TEACHER REFERENCE PAGES-FLY EYE PIGMENTS LAB (2009)

Introduction

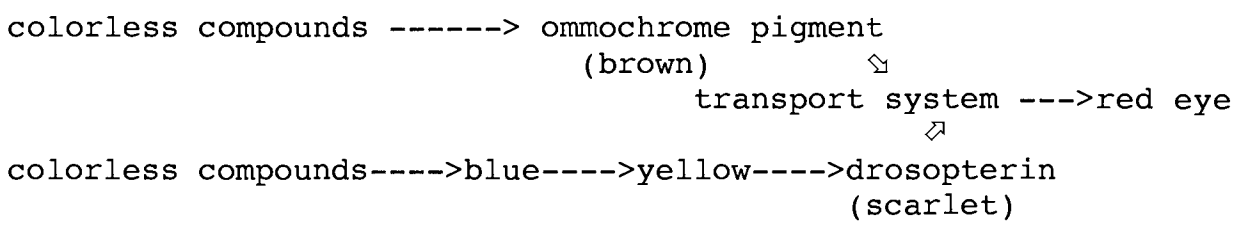
Background on fly eye pigments:

Genes have an effect on the appearance or phenotype of an organism. In most cases, a gene controls the production of a protein. These proteins control the synthesis/degradation, transport, and sorting of cellular molecules. Proteins that catalyze synthesis/degradation reactions are called an enzyme. Often, enzyme catalyzed reactions in the cell are arranged into sequential reactions leading to a particular product or set of products, called a biochemical pathway. In the eyes of Drosophila, the pigments responsible for eye color are produced by two biochemical pathways: the ommochrome pathway producing a brown pigment, and the pteridine pathway first passing through a pale blue then yellow pigment stages producing a bright red (scarlet) pigment called drosopterin. After the pigments have been produced in the cells, they are transported into the pigmented areas (crystals) of the eye cells. This is a multi-step process involving a large number of genes with the red-brown eye color of wild-type Drosophila due to the biosynthesis and deposition of the two pigment types. Several pigments use the same general transport mechanism, which requires a chemical pathway found on the X chromosome, at least in part. For example, brown, scarlet and white are all transporter subunits and white forms complexes with both brown and scarlet.

The study of mutant alleles has linked a number of eye color genes to their function in the pigment deposition pathway. The genes can be grouped into three broad functional categories: (1) synthesis of pathway intermediates (e.g. cinnabar, vermilion); (2) transport of precursor molecules across cellular membranes (e.g. white, brown and scarlet); and (3) protein sorting and biogenesis of pigment granules (e.g. garnet and deep orange). There is strong genetic and biochemical evidence indicating that white, scarlet and brown are involved in transport/production of pigment precursors (see flybase.org). These pathways are diagrammed below:

TEACHER REFERENCE PAGES-FLY EYE PIGMENTS LAB (2009)

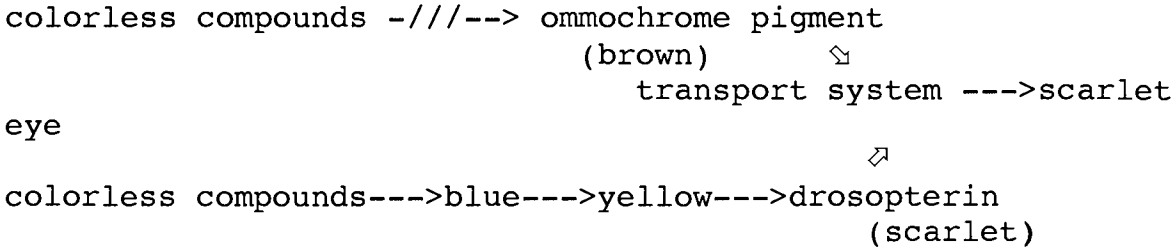
Normal Wild Eye Type (Red-brown Eye Fly)



There are additional mutants blocked at different points in the synthesis of pteridine pigments or ommochrome pigments. Pteridine pigments consist of at least five compounds. The different pigments in the eyes are all blended together and so a technique that can separate them is needed in order to find out how the genes of the flies affect their phenotypes. In the experiment given, the pteridine pigments are separated.

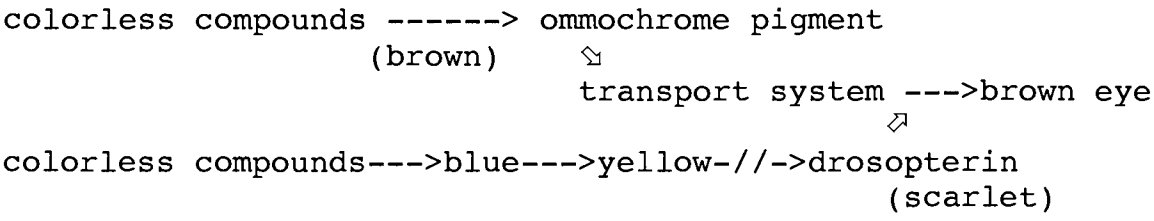
Scarlet Eye Type (stst)

A homozygous recessive mutant condition prevents the production/transport of brown pigment.



Brown Eye Type (bwbw)

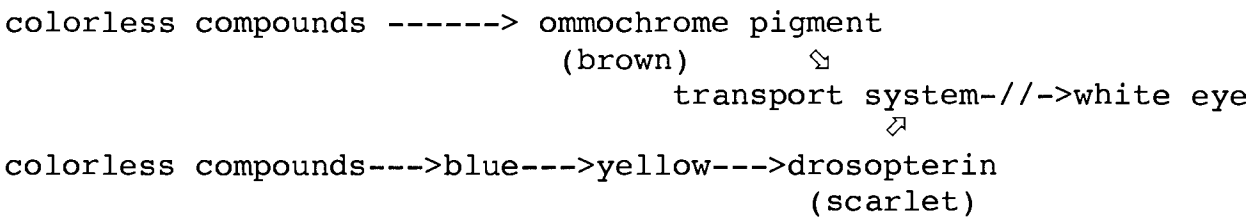
A homozygous recessive mutant condition prevents the production/transport of red pigment.



TEACHER REFERENCE PAGES-FLY EYE PIGMENTS LAB

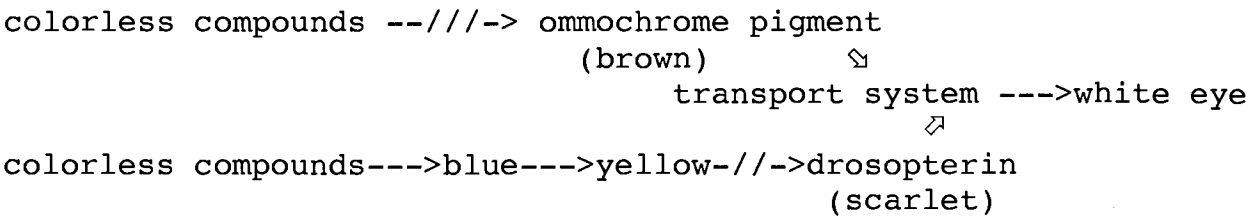
White Eye Type (X-linked, X^wX^w or X^wY)

Absence of any wild type allele of the white gene leads to white eyes because neither brown nor scarlet pigments can be produced/transported into the eye's pigment granules.



White Eye Type (Autosomal double mutant) (bwbw,stst)

A homozygous recessive condition for scarlet prevents scarlet pigment formation/transport and a homozygous condition for brown prevents brown pigment formation/transport so eyes are white due to lack of either pigment.



Thin Layer Chromatography

The technique used in this laboratory is a type of chromatography. **Chromatography** is a process in which compounds are separated by applying the compounds to a stationary material such as paper, and passing a moving solvent over the compounds. Depending on the relative properties of the stationary phase and the moving solvent, compounds will move fast, move slowly, or remain tightly bound at the point where they were applied. After a period of time, the process is stopped and the separated compounds are observed. This laboratory is using a variant of chromatography called **thin layer chromatography (TLC).** Here, the stationary phase is a thin layer of something (in our case cellulose ground up into tiny granules). The cellulose being used is smoothed over a

plastic sheet so that it can be handled without breaking. The cellulose is attractive to substances that dissolve well in water. The moving phase or solvent passes over the cellulose. The experiment uses a 1:1 mixture of n-propyl alcohol and 29% ammonium hydroxide in water ("ammonia"). This solvent is able to dissolve some substances that would not dissolve well in water. Such substances will move fast up the thin layer plate. During the thin layer chromatography, the plastic sheet with the thin layer on it (the plate) should be in an atmosphere saturated with the solvent for best results.

Equipment

UV illuminator

Supplies

20 600 ml beakers(glass)
20 Glass rods
10 tweezers
10 metric rulers
box UV goggles
2 Sharpie marking pens
32 Plastic vials for dispensing flies (8 for each type of fly)
10 fine tipped paintbrushes (to move flies)

Consumable supplies:

1L n-propyl alcohol/ 29 % Ammonium Hydroxide (solvent) 1:1
50 TLC plates 1 per group - always have 50 or more precut.
? fruit flies (brown (bw), scarlet(st),wild(+), white(w)
1 vial of each per class
50 Ziplock baggies 1 per group -always have 50 or more
50 Aluminum foil (need pre cut squares for wrapping TLC plates)
20 Filter paper (used as wick in TLC beaker)
48 Color film #669 (1 per group)
2 Kimwipes boxes (for cleaning glass rods)
2 pet ether in small vials (for killing flies)

TEACHER REFERENCE PAGES-FLY EYE PIGMENTS LAB

Procedure

Flies to use: Drosophila for this experiment are either: freshly anaesthetized from your own crossing experiments or anaesthetized this morning by the TOPS resource teacher. The different types available for testing can include *brown*, *scarlet*, *brown and scarlet (autosomal white)*, wild type, *white (X-linked)*. If you are using your own crosses, try a heterozygous fly for one or more eye pigment genes.

Preparing the TLC chamber: The solvent will have been mixed in advance and you should pour it into a 600mL beaker to a depth of 1 cm. If a fume hood is available, the beakers should be located there. If not, make sure the area is well ventilated. Cut out a section of a filter paper sheet and place it against one wall of the beaker to conduct the solvent up into the air in the beaker, so the solvent vapors can easily saturate the air. Cover the beaker with foil.

Preparing the TLC plate for chromatography. Use TLC plates that are cut to 5cm x 10cm in 600ml beakers. Measure 1.5 cm above the bottom of the plate and 1 cm from the edge, and lightly (do not gouge the cellulose!), using a lead pencil only, mark the spot with an x. Measure over from that x on the same line 1.0 cm and make another x. Repeat until no more space exists on the TLC plate. (Starting only 0.5cm from the edge has been used successfully.) **See Figure 1 in student handout.** Each x mark can be loaded with a fly sample. Decide what samples you will examine and mark the top of the TLC plate, directly over each x mark, with the type of flies to be placed there. Then select 3 or 4 flies of the type to be used. Put the first on the x for its type and gently but firmly squash the head onto the x. Using forceps, remove that fly and repeat with the second fly of that type. Try not to make a hole in the cellulose, and try to get both orange pigment spots on top of each other. (If you miss, it will still work). Before switching to another type of fly, clean the glass rod with a Kimwipe.

Running the TLC: After the flies are applied to the TLC plate, you may begin the chromatography. Place the TLC plate into the solvent so that the bottom edge dips into the solvent but NOT the spots where the flies are located. Ideally the solvent should be about halfway between the bottom and the line of fly eye pigment spots. You can add or take away solvent at this stage if needed. Keep the tank level during the chromatography. The solvent will move to within 2 cm of the top of the TLC plate in 30 minutes. It is the teachers choice whether the next class or the teacher will remove

the plates and mark the solvent front with a light pencil line after the class is over. The plates are light sensitive at this time and must be wrapped in foil at once.

Data collection

Observing the TLC chromatogram: In preparation for this part of the experiment, you may wish to have the students view a real chromatogram or to observe an audiovisual presentation about interpreting chromatograms. Wear eye protection for this part of the experiment because a long wave ultraviolet (UV) light will be used. The wavelengths used do not cause mutations, but may cause a sunburn-like reaction if you lean over the lamp a long time, so be quick.

In a room that can be darkened, place the plate with the cellulose side down over the UV lamp, and mask off with brown paper or foil the rest of the lamp area. Have a marking pen available. Turn on the lamp. For each x mark, mark where the different colored spots or streaks that you can see occur, using the marking pen on the plastic side of the TLC plate. You may want to demonstrate this procedure on the overhead. Note the colors the spots appear to be by placing abbreviations next to the spots (suggested colors: blue-green (BG), blue (B), violet (V), yellow (Y), orange (O)). Turn off the lamp.

Make a drawing of the chromatogram, noting colors, sizes and shapes of the spots, and recording distances traveled accurately.

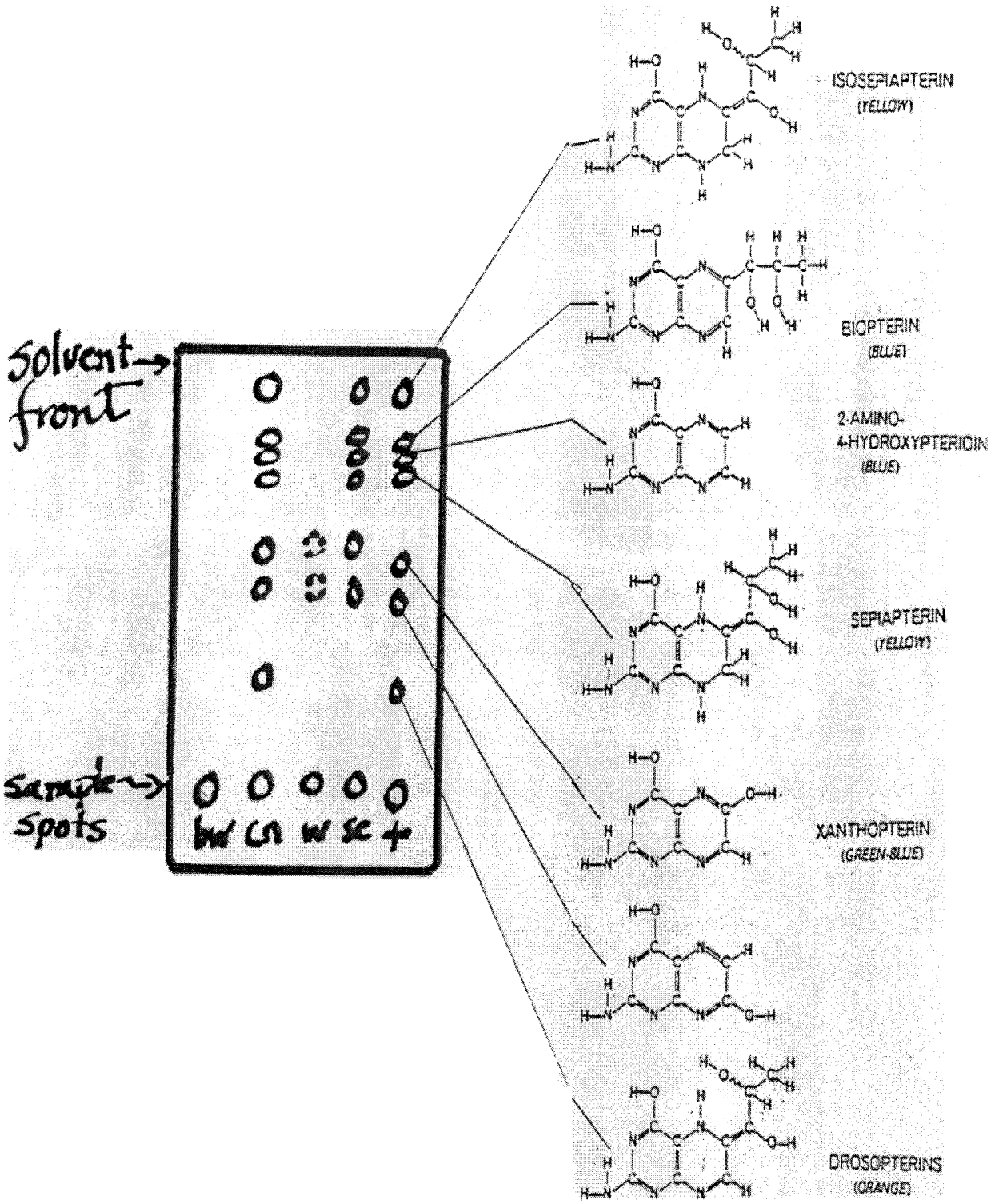


Figure 3. An example of Thin Layer Chromatography from a textbook. Your students' results will be slightly different.

TEACHER REFERENCE PAGES-FLY EYE PIGMENTS LAB

Example of typical student TLC results. The + and st fly columns will show 4-5 pigment spots; the bw and w fly columns will have no pigment spots.

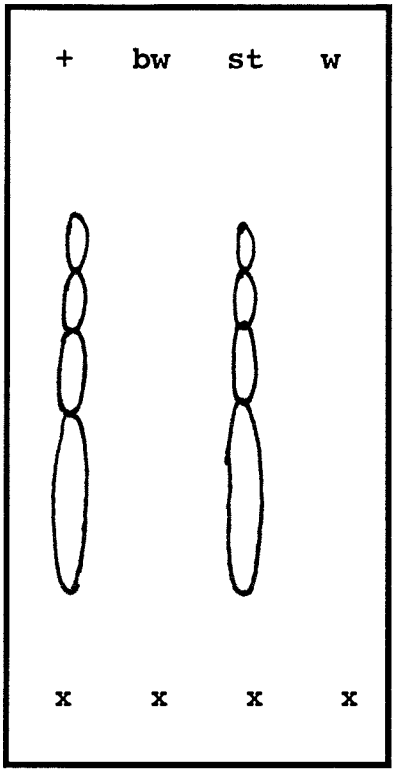


Figure 4.

Interpretation

Interpreting the results: Carefully observe all spots that are visible on the wild type fly lane(s) on your TLC plate. Then, compare these with each mutant lane in turn. See whether or not all the wild type pteridine pigments you can see are also present in each type of mutant. Make a table to record your results. Also record if there is less or more of any pigment than in wild type (the brighter and more intense the fluorescence, the more pigment there is). If the Polaroid camera is available, plates may be photographed with color film (Polaroid #669), 2 sec. exposure at 4.5 aperture.

TEACHER REFERENCE PAGES-FLY EYE PIGMENTS LAB

Questions

1. Which pigments are found in the *wild type* flies? ***pteridines***

2. Are there any other strains of flies on your plate which have the same pigments? ***yes*** Which strain(s)? ***st***

3. Are there one or more strains on your plate in which one or more of the pigments are missing? ***yes*** Which pigments are missing from which strain or strains? ***all pigments from bw & w***

4. Do all the pteridine pigments appear in all the mutants? ***No***

5. What pigment(s) are absent when the eyes are white in color? ***All***

6. Which of these mutants has more severely affected the pteridine pigments, *brown* or *scarlet*? ***Brown***

7. Reread the introduction to this laboratory, and answer this question: how can these mutations affect the presence of eye pigments?

Real World Tie-ins

Medical solution, chemical pathways i.e. respiration and photosynthesis, agriculture

Enrichment

Challenge options: One useful way to compare pigments on more than one TLC plate is using the Rf or ratio of migration to the solvent front migration. For one of the clearly defined spots, mark the center of the spot with a dot. Measure between this dot and the starting x. Then measure from the starting x to the solvent front. Divide the migration distance of the spot by the distance to the front. This number is the Rf and is characteristic of the chemical compound and the solvent. Determine the Rf of the same spot on a TLC plate prepared by another student group and see how close the two values are.

Another interesting thing to look at in this type of experiment is dominance at a more biochemical level. If you have been crossing

these fly mutants, you will know that the mutants are all recessive. One question that can be asked is: does a heterozygote between mutant and wild type have exactly the same type and amount of pteridines as wild type? An interesting heterozygote to look at in this regard is the *+/white* heterozygote. Another interesting one is the *wt/brown* heterozygote. If the heterozygote has the same intensity and type of pigment as the wild type, it suggests that the biochemical pathways can compensate for different doses of good or bad genes and manage to produce the same amount of pigment in the eye. If there is about half of some of the pigments, it suggests that the gene dose directly controls the pigment production, without regulatory compensation by the biochemical pathways.

Find flies of another species, or genus. Examine their eyes. What color are they? Could you formulate a hypothesis which can be tested using the equipment in this activity? Would such flies have the same pigments present in the same locations?

References

Eldon Gardner, Thomas Mertens, and Robert Hammersmith. Genetics: Laboratory Investigations, Eighth Ed., Macmillan, New York, 1985.

Dale Dorsett and K. Bruce Jacobson. Purification and biosynthesis of Quench Spot, a Drosopterin precursor in *Drosophila melanogaster.* Biochemistry 21:1238-1243, 1982; see also J. of Biol. Chem.242:565.

Ivor Smith. Experiment C16. Chromatography, ascending separation of *Drosophila melanogaster* (fruit fly) pigments (pteridines); pp 73-74, in Chromatographic and Electrophoretic Techniques.

Ernst Hadorn. Drosophila Eye Pigments. *Scientific American* 206:100-110, 1962.

M. Demerec, The Biology of Drosophila. John Wiley and Sons, New York, 1950.

TEACHER REFERENCE PAGES-FLY EYE PIGMENTS LAB

PROCEDURES TO KILL FLIES

1. Transfer flies from glass breeding container to plastic kill jar:
   a. Get properly labeled plastic kill jar and sponge from kit.
   b. Tap glass container on table to knock flies to bottom of jar.
   c. Quickly remove sponge and invert over plastic container, lining up lips so no flies escape.
   d. While holding the jars in this configuration, tap plastic jar against table to make the flies fall from the glass jar into the plastic one.
   e. Quickly put glass jar mouth down onto the table (near edge) and place other hand over mouth of plastic jar.
   f. Put sponge plug into plastic kill jar.
   g. With the sponge from the glass breeding jar in one hand, slide the glass jar off the edge of the table, stuffing the sponge into its mouth just as it leaves the edge. Try not to lose too many flies.

2. Killing flies:
   a. Place kill jar in freezer until flies stop moving (approx. 2 hrs.)

   OR

   b. Add 10 drops of Petroleum Ether to the sponge in the kill jar and slide it down to about 2cm from the bottom of the kill jar. Wait approximately 10 minutes before removing the sponge with a pair of tweezers. Remember all the flies should be suffocated, but some may be only anesthetized and may wake up during the class period. Keep sponge in kill jar to prevent these flies from escaping and reapply Pet. Ether if necessary.

3. Clean Up:
   When finished with lab (End of Day) please empty the dead flies from the kill jars. Eyes that have been dried out will not crush or spot easily.