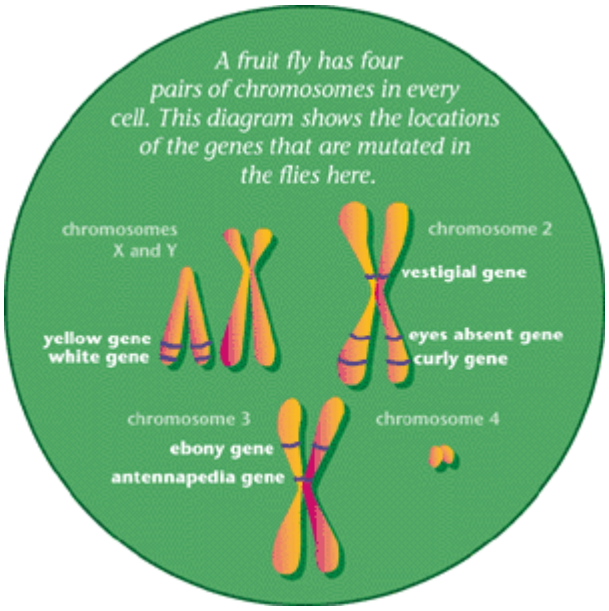
Mutations in Drosophila- Chromosome Squash

Background



Drosophila have 4 homologous chromosomes. Two large pairs of autosomes (chromosome 2 and 3), 1 pair of very small autosomes (chromosome 4) and a pair of sex chromosomes (chromosome X and Y). Females normally have two X chromosomes and males one X and one tiny Y. However, sex is determined by the X ratio not the presents of a Y chromosome. Two X chromosomes to two sets of autosomes = 1:1 ratio therefore the fly will be female. One X chromosome to two sets of autosomes = 1:2 ratio and the fly will be male. Hence, a fly that is XO is male and externally appears normal but is sterile because he lacks a Y chromosome.

Chromosome Squash

The salivary gland chromosomes (dissected from the third instar larvae) are large and easily visable. When a chromosomal squash is made, the chromosomes can be seen radiating out from a common center called the chromocenter, forming 5 arms

microscope slides, (depression and flat), and coverslips

fine forceps

isopropyl alcohol (for cleaning slide)

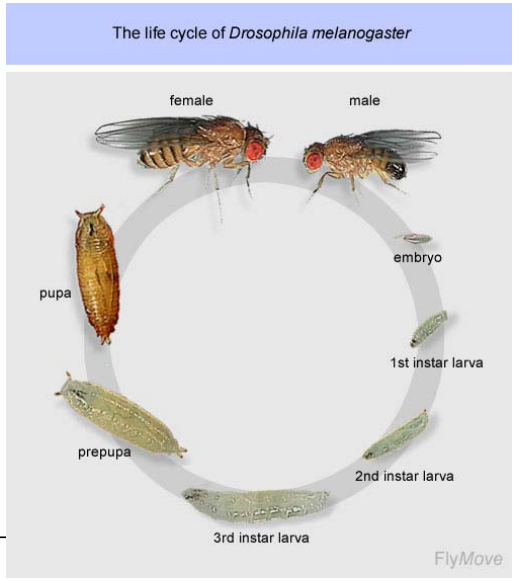
Saline solution

Silon wash (such as ﷺRain-Xﷺ)

45% Acetic acid (a fixative)

aceto-orcein stain

Kimwipes



Procedure

1. Pick the largest third instar larvae you can find (don't choose one in which you can see the pupal horns.
2. Drop the larvae on a depression slide onto which you have placed a few drops of saline solution. Leave it there briefly to wash the larvae.
3. Clean one slide and coverslip with alcohol, then rinse with silicon wash.
4. Prepare the slide and the coverslip with three drops of liquid arranged like this:

_____

X (Saline sol.)

_____

X aceto-orcein stain on top of cover slip)

_____

_ X (Acetic acid)

_____

5. Take the larvae from the wash slide and drop it into the saline solution drop.
6. Examine the larvae under the dissecting scope. Find the dark mouth parts moving in and out at the anterior end.(These structures need to be poking out for you to do the dissection; if your larvae is still, then gently nudge it.)
7. The idea is to use your forceps to grab the extended mouth part. Time your grab so that you have grabbed the mouth part when it is extended. Make sure that you only grab the mouth part and not any part of the body. (This takes time and patience and practice!) After you have grabbed the mouth part, hold the body about half way, and pull apart. The mouth part should pull cleanly from the body. The salivary glands are the symmetric extended bag-like things hanging off the mouth part.
8. Hold the salivary glands at the root near the mouth part and gently move them to the drop of acetic acid. (You are not so much holding the glands as "floating" them between your forceps tips.)
9. The glands are now being fixed in the acetic acid. Usually 1 minute is enough. Do not fix them too long or they will become brittle. While the glands are fixing, try to remove the fatty bodies attached to the glands. (These are small strips of greyish opaque bodies hanging off the glands.)
10. Move the glands to the drop of orcein stain on the coverslip. Wait 30 seconds for staining.
11.Take a clean slide and gently place it over the coverslip with the drop of orcein stain. The coverslip will stick up to the slide because of atmospheric pressure. In this way, lift the cover slip off the slide on which you have been dissecting.

12. Turn the slide over so that the cover slip is up. Take a piece of paper towel and gently blot off any excess stain. Squeeze gently over the cover slip and blot at the edges.
13. Take a blunt instrument and tap on the cover slip starting from the center. Make your way out towards the sides tapping in a concentric circle. This action loosens the chromosomes.
14.Turn the slide over with the coverslip facing down, and place on a paper towel. Place your thumb on one edge halfway over the coverslip and press and roll your thumb at the same time. The idea is to create a flow of the stain from one side of the slide to the other.
15. Turn the slide over again so that the coverslip is facing up. Place a paper towel over the coverslip. Put your thumb squarly over the coverslip, (holding it in place with the other hand,) and press really hard. (This is the "squash" part of this Lab activity.) Blot off excess liquid. Observe under the microscope.

Draw and describe what you see.

Genotypes of F1 generation: *v* + + (female) X *v cv ct* (male)

+ cv ct(*)

(*Remember the male has only one X, and the Y doesn't contain these genes.)

Look at the genotypes of the offspring (listed in this table by the phenotypes shown), to see the amount of crossing over that has occurred.

Offspring Number of offspring with each

phenotype phenotype

v + + 580 (vermillion eyes)

+ c v ct 592 (cross-veinless and cut, but not vermillion)

v cv + 45

References

http://www.exploratorium.edu/exhibits/mutant_flies/mutant_flies.html

http://www.yale.edu/ynhti/curriculum/units/1996/5/96.05.01.x.html

http://www.hoxfulmonsters.com/