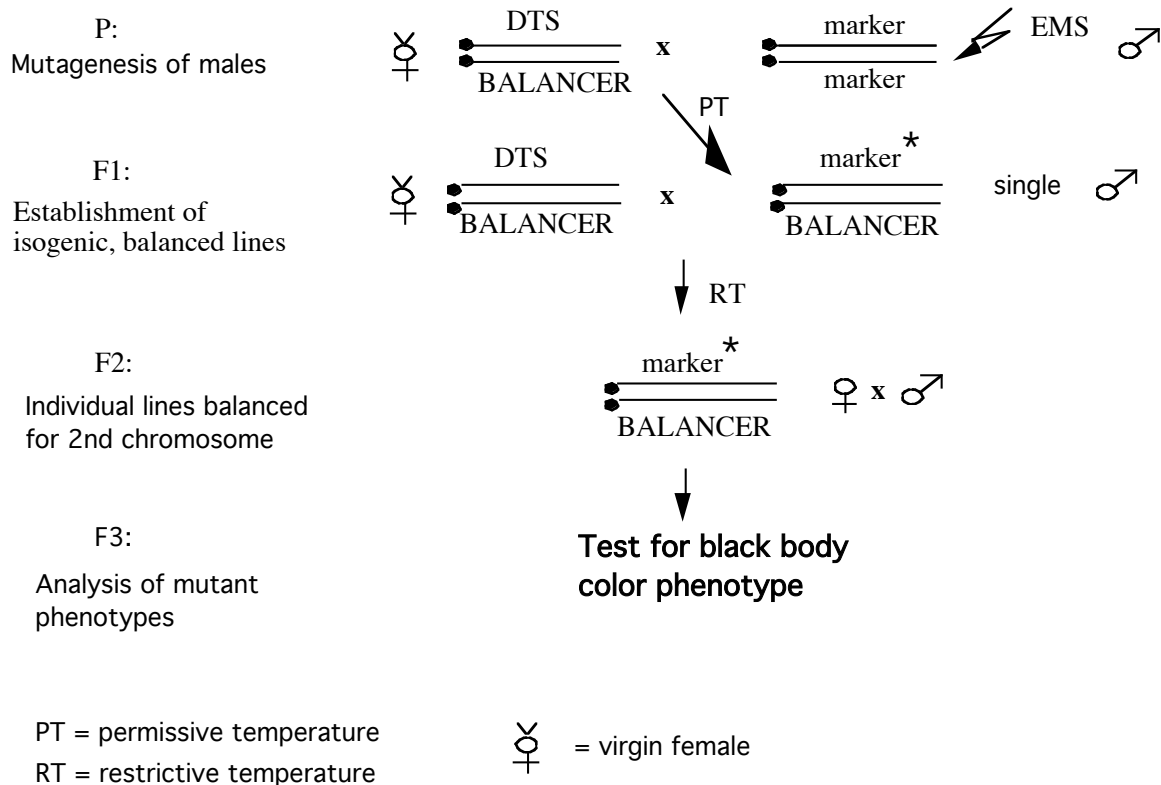


**Problems for Genetics-Mutagenesis,
9/24/03 Lecture**

Problem 1

You have conducted a screen for mutations affecting *Drosophila* body color. Your assay is to test for a “black” phenotype in the F3 generation. The mutagenesis scheme below shows only the 2nd chromosome for which balanced lines were established.



Balancer = Balancer chromosomes for chromosome 2. Balancer chromosomes are homozygous lethal. DTS = Conditional lethal mutation that maps to the 2nd chromosome, animals carrying the dominant-temperature-sensitive mutation die at the restrictive temperature (RT).

What will be the expected frequency of black flies among the F3 progeny of a stock derived from a single F1 male.

- and the black mutation was induced by mutagenesis on chromosome 2 and is recessive?
- and the black mutation was induced by mutagenesis on chromosome 2 and is dominant?
- and the black mutation was induced by mutagenesis on chromosome 3 and is recessive?
- and the black mutation was induced by mutagenesis on chromosome 3 and is dominant?

- e. -and the black mutation was induced by mutagenesis on the X chromosome and is recessive?

Problem 2

You have conducted a screen for mutations affecting cell death (apoptosis) in *Drosophila*. Your assay is to test for lack of increased apoptosis using acridine orange as an indicator for dying cells. Genes, which when mutated lead to death, are called pro genes, because their normal function is to protect cells from cell death, while genes which when mutated lead to lack of cell death are called kill, because their normal function is to cause death.

A) You have identified a number of recessive mutations, which affect apoptosis (pro and kill). Below are data from complementation analysis. Order the pro and kill mutants into complementation groups (the groups (genes) should be called (pro A, pro B.... kill A, kill, B etc.)...Assign complementation groups and indicate ambiguities.

Describe two genetic tests to determine whether the genes are mutations in the same gene. For each experiment describe the genetic crosses you would carry out and which results you would expect if the two mutations were in the same gene, or if they were in different genes.

Pro- Mutants: % dying cells (100% = all cells die, 10% ~ wildtype)

Allele/ Allele	1	2	3	4	5	6	7
1	100%	10%	100%	60%	10%	10%	10%
2		50%	10%	10%	10%	80%	10%
3			100%	60%	10%	10%	10%
4				40%	10%	10%	10%
5					80%	10%	80%
6						100%	10%
7							50%

Kill: Mutants: assay % dying cells (100%= wildtype)

Allele/ Allele	8	9	10	11	12	13
8	40%	100%	60%	100%	20%	100%
9		80%	60%	60%	100%	100%
10			30%	60%	60%	60%
11				0%	100%	40%

12					0%	100%
13						60%

A) From a large collection of deletions, you found the following results. Indicate the strength of the alleles within a complementation group. Indicate ambiguities.

Pro Mutants (more death than wild type): % dying cells of all cells (100% = all cells die, 10% ~ wild type)

Allele/ Df	Df I	Df II	Df III	Df IV	Df V	+
1	10	10	100	10	10	10
2	10	10	10	10	80	10
3	10	10	100	10	10	10
4	10	10	60	10	10	10
5	10	10	80	10	10	10
6	10	10	10	10	100	10
7	10	10	80	10	10	10

Kill Mutants (less death than wild type): % dying cells of cells that would normally die (0% none die, 100%= wildtype)

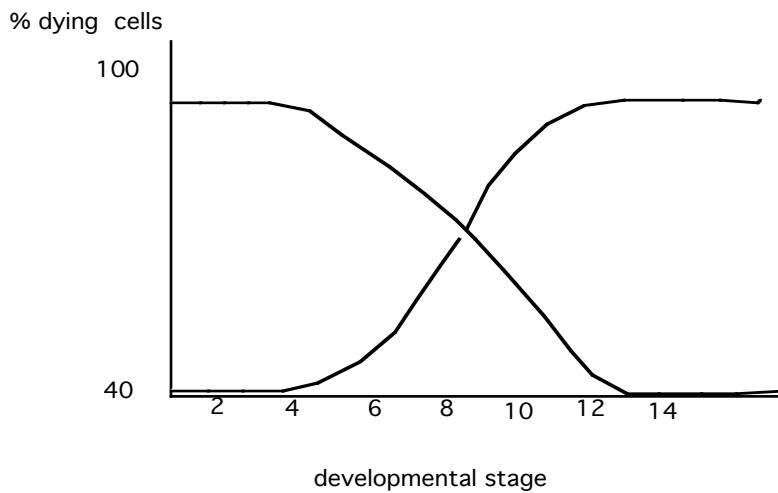
Allele/ Df	Df I	Df II	Df III	Df IV	Df V	+
8	100	100	100	20	100	100
9	100	20	100	100	100	100
10	60	60	60	60	10	60
11	100	0	100	100	100	100
12	100	100	100	0	100	100
13	100	40	100	100	100	100

B) Given all the information you have, determine what type of mutation # 10 is.

C) Are the data consistent with mutants 9, 11 and 13 affecting the same gene or of different genes? Provide a molecular model that is consistent with your answer.

D) Order alleles according to strength.

E) Mutant # 13 is temperature sensitive, at the permissive temperature cell death is normal, at the restrictive temperature only 40% of the normal number of cells die. Determine the temperature sensitive period and indicate which curve denotes the up shift and which the down shift.



F) To determine the epistatic relationship between these genes you make the following double mutant combination and observe the following phenotypes:

Double mutant	phenotype
1/1, 11/11	Less death than wild type
5/5,11/11	More death than wild type
6/6,11/11	Less death than wild type
1/1, 12/12	More death than wild type
5/5,12/12	More death than wild type
6/6,12/12	More death than wild type

Order the genes in a genetic pathway, which is consistent with the data.

G) You are also making double mutants with mutant #10.

1/1, 10/+	More death than wild type
5/5, 10/+	More death than wild type

Are these data consistent, or inconsistent with your conclusions about mutant #10 from (C)? Where could the gene mutated in #10 act in the pathway? Briefly explain your answer and propose an experiment to test your hypothesis.

E) You have cloned the proA gene and have used the alteration in mutation 1 to identify the gene. You find that overexpression of the proA wild type gene with a heterologous promoter leads to a reduction in the number of cells that die. You would like to identify dominant enhancers and suppressors of this phenotype. To determine the feasibility of this experiment you use the deficiencies I-V to test for suppression of enhancement of the over-expression phenotype. From the genetic analysis above, which deficiencies would you expect to enhance the phenotype, which ones to suppress (assume that in this

“sensitized” background the five deficiencies as well as the strong lack of function mutations modify the phenotype). Explain the results consistent with the pathway outlined in (F). Could this experiment resolve any ambiguities?

Problem 4 Mutagenesis protocols

- a) How would delayed mismatch repair (mosaicism) affect the recovery of mutations in *C. elegans*?
- b) Weigh the use of mutagenizing spermatogonia versus sperm with respect to recovery of mutations.

Problem 5 Mutagenesis in zebrafish

Follow meiosis I and meiosis II for two heteroallelic loci A/a and B/b. A maps close to the centromere and no recombination between the centromere and A occurs. B maps distant from the centromere and recombination occurs in meiosis I between B and the centromere. Begin by drawing all sister chromatids. Draw the polar bodies and the products of meiosis and the genotype of the diploid nucleus after EP with reference to the alleles transmitted to the embryo.

Problem 6 Saturation mutagenesis

Why do most mutagenesis experiments aim to identify multiple alleles/ locus?