

Genetics 201
Extra *C. elegans* problems

1). The nematode *Caenorhabditis elegans* is diploid. It can reproduce as a result of either hermaphrodite self-fertilization or of matings between males and hermaphrodites. Hermaphrodites have two X chromosomes (XX) and males have one X chromosome (XO).

You are given four true-breeding hermaphrodites strains (A-D), each carrying a *dumpy* mutation. All four are known to result from single gene mutations. You cross wild-type males with *dumpy* hermaphrodites of each strain and obtain F1 progeny of the following phenotypes (remember that *C. elegans* hermaphrodites will produce self-progeny as well as cross-progeny).

	←	F1 progeny	→	
<u>hermaphrodite strain</u>		<u>hermaphrodites</u>		<u>males</u>
A		<i>wt, dpy</i>		<i>wt</i>
B		<i>dpy</i>		<i>dpy</i>
C		<i>wt, dpy</i>		<i>dpy</i>
D		<i>wt, dpy</i>		<i>wt</i>

wt = wild type phenotype

dpy = dumpy phenotype

- a). Which of the progeny listed are self-progeny?
- b). List the mutation(s) with a recessive phenotype.
- c). List the mutation(s) with a dominant phenotype.
- d). List the mutations that could be sex-linked.
- e). You mate a true-breeding *dpy* male from strain A to a true-breeding *dpy* hermaphrodite from strain D. The F1 progeny are *wt* males, *wt* hermaphrodites, and *dpy* hermaphrodites. Are mutations A and D in the same or different genes? Explain.

2). During your proverbial rotation project, a post-doc in your *C. elegans* lab mentions that he has been conducting large screens for new mutant alleles with dominant phenotypes affecting the cell death pathway. He generously gives you a strain of worm which is heterozygous for one of these new mutations at a previously uncharacterized gene and asks you to look for perturbations in cell death during development. After hours of staring at perfectly normal adult worms under the microscope, you come to the conclusion that this strain has no apoptosis defects and you turn on the radio for some music as you prepare to clean up the lab and go home for a hot bath.

Miraculously, as soon as the music begins in the lab, the worms of your strain begin to gyrate with a lithe yet spunky sinuousness that can only be described as “bouncy.” Jubilant and inspired, you name this new phenotype britney (Brt) and the corresponding gene *brt-1* and decide to spend your rotation characterizing it. (Does the world really need another apoptosis project, anyway?)

a). Your excited PI urges you to pursue this new project and asks you first to propose four general classes of mutations to which the *brt-1* allele might belong, based on what you *already* know about the allele. What do you tell her?

b). Your advisor looks over your proposed classes of mutations and nods in approval. “Now do some experiments to figure out which one of these *brt-1* really is,” she says. Given the worm stocks listed below, please describe how you will use them to distinguish among your four possible types of mutations. The worm stocks you may use are:

<u>Genotype at <i>brt</i> locus</u>	<u>Explanation</u>
+ / +	wild type (wt).
<i>brt-1</i> / <i>brt-1</i>	homozygous mutant allele at the <i>brt-1</i> locus.
<i>Df</i> / <i>Df</i>	homozygous for deficiency at <i>brt-1</i> locus.
<i>Dp</i> (+ +) / <i>Dp</i> (+ +)	homozygous for a duplication at the <i>brt-1</i> locus.

Note: You may assume both males and hermaphrodites are readily available for each strain.

c). A collaboration with a neighboring biochemistry lab shows that the Brt phenotype results from changes in the *C. elegans* nervous system caused by *brt-1* (your mutant allele), but it remains unclear whether the mutant *brt-1* gene product itself functions in nerve cells or in the surrounding musculature. Your advisor, predictably, asks you to resolve this ambiguity, and suggests you pursue mosaic analysis of your *brt-1* allele.

Your lab has a worm strain which is wild-type at the *brt-1* locus and homozygous mutant at both the *unc* and *fat* genes, two phenotypic markers commonly used in mosaic

analyses. The gene *unc*, which confers a recessive Unc phenotype easily distinguishable from the Brt phenotype, is known to function in the nervous system in cells derived from the AB precursor cell. The gene *fat*, which confers a recessive Fat phenotype also easily distinguishable from the Brt phenotype, is known to function in the musculature in cells derived from the P1 precursor cell.

Your advisor says that a tech in the lab has created a free duplication, Dp, which carries your mutant allele of *brt*. The free duplication also carries the wild type alleles of the *unc* and *fat* genes. When inserted into worms, Dp is ordinarily maintained very stably. You insert the Dp duplication into your Unc/Fat worm strain from above to create the following strain:

$+/+$; *unc/unc* ; *fat/fat* ; Dp (*brt-1/brt-1* ; $+/+$; $+/+$)

These worms are phenotypically non-Unc, non-Fat and Brt. (Assume that all three phenotypes can easily be distinguished from each other, even when in combination). You then self the hermaphrodites of this newly created worm strain.

-What are the genotypes and phenotypes of the two major classes of progeny you expect from this selfing?

d). The lab tech (who deals with many many worms) tells you he has noticed that, rarely, Dp is not stably maintained, creating animals which are genetic mosaics when Dp is lost at the first cell division. That is, Dp is retained in only one of the two precursor cells of the embryo: AB or P1. You suddenly realize these rare mosaic animals will allow you to deduce whether the mutant *brt-1* gene product itself acts in the nervous system or the musculature.

-What would be the Unc and Fat phenotypes of the mosaic animals which are created by the loss of Dp after the first cell division ?

-What would be the Brt phenotypes of these animals if the mutant *brt-1* gene product acts in the nervous system? In the musculature?

3). During a mutant screen, you obtain a strain of worms with a recessive phenotype that results in a pigmented hypodermis. You are excited at the prospect of having a new visible marker to use for genetics, and at the possibility of studying hypodermal pigmentation. You name your mutant *red-1*.

a). You embark on the mapping of *red-1*. You cross *red-1* males to two strains:

- 1) *lin-5* (I); *dpy-4* (IV); *daf-6* (V)
- 2) *ced-10* (II); *ncl-1* (III); *sup-56* (X)

You choose wild type F1's, single hermaphrodites and allow self-fertilization, then from the progeny single 135 Red nonLin nonDpy nonDaf and single 135 Red nonCed nonNcl nonSup F2 hermaphrodites and allow self-fertilization. You examine the F3 progeny on these 270 plates; the phenotypic classes found on each plate are listed below. Of course, all F3 animals are Red.

Cross 1:	<u>phenotypes</u>	<u># plates containing this phenotype</u>
	Lin, Dpy and Daf	39
	Lin	8
	Lin and Daf	22
	Lin and Dpy	21
	Dpy and Daf	19
	Dpy	12
	Daf	9
	none	5
Cross 2:	Ced Ncl Sup	1
	Ced	32
	Ced and Sup	57
	Ced and Ncl	0
	Ncl and Sup	2
	Ncl	0
	Sup	29
	none	14

Which chromosome is *red-1* on? How do you know?

b). You wonder if there might be other genes besides *red-1* that affect visible hypodermal pigmentation. Design a screen that would allow you to isolate recessive alleles in a different gene that mutates to the Red phenotype. Be sure to specify how you would prove that these mutations were not additional alleles of the *red-1* gene. Specify the sex, generation, genotype and phenotype of each animal in your screen. You may use any strains from part a.

c). Based on the screen you have proposed in part b), how many worms would you have to screen if you wish to find 4 mutations in each gene that readily mutates to the Red phenotype? Assume that you use 50 mM EMS for four hours (the standard mutagenesis protocol). Justify your answer.

d). How would you modify your screen in part b), above, if you wished to isolate dominant alleles in a different gene that mutates to the Red phenotype?