Teaching Notes

Reduction in fitness and possible population extinctions due to the accumulation of deleterious mutations on non-recombining X chromosomes in *Drosophila melanogaster* males.

Schimmoeller, Christopher J., Lindsay A. Honigford, Daniel R. Rochester, Michael A. Balinski, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403.

Background

H.J. Muller (1964) predicted that organisms that do not undergo recombination (for example, asexual organisms) will accumulate deleterious mutations over time and will not be able to eliminate them by crossing over leading to recombinant chromosomes with fewer or no mutations. If the least-loaded chromosome (the chromosomes with the fewest mutations) is lost by drift, a "ratchet" occurs and the population has a reduction in fitness. Felsenstein (1974) called this drop in fitness "Muller's ratchet" (see reviews of this topic in Maynard Smith, 1978; Charlesworth and Charlesworth, 1997; Arjan *et al.*, 2007; Loewe and Hill, 2010; Charlesworth, 2012, 2013). This ratchet process could also lead over time to the extinction of some populations or species with low numbers. There is theory on how fast Muller's ratchet clicks and on the expected time to extinctions, which is mainly based on population size, deleterious mutation rate, and selection against deleterious mutations (Felsentein, 1974; Haigh, 1978; Maynard Smith, 1978; Wagner and Gabriel, 1990; Gabriel *et al.*, 1993; Stephan *et al.*, 1993; Butcher, 1995; Gessler, 1995; Gessler and Xu, 1999; Gordo and Charlesworth, 2000a,b, 2001; Bachtrog and Gordo, 2004; Jain, 2008; Loewe and Cutter, 2008; Neher and Shraiman, 2012). Yet, no direct measures of Muller's ratchet that include extinctions in multicellular organisms have been performed, leaving many unanswered questions on the role of the ratchet in metazoans. Hence, this proposed study is an attempt to identify reductions in the fitness of males due to the accumulation of X-linked mutations and possible extinctions of *D. melanogaster* populations.

Mutational accumulation studies with higher organisms, including *Daphnia pulex*, *Caenorhabditis elegans*, and *D. melanogaster*, have shown consistent decreases in fitness over time and occasional extinctions of lines (see discussions in Garcia-Dorado *et al.*, 1998; Vassilieva *et al.*, 2000; Estes *et al.*, 2004; Gong *et al.*, 2005; Baer *et al.*, 2007; Arjan *et al.*, 2007; Halligan and Keightley, 2009; Schaack *et al.*, 2010; Mallet *et al.*, 2011). These mutational accumulation experiments, however, were not set up to identify reductions in fitness and extinctions of lines in the same experiment. In this study, changes in fitness will be measured by comparisons of male to total progeny ratios over generations, and extinctions will be identified by the elimination of males in lines that contain sibling females.

Hypotheses

This proposed study will allow for the testing of two hypotheses regarding the role of mutation accumulation of X-linked recessive mutations on male fitness and population extinctions.

Hypothesis 1: The fitness of males is expected to decrease over time due to the accumulation of recessive deleterious mutations on X chromosomes that remain only in males, where they cannot be removed by recombination (there is no pairing partner for the single X chromosome in males). Tests of this hypothesis will provide experimental insights into the importance of recombination on the accumulation of deleterious mutations and its potential fitness consequences in small populations or species.

Hypothesis 2: In addition, mutation accumulation over time may cause some lines to go extinct toward the end of the experiment because of the loss of males with low fitness due to the expression of

recessive, X-linked, deleterious mutations. On the other hand, their female siblings with two X chromosomes survive because they do not express recessive, X-linked, deleterious mutations. This proposed protocol will reduce the chance that extinctions occur by handling accidents or poor fly husbandry.

Experimental Plan

The accumulation of recessive deleterious mutations on the X chromosomes of males in small populations of one male and one female over four generations was measured in each of 17 coded vials (Figure 1). In these crosses, w^{1118} is a white-eyed mutation that marks the X chromosome in w^{1118} / Y males, and C(1)DX, *y f* / Y are females that contain two X chromosomes attached to a single centromere, with the markers *y* for yellow body color and *f* for small, forked bristles (Lindsley and Zimm, 1992). Hence, from these crosses patroclinous male progeny receive their w^{1118} X chromosome from their fathers (where there is no recombination) and female progeny receive the attached-X chromosome from their mothers. Recessive deleterious mutations on the X will be expressed in the hemizygous males, but not in the diplo-X females. One male and one female sibling were used in each cross each generation to increase the probability of population (vial) extinctions.

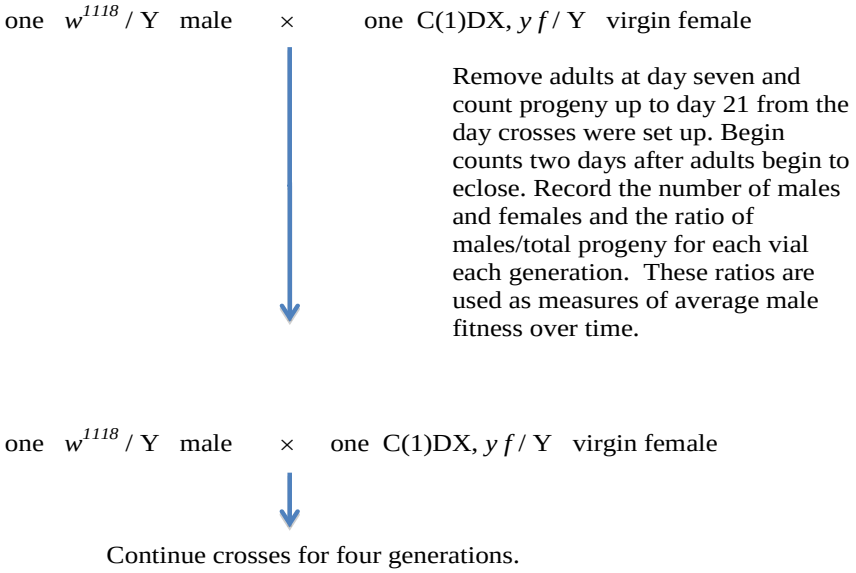

Figure 1. Crossing scheme to accumulate deleterious, X-linked, recessive mutations.

Hypothesis 1 was tested by measuring changes in the ratios of males/total progeny per line over generations as a measure of male fitness. The accumulation of new recessive deleterious mutations on the X chromosome in males will cause the ratio of males/total progeny to decrease with time, because diplo-X females will not express new recessive deleterious X-linked mutations.

Hypothesis 2 was tested by measuring for extinctions of vial populations, which will be identified by the absence of w^{1118} / Y males in lines that contain sibling females. It is expected that line extinctions will occur in latter generations of this proposed study.

Since Haag-Liautard, *et al.* (2007) observed 1.2 deleterious mutations per diploid genome each generation in *D. melanogaster*, and the X is about 15 percent of the genome (National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov/genome/47), we expect about 0.18 (0.15 × 1.2) deleterious mutations per fly. Hence, about one new X-linked deleterious mutation is expected in three of the 17 total vials each generation (0.18 × 17).

Results

Although we expected about one new deleterious mutation per line, there was a non-significant reduction in the fitness of males over four generations due to the accumulation of mutations on their X chromosomes (Figure 2). In addition, no extinctions of lines due to the absence of males were observed. These results indicate that for fitness decreases and population extinctions to be observed using this experimental design, a much larger number of crosses must be initiated and the experiment run for more generations. For example, with the expected reduction in fitness of about three percent for each new mutation (Simmons and Crow, 1977) and an expected X-chromosome mutation rate of 0.18 each generation (Haag-Liautard *et al.* (2007), after 20 generations of a larger experiment the expected average drop in male fitness per line would be about 10 percent, with some lines showing a larger reduction in fitness. This expected drop in fitness from the accumulations of recessive deleterious mutations should allow for the identification of population extinctions.

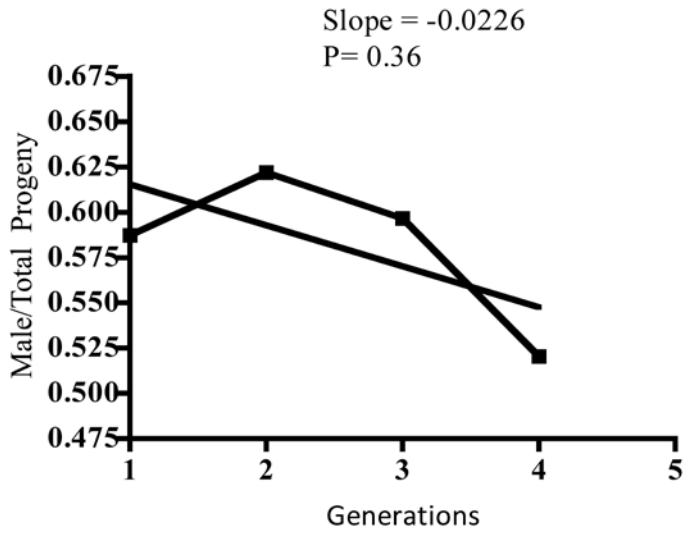

Figure 2. Mean Ratio of Male to Total Progeny. Each point represents the mean of 17 vials from that generation, showing a non-significant decrease in male fitness over four generations.

References: Arjan, J., G.M. de Visser, and S.F. Elena 2007, Nature Reviews Genetics 8: 139-149; Bachtrog, D., and I. Gordo 2004, Evolution 58: 1403-1413; Baer, C.F., M.M. Miyamoto, and D.R. Denver 2007, Nature Reviews Genetics 8: 619-631; Butcher, D., 1995, Genetics 141: 431-437; Charlesworth, B., 2012, Genetics 190: 5-22; Charlesworth, B., 2013, J. Heredity 104: 161-171; Charlesworth, B., and D. Charlesworth 1997, Genet. Res. Camb. 70: 63-73; Estes, S., P.C. Phillips, D.R. Denver, W.K. Thomas, and M. Lynch 2004, Genetics 166: 1269-1279; Felsenstein, J., 1974, Genetics 78: 737-756; Gabriel, W., M. Lynch, and R. Burger 1993, Evolution 47: 1744-1757; Gong, Y., R.C. Woodruff, and J.N. Thompson, jr. 2005, Biol. Lett. 1: 492-495; Gordo, I., and B. Charlesworth 2000a, Genetics 154: 1379-1387; Gordo, I., and B. Charlesworth 2000b, Genetics 156: 2137-2140; Gordo, I., and B. Charlesworth 2001, Genet. Res. Camb. 78: 149-161; Garcia-Dorado, A., J.L. Monedero, and C. Lopez-Fanjul 1998, Genetica 102/103: 255-265; Gessler, D.D.G., 1995, Genet. Res. Camb. 66: 241-253; Gessler, D.D.G., and S. Xu 1999, Genet. Res., Camb. 73: 119-131; Gong, Y., R.C. Woodruff, and J.N. Thompson, jr. 2005, Biol. Lett. 1: 492-495; Haigh, J., 1978, Theoretical Population Biology 14: 251-267; Haag-Liautard, C., M. Dorris, X. Maside, S. Macaskill, D.L. Halligan, B. Charlesworth, and P.D. Keightley 2007, Nature 445: 82-85; Jain, K., 2008, Genetics 179: 2125-2134; Halligan, D.L., and P.D. Keightley 2009, Annu. Rev. Ecol. Syst. 40: 151-172; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York; Loewe, L., and A.D. Cutter 2008, BMC Evolutionary Biology 8: 125; Loewe, L., and W.G. Hill 2010, Phil. Trans. R. Soc. B 365: 1153-1167; Mallet, M.A., J.M. Bouchard, C.M. Kimber, and A.K. Chippindale 2011, BMC Evolutionary Biology 11: 156; Maynard Smith, J., 1978, *The Evolution of Sex*. Cambridge University Press; Muller, H.J., 1964, Mut. Res. 1: 2-9; Neher, R.A., and B.I. Shraiman 2012, Genetics 191: 1283-1293; Schaack, S., E. Choi, M. Lynch, and E.J. Pritham 2010, Genome Biology 11: R46; Simmons, M., and J.F. Crow 1977, Annu. Rev.

Genet. 11: 49-78; Stephan, W., L. Chao, and J.G. Smale 1993, Genet. Res. Camb. 61: 225-231; Vassilieva, L.L., A.M. Hook, and M. Lynch 2000, Evolution 54: 1234-1246; Wagner, G.P., and W. Gabriel 1990, Evolution 44: 715-731.

New species of *Drosophila* or not.

Rochester, Daniel R., Lindsay A. Honigford, Christopher J. Schimmoeller, Michael A. Balinski, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403

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