An attempt to select for increased recombination in *Drosophila melanogaster*.

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There are four main reasons why genetic recombination, the exchange of genetic material between homologous chromosomes during meiosis, is so important for the survival and fitness of most organisms (for reviews of this topic see Muller, 1964; Crow and Kimura, 1965; Maynard Smith, 1978; Bell, 1982; Charlesworth, 1989, 1993; Michod and Levin, 1988; Michod, 1995; Barton and Charlesworth, 1998; Otto and Michalakis, 1998; West *et al.*, 1999; Burt, 2000; Otto and Barton, 2001; Rice and Chippindale, 2001; Otto and Lenormand, 2002; Rice, 2002; Gillespie, 2004; Agrawal, 2006; Charlesworth and Charlesworth, 2010; Becks and Agrawal, 2012):

1) Recombination can give rise to new multiple-gene variation that can allow organisms to adapt to changing environmental conditions faster than if there was no recombination (Fisher, 1930; Muller, 1932; Roze, 2012).

2) Recombination allows for the removal of deleterious mutations faster than if there was no recombination. This is because recombination can place different deleterious mutations on the same chromosome, where they can be eliminated together and can generate chromosomes with no, or fewer, deleterious mutations (Kondrashov, 1988, 1993).

3) Recombination is essential for the proper segregation of homologous chromosomes during meiosis, because recombination physically serves to interlock homologous chromosomes and ensure their proper segregation during the first meiotic cell division. Therefore, there is at least one recombination event for each arm of every chromosome during meiosis in humans (Hassold *et al.*, 2004). Failure of recombination can lead to either germ cell death or chromosome aneuploidy (missing or extra chromosomes) (Hawley, 2011).

4) Recombination is needed for repair of double-strand chromosome breakage, which occurs about ten times each day in each cell of higher organisms (Whyman *et al.*, 2004; Lieber, 2010).

Because recombination is essential for the long-term survival of most organisms, it is of interest to determine if selection can act on standing and new genetic variation to modify the frequency of recombination. For example, Chinnici (1971) was able to increase and decrease recombination significantly in *Drosophila melanogaster* for the X chromosome, although others have not been so successful (see discussions in Detlefsen and Roberts, 1921; Parsons, 1958; Acton, 1961; Charlesworth and Charlesworth, 1985; Otto and Barton, 2001). Selection for DDT resistance, geotaxis, and temperature fluctuation has also led to concomitant significant increases in recombination in *D. melanogaster* (Flexon and Rodell, 1982; Zhuchenko *et al.*, 1985; Korol and Lliadi, 1994). Finally, there are known differences in recombination rates in individual humans and *D. melanogaster*, and genes have been identified in both humans and *D. melanogaster* that affect recombination (Ashburner, 1989; Baudat *et al.*, 2010; Kong *et al.*, 2010; Chan *et al.*, 2012).

In this study we have attempted to increase the frequency of recombination for the X-chromosome region between the genetic markers white (white eyes, *w*, map position 1.5) and singed-3 (singed, short, bristles, sn^3, map position 21.0), by selecting for increased

recombination in each generation for up to five generations.

The following crosses were performed to initially increase the amount of genetic variation in the tested F1 + + / *w* sn^3 females. The OBL1&2 wild-type stock was initiated by mixing six mated females from a Perrysburg, Ohio, population that were captured by sweeping bananas in 2010.

w sn^3 / *w* sn^3 females × OBL1&2 males

For each of the five participants in this study, a total of ten or eleven vials of single F1 + + / *w* sn^3 virgin females were mated with one or two *w* sn^3 / Y males from the *w* sn^3 stock. The F2 progeny from these vials were then screened as:

1) Non-recombinants females that were + + / *w* sn^3 (red eyes and long bristles) or *w* sn^3 / *w* sn^3 (white eyes and short bristles); or non-recombinant males that were + + / Y (red eyes and long bristles) or *w* sn^3 / Y (white eyes and short bristles);

2) Or as recombinants females that were + sn^3 / *w* sn^3 (red eyes and short bristles) or *w* + / *w* sn^3 (white eyes and long bristles), or recombinant males that were + sn^3 / Y (red eyes and short bristles) or *w* + / Y (white eyes and long bristles).

From the vial with the highest frequency of recombination in the F2 generation among the ten to eleven tested vials, each of the five participants in this study set up five to ten new vials of F2 single virgin + + / *w* sn^3 females mated with one or two F2 sibling *w* sn^3 / Y males. The F3 progeny were then screened as non-recombinants or recombinants. This mating and selection procedure was then continued for four or five generations. Since the first generation was not selected, the selection scheme occurred for four or five generations only. It should be noted that in some generations, fewer than ten vials produced progeny in some experiments.

The results of the five selection experiments for increased recombination are shown in Figures 1-5. The beginning frequency (generation one) of recombination for the *w* to sn^3 region was 18% (589/3,270), which was not significantly different (P = 0.12) from the frequency of recombination of 19.5% reported in Lindsley and Zimm (1992). In one of the five selection experiments there was a significant (P = 0.01) increase in the mean frequency of recombination over generations (see Figure 1), and in another the frequency was significantly (P = 0.02) decreased (see Figure 5). The reason for the lack of a significant response of selection for increased recombination in three of the experiments (Figure 2, P = 0.86; Figure 3, P = 0.18; Figure 4, P = 0.17) is not known, but may be because only four or five generations of selection were completed in this study, compared with 33 generations in Chinnici (1971). The single experiment (Figure 5) that had a significant decrease in the frequency of recombination over time was due to the high frequency of recombination in generation one (P = 0.67 for generations two through six).

A class discussion of the results of this teaching exercise could include the following: 1) Under what conditions in nature would one expect the frequency of recombination to increase? This might happen under a changing and stressful environment, such as in the presence of a new parasite or increased temperature (Parsons, 1988). 2) Is the frequency of recombination the same in human females and males? The frequency is almost twice as high in females (Lenormand and Dutheil, 2005). 3) What is the maximum frequency of recombination possible between two genetic markers in an experiment? The answer is fifty percent, because recombination occurs at the four-strand stage of meiosis after DNA synthesis. Even if an exchange occurred in every tetrad, only one-half of the four products of meiosis would be a recombinant. 4) What could be

done to increase the ability of observing an increased response of selection on the frequency of recombination? One could discuss using larger numbers of selected lines in each experiment (Chinnici, 1971, used 20 to 40 lines), additional generations of selection, and genetic markers with greater map distances. 5) Why was recombination not measured in males? There is no recombination in *D. melanogaster* males (Morgan, 1912). 6) Are there any organisms that have survived without sex and recombination? This is true for some species of rotifers (Welch and Meselson, 2000).

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Figure 1. Average percent recombination between the X-linked markers *w* and *sn*^{3} over five generations. There was a significant (P = 0.02) increase in the frequency of recombination over time.

Figure 2. Average percent recombination between the X-linked markers *w* and *sn*^{3} over five generations. Not significant (P = 0.86).

Figure 3. Average percent recombination between the X-linked markers *w* and *sn*^{3} over five generations. Not significant (P = 0.18).

Figure 4. Average percent recombination between the X-linked markers *w* and *sn*^{3} over five generations. Not significant (P = 0.17).

Figure 5. Average percent recombination between the X-linked markers *w* and *sn*^{3} over six generations. There was a significant (P = 0.04) decrease in the frequency of recombination over time.