References: Buri, P., 1956, Evolution 10: 367-402; Frankham, R., J.D. Ballou, and D.A. Briscoe 2010, *Introduction to Conservation Genetics*. Cambridge University Press; Hedrick, P.W., 2011, *Genetics of Populations*. Jones and Bartlett Publishers, Sundbury, MA; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York; Woodruff, R.C., and A.M. Boulton 2011, Dros. Inf. Serv. 94: 167-169.

Heterosis and the recovery of *Drosophila melanogaster* triplo-X females.

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Heterosis (hybrid vigor) improves the fitness of hybrids over their parents, including an increase in reproductive success (Lippmann and Zamir, 2006). We used *D. melanogaster* to test the hypothesis that heterosis also allows for an increased recovery of hybrid progeny that have an extra X chromosome (triplo-X females). Our hypothesis is that triplo-X females, which usually do not survive as adults (Lindsley and Zimm, 1992), will be recovered at a significantly higher frequency in first generation progeny of crosses between males and females from separated, unrelated, stocks, than in subsequent generations. We also predict that hybrid progeny from crosses between separate stocks will have a higher frequency of triplo-X progeny than progeny from crosses between males and females from the same, long-term stock.

To test this hypothesis, we first crossed C(1)DX, *y f* / Y females with w^{1118} / Y males and screened for F1 triplo-X (XXX) females. In this cross, parental females have two X chromosomes attached to a single centromere and contain the markers *y* (yellow body color) and *f* (forked, short bristles). In addition, the males contain the w^{1118} X-linked mutation that causes a white-eyed phenotype. Because of the attached-X chromosome in females, and the Y chromosome, which is present in both males and females, female progeny receive the attached-X chromosome from their mothers and the male progeny receive the w^{1118} X chromosome from their fathers. Females receive their Y chromosome from their fathers, and males receive one from their mothers. See Lindsley and Zimm (1992) for discussions of mutants and the attached-X stock. The F1 triplo-X females from this cross have red eyes, long bristles, and grey body color. The frequency of triplo-X female progeny from this cross was significantly reduced over seven generations (Figure 1, P = 0.04 for the slope of the regression line being zero).

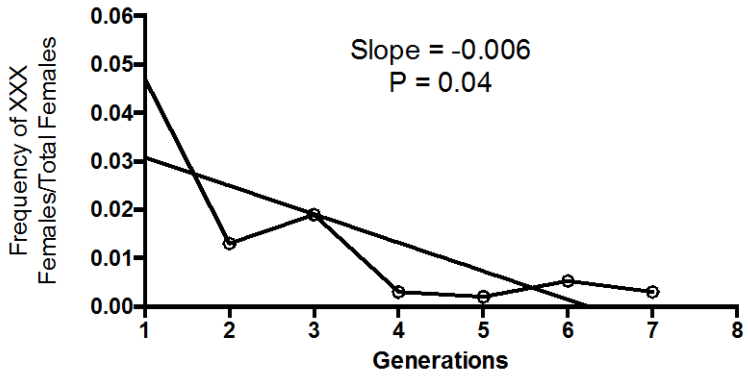

Figure 1. Frequency of triplo-X progeny over time.

We next tested our heterosis hypothesis with five additional crosses. First, we mated C(1)DX, *y f* / Y females with Canton-S (wild type) males that had been maintained in separate stocks and again observed that the triplo-X females were recovered in a significantly higher frequency in the F1 progeny than in the F2 progeny [44/679(6.5%) *vs*. 11/619 (1.8%); P < 0.0001] or the F3 progeny [44/679(6.5%) *vs*. 3/513(0.58%); P < 0.0001) (Figure 2), although the slope of the regression line for the three generations was not significantly different from zero (P = 0.21).

As controls, we also measured the frequency of triplo-X females within two stocks (interline crosses): 1) C(1)DX, *y f* /Y females with Binscy / Y males (the Binscy X contains the Bar-eyed dominant mutation), and

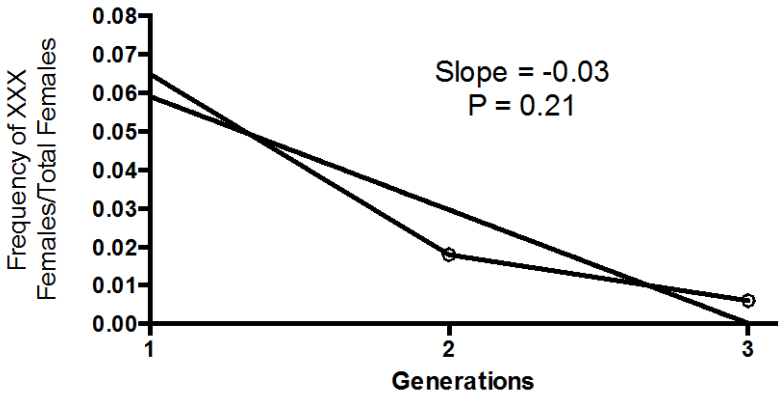

Figure 2. Frequency of triplo-X progeny over time.

2) C(1)DX, *y w f* / Y females with *Per*$^{+}$ (wild type) males. We observed 0 XXX in 1298 progeny in stock 1 and 0 XXX in 673 progeny in stock 2, showing that triplo-X females were recovered within stocks, in the absence of heterosis, at a low frequency.

We then performed inter-stock crosses of stock 1 females (C(1)DX, *y f* / Y) with stock 2 males (*Per*$^{+}$) (Cross 1/2), and stock 2 females (C(1)DX, *y w f* / Y) with stock 1 males (Binscy) (Cross 2/1). It was our hypothesis that the frequencies of triplo-X progeny would be significantly higher in the inter-stock crosses than in the interline controls. In support of this hypothesis we observed 7/309 (2.3%) XXX progeny in Cross 1/2 ($P < 0.0001$, in comparison with 0/1971 from the interline crosses) and 1/317 (0.3%) triplo-X progeny in Cross 2/1 ($P = 0.41$, in comparison with 0/1971 from interline crosses). The latter non-significant results are probably due to the low observed viability of the C(1)DX, *y w f* / Y females, which did not allow for development of the triplo-X progeny. For example, the average number of progeny per vial for C(1)DX, *y f* / Y females was 17.40, whereas for C(1)DX, *y w f* / Y females it was 11.36.

In addition, the frequency of triplo-X progeny from generation one was significantly higher ($P = 0.003$) than from generation two in Cross 1/2, but was not significantly higher in Cross 2/1 ($P = 0.41$) (Figure 3).

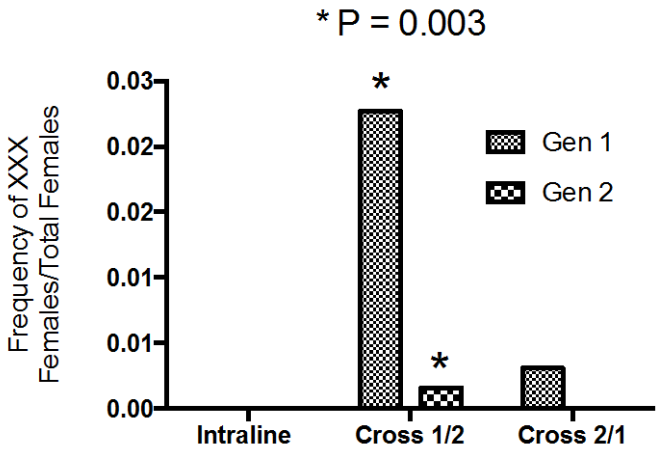

Figure 3. Frequency of triplo-X progeny from crosses within a line and between lines.

In summary, the results from this study support the hypothesis that heterosis increases the recovery of *D. melanogaster* females with an extra sex chromosome (triplo-X females).

A class discussion of the results of this teaching exercise could include: 1) What are the possible genetic mechanisms of heterosis? There are two main mechanisms: the superior fitness of heterozygotes and/or the masking of recessive deleterious mutations in hybrids (Crow, 2008). 2) It is of interest to consider: If heterosis occurs in humans, would this increase the survival of offspring with extra chromosomes, such as trisomy-21 (Down syndrome), trisomy-13 (Patau syndrome), and trisomy-18 (Edwards syndrome) (Schaefer and Thompson, 2014)?

References: Crow, J.F., 2008, Annual Review of Genetics 42: 1-16; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster: Academic Press, New York; Lippman, Z.B., and D. Zamir 2006, TRENDS in Genetics 23: 60-66; Schaefer, G.B., and J.N. Thompson, jr. 2014, *Medical Genetics: An Integrated Approach*. McGraw-Hill, New York.