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New species of *Drosophila* or not.

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Three undergraduate students (Honigford, Rochester, and Schimmoeller) were given a stock of *Drosophila* that had white eyes and were told that it was sent to the Department of Biological Sciences at Bowling Green State University as a possible new species of *Drosophila*. The students were asked to determine if this were true, or was the white-eyed stock just *Drosophila melanogaster* with a new mutation. The students were told to determine from the literature mechanisms of speciation and the definition of a species (Coyne and Orr, 2004; Price, 2008) and to use this information to compare the white-eyed stock with the Canton-S wild type stock of *D. melanogaster*.

The students decided to test the hypothesis that the white-eyed stock was a new species based on the biological species concept: groups of interbreeding natural populations that are reproductively isolated from other groups (Mayr, 1966, 1982). Accepting the biological species concept, species are defined by reproductive isolation, including premating isolation mechanisms (for example, the prevention of the formation of hybrid offspring due to ecological or habitat isolation, seasonal or temporal isolation, sexual isolation, and mechanical isolation), or by postmating isolation mechanisms (for example, reduced viability or fertility of hybrid offspring) (Dobzhansky, 1937; Klug *et al.*, 2013). In his famous 1859 book *On the Origin of Species by Means of Natural Selection*, Darwin said: "Nor shall I here discuss the various definitions which have been given of the term species. No one definition has as yet satisfied all naturalists." (Darwin, 1859). Yet, Darwin did anticipate the biological species concept where species are defined by reproductive isolation: "This view generally entertained by naturalists is that species when intercrossed, have been specially endowed with the quality of sterility." (Darwin, 1859).

At first the students in this study looked for any morphological differences between the white-eyed and Canton-S stocks. Other than the color to the eyes, they observed no obvious differences in the two stocks. Hence, these two stocks did not fit the morphological species concept, where different species are different in appearance (Coyne and Orr, 2004; Herron and Freeman, 2014). They did, however, note that the number of progeny in vials of the Canton-S stock was greater than in vials of the white-eyed stock and that development time to adult was slower in the white-eyed stock.

To determine if the two stocks were different species based on the biological species concept, the students next mated white-eyed virgin females with Canton-S males and white-eyed males with virgin Canton-S females. Flies from the white-eyed stock were observed to freely mate with flies from the Canton-S stock in both crosses. Therefore, there was no premating isolation between these two stocks.

In the first cross (white-eyed females with Canton-S males), some progeny were produced in one vial, but the progeny had white eyes, suggesting that non-virgin females were used in the cross. In additional crosses, no adult progeny were observed and the progeny were observed to die as pupae.

After reading articles on stocks of *D. melanogaster* with rearranged chromosomes (Ashburner, 1989; Holm *et al.*, 1980; Boulton and Woodruff, 2010), the students were told that the white-eyed stock was *D. melanogaster* and it had two attached 2L chromosomes and two free 2R chromosomes; C(2L), *dp*; F(2R), *cn c bw*, giving the flies white eyes due to the interaction of the *cn* and *bw* mutant genes (Grell, 1970; Ashburner, 1989; Lindsley and Zimm, 1992); *D. melanogaster* with a normal karyotype have two 2L.2R chromosomes (the period represents the centromere). The students also determined that the reason for the low progeny number in the white-eyed stock was because one-half of the progeny have four 2L chromosomes or no 2L chromosomes

(Boulton and Woodruff, 2010, see their Figure 5). In addition, the reason why no adult progeny were recovered from crosses of the white-eyed stock with Canton-S was because the progeny either had three 2L chromosomes or one 2L chromosome, leading to chromosomal imbalance (Ashburner, 1989; Boulton and Woodruff, 2010, see their Figure 4).

In summary, the students concluded that the white-eyed stock was not a new species, but was a *D. melanogaster* stock with rearranged chromosomes. It is known that chromosome rearrangements can lead to reproductive isolation and speciation (White, 1978; King, 1993). We also observed that flies with three 2L chromosomes or only one 2L chromosome lived until the pupal stage. Holm *et al.* (1980) stated that monosomic 2L flies die during early embryogenesis while the trisomic 2L flies survive to late pupae. It seems, therefore, that flies with an extra 2L chromosome in this study were able to develop up to the pupal stage, but were not able to eclose as adults. The reason for this is unknown.

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Chemical stress and recombination in *Drosophila melanogaster*.

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It has been reported that environmental stresses can influence frequencies of recombination. For example, temperature, nutrition, bacterial infections, and wasp predation can increase the frequency of recombination in *Drosophila melanogaster* (Gowen, 1919; Stern, 1926; Bergner, 1928; Neel, 1941; Singh *et al*., 2015; for a review of this topic, see Parsons, 1988). These observations suggest that organisms can respond to stresses by increasing the frequency of recombination, producing a quick increase in genetic variation that may allow for adult survival (Badyaev, 2005).

It is important, therefore, to determine if exposure to environmental chemical stressors can also influence recombination frequencies. Hence, in this proposed study we tested if copper sulfate in the food of *D. melanogaster* can alter the recombination frequency for X-chromosome linked markers. Copper sulfate is known to be toxic to *D. melanogaster* at moderate to high concentrations (Egli *et al*., 2006).

The following crosses resulted in F1 females that were heterozygous for X-linked visible mutant markers *w* (white eyes; map position 1.5) and sn^3 (singed, small bristles; map position 21) and F1 males that have these two markers on their single X chromosome.