The identification of unequal crossing-over events at the Bar (*B*) locus of *Drosophila melanogaster*.

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Unequal crossing over at meiosis can lead to an extra copy of a gene that evolves into a new function or one of the tandem duplicated genes mutates into a pseudogene that is not functional (Chen *et al*., 1990; Lynch and Conery, 2000; Zhang *et al*., 2003; Hurles, 2004). For example, in humans the five beta globin genes (and one pseudogene) arose by duplication events (Hurles, 2004), as did the genes giving humans red-green color vision (Botstein, 1986). In addition, unequal crossing-over events can lead to human genetic disorders (Emanuel and Shaikh, 2001). For example, Charrot-Marie-Tooth disease and red-green color blindness are caused by unequal recombination events (Purandare and Patel, 1997; Nathans *et al*., 1986; Drummond-Borg *et al*., 1988; Jagla *et al*., 2002).

The classical example of a change in phenotype associated with unequal crossing-over is the X-linked Bar (*B*) locus of *Drosophila melanogaster*. A duplication of the 16A region of the X chromosome gives the Bar-eye mutation, where the eye is reduced in size to a narrow vertical bar (Tice, 1914; Sturtevant and Morgan, 1923; Sturtevant, 1925; Bridges, 1936; Muller, 1936; Lindsley and Zimm, 1992). Unequal crossing over at the Bar locus is shown in Figure 1 and the Bar and wild-type structure of the eye are shown in Figure 2.

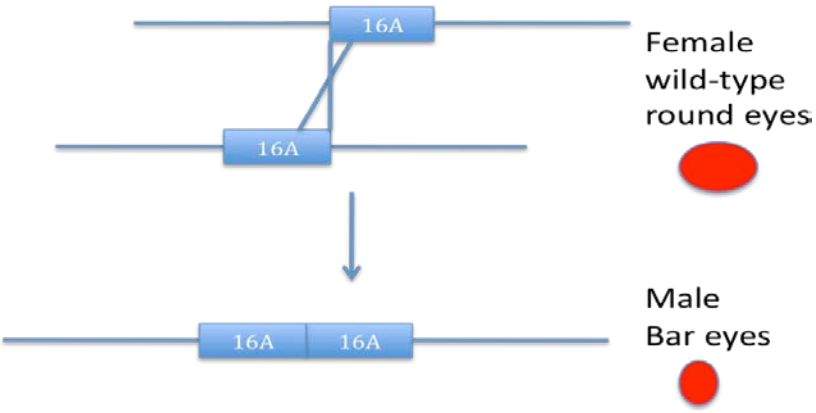


Figure 1. Unequal crossing over leading to a tandem duplication of the 16A region of the X chromosome of *Drosophila melanogaster* and to the Bar eye phenotype.

In this teaching exercise, we will attempt to verify the hypothesis that the narrow-eye Bar mutation reverts to wild type (*B* to B^+) by unequal crossing-over, as shown in Figure 3.

First, we demonstrated that reversion of the Bar mutation to wild type (*B* to B^+) only occurs in females; *D. melanogaster* males do not undergo recombination (Morgan, 1912). Second, we

confirmed that these Bar reversion events in females are always associated with recombination of outside genetic markers on either side of the *B* locus on the X chromosome.

Figure 2. Bar (*B*) eye (left) and wild type (B^+) eye (right) of *D. melanogaster*.

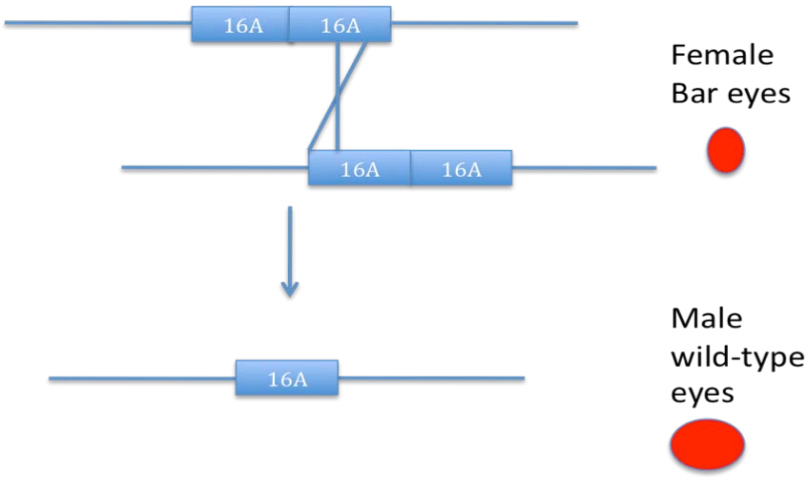


Figure 3. Unequal crossing over in *B*/*B* females can lead to reversion of *B* to B^+.

Reversion of B only occurs in females

We began this study by measuring the occurrence of B^+ revertants in a *f B* stock (*f* = forked bristles, X linked). We used the *f* marker to eliminate wild-type contaminants in this cross. Any true B^+ revertants will also be *f* and have forked bristles. We recovered two B^+ revertants out of 7,917 total flies (0.00025 or about one revertant in 3,959 flies). One of the revertants was a female and one was a male; both had forked bristles, *i.e.*, they were *f* B^+. We also tested each of these B^+ revertants and they bred true for the B^+ phenotype. This frequency of observed B^+ revertants (1/3,959) is similar to the previous report of about one revertant in 2,500 progeny (Sturtevant, 1925). To verify that these B^+ revertants came from unequal crossing over, we also measured the frequency of B^+ revertants from males in the following cross. The C(1)DX, *y f* chromosome is two X chromosomes attached to a single centromere and contains the recessive markers *y* (yellow, yellow body color), and *f* (forked, short bristles). In this cross, the X chromosomes in the F1 male progeny come from the parental males. If B^+ reversions were caused by unequal crossing over, there would be no expected B^+ revertants recovered in the male progeny of this cross.

C(1)DX, *y f* / Y females × *f B*/Y males

↓

Score the *f B* / Y patroclinous male progeny for B^+ revertants

We observed no B^+ revertants out of 15,849 F1 male progeny from this cross. Although the frequencies of revertants from females (2/7,917) and males (0.15,8459) are not significantly different (P = 0.21), the results support our hypothesis that B^+ reversion events only occur in females.

Reversions of B are associated with recombination

To confirm the hypothesis that B^+ revertants occur by unequal crossing over, we also determined if B^+ revertants from females were always associated with recombination. In the cross of this experiment (shown below), *f* (map position 56.7, distal from the centromere of the X chromosome) is located to the left of *B* (map position 57.0) and Bx^3 (a dominant marker that causes narrow, excised wings, map position 59.4, proximal to the centromere of the X chromosome) is located to the right of *B*. We measured the map distance between *f* and Bx^3 in f + / + Bx^3 females and got 1.5% recombination, which is similar to the reported distance of 1.7% (Lindsley and Zimm, 1992). The females in this cross were mated with w^{1118}/Y males that have a white eye due to a deletion of part of the white gene (http://flybase.org/reports/FBal0018186.html).

f B + / + *B* Bx^3 females × w^{1118} / Y males

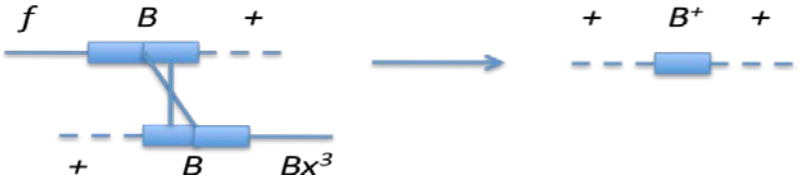
↓

Female and male F1 progeny were scored for B^+ revertants
and for the *f* and Bx^3 outside markers.

All presumptive revertants were mated to make sure that the B^+ revertant phenotypes bred true and that the F1 B^+ revertant females were heterozygous for the w^{1118} containing X chromosome.

From this cross, we recovered eight B^+ revertants out of 23,064 progeny. Six of these B^+ revertants were females, which all gave some F2 white-eyed male progeny, and two were males, which bred true as F2 B^+ males when mated with C(1)DX, *y f* / Y females. Hence, all eight were true B^+ revertants.

All eight of the B^+ revertants were also recombinant for outside markers, supporting the hypothesis that B^+ reversions are caused by unequal crossing-over events. All eight of these B^+ revertants were + B^+ + recombinants, *i.e.*, they were wild type for the *f* focus, B^+ and wild type for the Bx^3 locus. The unequal crossing-over event that gave rise to the + B^+ + chromosomes are shown below.



We do not know why the reciprocal *f* B^+ Bx^3 revertants were not recovered in this experiment.

In summary, the results from two experiments supported the hypothesis that reversions of the *B* mutation occur by the mechanism of unequal crossing over. 1) Bar reversions only occurred in females and 2) B^+ revertants were always associated with exchange of closely linked outside markers.

Class discussions may include the following. 1) About 8% of males and 0.5% of females of European origin have red-green color vision defects (Drummond-Borg et al., 1988). Why is there such a higher frequency of color blindness in males? The reason is that this is a sex-linked trait and hemizygous (XY) males are expected to have a higher frequency than females who must be homozygous (XX) for the defective region. 2) If the red-green sequences are in Hardy/Weinberg equilibrium and the frequency of the defect is 8% in males, what is the expected frequency in females? The answer is 0.08 × 0.08 = 0.6%, close to the reported 0.5%. 3) The assumption that humans are in Hardy/Weinberg equilibrium for the red-green sequences also assumes that females and males that have red-green color blindness are as fit as humans without color blindness, *i.e*., humans with and without the red-green defect have about the same number of offspring. One might ask students if they think this would be true in prehistoric and modern times. What is known is that a higher frequency of red-green color blindness is found in more advanced societies than in some primitive societies (Malhotra, 1978; Narahari, 1993). This may suggest relaxed natural selection for red-green color blindness in modern societies.

References: Botstein, D., 1986, Science 232: 142-143; Bridges, C., 1936, Science 83: 210-211; Drummond-Borg, M., S. Deeb, and A.G. Motulsky 1988, Am. J. Hum. Genet. 43: 675-683; Hurles, M., 2004, PLoS Biology 2(7): e206; Jagla, W.M., H. Jagle, T. Hayashi, L.T. Sharpe, and S.S. Deeb 2002, Human Mol. Genet. 11: 23-32; Lindsley, D., and G. Zimm 1992, *The Genome of* Drosophila melanogaster, Academic Press, Inc., NY; Lynch, M., and J.S. Conery 2000, Science 290: 1151-1155; Malhotra, K.C., 1978, Genetical Research 31: 203-207; Morgan, T.H., 1912, Science 36: 718-720; Muller, H.J., 1936, Science 83: 528-530; Nathans, J., T.P. Piantanida, R.L. Eddy, T.B. Shows, and D.S. Hogness 1986, Science 232: 203-210; Narahari, S., 1993, Arthropologischer Anzeiger 51: 169-171; Sturtevant, A.H., 1925, Genetics 10: 117-147; Sturtevant, A.H., and T.H. Morgan 1923, Science 57: 746-747; Tice, S.C., 1914, Biol. Bull. 26: 221-230; Zhang, P., G. Zhenglong, and W.H. 2003, Genome Biology 4: R56.

White eye phenotypes and their genetic analysis.

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An interesting case for undergraduate students of general Genetics is to consider that different genes can produce the same or similar phenotypes. We present here an experiment to discover that the same phenotype could be produced by different genes, and then, to carry out the genetic analysis of these genes. For this laboratory study we have used the following *Drosophila melanogaster* strains: *white* (white eyes) and *scarlet – brown* (white eyes).

Initially, students have a couple of strains (named mutant 1 and mutant 2) showing the same phenotype (white eyes) and the first question is, are they mutations from the same gene or from different genes? The classical approach is to carry out reciprocal crosses between them. The crosses and results that would be obtained are:

(P) ♂ mutant 1 × ♀ mutant 2

↓

(F_1) All individuals present normal eyes