Mosaic Analysis

Reading: Chapter 5, pp140-141; Reference chapter D, pp820-823
Problem set F

Twin spots in *Drosophila*

Although segregation and recombination in mitosis do not occur at the same frequency as in meiosis, under the proper conditions, crossing over can be observed in mitosis. Curt Stern was the first to describe mitotic recombination. In females heterozygous for the recessive X-linked yellow body (*y)* and the singed bristle (*sn*) alleles (*y+ sn*/ *y sn+*), Stern noted that most females are wild type with grey bodies and normal bristles. However, some females had patches of yellow or singed (short twisted bristles). Other females had twin spots, or two adjacent regions, one exhibiting a yellow phenotype and the other exhibiting a singed phenotype. Since the two spots were adjacent, Stern reasoned that the twin spots were reciprocal products of the same event, the product of mitotic crossing over. Since the order on the X chromosome is centromere-*sn*-*y*, a crossover between the centromere and the *sn* gene will result in a twin spot. A single singed patch can be explained by mitotic nondisjunction and a yellow patch can be explained by mitotic nondisjunction or by a mitotic crossover between the *sn* and *y* genes. Individuals that are composed of cells of more than one genotype are referred to as genetic mosaics.

The use of genetic mosaics to determine the site of gene function

It turns out that the Sevenless protein is expressed in many cells of the developing ommatidium. How then can we determine in which cells sevenless functions? Drosophila geneticists can use mosaic animals to address this question. Let's consider the experiments to determine the site of Sevenless function first, and then we will turn to Boss.

The basic approach is to make mosaic animals that have some cells in the eye that are functionally wild type for Sevenless and others that are mutant (Figure 1). X rays stimulate mitotic recombination, causing frequent crossing over between homologs during mitosis; it is thought that induced chromosomal breaks stimulate mitotic recombination. When mitotic recombination is stimulated in animals that are heterozygous for the *sev* mutation, clones of homozygous mutant cells are produced. We also need to know which cells are mutant, and the chromosome bearing the *sev* mutation also contains a *white* mutation. Mosaic eyes will have patches of white tissue. These eyes are sectioned and the cells that are wild type for *white*, and hence wild type for *sev*, contain pigment granules, while cells that are mutant for *white*, and hence mutant for *sev*, don't contain pigment granules. The *white* gene is necessary for the transport of pigment granules.

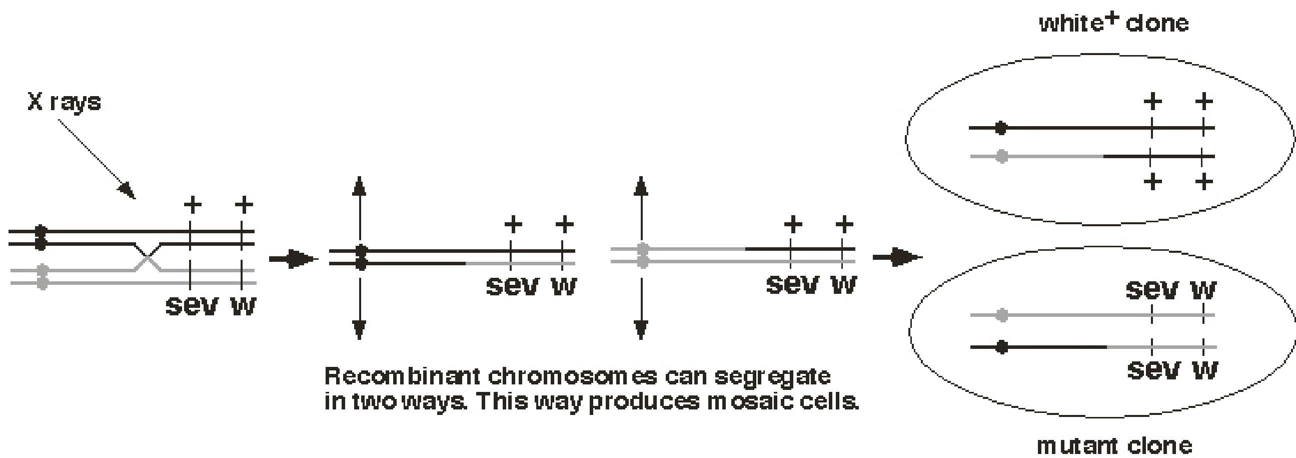


Figure 1. X-ray induction of a mitotic recombination event that will lead to a mosaic clone. Note that the alternate orientation of the homologous chromosomes would result in a segregation pattern that would not lead to mosaic clones.

In the mosaic eyes, some ommatidia will consist of mixtures of wild-type and mutant cells. There were no ommatidia in which the R7 cell was *white*. Moreover, as long as the R7 cell was wild-type for *white*, any or all of the cells could be mutant (Figure 2). These results indicate that the requirement for the wild-type *sev* gene product is in the R7 cell, and we say that the requirement for *sev* is **cell autonomous**. In other words, to develop into an R7 cell (and not a cone cell) a presumptive R7 cell must make the Sev protein, and it doesn't matter whether other cells of the same ommatidium make Sev protein.

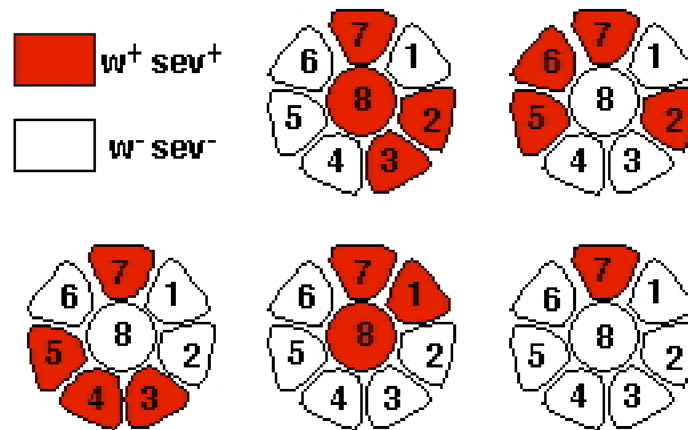


Figure 2. Examples of *sev* mosaic ommatidia where the R7 cell is produced. Only the photoreceptors cells are shown in the diagram. 1 is R1, 2 is R2, etc.

When researchers performed mosaic analysis for the *boss* gene, the results were different. An R7 cell develops properly only if the R8 cell is wild type for *boss*. All of the other cells of the can be mutant for

boss, including R7, and R7 will develop normally as long as R8 is wild type. Conversely, if R8 is mutant, even when all of the other cells of the ommatidium are wild type, the presumptive R7 cell will develop into a cone cell. These results indicated that the wild type *boss* gene functioned in the R8 cell to specify the R7 fate, and we say that the requirement for the gene is **cell nonautonomous**. This is consistent with the view that Boss is the Sev ligand.

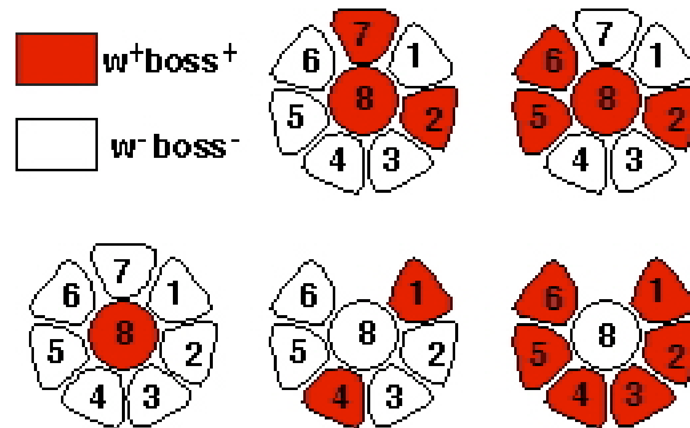


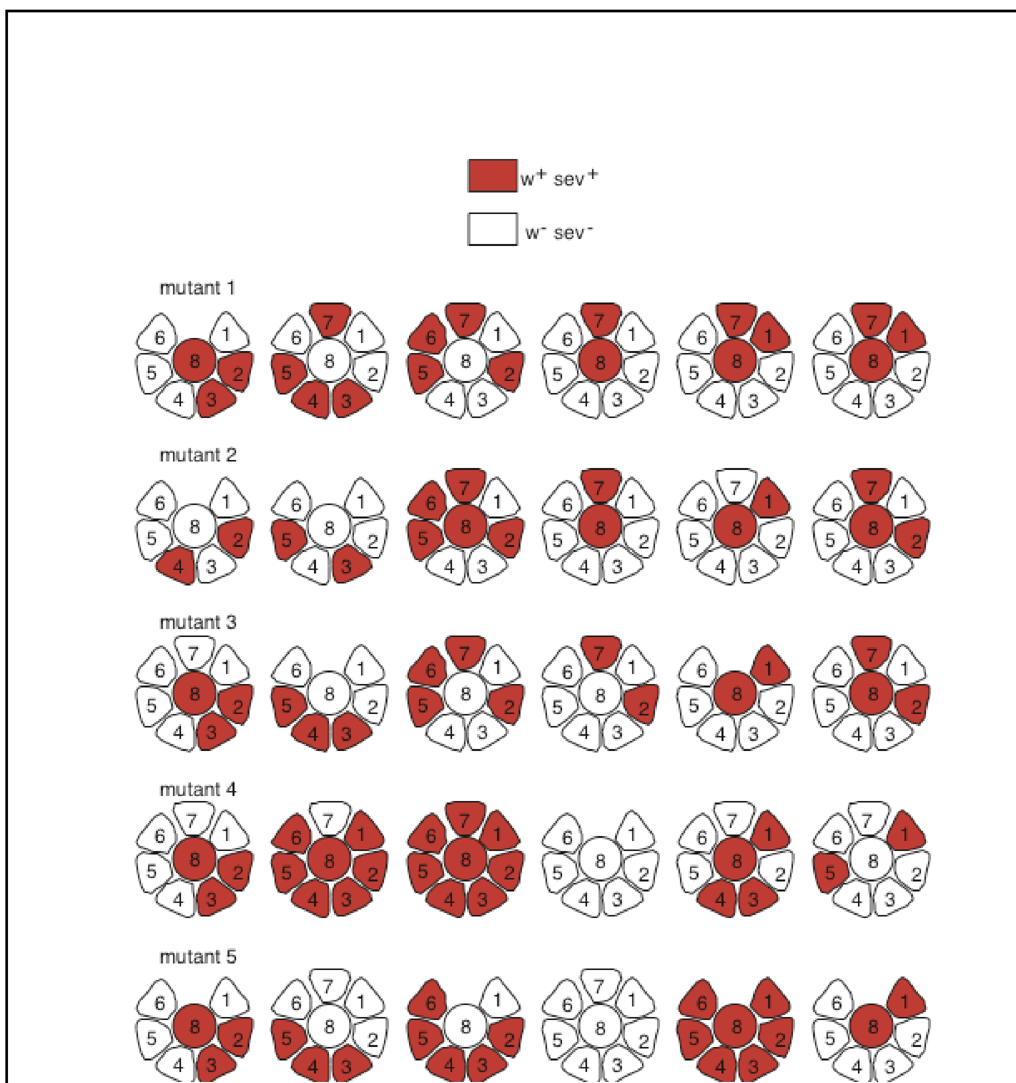
Figure 3. Examples of ommatidia mosaic for *boss*. Note that there is a correlation between R8 being wild type for *boss* and the presence of R7.

Problem set F

1. Although the mechanism for generating mosaic animals in *C. elegans* and *Drosophila* is different, the principle is the same: a mosaic animal is generated, and a cell autonomous marker (like *white* in the fly eye), is used to determine which cells are wild-type and which cells are mutant for the gene being studied. Acetylcholine is the neurotransmitter that is released from motor neurons in *C. elegans* and stimulates muscles that control movement to contract by binding to acetylcholine receptors on the muscle cells. Acetylcholinesterase is the enzyme that breaks down acetylcholine in the synapse to ensure that the neurotransmitter acetylcholine does not accumulate. *ace-1* is the gene that encodes the acetylcholinesterase enzyme and recessive mutations in the gene lead to animals that move abnormally. This abnormal or uncoordinated movement is caused by the buildup of acetylcholine in the neuromuscular synapse. You are interested in determining whether *ace-1* gene function is required in motor neurons, in muscles, or in both motor neurons and muscles. You generate mosaic animals that have the recessive *ncl-1* mutation and the *ace-1* mutation linked, so that when cells loose the wild-type *ncl-1* gene, they also loose the wild-type *ace-1* gene. The loss of wild-type *ncl-1* function is cell autonomous and leads to cells with large nucleoli. Thus, *ncl-1* can be used as a cell autonomous marker in this strain for the loss of *ace-1* in specific cells.

The division of the *C. elegans* zygote produces the AB and P1 cells. All of the neurons in *C. elegans* are derived from the AB cell and all of the muscles are derived from the P1 cell. Describe the results from your mosaic analysis if *ace-1* acetylcholinesterase is produced by motor neurons; by muscle; by both neurons and muscles. Be sure to include which cells contain normal nucleoli (Ncl^+) and which cells contain enlarged nucleoli (Ncl^-) in the different mosaic animals and which animals are uncoordinated (Unc).

2. In a screen, you identify several new recessive mutations that lead to a loss of R7. You are interested in defining where the genes function, and carry out a mosaic analysis using *white* as a cell autonomous marker. The results for mosaic ommatidia are shown below for each mutant. What can you say about the site of function for each gene?



3. 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. You conduct a mosaic analysis of *numb* function and generate both types of mosaics. Those where dMP2 is wild type for numb and vMP2 is mutant, and those where vMP2 is wild type for numb and dMP2 is mutant. From what you know about Numb, what do you think will be the phenotypes of the two types of mosaics.