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The role of sexual reproduction and recombination in adaptive evolution.

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The evolution and maintenance of sexual reproduction that leads to recombination of chromosomes at meiosis is a evolutionary puzzle. Why should a fit organism give up half of its genes and experience possible harm (for example, sexually transmitted diseases and increased risk of predation) when it could reproduce asexually? With asexual reproduction all of the genes of an individual are passed to its offspring, and there is no harm that occurs in the search for a mate (Muller, 1964; Maynard Smith, 1978; Ridley, 1993; Michod, 1995; Barton and Charlesworth, 1998).

There are two main hypotheses for the evolution and maintenance of sexual reproduction: 1) Sexual reproduction brings together favourable alleles of different genes by recombination, increasesing the fitness of offspring and the rate of adaptation to new environments; 2) Sexual reproduction can bring together deleterious alleles of different genes by recombination. These deleterious alleles can then be eliminated from the population in bunches by negative selection more quickly than can a combination of deleterious alleles that are removed one at a time in the absence of recombination (for reviews of this topic see Crow and Kimura, 1965; Barton and Charlesworth, 1986; Kondrashov, 1988; Otto and Lenormand, 2002; Rice, 2002; Gillespie, 2004).

The objective of this proposed study is to test the first hypothesis listed above (combining favourable genes by recombination) by measuring the rates of selection response in the presence and absence of recombination in the model system *Drosophila melanogaster*. Rice and Chippindale (2001) have shown that beneficial alleles that increase offspring numbers in *D. melanogaster* accumulate faster in populations with recombination than in populations without recombination.

We took advantage of the natural lack of recombination in *D. melanogaster* males and the aviability of balancer chromosomes with multiple inversions that eliminate recombinant gametes in *D. melanogaster* females. With the appropriate crosses, as shown below, we tested a model for adaptive evolution, selection response for bristle numbers, in the presence and in the absence of recombination. As part of this model, it was assumed that flies with decreased or increased bristle numbers are more fit. This proposed study is partially based on the materials, methods, and results in

McPhee and Robertson (1970). Figure 1 shows the sternoplueral bristles (eleven here) that will be counted in this study.

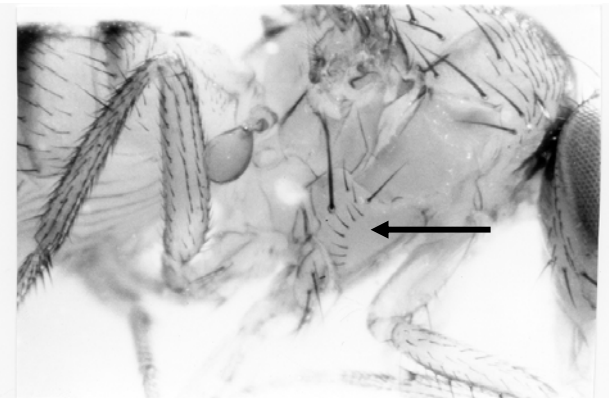


Figure 1. Sternopleural bristles of *Drosophila melanogaster*.

The following mating scheme includes two crosses (Rec with recombination and NoRec without recombination) to measure the role of recombination in evolution (rate of response to selection). In this mating scheme, "+" stands for a wild-type chromosome, T(2;3)A1-W, *Cy L* is a translocation for the second and third chromosomes that is also a balancer (eliminates recombinant gametes) for the second and third chromosomes, *Cy* = curled wings (dominant visible and recessive lethal mutation), and *L* = lobed eyes (dominant visible and recessive lethal mutation) (after the first generation below, this translocation will be given the symbol A1); T(2;3)B18, *Pm Sb* is a translocation for the second and third chromosomes, with *Pm* = plum eye color (*Pm* is also called bw^{V1}) and *Sb* = stubble bristles; and +/+;+/+ is a wild-type stock that was derived from a mixture of four isofemale lines captured from nature (Perrysburg, OH) on July 30, 2010. For details on the mutant genes, rearrangements, and balancer chromosomes used in this study, see Lindsley and Zimm (1992). Again, we emphasize that recombination does not occur in the males of these crosses. Virgin females were used in all crosses.

+/+;+/+ wild-type females × T(2;3)A1-W, *Cy L*/T(2;3)B18, *Pm Sb* males

F1: select for +;+/T(2;3)A1-W females × +;+/T(2;3)A1-W males

Cross Rec Cross NoRec

F2: +/+;+/+ females × +;+/A1-W males (recombination in +;+/+;+ females)

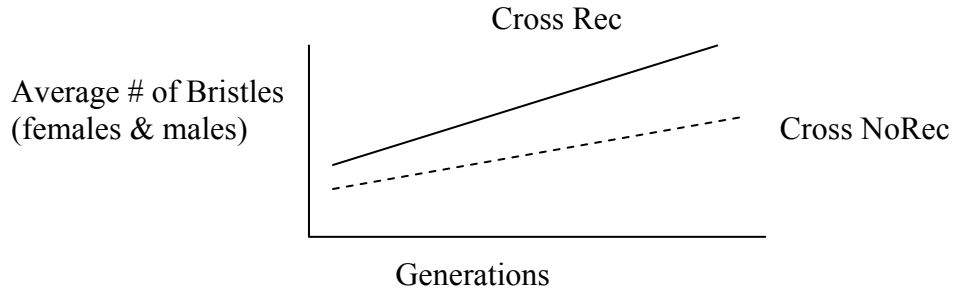
F2: +;+/A1-W females × +/+;+/+ males (no recombination in +;+/A1-W females)

Selection for Lower Bristle Numbers: For Cross Rec and NoRec, we picked eight of 20 F3 +/+;+/+ virgin females and eight of 20 F3 +;+/A1-W males with the lowest number of bristles and

mated them for the next generation. We then repeated these crosses and bristle counts for ten generations.

Selection for Increased Bristle Numbers: For Cross Rec and NoRec we picked eight of 25 F3 +;+/A1-W virgin females and eight of 25 F3 +;+/+;+ males with the highest number of bristles and mated them for the next generation. We then repeated these crosses and bristle counts for six generations.

It is our hypothesis that recombination in Cross Rec will increase the coupling of beneficial alleles of different genes on autosomes that will increase the rate of selection response above that of Cross NoRec, where there is no recombination. Hence, the response to selection will be faster in the flies in Cross Rec compared to flies in Cross NoRec as shown below for an increased bristle number experiment.



We will determine if the slopes of the lines from Cross Rec and Cross NoRec are significantly different using the Prisim Statistical Program.

The results of the two selection experiments for decreased bristle numbers in the presence and absence of recombination are shown in Figure 2, whereas the results of the two selection experiments for increased bristle numbers in the presence and absence of recombination are shown in Figure 3. There was a significant (P = 0.002) decrease in bristle numbers in the presence of recombination in one experiment (Figure 2a), and a significant (P = 0.01) increase in bristle numbers in the presence of recombination in one experiment (Figure 2b). These results support the theory that adaptive evolution (selection response in this study) is faster in the presence of recombination.

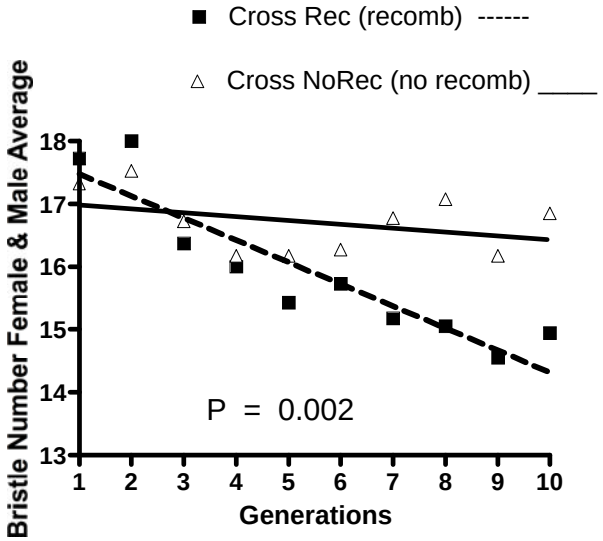


Figure 2a. Response to selection for decreased bristle numbers in the presence and absence of recombination.

In relation to a teaching exercise, the increased bristle number experiment might be more appropriate in a teaching environment, since the positive response occurred in fewer (six) generations. Part of the reason this experiment gave a faster significant increase in bristle numbers than that observed in the decreased bristle number experiment was because there was a larger selection differential in the

increased bristle experiment (eight of 25 flies were selected each generation) as compared to the decreased bristle experiment (eight of 20 flies were selected each generation). In a teaching environment with a shorter time frame, one could also increase the selection differential.

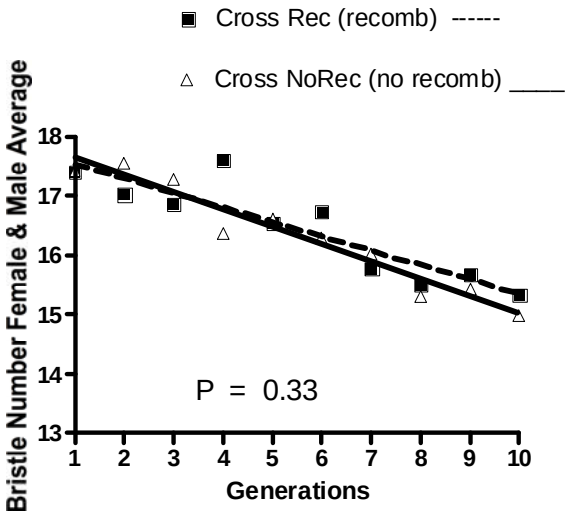
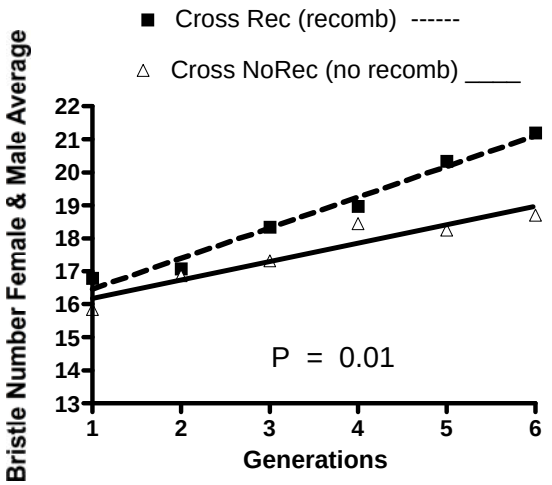


Figure 2b. Response to selection for decreased bristle numbers in the presence and absence of recombination.



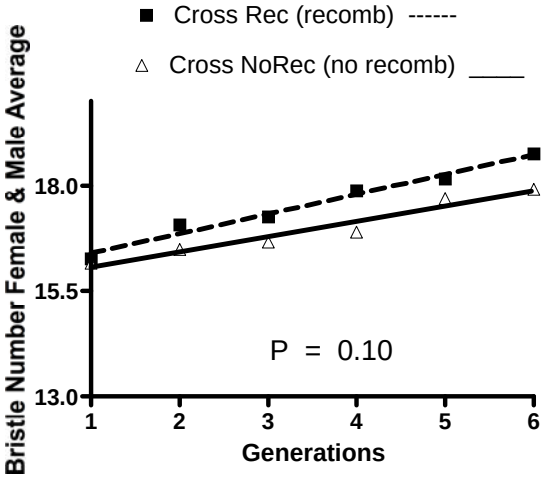


Figure 3a and b. Response to selection for increased bristle numbers in the presence and absence of recombination.

Part of a class discussion of the results of this teaching exercise could include: 1) What would have been the expected results of this experiment if a highly inbred stock with no genetic variation had been used instead of the wild-type line? All responses to selection over time would have been non-significant (see Woodruff and Thompson, 2005). 2) The class might be asked to read Goddard *et al*. (2005), which shows that sex increases the rate of adaptation of yeast to a new harsh environment. 3) The mutants *Cy*, *L*, *Pm*, and *Sb* used in this experiment are homozygous dominant visible mutations and recessive lethal mutations in *D. melanogaster*. Are there similar acting mutations in humans? One might ask students to go to National Center for Biotechnology Information (NCBI) (www.ncbi.nim.nih.gov) and then to Online Mendelian Inheritance in Man (OMIM) (www.ncbi.nim.nih.gov/omim) and search for achondroplasia (#100800). This form of short-limb

dwarfism is caused by a dominant autosomal mutation that also has more drastic effects in homozygotes.

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Spontaneous and gamma ray induced chromosome breakage in *Drosophila melanogaster*.

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Chromosomal rearrangements are more frequent in humans than previously thought. For example, the diploid genome sequence of J. Craig Venter (his company, Celera Genomics, and the Human Genome Sequencing Consortium first sequenced the human genome) contained 292,102 heterozygous insertion/deletion events (1 to 571 base pairs), 559,473 homozygous indels (insertions and deletions of one to 82,711 base pairs), 90 inversions, and numerous duplications (Levy *et al.*, 2007). The rate of new chromosome aberrations in humans is about 4/1000 live births (Sankaranarayanan and Wassom, 2005), with one in 500 humans carrying a new reciprocal translocation (Gajecka *et al*., 2008). In addition, many chromosome rearrangements are associated with human genetic defects and cancer (Strachan and Read, 2004; Lupski 2007; Hastings *et al*., 2009). Hence, it is important to identify spontaneous and induced chromosome breakage events and to estimate their rates in a model organism such as *Drosophila melanogaster*.

It is the objective of this study to measure spontaneous and gamma ray induced X-chromosome breakage events in an F1 assay in *D. melanogaster*. This hyperploidy chromosome breakage assay involves the identification of breakage events that delete segements of the X chromosome in males, which are then recovered as extra chromosomal fragments (hyperploidy) in F1 females. This assay is shown in Figure 1, and is discussed in Auerbach (1962) and Blount and Woodruff (1986). The C(1)DX, *y w f* chromosome is two X chromosomes attached to a single centromere and containing the recessive markers *y* (yellow, yellow body color), *w* (white, white eyes), and *f* (forked, short bristles) (Lindsley and Zimm, 1992); *D. melanogaster* that have two X chromosomes and a Y chromosome are fertile females. In this cross, y^+, w^+ and f^+ denote the wild-type alleles of the three genes; Canton-S is a wild-type stock (containing a y^+ w^+ f^+ X chromosome), and O is a centromere. It should be noted that some exceptional C(1)DX, $y\ w\ f\ /\ y^+\ w^+\ f^+$ triplo-X female progeny occur in older cultures in this breakage assay. These XXX females have grey bodies, red eyes, and long bristles, plus they usually have slightly deformed wings, move slowly, have slow