

## Separation of *Drosophila* Eye Pigments with Chromatography

**Summary:** My group and I tested whether there were any differences in *Drosophila* eye pigments, called pteridines, between males and females having the same eye colors. We did this by creating many different samples of water combined with mashed and mixed fruit fly eyes of a single color and sex and then employed thin layer chromatography to find the different pteridines contained for each. Our results show that males and females with the same eye color frequently have different pteridines. These techniques are supported by the wide use of thin layer chromatography as a way to isolate pteridines in fruit flies (Tomic-Carruthers *et al.*, 1996). Pteridines are also used to determine fruit fly age and certain physical properties, indicating their usefulness in fruit fly experiments (Millest *et al.*, 1992).

**Methods & Results:** My group and I obtained samples of postmortem fruit flies with the following eye colors: red (wild type), white, sepia and vermillion. We used magnification to separate males and females of particular eye colors, knowing that males have a pointed and darkened abdomens. For our experiment only females were retrieved

The heads were carefully removed from each eye color sample with a pin and transferred to separate depressions in a porcelain spot plate. Using clean pins for each color sample, we mashed the fly heads into a paste-like substance and then mixed each with a single drop of distilled water. Next, we obtained a silica-gel coated strip and, using capillary tubes, spotted it about  $\frac{3}{4}$ " from the bottom with each eye color sample at equidistant points. The TLC plate was then developed in a darkened container for more than an hour.

Upon completion, the solvent front position was marked and the plate was left to dry. After exposing the results to long wavelength ultraviolet light under a UV light box, the marks left by the pteridines were circled with a pencil and then measurements were made for each. These results were used to determine the retardation factor for each pteridine, for which we used the following formula  $R_f = (\text{Distance Traveled by Solvent}) / (\text{Distance Traveled by Solvent Front})$ . We compared our results for the female sex with another group's results for the male sex, which produced the following table of values:

Pteridines	Wild-Type ( $R_f$ )		White ( $R_f$ )		Sepia ( $R_f$ )		Vermillion ( $R_f$ )	
	Male	Female	Male	Female	Male	Female	Male	Female
Isosepiapterin (yellow)	0.91*					0.98*	0.91*	
Biopterin (blue)		0.91*				0.93*		0.87*
2-amino-4-hydroxypterin (blue)		0.91*				0.93*		0.87*
Sepiapterin (yellow)	0.91*					0.98*	0.91*	
Xanthopterin (green-blue)								
Isoxanthopterin (violet-blue)								
Drosopterin (orange)	0.40	0.29					0.49	0.49
Unknown (brown)					0.30		0.70	

\* Indicates a value for a color that could be more than one pteridines

We found that making accurate determinations for the specific pteridines to be difficult, given that there are two pigments for yellow and four pigments that are blue or contain blue. Also, an unexpected brown pigment appeared twice: in the sepia male and the vermillion male samples. This suggests that there are perhaps additional pteridines to consider. More thorough experimental procedures in this area are required. However, our data does indicate that pigments are oftentimes different between male and female fruit flies with the same eye colors, such as with the wild-type males and females, which have yellow with orange pigments and blue with orange pigments, respectively.

### References:

Tomic-Carruthers, N. & Robacker, D.C. & Mangan, R.L., 1996. Identification and Age-Dependence of Pteridines in the Head of Adult Mexican Fly *Anastrepha ludens*, *Journal of Insect Physiology*, 42(4), pp. 359-366.

Millest, A.L & Cheke, R.A & Howe, M.A. & Lehane, M.J. & Garmes, R, 1992. Determining the Ages of Adult Females of Different Members of the *Simulium damnosum* complex (Diptera: Simuliidae) by the Pteridine Accumulation Method, *Bulletin of Entomological Research*, 1992(82), pp.219-226.