the positions of the three deficiencies and the *ced-3, ham-1, unc-30* and *unc-31* genes.




**OR**




3. A dwarf variant of tomato appears spontaneously, and it is used to generate a pure breeding stock. A dwarf plant is crossed as a female to a normal plant, and all of the progeny are dwarf. What can you conclude?

**The dwarf phenotype is caused by a dominant nuclear mutation, a maternal effect mutation, or a maternally inherited mutation.**

The F1 dwarfs are selfed and all of the progeny are normal. What can you conclude?

**The mutation causing the dwarf phenotype is recessive and maternal effect.**

4. A deletion in the p53 gene causes a dominantly inherited form of cancer. Normal cells of an individual with cancer contain both the normal and deleted forms of the p53 gene, but the cancer cells contain only the deleted version. Explain these observations.

**p53 is a tumor suppressor gene. While the cancer trait is inherited in a dominant fashion, all p53 function must be lost for cells to become cancerous. The loss of heterozygosity is caused either by mitotic recombination, loss of the normal chromosome or a spontaneous mutation in the wild-type p53 gene.**

5. You are given a Drosophila strain that white eyes caused by the insertion of a P-element into the *white* gene on the X chromosome. The strain has only this single P element insertion, and the *white* allele is stable. Thus, all of the animals in the strain have white eyes.

You cross this strain as a female to a P strain male with normal red-colored eyes. What will be the phenotypes of the F1 progeny?

**All of the males will have white eyes and all of the females will have red eyes. (Remember that the *white* gene is on the X chromosome.)**

You now cross the F1 males with white-eyed females of the M cytotype. What will be the phenotypes of the progeny?

**Most of the progeny will have white eyes, but some will have reverted to red because the P element has been mobilized and excised in the germline of the F1 males.**

6. You cross two haploid petite yeast strains, and the diploids are grande. When you sporulate the diploids all of the tetrads have two grande spores and two petite spores. What type of petites did you cross? Describe your reasoning.

**One of the petites was a nuclear petite and the other was a rho$^0$, or had no mtDNA. Since this latter strain doesn't contribute any mtDNA to the cross and doesn't affect the phenotype of the diploid or its haploid progeny, the only phenotype we will see in the tetrads is the segregation of the nuclear petite in two of the spores.**

7. You cross a petite haploid strain and a grande strain, and find that all of the diploids from independent mating events are petites. Give a possible explanation for this result, and describe a simple way to distinguish between them.

**Because all of the diploids are mutant, the petite could be caused by a dominant mutation in a nuclear gene. Alternatively, it could be a prion form of a protein that has mitochondrial functions.**

8. The *Drosophila* MP2 neuroblast divides to produce two daughter cells, known as vMP2 and dMP2. The Numb protein is produced and distributed asymmetrically in MP2 so that it is segregated into dMP2. In the absence of *numb* function, dMP2 is transformed into an additional vMP2 neuron. The Notch receptor is also required for the MP2 division, but loss of *Notch* function results in the transformation of vMP2 into an additional dMP2 neuron, the opposite phenotype caused by loss of *numb*. The Numb and Notch proteins physically interact, suggesting that they function in the same pathway.

a) Do you think that one protein activates or inhibits the function of the other protein?

**Inhibits, because the mutants have opposite phenotypes.**

b) It turns out that Numb regulates the activity of the Notch receptor. A genetic result supports this. What was the result?

**The *numb, Notch* double mutant produces two dMP2 neurons, the Notch phenotype. *Notch* is epistatic to *numb*.**

c. Delta is a ligand for the Notch receptor, and like *Notch* mutants *Delta* mutants also produce two dMP2 neurons. Using antibodies to Delta protein, you can detect its expression in the overlying mesoderm but not in the MP2 lineage, so you propose a model where Delta from the mesoderm activates Notch in vMP2. To test this hypothesis, you generate two types of animals. One type is heterozygous for a null Notch mutation, and the chromosome containing the wild-type Notch allele has a transgene linked to Notch that expresses green fluorescence protein (GFP) in both the nervous system (the MP2 lineage) and the mesoderm. The other type is heterozygous for a null Delta mutation, and the chromosome containing the wild-type Delta allele has a transgene linked to Delta that expresses GFP in both the nervous system and the mesoderm. Using these two types of animals, describe the type of experiment you will do to test your model and describe the outcomes if your model is correct.

**By irradiating the animals with X rays, you will generate mosaic animals. If your model is correct, then Delta should be required in the mesoderm, and Notch should be required in the MP2 lineage to generate a normal MP2 division.**

**Thus, Delta-/Delta+ GFP animals that had lost GFP in the mesoderm should be functionally defective for Delta in the mesoderm but normal for Delta in the MP2 lineage and have MP2 divisions that produce two dMP2 neurons. When these animals lost GFP in the MP2 lineage but not the mesoderm, they should have normal MP2 divisions because Delta is not required in the MP2 cells.**

**Conversely, Notch-/Notch+ GFP animals that had lost GFP in the mesoderm should be functionally mutant for Notch in the mesoderm but normal for Notch in the MP2 lineage and should have normal MP2 divisions. When these animals have lost GFP and hence Notch function in the MP2 lineage but not the mesoderm, they should have MP2 divisions that produce two dMP2 neurons.**

9. In *C. elegans*, six mechanosensory neurons known as touch cells mediate the worm's response to light touch. Screens for mutants that failed to respond to light touch (the Mec phenotype) identified a number of *mec* genes. Below are results for three of the *mec* alleles. Hermaphodites homozygous for a *mec* mutation and an unlinked mutation in *dpy-5* are crossed to wild-type males. Below are the results of the cross. *mec-4(a)* and *mec-4(b)*are different mutant alleles of the *mec-4* gene.

Pure breeding *dpy-5; mec-4(a)* ♀ crossed with wild-type ♂ produced Dpy, Mec h, NonDpy, Mec ♀ and NonDpy, Mec ♂ progeny.

Pure breeding *dpy-5; mec-4(b)* ♀ crossed with wild-type ♂ produced Dpy, Mec ♀, wild-type (NonDpy, NonMec) ♀, and NonDpy, Mec ♂ progeny.

Pure breeding *dpy-5; mec-3* ♀ crossed with wild-type ♂ produced Dpy, Mec ♀, wild-type ♀, and wild-type ♂ progeny

a. How do the Dpy, Mec ♀ progeny arise?

**These are hermaphrodite self progeny.**

b. Which genes are X linked?

***mec-4***

c. Which mutations are dominant?

***mec-4(a)***

d. While all of these mutations result in the loss of the touch response, only the *mec-4(a)* mutation causes the touch cells to degenerate. In *mec-4(a)/Df, mec-4(a)/+* and *mec-4(a)/+/Dp(+)* hermaphrodites, all of the touch cells degenerate at the same time after they are generated. How do you think the *mec-4(a)* mutation affects gene function?

**Because the dose of the wild-type *mec-4* gene doesn't affect the phenotype, *mec-4(a)* is neomorphic.**

e. In *mec-4(b)/Df* hemizygous and *mec-4(b)* homozygous hermaphrodites, the touch cells do not degenerate. Both types of animals have a similar touch-insensitive phenotype in terms of its severity and penetrance. How do you think the *mec-4(b)* mutation affects the function of the *mec-4* gene?

**Because the Df doesn't enhance the phenotype, *mec-4(b)* is amorphic (null, complete loss of function) allele.**

f. Double mutants homozygous for *mec-4(a)* and homozygous for *mec-3* are insensitive to touch, but their touch neurons do not degenerate. *mec-3*

encodes a transcription factor. Describe a simple model that would explain this genetic interaction.

**MEC-3 regulates the expression of the *mec-4* gene. In the absence of *mec-3* function, the neomorphic product of *mec-4(b)* isn't produced.**

g. The touch-sensitive phenotype caused by the *mec-3* mutation is suppressed by a recessive mutation in another gene. This suppressor can also suppress some mutant alleles of *ced-3* and *unc-54*. What type of suppression is this? Explain your reasoning.

**Because the suppression is gene nonspecific, it is informational suppression.**

h. The *mec-4* gene encodes a subunit of an ion channel required in the mechanosensory neurons for the touch response, and the *mec-4(a)* mutation causes the channel to be locked in an open state; this leads to a constant influx of ions and neurodegeneration. The ion channel is conserved and has been studied in other organisms. In other organisms, each of the subunits, which are encoded by separate genes, is required for the function of the channel. You look at the sequence of the *C. elegans* genome and find that homologs for the other genes also exist. Since you know that one of the subunit genes, *mec-4*, is required for mechanosensation, you propose that the *C. elegans* homologs of the other channel subunit genes are also required for mechanosensation.

What approach could you use to test this hypothesis, and how would you test it?

**RNAi. You would inject dsRNA from the genes into wild-type worms (or feed them bacteria the express the dsRNA) and test the progeny of the treated worms for the Mec phenotype.**

How would you use this approach to show that the other channel subunits are also required for the neurodegeneration caused by *mec-4(a)* mutation?

**The same experiment as above, but now you would treat *mec-4(a)* mutants with RNAi and look for the loss of neurodegeneration.**

10. You decide that you want to test whether there are additional genes in the Sevenless pathway using two approaches. In the first approach, you express from the Sevenless promoter a mutant form of Ras that has a defective GTPase. This promoter will drive the expression of this mutant form of Ras in the R7 cell and the cone cells. You find that animals carrying

this Sevenless Ras transgene have several R7 cells and are missing cone cells.

a. What do you think that the mutation did to the activity of Ras, and how does this explain the extra R7 neurons?

**It activates Ras independent of Sevenless signaling. Now the cells that would normally become cone cells instead become R7 neurons.**

b. You want to identify genes that encode proteins that are normally activated by Ras in the R7 cell. You screen the F1 progeny of animals that contain the Sevenless-Ras transgene described in a. For what phenotype will you be screening.

**Loss of the R7 cells.**

c. In the second approach, you want to screen using a mosaic animals. To see if this will work, you first carry out a control to see if you would isolate Ras mutants in such a screen. You generate animals that are homozygous mutant for *white*, express FLP from the eyeless promoter, and the chromosomes that contain the Ras gene also have FRT sites near the centromere. Finally, one of these chromosomes contains a null allele of Ras and the other chromosome contains the wild-type Ras gene and a transgene bearing the wild-type *white* gene.

What phenotype would you expect?

**The mutant *white* clones would lack the R7 neuron.**

11. You cross a female fly with a male and find that all of her progeny die in early embryogenesis. A friend thinks that the F1 phenotypes could be explained if the female fly was heterozygous for a dominant lethal mutation, was homozygous for a recessive maternal effect mutation, or carried a mitochondrial mutation that led to the lethality. Explain whether you agree or disagree with each of your friend's ideas.

**The female fly can't carry a dominant lethal mutation because she is alive (3 points), and for the same reason can't have mutant mitochondria (2 points). She could be homozygous for a recessive maternal effect mutation that leads to lethality because we would expect that she would survive and all of her progeny die.**

What will be the genotype of her progeny?

**m/+**

12. Several dominant mutations in the *C. elegans egl-1* gene cause the hermaphrodite's HSN neurons to undergo programmed cell death, the normal fate of the HSNs in males. In hermaphrodites, the HSNs normally survive and innervate egg-laying muscles. Recessive mutations in *egl-1* cause all of the 131 cells that normally die in the hermaphrodite to survive, and they cause the male HSNs to survive.

Hermaphrodites hemizygous for a deficiency (Df/+) of the *egl-1* gene have HSNs. What can you conclude about the dominant mutations.

**They are gain-of-function mutations, or they are not haploinsufficient.**

The penetrance of the HSN defect is more severe in hermaphrodites homozygous for any of the dominant mutation compared with hermaphrodites heterozygous for the mutation, and the heterozygotes are more severely affected than animals that carry a dominant and a recessive allele of gene. How do the dominant alleles affect the function of the *egl-1* gene. Explain your reasoning.

**They increase its activity (or they are hypermorphic) because adding a wild-type allele increases the severity of the phenotype.**

A gain-of-function mutation in *ced-9* cause the HSNs to survive in males. Genetic epistasis experiments indicated that the *egl-1* is a negative regulator of *ced-9*. From what has been described here, describe a result that led to this conclusion.

**The HSNs survive in *ced-9 (gf); egl-1(lf)* hermaphrodites.**