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Use of the *Drosophila melanogaster* zeste screen to identify aneuploidy induced by cold treatment.

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Nondisjunction or chromosome breakage can lead to missing or extra chromosomes in gametes and subsequently in offspring. The result is aneuploidy, a deviation from the normal chromosome complement. For example, in humans there are usually 23 pairs of chromosomes, with 22 autosomal pairs and the sex chromosomes (XX in females and XY in males). However, mistakes in chromosome movement during meiosis are surprisingly common. Examples of anueploidy in humans, which is caused by chromosome gain events (nondisjunction of chromosomes) or chromosome loss events (nondisjunction or chromosome breakage), include Turner Syndrome females that have only one X chromosome, Klinefelter Syndrome males that have two or more X chromosomes and a Y, and Down Syndrome individuals (female or male) that have three number 21 chromosomes. Chromosome gains or losses for most of the other chromosomes cause early embryonic death, developmental abnormalities and/or sterility. For a discussion of the effect of aneuploidy in humans see Cummings (2000).

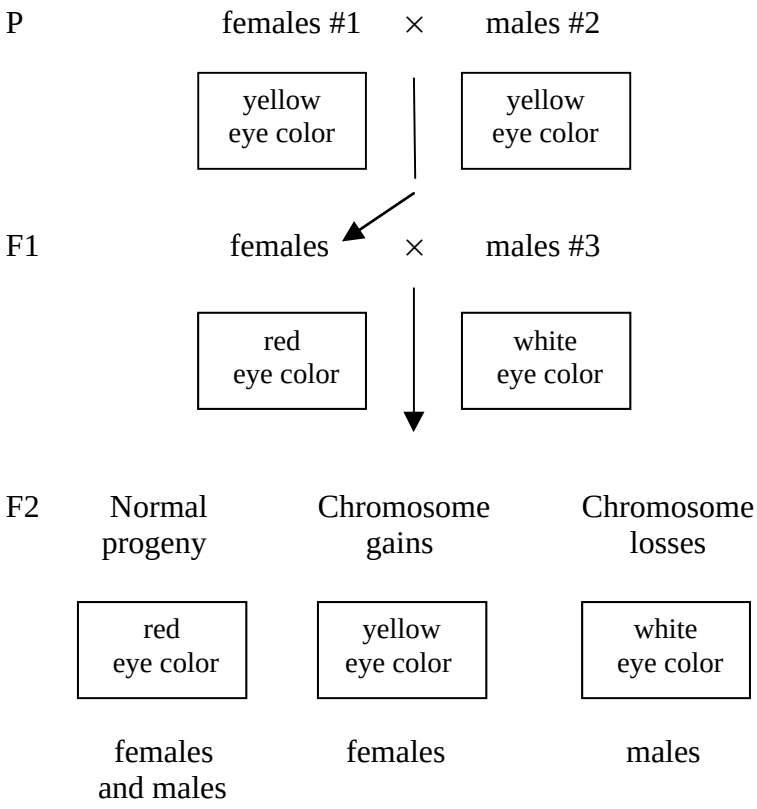

Figure 1. Eye phenotypes observed in the zeste test for aneuploidy in the gametes of female *Drosophila melanogaster*.

Aneuploidy occurs in at least five percent of all clinically recognized pregnancies, is the leading cause of pregnancy loss, and is the most common cause of mental retardation in humans (Hassold *et al*., 1996; Hassold and Hunt, 2001). Hence, it is important to have effective assays in model systems to identify physical and chemical agents that may increase aneuploidy in humans. Positive aneugens can then be excluded, if possible, from human environments. For example, although controversial, there are

reports that irradiation, oral contraceptives, fertility drugs, alcohol and smoking may increase aneuploidy in humans (Hassold and Hunt, 2001). With this in mind, a number of assays for aneuploidy have been developed in *Drosophila melanogaster* (Zimmering, *et al*., 1990). Herein, we would like to introduce a one-generation zeste test for the identification of aneuploidy in female *D. melanogaster* that gives chromosome gain or loss progeny with distinct eye phenotypes. We illustrate this test with one example of an easy way to induce aneuploidy by cold treatment. For a discussion of the development and use of the zeste test, see Zimmering *et al.* (1990) and Osgood (1991). In Figure 1 we will first give an eye color phenotype summary of the crosses used in the zeste test and will then give details of the genotypes used in the assay.

The three stocks used in the zeste test of Figure 1 are as follows:

Females #1: $y\ z\ /\ y\ z\ ;\ spa^{pol}\ /\ spa^{pol}$ (y = yellow body color and z = zeste/yellow eye color, which are located on the X chromosome; spa^{pol} = sparkling poliert (rough eye surface, which is on the fourth chromosome and is used to identify outside contamination of crosses with extraneous flies).

Males #2: $y^2\ z\ f\ .\ Y^L\ /\ sc^{V1}.\ Y^S,\ y^+\ ;\ spa^{pol}\ /\ spa^{pol}$ (f = forked bristles on the X chromosome; sc^{V1} = bristle mutant that is ignored in this assay; Y^L = long arm of the Y chromosome that is attached by a single centromere to the X; $sc^{V1}.\ Y^S,\ y^+$ = short arm of the Y chromosome marked by the wild type, dominant allele of yellow).

Males #3: $XY^L.YS,\ w\ ;\ net\ /\ net$ ($XY^L.YS$, w = attached X and Y chromosomes with one centromere and marked with the white-eyed mutation; *net* = netted wing veins on the fourth chromosomes and is used to identify outside contamination of crosses with extraneous flies; this stock does not have a free Y chromosomes).

To generate the F1 $y\ z\ /\ y^2\ z\ f\ .\ Y^L\ ;\ spa^{pol}\ /\ spa^{pol}$ females that are treated with cold and screened for aneuploidy offspring, virgin Females #1 are mated with Males #2. The F1 females are automatic virgins, since the F1 male siblings are sterile due to the lack of male fertility factors on the missing long arm of the Y chromosome. These females are then mated with Males #3 individually in vials or in groups in bottles as follows.

$$\text{F1}\ y\ z\ /\ y^2\ z\ f\ .\ Y^L\ ;\ spa^{pol}\ /\ spa^{pol}\ \text{females} \quad \times \quad XY^L.Y^S,\ w\ ;\ net\ /\ net\ \text{males}$$

The F2 progeny are then screened for the three eye phenotypes shown in the crosses above. It should also be noted that 1) another very rare class of F2 progeny may appear: yellow-eyed males that have two X chromosomes and three sets of autosomes due to nondisjunction for the X and the autosomes; 2) no X-chromosome gains occur in F1 male gametes; and 3) X-chromosome losses in F1 male gametes are not identified because they give rise to red-eyed F2 males that have the same phenotype as F2 males with normal chromosomes.

To induce aneuploidy, three day old F1 female adults are placed at 4-5^{o}C (in a refrigerator) for four days, removed and mated with Males # 3. For a control, F1 females are kept at room temperature for four days (21-24^{o}C).

An example of data from the zeste assay are given in Table 1 (one yellow-eyed, 2X;3A male was also recovered).

A total of 18/7,000 (0.26%) aneuploids were recovered from the cold treatment and 0/6,984 aneuploids were recovered from room temperature. These frequencies are significantly different ($p < 0.001$, Fisher's exact test).

As additional teaching exercises, the F1 females could be treated with a chemical or physical agent of choice by students or one could determine if there is a maternal age effect on aneuploidy in *Drosophila* females as was first observed in humans nearly 70 years ago (Penrose, 1933). A hint: *Drosophila* females do not maintain eggs at diplotene of meiosis until their release as humans do.

Acknowledgments: We decided to develop this breeding program as a teaching exercise after using it to test the effects of hypergravity and vibration on aneuploidy in *Drosophila*, as part of a study of genetic responses to stress, funded by NASA grant NAG2-1427.

References: Cummings, M., 2000, *Human Heredity*. Brooks/Cole, Pacific Grove, California; Hassold, T. *et al*., 1996, Environ. Mol. Mutagen. 28: 167-175; Hassold, T., and P. Hunt 2001, Nature Reviews 2: 280-289; Osgood, C., S. Zimmering, and J.M. Mason 1991, Mutat. Res. 259: 147-163; Penrose, L., 1933, J. Genet. 27: 219-224; Zimmering, S., C. Osgood, and J.M. Mason 1990, Mutat. Res. 234: 319-326