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Effects of food coloring on longevity and viability of *Drosophila melanogaster.*

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We carried out a number of small experiments to investigate the effects of feeding food coloring on the viability and longevity of *Drosophila melanogaster*. We were interested in using food coloring as a marker for distinguishing phenotypically identical flies in behavior assays. With food coloring, it is possible to distinguish two identical flies from one another without having to paint, cut, or otherwise physically alter the flies. Although using food coloring is this way is certainly not a novel idea (*e.g.*, Wu *et al.* 1995; Hollocher *et al.* 1997), little specific information about the effects of feeding food coloring to *Drosophila* appears to be available in the literature.

Effects on viability: We tested the effect of food coloring on viability. We mixed standard banana fly food with food coloring (McCormick Assorted Food Colors and Egg Dye) and allowed eggs to be laid on the colored media. We used three different colors (red, blue, and green) that were in different concentrations per 8-dram vial (1, 2, and 3 drops). We put flies into vials sorted by color and concentration as well as vials with no coloring as controls. After 1-2 days we cleared the vials of egg-laying adults, leaving only eggs and early larva. We examined third instar larva and found that they had taken up the colored food, which was evident from the dense coloration of internal organs. After metamorphosis, eclosed mature flies had no internal trace of food coloring and seemed to be fully developed and qualitatively "normal". We conclude that feeding larvae food coloring is not obviously detrimental, and that the method here could be used to mark larvae. However, it appears useless as a method for marking adult flies.

Effects on longevity: We tested the effect of feeding food coloring on longevity of adult flies. In each of two experiments, we isolated about 10 wild-type Oregon-R adults in four vials (blue, red, green, and control) with 5 drops of food coloring in each vial. We observed the flies in the vials for four days. In total, the blue vial had 12 deaths per 20 flies, red vial had 8/20 dead, the green vial had 14/20 dead, and the control had 0/20 dead. In every case, deaths only occurred in the first two days. Feeding food coloring for long periods of time (24+ hours) appears to be very toxic.

Duration of retention of color: We tested flies for their capacity to retain food coloring in their system. We put three drops of food coloring (blue, red, or green) onto Kimwipes in banana food vials. We exposed adult Oregon-R adults to the food coloring for one hour, and then checked the vials under a microscope every hour after that. We found that for the first hour after exposing the flies in the food coloring, all the flies still had the food coloring in their gut. We checked the vials 2.5 hours after exposing the flies to food coloring and about half of the flies had already removed the food coloring from their system. The third hour after being exposed to food coloring, no flies appeared to have food coloring in their system. We conclude that after feeding food coloring to adult flies, they must be quickly used in an assay.

Current protocol: Based on the above data, we are currently using the following protocol in our experiments. Approximately 10 adult males are placed in a banana food vial with 3 drops of food color

on a strip of Kimwipe. They are allowed to feed for 3 hours, at which time they are hand-transferred into a clean vial, and then mouth-pipetted into a behavior assay. In this way, most flies have food color, and appear to behave normally, or in the case of mutants, as expected.

We note that blue coloring appears to work far better than either red or green. For experiments in which only one marker need be used, we strongly advise using blue. We also note that it is important to use each color in each treatment in independent experiments (*e.g.*, red with mutant and blue with control, and then blue with mutant and red with control) to show the robustness of results and demonstrate that coloration did not affect the relative behavior of the genotypes being compared. We have not found "color effects" in any of our experiments.

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