# Alfred Sturtevant Walks into a *Bar*: Gene Dosage, Gene Position, and Unequal Crossing Over in *Drosophila*

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**ORIGINAL CITATION**

The effects of unequal crossing over at the *Bar* locus in *Drosophila*
Alfred H. Sturtevant
*GENETICS* March 1, 1925 **10**: 117–147

By the early 1920s, the existence of mutations was well established, but how they could be generated remained a topic of lively speculation. One interesting case was the *Drosophila Bar* mutation (Tice 1914). While normal flies have round eyes, the X-linked mutation *Bar* (*B*) caused the eyes to be small and slit-like in males and homozygous females; female heterozygotes had kidney bean-shaped eyes (Figure 1A). Intriguingly, the *Bar* mutation was somewhat unstable: it tended to revert to wild-type spontaneously (May 1917). In 1919, Zeleny reported that females homozygous for *Bar* had progeny with round eyes at a frequency of ~1/1000 (Zeleny 1919). What could be going on? Why was this mutation so unstable? And what were the more extreme "*ultra-Bar*" progeny of *Bar* females, whose eyes were even more severely reduced than *Bar* mutants', and which appeared at a frequency similar to that of the apparent revertants?

Having figured out as an undergraduate how to map gene positions using three-point crosses, Alfred Sturtevant turned his talents to exploring the *Bar* mutation. He realized that he could apply similar logic to test the hypothesis that revertant *Bar* alleles reflected a structural change in the gene or chromosome caused by recombination; one simply had to examine the progeny of flies carrying *Bar* alleles flanked by other markers. In a 1923 *Science* paper (Sturtevant and Morgan 1923), he and his mentor T. H. Morgan reported that females heterozygous for *Bar* and a *Bar* allele flanked by *forked* (*f*) and *fused* (*fu*) alleles gave round-eyed (*Bar*-revertant) progeny that carried only one of the two flanking mutations. Females heterozygous for one *Bar* allele flanked only by *f* and another *Bar* allele flanked only by *fu* gave revertant progeny that were either wild-type for both flanking markers or carried both flanking mutations. These two results suggested that the revertants arose from crossovers within or very near to the *Bar* allele.

In his 1925 *GENETICS* paper (Sturtevant 1925), Sturtevant proved that unequal crossing over was responsible for generating not only the revertants but also the reciprocal crossover products: the new, stronger *ultra-Bar* alleles (which he renamed *double-Bar*). He showed that unequal recombination between two *Bar* alleles could generate non-*Bar* and *double-Bar* alleles at roughly equal frequencies, suggesting that the *Bar* mutant-allele was comprised of two units that could be separated by recombination. In this model, the wild-type condition was a single unit at *Bar*, and the *Bar* mutation was a duplication. If one of the two *Bar* units on one homolog paired out-of-register with a unit on the other homolog, a crossover could generate one "revertant" product with a single unit, and one *double-Bar* product that contained three units (Figure 1C). This was a remarkable insight that would take nearly 90 years to confirm at the molecular level (Miller *et al.*


doi: 10.1534/genetics.116.195891

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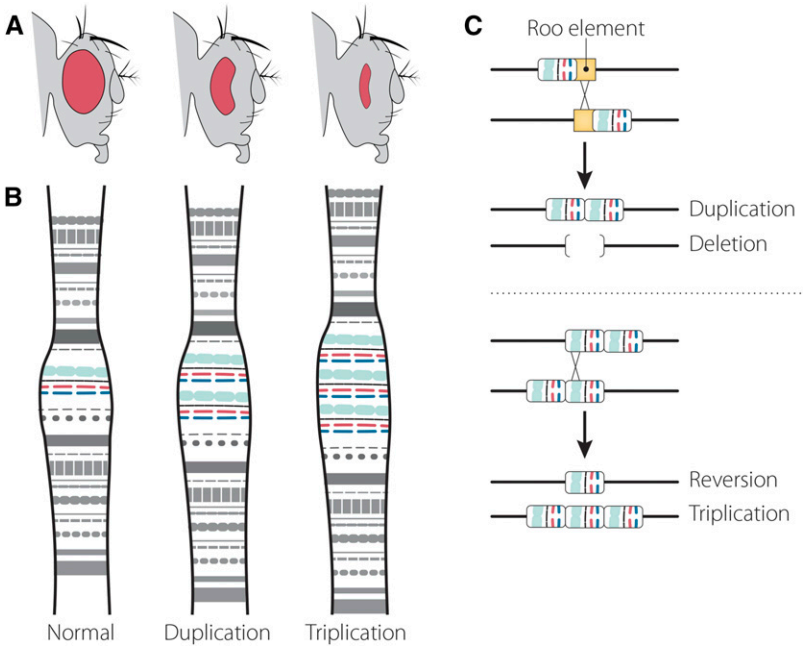


**Figure 1** *Bar* alleles and their phenotypes. (A) Wild-type *Drosophila* have round eyes. Flies homozygous (or hemizygous) for the *Bar* mutation have thin, slit-like eyes. Flies homozygous (or hemizygous) for the *double-Bar* mutation have even smaller eyes. (B) Schematic of the *Bar* region of polytene chromosomes. The *Bar* mutation is a tandem duplication and *double-Bar* a tandem triplication of the region. (C) The *Bar* mutation arose by unequal crossing over between two *Roo* transposable elements (yellow), resulting in a tandem duplication. Reversion and triplication alleles arose from the *Bar* mutant by unequal crossover between duplicates that had aligned out of register.

2016b). The crossovers between *Bar* alleles only occurred in females; in a screen of the progeny from over 10,000 males, Sturtevant recovered no evidence of intra-allelic crossovers.

To further test his hypothesis that the alleles at *Bar* encompassed varying numbers of linked units, Sturtevant used an allele named *infraBar* that gave a weaker phenotype. Using the *f* and *fu* flanking markers, he showed that recombination in a *Bar/infraBar* female could occasionally generate round-eyed revertants or *Bar-infraBar* chromosomes (analogous to how *Bar/Bar* females could occasionally generate round revertants or *double-Bar* chromosomes). Interestingly, the *Bar* and *infra-Bar* units in double-mutant chromosomes could be in either order (*Bar-infraBar* or *infraBar-Bar*) and would retain this order. Therefore, Sturtevant's work showed that *Bar* alleles were comprised of units that could be added together or separated by recombination. All of this was achieved without sequencing, PCR, or knowing that Tice's original *Bar* mutation—still in use in hundreds of fly labs today—arose from unequal crossing over between two *Roo* elements (Figure 1C) (Tsubota *et al.* 1989), let alone the knowledge that the *Bar* gene encodes homeodomain transcription factors (Higashijima *et al.* 1992) important for neural and sense organ development. About 10 years after Sturtevant's paper, cytological studies by Bridges (1936), in the context of interpretations by Muller (1936), confirmed that the *Bar* mutation was indeed a tandem duplication (Figure 1B).

Sturtevant's 1925 study was huge (> 100,000 flies) and carefully controlled for genetic background and temperature, factors he noted affected the severity of *Bar* phenotypes. Though he recognized that unequal crossing over is not the only mechanism for generating mutations, Sturtevant's discovery was extremely influential. His findings are highly relevant to many areas of current study. Recent work has shown that unequal crossing over among repeats in tandem units analogous to *Bar* occurs *de novo* in about 1% of *Drosophila* meioses (Miller *et al.* 2016a). This mechanism also underlies the expansion of gene families like *Hox* genes, key events in the evolution of different body plans (Holland 2015). The influence of gene copy number on phenotype is now evident in many other cases, including for copy number variants in humans (Zhang *et al.* 2009). Indeed, a study looking at *de novo* duplications and triplications in Charcot-Marie Tooth disease identified a similar phenomenon: individuals with a triplication at the disease locus have a stronger phenotype than those with a duplication (Liu *et al.* 2014). Finally, Sturtevant made a very important finding concerning the effects of gene position/context on phenotype: the phenotypic severity of *Bar* alleles depended on the relative *cis/trans* position of their repeat units. Measurements of eye-facet number showed that the phenotype of a female with four *Bar*-locus units depends on how they are distributed across chromosomes: a female with *double-Bar* (three units) on one X chromosome that is wild-type (one unit) on the other X has a more severe *Bar* phenotype than a female who has four *Bar*-locus units arranged as *Bar/Bar* (two units on each X chromosome). Sturtevant proposed that this could reflect a "different balance of modifying genes in the (duplicated) section of the chromosome;" we would now phrase this as creation of new enhancers. Alternatively, he suggested, the difference could reflect "localized regions of activity" inside what we now know to be the nucleus. Both ideas foreshadowed molecular findings to be made decades later (Gonzalez-Sandoval and Gasser 2016), phenomena that remain under intense study nearly a century after Sturtevant crossed his first bar-eyed flies.

## Acknowledgments

Many thanks to Scott Hawley for insightful comments, and to Angela Miller for drawing the figure.

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*Communicating editor: C. Gelling*