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Lack of chromosome breakage and altered sex ratios by copper sulfate in *Drosophila melanogaster.*

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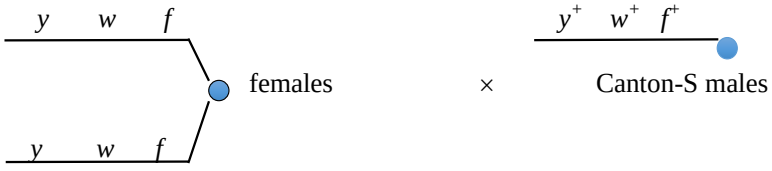
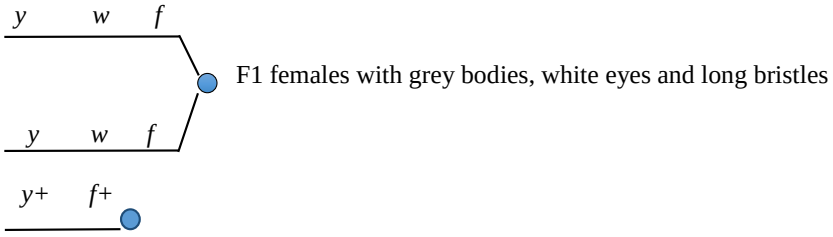
Although copper plays an important metabolic role in all organisms, high concentrations can have toxic and mutagenic effects (Pra *et al.*, 2008; Balinski and Woodruff, 2017, and references therein). One common source of excess copper concentrations in the environment is copper sulfate, which is a fungicide used to kill bacteria, fungi, and snails and is a potential producer of genetic damage in exposed humans (National Pesticide Information Center: http://npic.orst.edu/factsheets/cuso4gen.html). Copper sulfate induces chromosome breakage events in mice and increases the rate of recessive sex-linked lethal mutations in *Drosophila melanogaster* (Law, 1938; Agarwal *et al*., 1990). It is the objective of this study, therefore, to determine if copper sulfate induces chromosome breakage events in the model system *D. melanogaster*. It is our hypothesis that this chemical will significantly increase X-chromosome breakage events. Since copper can alter sex ratios (Niklasson *et al*., 2000), we also investigated the ability of copper sulfate to alter sex ratios and the recovery rate of XXX (triplo-X) female progeny.

We screened for the ability of copper sulfate to induce chromosome breakage by treating adult wild-type (Canton-S) *D. melanogaster* males with 0.5 mM of copper sulfate mixed in *Drosophila* instant food (Wards Natural Science) and mating these males to C(1)DX, *y w f* / Y females possessing two X chromosomes attached to a single centromere and the recessive genetic markers *y* (yellow body color), *w* (white eyes), and *f* (forked bristles). We have previously observed that 0.5 mM of copper sulfate is just below the toxic level for *D. melanogaster* males (Balinski and Woodruff, 2017, and unpublished results). The attached-X chromosome and visible mutants are further discussed in Lindsley and Zimm (1992).

As shown in the mating scheme below, y^+ flies have wild-type grey body color, w^+ flies have wild-type red eyes, and f^+ flies have wild-type long bristles. The Y chromosomes in females and males will be ignored, since we did not identify Y-chromosome breakage events. This assay was previously used to identify chemical-induced and gamma-ray-induced chromosomal breakage in *D. melanogaster* males (Blount and Woodruff, 1986; Woodruff and Russell, 2011).



If no X-chromosome breakage occurs, the F1 females will have yellow body color, white eyes, and forked bristles. If, for example, a break occurs in males between y^+ and w^+ and another break between w^+ and f^+, the rejoined fragment (y^+ and f^+) will be recovered in F1 females, resulting in grey body color, white eyes, and long bristles, as shown below.



Other F1 female phenotypes can occur from different breakage events in the parental male X chromosome, including w^+ f^+ fragments, which give yellow bodies, red eyes, and long bristles, y^+ w^+ fragments that result in grey bodies, red eyes, and forked bristles, and y^+ fragments that produce grey bodies, white eyes, and forked bristles (see Figure 1 in Woodruff and Russell, 2011).

Since all of these breakage events result in females with extra X-chromosome fragments, this assay has been called the hyperploidy test (Auerbach, 1976). In addition, XXX females that are grey in body color, have red eyes, and long bristles were observed in this study, independently of chromosome breakage events.

We hypothesized that 0.5 mM of copper sulfate would significantly increase our historical laboratory frequency of spontaneous chromosome breakage events (2/97,895 = 0.002%) (Woodruff and Russell, 2011), suggesting that exposure to copper sulfate in humans may cause genetic changes. As a positive control, we previously observed that 2,010 rads of gamma rays induced two breakage events out of 1,521 flies using this hyperploidy assay (2/1,521 *vs* the spontaneous frequency of 2/97,895; P = 0.001) (Woodruff and Russell, 2011). We also predicted that copper sulfate will alter sex ratios by reducing the number of hemizygous F1 males that inherit treated X chromosomes. In addition, we determined if copper sulfate treatment alters the recovery of XXX F1females as compared to the untreated controls; some XXX F1 flies may be due to recovered breakage events that do not include the y^+, w^+ or f^+ markers.

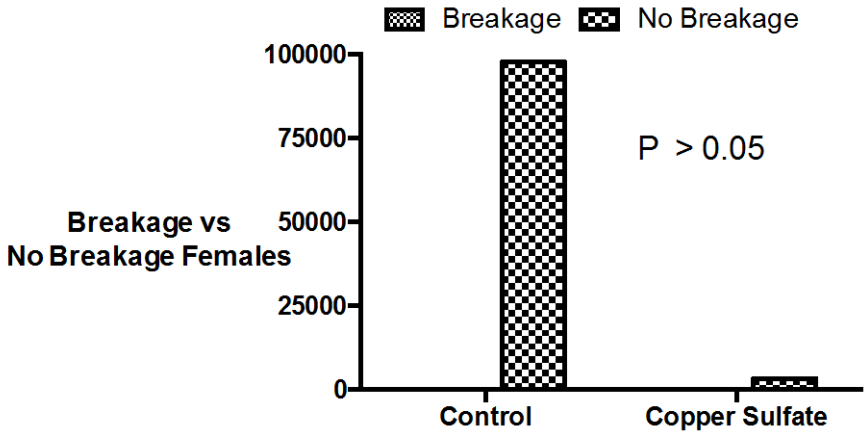


Figure 1. Chromosome breakage events in the presence and absence of copper sulfate.

Copper sulfate did not induce chromosome breakage events in this study (two breakages in 97,895 flies in our historical laboratory control and no breakage events in 3,335 flies in this study; P > 0.05; see Figure 1). In addition, sex ratios were not altered by copper sulfate (treatment: 2,784 males and 2,119 females recovered, 0.57 males to total progeny; control: 4,438 males and 3,335 females recovered, 0.57 males to total progeny; P = 0.75; see Figure 2). Finally, we observed 16 F1 XXX females out of 2,312 total females in the

control crosses, whereas 40 XXX females were recovered out of 3,375 total females in the treated flies (P = 0.09). Hence, copper sulfate did not significantly change the recovery of XXX females (see Figure 3).

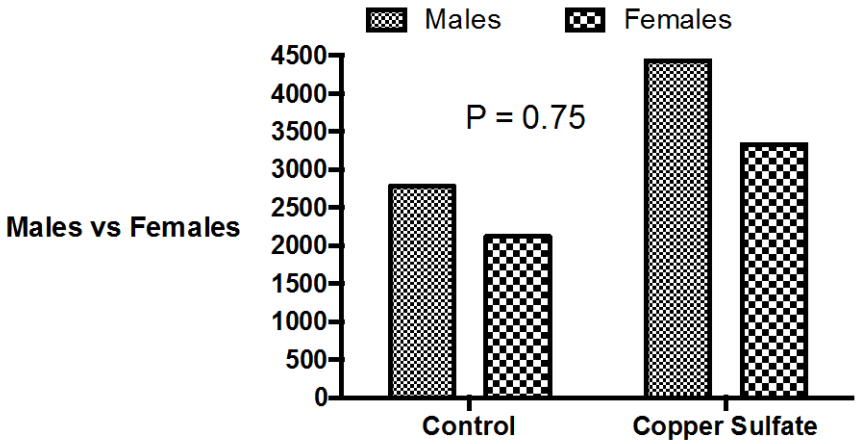


Figure 2. Sex ratios in the presence and absence of copper sulfate.

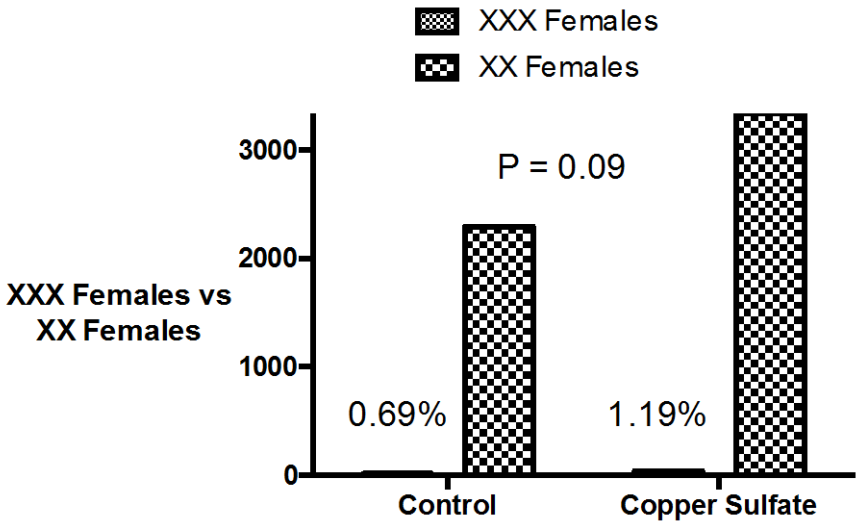


Figure 3. The recovery of XXX females in the presence and absence of copper sulfate.

In summary, copper sulfate did not cause chromosome breakage, did not alter the sex ratio, and did not alter the recovery of XXX females. It would be of interest to increase the number of copper sulfate treated flies in a follow up experiment to increase the recovery of low frequency breakage events. It would also be of interest to follow the procedure of larval injections and immersion of eggs with copper sulfate, which was observed to increase the frequency of recessive sex-linked lethal mutations in *D. melanogaster* (Law, 1938), to see if these procedures induce chromosome breakage events.

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An undergraduate cell biology lab: Western Blotting to detect proteins from *Drosophila* eye.

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Abstract

We have developed an undergraduate laboratory to allow detection and localization of proteins in the compound eye of *Drosophila melanogaster*, a.k.a fruit fly. This lab was a part of the undergraduate curriculum of the cell biology laboratory course aimed to demonstrate the use of Western Blotting technique to study protein localization in the adult eye of *Drosophila*. Western blotting, a two-day laboratory exercise, can be used to detect the presence of proteins of interests from total protein isolated from a tissue. The first day involves isolation of proteins from the tissue and SDS-PAGE (sodium dodecyl sulfate-polyacrylamide) gel electrophoresis to separate the denatured proteins in accordance to their molecular weight/s. The separated proteins are then transferred to the Nitrocellulose or Polyvinylidene difluoride (PVDF) membrane in an overnight transfer. The second day lab involves detection of proteins (transferred to the membrane) using Ponceau-S stain, followed by immunochemistry to detect the protein of interest along the total protein transferred to the membrane. The presence of our protein of interest is carried out by using a primary antibody against the protein, followed by binding of secondary antibody which is tagged to an enzyme. The protein band can be detected by using the kit, which provides substrate to the enzyme. The protein levels can be quantified, compared, and analyzed by calculating the respective band intensities. Here, we have used fly eyes to detect the difference in level of expression of Tubulin (Tub) and Wingless (Wg) proteins in the adult eye of *Drosophila* in our class. The idea of this laboratory exercise is to: (a) familiarize students with the underlying principles of protein chemistry and its application to diverse areas of research, (b) to enable students to get a hands-on-experience of this biochemical technique. Keywords: *Drosophila melanogaster*, eye, Western Blot, protein estimation. localization of proteins, SDS-PAGE gel electrophoresis.

Introduction

Recent educational research on teaching biology to undergraduates has raised concerns about how traditional approaches in large classes fail to reach many students and thereby emphasized on the need for more hand-on experiential learning instructions (Puli and Singh, 2011; Tare *et al.*, 2009; Tare and Singh, 2008; Uman and Singh, 2011; Wood, 2009; Woodin *et al.*, 2009). One of the hallmarks of the modern day science education is experiential learning, which allows students to get a hands-on-experience to understand latest scientific research and concepts. In modern day undergraduate curriculum, research is an important component of habits of inquiry and learning (Puli and Singh, 2011; Tare *et al.*, 2009; Tare and Singh, 2008; Uman and Singh, 2011). Efforts have been channeled to develop a repertoire of laboratory courses to expose undergraduates to modern day biology concepts and techniques used in biomedical research. The new text books provide exhaustive and detailed information through movies and illustrations on how proteins play a role in a biological function and what approaches can be used to determine their localization as well as