**Chapter 4 Study Questions**

*Genetic Analysis: Genes, Genomes, and Networks in Eukaryotes*

1. A recessive mutation in *C. elegans* in the gene *age-1* results in worms whose lifespan is approximately twice as long as wild-type. *age-1* maps very close to *daf-3*, a gene that affects the formation of dauer larvae; a recessive mutation in *daf-3* cannot form dauer larvae even under stress conditions. (Dauer larvae are discussed in Chapter 3 as the alternative stress resistant stage of the life cycle.) The *age-1* mutant has the ability to form dauer larvae under stress conditions. However, if *age-1* males are mated with *daf-3* hermaphrodites (females), the F1 offspring do not form dauer larvae. They also have unusually long lifespans.
2. How do you explain this result?
3. Which of the two mutant phenotypes discussed in part a is more likely to be the null phenotype and which is more likely to be a hypomorphic phenotype? Why did you come to this conclusion?
4. Sex determination in *Drosophila melanogaster* depends upon numerous genes. Three of these are discussed below. The phenotype of recessive mutations in these genes is summarized in the table.

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| --- | --- | --- |
| **Genotype** | **XY Phenotype** | **XX Phenotype** |
| wild-type | males | females |
| *tra-2/tra-2* | males but sterile | male  |
| *tra/tra* | males and fertile | male  |
| *dsx/dsx* | intersexes | intersexes |

The phenotype of *dsx/dsx* flies is identical in XX and XY genotypes, and shows aspects of both male and female sexual development, referred to as an intersex.

1. Briefly explain the normal function of each of these genes in wild-type flies.
2. There is an unusual dominant mutation known as *dsxdom.* XY flies that are heterozygous or homozygous for this mutation are normal males. XX flies that are *dsxdom/+* are intersexes. In addition, XX flies that *dsxdom/Df* (that is, with a deficiency of the *dsx* gene) are phenotypically males, and XY flies that are *dsxdom/Df* are normal males. Postulate the nature of the defect in the d*sxdom* mutation.
3. Arabidopsis, like most broad-leaf plants, is sensitive to the widely used herbicide glyphosate (the ingredient in Round-up™). Briefly outline a screen to find Arabidopsis mutations that are resistant to this herbicide. Your screen (or selection) can induce mutations with any appropriate mutagen (although you should identify which one), and might look for either recessive or dominant mutations.
4. The Case Study provides a brief introduction to maternal effect mutations as they were found to affect segmentation in Drosophila. Many different processes in embryos have been analysed using maternal effect mutations, including the specification of the germ line. In Drosophila, C. elegans, and many invertebrates, the nuclei or cells that will specify the germ line are set aside from the presumptive somatic cells very early in development. In order to study this process in Drosophila, several different investigators conducted maternal effect screens for sterile mutants; two examples are Boswell and Mahowald 1985 Cell 43: 97-104 and Schupbach and Wieschaus 1986 Develop. Biol. 113:443-448, both of which describe maternal effect mutations in a gene known as *tudor*. You are welcome to access either or both of these papers, although the questions do not require detailed information from them.
5. Briefly outline the expected phenotype of a maternal effect mutation that affects the specification of the germ line in females.
6. Diagram a genetic screen that might be used to find maternal effect mutations affecting the germ line such as *tudor*.
7. Do you expect *tudor* homozygous mutant females to be fertile when mated with wild-type *tudor+* males? Why or why not? Is this even a useful experiment to conduct? .
8. Suppose that you have a *tudor* mutant, but you want to find more mutant alleles of *tudor*. Briefly describe how you would carry out a screen specifically designed to find more alleles of *tudor*.
9. As will be described in more detail in Chapter 5 and particularly in the Case Study for Chapter 9, the gene for α-tubulin was originally cloned from chickens, and then used to identify two α-tubulins genes in yeast, known as TUB1 and TUB3. (TUB2 is β-tubulin.) TUB1 and TUB3 differ slightly in nucleotide and amino acid sequence but both appear to be most closely related to α-tubulins.
10. Are the TUB1 and TUB3 genes paralogs or orthologs of each other?
11. Very briefly describe the way that you make a gene disruption of the TUB1 gene in yeast.
12. Cells that have a gene disruption of TUB1 do not divide. You want to test the ability of wild-type TUB3 to “rescue” or complement the mutant *tub1* defect. The wild-type TUB3 gene is placed on a 2-micron yeast episomal plasmid as shown. 

Based on this diagram, how would you set up an experiment to select for host yeast cells to test the effect of the transforming TUB3 plasmid on a tub1 mutant?

1. You discover that a yeast cell with *tub1* gene disruption and the wild-type TUB3 on a 2-micron plasmid is able to grow and divide normally. This is a paradox since yeast cells with disrupted *tub1* but retaining their normal TUB3 gene on the chromosome cannot grow. Yet when you put the normal TUB3 gene on a 2-micron plasmid, the yeast cells with *tub1* mutation grow normally. How do you explain this result? (In other words, why does TUB3 on a 2-micron plasmid complement or rescue the *tub1* defect whereas TUB3 on the chromosome does not rescue it?)