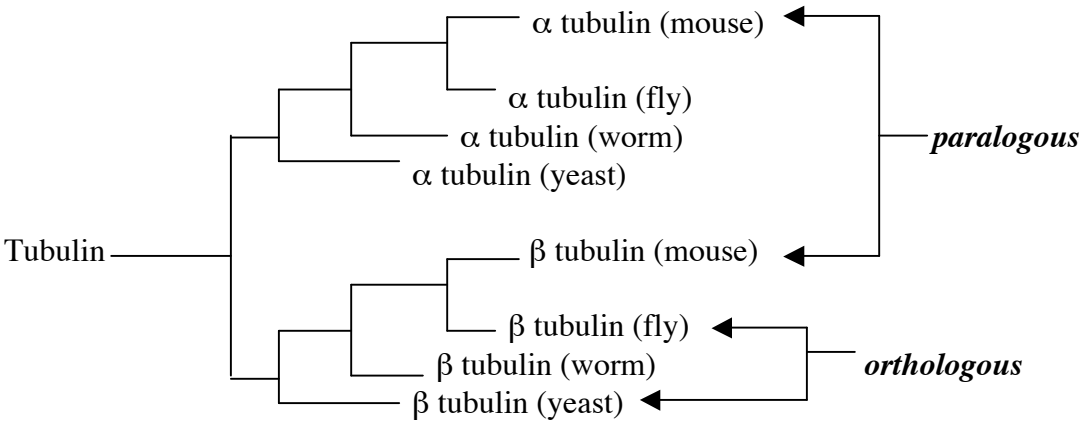
7.22
ANSWERS to Problem set #1

1. List whether the pairs marked by the arrows are orthologous or paralogous.



2. You would like to determine the phylogeny of the species depicted below, A thru D. Based on other data (sequence analysis, fossil record) you know that there is the existence of a distantly related outgroup.

A. Identify the autopomorphies in species A thru D.

B. Identify the synapomorphies across species A thru D.

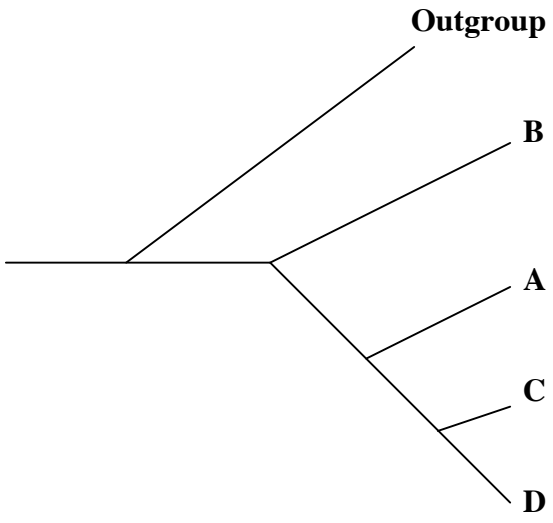
C. Using maximum parsimony and synpomorphies, place the animals into a phylogenetic tree.

<u>Synampomorphies</u>

- ***Outgroup and B: 7 body segments***
- ***Outgroup and B: short tail***
- ***A, C, D: 3 body segments***
- ***A, C, D: curly tail***
- ***C, D: pincers***

<u>Autopomorphies</u>

- ***C: wings***
- ***B: paddle "paws"***



3. While working with *C. elegans* you isolate a recessive cell lineage mutant *lin* with phenotype "Lin". In order to map your mutation you mate your *lin* mutants to animals homozygous for mutations in 2 linked genes, *bli* and *lon*. The *bli* and *lon* genes reside on the same chromosome as *lin*. The F1 generation is phenotypically wild type and allowed to self-fertilize. You select the rare phenotypically "Bli non-Lon" animals and "Lon non-Bli" animals in the F2 generation and place each animal on a separate plate.

***lin*/*lin* X *bli*/*bli lon*/*lon* (linked)**

↓

F1 WT phenotype

↓ **Self-fertilized**

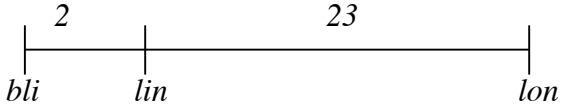
F2 Bli, non Lon (none are "Lin")
Lon, non Bli (none are "Lin")

A. 3/25 Bli, non Lon animals have 1/4 "Lin" progeny. The other 22 have no "Lin" progeny. Explain.

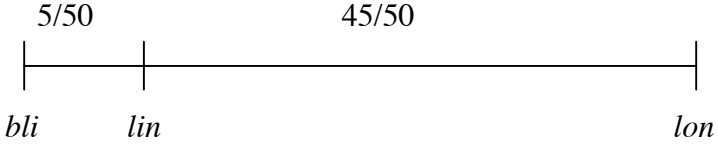
We already know that the three genes reside on the same chromosome. As the mutations start on chromosomes from two different animals, there must be recombination to get any Bli Lin or Lon Lin progeny. 3/25 Bli animals were bli lin recombinants. 1/4 of the progeny from these animals are homozygous for both bli and lin. 22/25 were recombinants between bli and lon but NOT between bli and lin.

B. 23/25 Lon, non Bli animals have 1/4 "Lin" progeny. The other 2 have no "Lin" progeny. Explain.

23/25 recombination events between lon and bli were also between lon and lin. 2 were not.



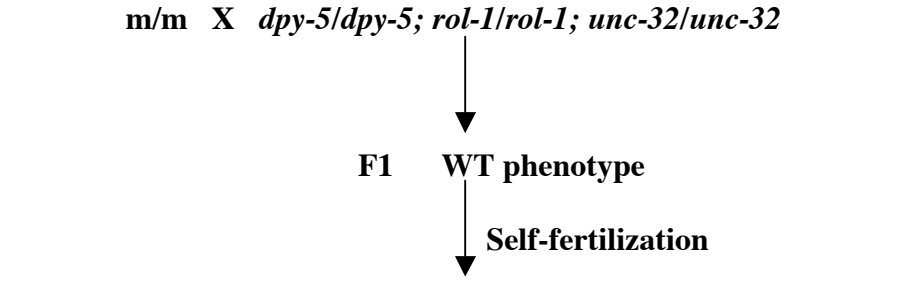
C. Draw a map containing the 3 genes. There is no need to indicate centimorgans, but indicate the TOTAL number of recombination events observed between each marker and the "Lin" mutation and show percentages (ratios are fine).



D. You have a series of polymorphisms that are in between *bli* and *lon*. Assuming your *lin* strain and your *bli lon* strain were polymorphic to begin with, how would you use these "new" polymorphisms? Which recombinants would you use to gain more fine mapping detail?

You could use ALL recombinants and compare where recombinations occur (SNPs, SSLPs) to more finely map the genes.

5. You want to map the recessive mutation "m" to one of the 3 chromosomes in *C. elegans*. You mate "m" males with a hermaphrodite homozygous for mutations conferring distinct recessive phenotypes on each of three chromosomes (*dpy-5*, *rol-1*, *unc-32*; on chromosomes I, II, and III, respectively). The F1 animals are separated to individual plates and allowed to self-fertilize to give an F2 generation. All animals with the "m" phenotype, and without the "Dpy", "Unc", or "Rol" phenotypes are picked to separate plates and allowed to self-fertilize.



A. 2/25 "m" F2 animals have F3 progeny that have a "Dpy" phenotype.
(i) Please explain.

The m and dpy genes are linked. If they were completely unlinked, one would expect independent segregation of each of the mutations. In order for the F3 animals to have the Dpy phenotype, the F2's would have to be heterozygous carriers (as only phenotypically "m" animals were picked). 2/3 of non Dpy F2 animals would be heterozygous carriers of dpy, if unlinked (see schematic below). Therefore, if m and dpy were unlinked then 66% of the 25 plates should give Dpy animals. Since only 8% of the plates give Dpy animals, m and dpy must be linked.

If unlinked: F1 *m*/+ *dpy*/+

↓ Self-fertilize

F2 *m/m;* [*dpy/dpy* : *dpy*/+ : +/+]
1 : 2 : 1

(ii) On the 2 plates with "Dpy" animals, what percentage of the animals would you anticipate to be "Dpy"?

25%: In order to have F3 Dpy animals, but F2 non Dpy animals, the F2's would have to be heterozygous carriers of dpy.

F2 *dpy*/+ (x) *dpy*/+

↓ Self-fertilize

F3 [*dpy*/*dpy* : *dpy*/+ : +/+]
1 : 2 : 1

B. Predict the number of plates generated from the individual 25 F2 "m" animals that have progeny with the "Unc" phenotype. What about "Rol"?

If m and dpy are linked, then they are on the same chromosome. As dpy, unc and rol all reside on different chromosomes, m is completely unlinked to unc and rol. Therefore, if phenotypically "m"(non-Unc) F2 animals are separated, they are either heterozyzgous carriers for unc (unc/+) or homozygous WT (+/+). 2/3 will be the former and 1/3 will be the latter. So, ***66% or ~17/25 plates will have F3 Unc progeny****. The same is true for rol.*

F1 *m*/+ *unc*/+

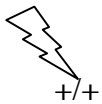
↓ Self-fertilize

F2 *m*/*m* [*unc*/*unc* : *unc*/+ : +/+]
1 : 2 : 1

C. One of the 2 plates that has phenotypically "Dpy" animals also has "Unc" animals. You select a single animal that is both "Dpy" and "Unc." Write the genotype of this animal. Be sure to show the linkage group and both alleles. Note that mutations on separate chromosomes can be indicated with a ";" and mutations on the same chromosome do not need a semicolon.

m dpy/*m dpy; unc*/*unc*

6. A. You perform a Zebrafish (*Danio rerio*) screen using ENU mutagenesis and isolate recessive developmental mutants. Draw out the crosses you would perform to attain your goal. Balancer chromosomes are not available.



+/+ (x) +/+ Po

↓

m_1/+ (x) +/+ F1

↓

m_1/+, +/+ F2

↓ Random sibling crosses

m_1/m_1, m_1/+, +/+

Alternatively, you could utilize a haploid screen, fertilizing F1 females with UV-treated sperm and analyzing the haploid embryos that result OR one could utilize a gynogenesis screen.

B. You classify the mutants according to phenotypes and get 2 groups: those with abnormal brain development and those with abnormal heart development. Animals in neither class are homozygous viable.

(i) Explain, with crosses, how you would maintain strains containing a given mutation.

m/m die, so left with 2/3 m/+, 1/3 +/+. Cross these F3 siblings and look at progeny. If offspring have embryonic mutant phenotype (1/4), then you mated heterozygotes and you should keep these fish to breed. If embryos don't have the mutant phenotype, you can toss the fish.

(ii) Explain why balancers are useful.

Balancers allow you to sort through the genotypes of a cross very quickly and efficiently, so that the only animals you keep are the ones that have or carry your mutation. If you don't have balancers you constantly have to look at the offspring phenotype ratios to determine what the genotypes of the parents are, for example, to simply maintain a strain.

C. You want to perform complementation test crosses with each group to determine how many genes are mutated. What crosses would you perform to accomplish these tests? You have a WT strain and the parents of the dead embryos. (The parents of the dead embryos are viable and can still mate.)

Go back to F2 (parental) generation and mate two carriers from different mutant lines. For example, if you wanted to see if m_1 allelic to m_2,

F2 $m_1/+$ (x) $m_2/+$

m_1/m_2, $m_1/+$, $m_2/+$, $+/+$

If you see the same mutant phenotype in ~1/4 of the offspring, the mutations do not complement each other.

D. Once you perform the correct crosses for the heart development group, you obtain the following data: (+ indicates normal phenotype, - indicates mutant phenotype, + indicates an independent mutation)

	WT	H1	H2	H3	H4	H5
WT	**+**	**+**	**+**	**+**	**+**	**+**
H1		**-**	**+**	**+**	**-**	**+**
H2			**-**	**-**	**+**	**+**
H3				**-**	**+**	**+**
H4					**-**	**+**
H5						**-**

What are the complementation groups?

H1, H4
H2, H3
H5

7. You would like to perform an ENU mutagenesis screen for mutations on chromosome 3 that confer a recessive thorax-specific phenotype. You know from other existing mutants that this phenotype typically results in lethality.

A. Describe, with crosses, how this screen could be performed using the chromosome 3 balancers TM6b and TM3. (You can use the balancers in any "format" that you wish: homozygotes, compound heterozygotes, etc.) TM6b carries the dominant Tubby marker, which confers a tubby body phenotype. TM6b is recessive lethal very early in fly development. TM3 carries a dominant Serrate marker which confers serrated wings. TM3 is recessive for a "pink peach" phenotype.

+/+ male (x) TM3/TM6b female *Po*

m/TM6b (x) TM3/TM6b *F1*

m/TM6b "tubby" (as well as m/TM3, TM3/TM6b) *F2*

m/TM6b (x) m/TM6b (F2 sibling crosses)

m/m : m/TM6b : TM6b/TM6b *F3*
1 : 2 : 1
"tubby" : dead

B. Describe how you would maintain your screen isolates (show crosses).

m/TM6b F3 animals form balanced stock of mutation and only animals with tubby bodies. Cross together and keep all live, tubby animals. Continue these crosses to maintain mutant stock.

m/TM6b (x) m/TM6b (F3 tubby siblings)

m/m : m/TM36b: TM6b/TM6b
mutant (dead) : 2 tubby body : dead

C. You isolated numerous lines (*mut1-mut25)*. You want to determine whether any of your mutant lines are allelic with the *pannier (pnr)* gene, a gene on chromosome 3 required for normal thoracic development. *pnr*/*pnr* flies are also embryonic lethal. As such, they are maintained as *pnr*/TM6 stock. What experiment would you do to determine whether *mut1* is or is not allelc to *pnr*? Draw out your predicted outcomes of your crosses.

mut1/TM6b (x) *pnr*/TM6b

mut1/*pnr*, *mut1*/TM6b, *pnr*/TM6b, TM6b/TM6b

The desired animals are the only ones without a balancer "phenotype." If *mut1* is allelic to *pnr* then these animals will fail to complement and show the mutant phenotype.