Teaching Notes

Experimental observation of inbreeding depression and heterosis in *Drosophila melanogaster*.

Onasch, Katherine D., and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403.

In one of the early statements on heterosis (hybrid vigor) and inbreeding depression, Charles Darwin in *On the Origin of Species by Means of Natural Selection or the Preservation of Favored Races in the Struggle for Life* (Darwin, 1859) stated:

"In the first place, I have collected so large a body of facts, showing, in accordance with the almost universal belief of breeders, that with animals and plants a cross between different varieties, or between individuals of the same variety but of another strain, gives vigour and fertility to the offspring; and on the other hand, that close interbreeding diminishes vigour and fertility;" (Darwin, 1859, p. 96).

W. E. Castle supported this statement using *Drosophila melanogaster*, by stating:

"The cross-breds produced by the mating, B female × A male, are all of high productiveness." "Inbreeding probably reduces very slightly the productiveness of *Drosophila*…" (Castle, 1906).

Sewell Wright (1977) observed that the progeny of sibling matings of guinea pigs had a significantly smaller litter size than the non-sibling control (2.54 *vs*. 3.03). Infant mortality is also significantly higher in the children of cousin marriages in humans than among marriages of unrelated individuals (Schull and Neel, 1965; Freeman and Herron, 2007). In support of the widespread occurrence of inbreeding depression, Lynch and Walsh (1998) state: "While substantial variation of inbreeding depression exists among species (and among characters within species), almost all organisms exhibit it to some degree." They give a survey of inbreeding depression for numerous

characters, including egg-to-adult viability, female fertility, male mating ability, longevity, and wing length in *Drosophila*, and for birth weight, adult weight, IQ, and prereproductive survival in humans.

Inbreeding depression usually results from the expression of recessive deleterious alleles that are present in all diploid organisms and that are made homozygous by inbreeding (see reviews of this topic in Hedrick, 2005; Lynch and Walsh, 1998). Heterosis (hybrid vigor) that is observed in the progeny of crosses between highly inbred lines is usually due to the masking of expression of deleterious recessive alleles that are in the heterozygous state in the hybrids (see discussions in Falconer and Mackay, 1996; Hartl and Clark, 2007).

Azad, Woodruff and Thompson (2003) have previously shown how to identify deleterious genetic variation in natural populations of *D. melanogaster*; they observed that preexisting recessive deleterious alleles are present in most, if not all, *Drosophila*. There is also up to one new deleterious mutation per diploid genome in each *D. melanogaster* (see a discussion of this topic in Gong, Woodruff and Thompson, 2005). Hence, it is not surprising that, when the genome of *Drosophila* is made homozygous, recessive deleterious mutations are also made homozygous, causing a reduction in fitness.

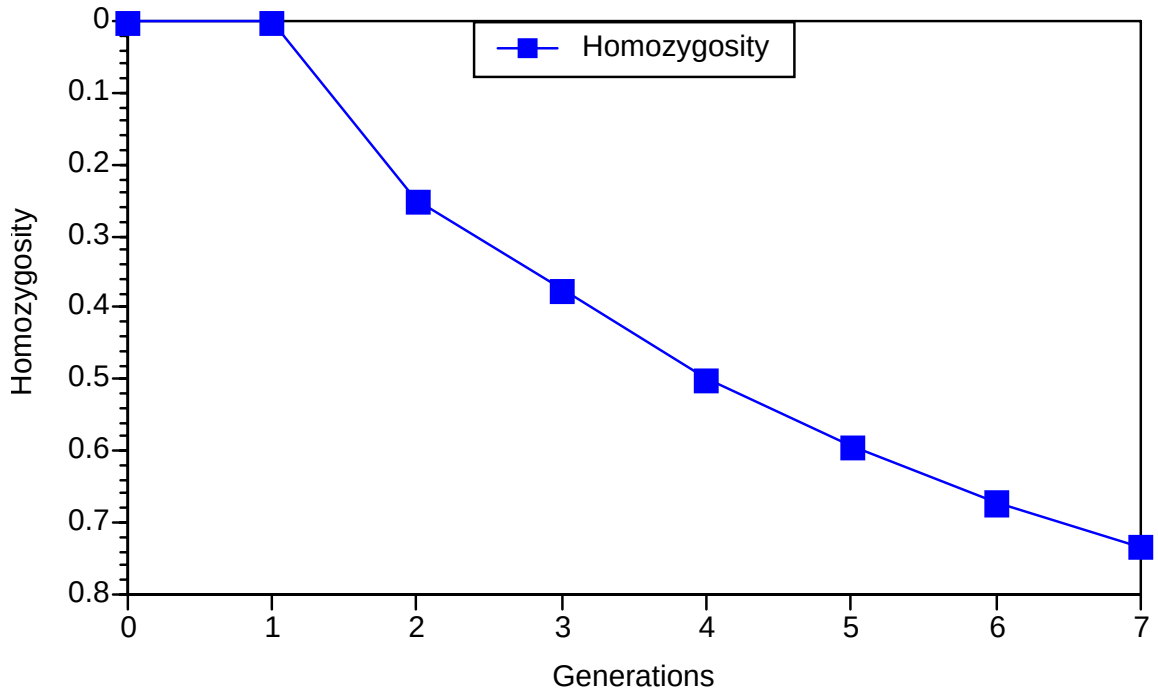

Figure 1. Expected increase in the frequency of homozygous loci over generations with single-pair, sib matings of *Drosophila melanogaster*, assuming that all genes begin as heterozygotes (for a discussion of this topic see Crow and Kimura, 1970; Hedrick, 2005). There is a one-generation delay in the increase in homozygosity at the beginning, because it is assumed that the original flies from nature are unrelated. If the sampled *Drosophila* from nature are related, the increase in homozygotes per generation would be faster.

It is the objective of this teaching exercise to attempt to identify inbreeding depression and heterosis by determining the progeny number over generations from single-pair, sibling matings of natural population lines of *D. melanogaster*, and from crosses of separate inbred lines. We

hypothesize that sibling matings will lead to rapid homozygosis of genes, including deleterious ones (see Figures 1 and 2), and will cause a significant drop in fitness as measured by progeny counts. We also hypothesize that crosses between separate inbred lines at the end of this experiment will show heterosis, with a concomitant significant increase in average progeny count per cross. This hypothesis is shown in Figure 2.

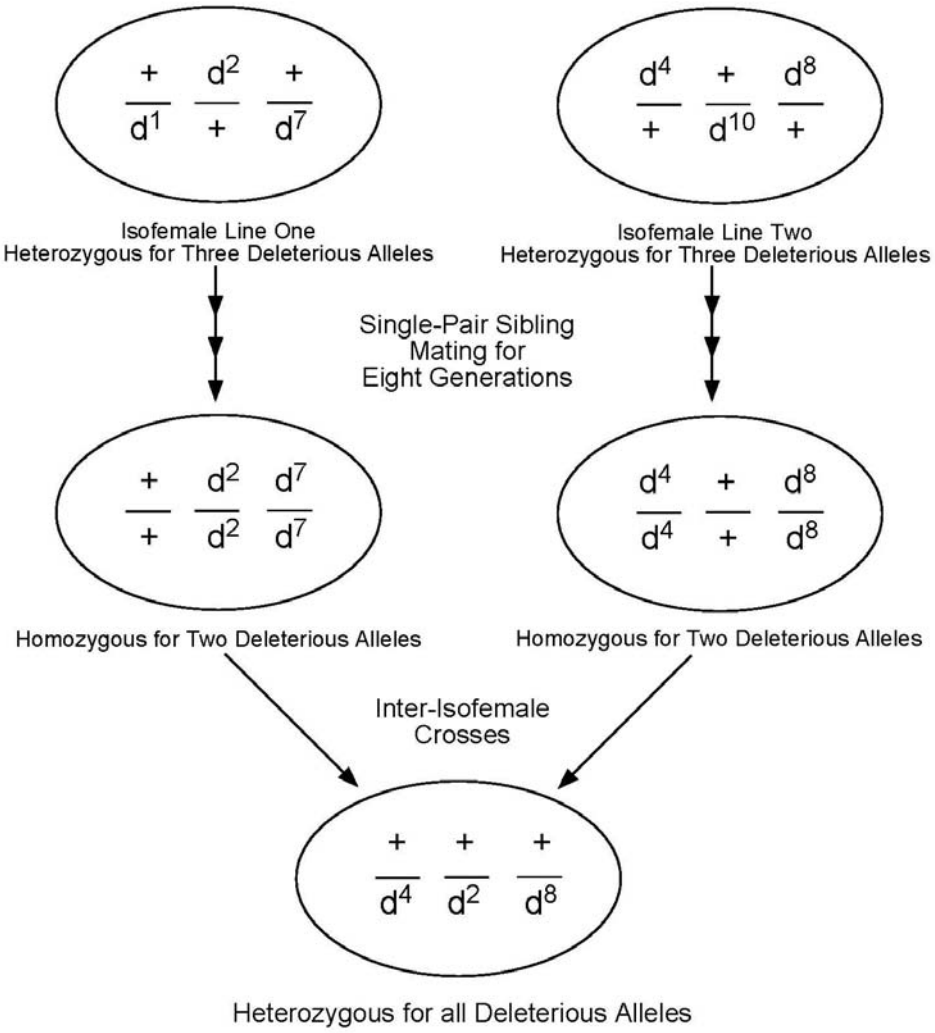

Figure 2. Predicted results of single-pair, sibling crosses leading to homozygosis of deleterious alleles and a reduced viability—inbreeding depression. The progeny of inter-isofemale crosses, however, will have all deleterious alleles in the heterozygous state and should have increased fitness above that of the homozygous lines (heterosis). The deleterious alleles are assumed to be recessive, or almost recessive.

Twenty-two *D. melanogaster* females were captured on October 5, 2007 in Perrysburg, Ohio (Wood County) by sweeping rotting fruit. Each female was placed in a separate vial of *Drosophila* food and 19 females produced offspring. From each of the 19 isofemale lines (PI-1 to PI-19), four vials were set up with a single virgin female mated with a single sibling male per vial at 25°C. The

progeny were then counted from three of these vials and the subsequent generations were again initiated by four single-pair sibling matings per line for a total of eight generations.

In addition, inter-isofemale line crosses were set up from generation eight flies, by mating at random single virgin females from one line (say, PI-1) to a single non-sibling male from another line (say, PI-2). A total of four vials were set up for each inter-isofemale line cross. The inter-isofemale line progeny were then counted for three of the vials.

The results of the inbreeding and inter-isofemale line crosses are shown in Figure 3. For generations one through eight there is a significant ($P < 0.0001$ for two-way ANOVA) decrease in viability over generations. There was also a significant difference in mean viabilities for the 19 tested lines ($P < 0.0001$). In addition the generation nine inter-isofemale crosses had an increase in mean viability (206 progeny) that is between the viabilities of generation one (211) and generation two (205). The inter-isofemale crosses gave a significant ($P < 0.0001$ for t-test) increase in viability over generation eight, presumably due to the masking of recessive deleterious mutations in heterozygotes that were homozygous in the generation eight flies.

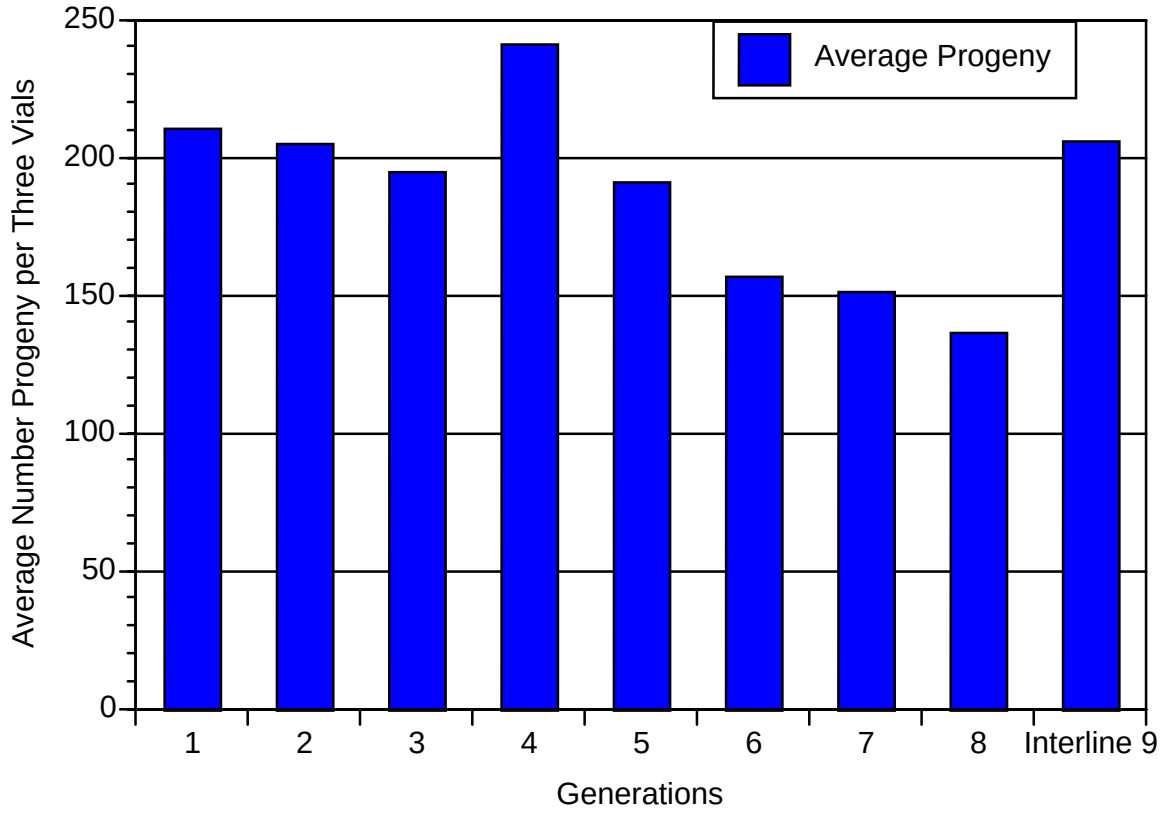

Figure 3. The average number of progeny for three vials from single-pair sibling crosses for eight generations and for inter-isofemale line crosses (generation nine). For generations 1-8, there is a significant ($P < 0.0001$) decrease in mean viability. The viability of lines eight and nine were also significantly different ($P < 0.001$).

In summary, we were able to detect inbreeding depression on viability by single-pair sibling crosses over eight generations, and were able to show that by inter-isofemale crosses the deleterious effect of recessive mutations in each line was hidden in the heterozygous state against their wild-type alleles.

A class discussion of the results of these crosses could include the following topics: 1) Why was the inter-isofemale average viability of generation nine (206 progeny) below the average viability of the generation one flies (211)? The 19 lines used in this study all came from one location and may have shared some deleterious alleles. Hence, in some inter-isofemale crosses a few deleterious mutations may have still been homozygous. 2) If crosses had been made for 19 lines collected from 19 separate locations, how might this have changed the generation one and inter-isofemale results? In this case, one would expect few, if any shared deleterious alleles in the lines. 3) Discuss how organisms in nature, including mammals, avoid inbreeding depression. How do humans avoid inbreeding depression?

References: Azad, P., R.C. Woodruff and J.N. Thompson, Jr. 2003, Dros. Inf. Serv. 86: 165-168; Castle, W.E., 1906, Science 23: 153; Darwin, C., 1859, *On the Origin of Species by Means of Natural Selection*. London, John Murray; Falconer, D.S., and T.F.C. Mackay 1996, *Introduction to Quantitative Genetics*. Longman House, Essex, Longman Group Limited; Freeman, S., and J.C. Herron 2007, *Evolutionary Analysis*. Upper Saddle River, NJ, Pearson Prentice Hall; Gong, Y., R.C. Woodruff, and J.N. Thompson, Jr. 2005, Biology Letters 1: 492-495; Hartl, D.L., and A.G. Clark 2007, *Principles of Population Genetics*. Sunderland, MA., Sinauer Associates, Inc.; Hedrick, P.W., 2005, *Genetics of Populations*. Sudbury, MA, Jones and Bartlett Publishers; Lynch, M., and B. Walsh 1998, *Genetics and Analysis of Quantitative Traits*. Sunderland, MA. Sinauer Associates, Inc.; Schull, W.J., and J.V. Neel 1965, *The Effects of Inbreeding on Japanese Children*, New York, Harper & Row Publishers, Inc.; Wright, S., 1977, *Evolution and the Genetics of Populations*, Volume 3, Chicago, The University of Chicago Press.

Genotyping and enzyme activity measurements of the *Adh* polymorphism: a simple exercise in population genetics, biochemistry, and the connection of genotype and phenotype.

Lum, Thomas E.C.H., and Thomas J.S. Merritt. Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario P3E 2C6.

Introduction

The study of genetic variation at enzyme loci offers students insight into population genetics, protein molecular evolution and structure-function relationships, and the connection between genotype and phenotype. An excellent teaching example is the *Alcohol dehydrogenase* (*Adh*) amino acid polymorphism in *Drosophila melanogaster*. This polymorphism is one of the most studied in modern molecular genetics; a textbook example of genetic variation likely under selection, it has been the focus of a number of seminal works in the study of molecular evolution (*e.g.*, Oakeshott *et al.*, 1982; McDonald and Kreitman, 1991). There are two *D. melanogaster Adh* alleles that can be resolved by protein electrophoresis. The fast (ADH-F) and slow (ADH-S) allozymes, named so for their electrophoretic migration rates in standard starch-gel electrophoresis, differ by a single threonine to lysine substitution (Fletcher *et al.*, 1978; Kreitman, 1983). The alleles also differ in V_{max}: ADH-F alleles generally have higher activity than ADH-S alleles, but possibly at the expense of decreased stability in higher temperatures (Fletcher *et al.*, 1978). Reciprocal latitudinal clines and non-random patterns of inter- and intra-specific genetic variation suggest the alleles are under selection (Oakeshott *et al.*, 1982; McDonald and Kreitman, 1991).