

REVIEW

# The joy of balancers

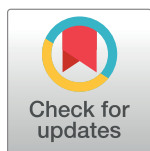
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## Abstract

Balancer chromosomes are multiply inverted and rearranged chromosomes that are widely used in *Drosophila* genetics. First described nearly 100 years ago, balancers are used extensively in stock maintenance and complex crosses. Recently, the complete molecular structures of several commonly used balancers were determined by whole-genome sequencing. This revealed a surprising amount of variation among balancers derived from a common progenitor, identified genes directly affected by inversion breakpoints, and cataloged mutations shared by balancers. These studies emphasized that it is important to choose the optimal balancer, because different inversions suppress meiotic recombination in different chromosomal regions. In this review, we provide a brief history of balancers in *Drosophila*, discuss how they are used today, and provide examples of unexpected recombination events involving balancers that can lead to stock breakdown.



## OPEN ACCESS

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The tools and techniques of the *Drosophila* genetics trade have evolved dramatically over the last century, but one instrument has stood the test of time—the balancer chromosome. Balancers are now an omnipresent and indispensable tool in the fly lab, and their importance has been recognized in other organisms as well. The multiple inversions and rearrangements that make up a balancer chromosome work to constrain recombination and impede the recovery of recombinant products. This allows for single deleterious alleles to be easily maintained in stock and also allows for the maintenance of mutations, transgenes, and/or chromosomal aberrations that are linked together in cis on the same chromosome. The presence of recessive lethal or sterile mutations on balancers assures that balancers never displace homologous chromosomes from stock populations while maintaining heterozygosity of deleterious mutations on the homologs. This combination of recombination suppression and enforced heterozygosity is what makes balancers so valuable to geneticists.

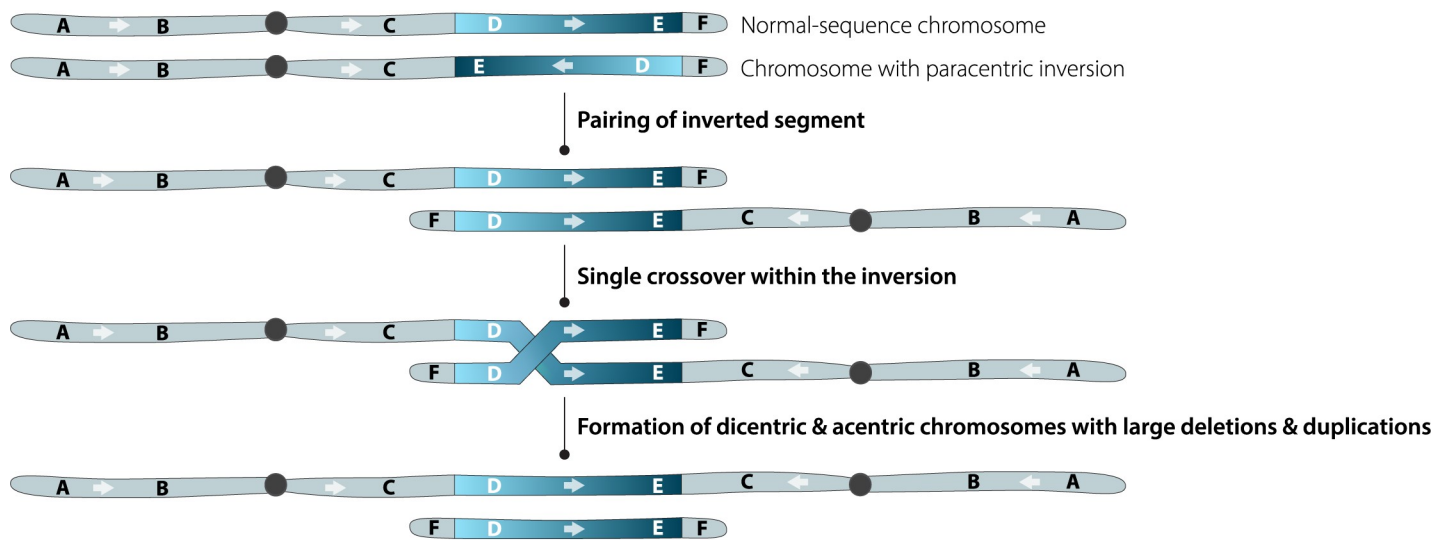
When a normal chromosome is combined with a balancer, the inversions prevent meiotic DNA double-strand breaks from being repaired as crossovers [1]. On those rare occasions that crossovers do form, balancers can prevent the recovery of recombinant chromosomes by one of two mechanisms, depending on the nature of the component inversion. A single exchange event within a paracentric inversion, which does not span the centromere, results in the

formation of an acentric and a dicentric chromosome, neither of which will segregate properly during the subsequent meiotic divisions (Fig 1A). Single exchange events within pericentric inversions (those spanning the centromere) generate large deletions and duplications that are usually lethal to a developing embryo (Fig 1B). However, within an inverted segment, a double crossover between the same two chromatids does not lead to aneuploidy and, consequently, does not affect embryonic viability [2,3].

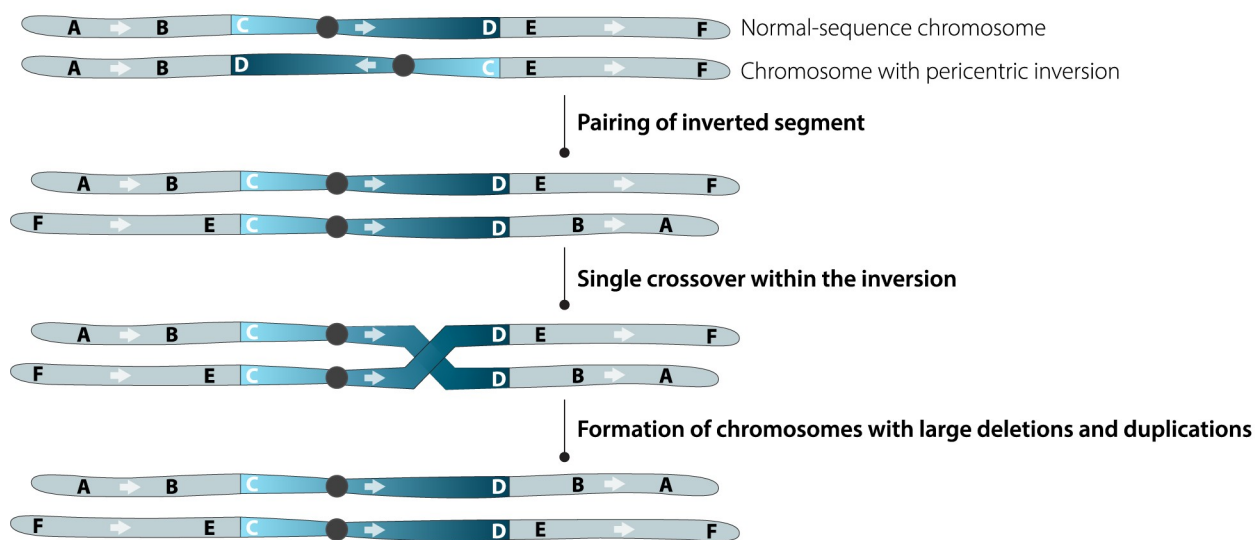
### A brief history of balancer chromosomes

The idea that heterozygosity for an inversion could suppress exchange was first proposed by Sturtevant [4] as a simple way to explain the observation that some chromosomes show a

#### A Products of crossing over between a paracentric inversion and its normal-sequence homolog

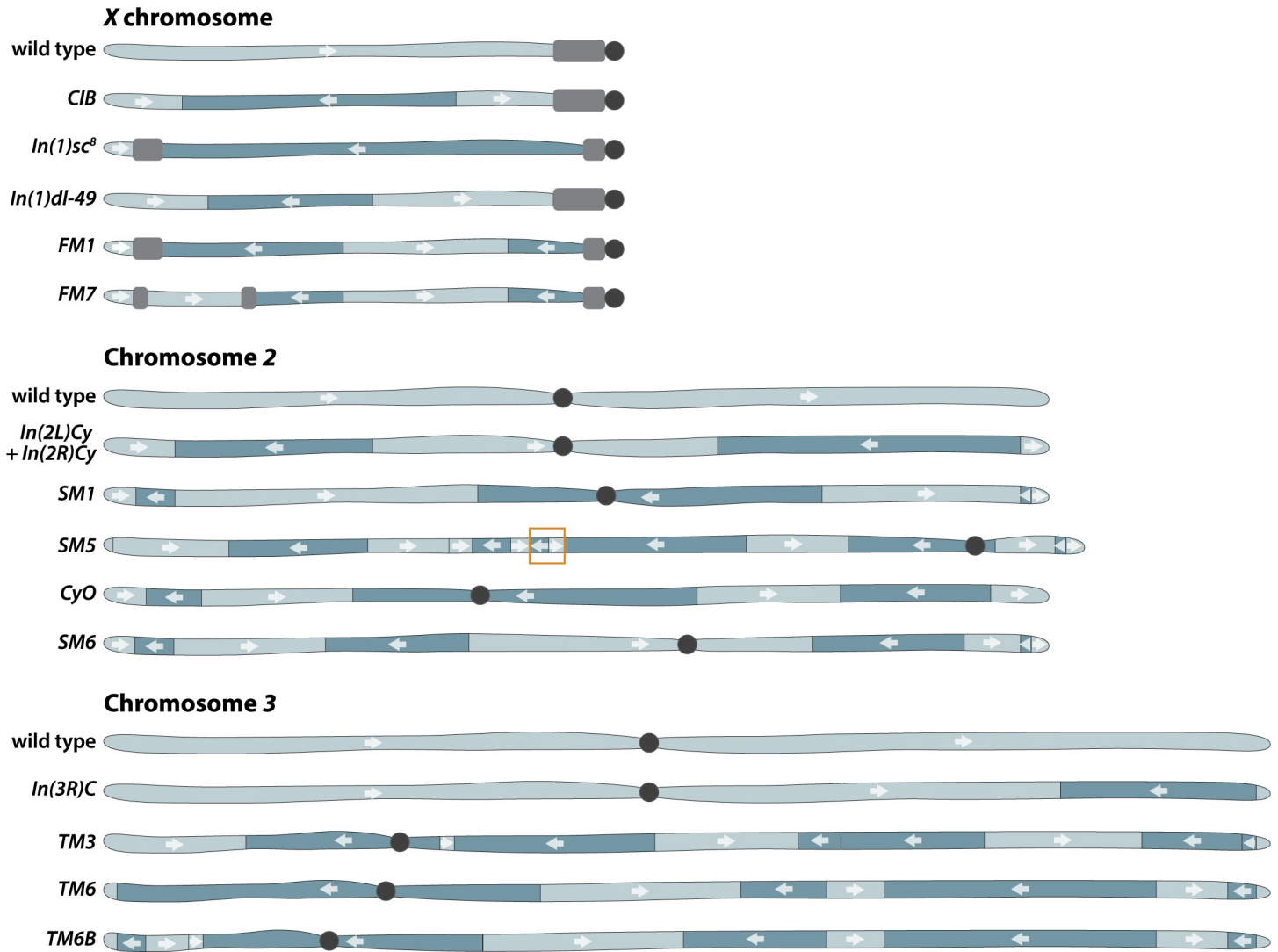


#### B Products of crossing over between a pericentric inversion and its normal-sequence homolog



**Fig 1. Common types of inversions.** Paracentric inversions (A) do not encompass the centromere whereas pericentric inversions (B) do. Recombination between either type of inversion and a structurally wild-type homolog produces aneuploid chromosomes, which cause embryonic lethality.

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**Fig 2. Commonly used balancer chromosomes in *Drosophila* showing approximate sizes of inverted segments.** X chromosome balancers invert an uncertain amount of pericentric heterochromatin (gray). Second and third chromosome balancer inversions do not bisect centric heterochromatin, and thus heterochromatin is not represented here. The large duplication present on SM5 is indicated (orange box).

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reduction of crossing over in a single region: “. . . individuals bearing one normal chromosome and one chromosome with an inverted section would probably show no crossing over in the region in question. . . .” As proof, he demonstrated that a crossover suppressor known as *C<sub>III</sub>* [5] on Chromosome 3 was in fact an inverted segment later renamed *In(3R)C* [4,6]. This inversion, which involves the distal one-third of chromosome arm 3R, is present on most third chromosome balancers used today (Fig 2).

Hermann Muller was likely the first to use the term “balanced” to describe enforced heterozygosity in a stock with a recessive lethal mutation on one chromosome and a crossover suppressor plus a recessive lethal on a homolog [7,8]. The first balanced stock involved *In(3R)C*, which carried a recessive lethal, in stock with a homolog carrying a recessive lethal allele of *Ser* with a dominant visible wing phenotype called *Beaded* (*Ser<sup>Bd-1</sup>*) [7]. Had there been free recombination between *In(3R)C* and the homolog, *Ser<sup>+</sup>* from *In(3R)C* would have replaced *Ser<sup>Bd-1</sup>*, and the newly generated mutation-free chromosome would have quickly

outcompeted both progenitor chromosomes to eliminate them from the stock population. By the 1920s, Muller had also characterized a large inversion of the middle of the X chromosome that carried several recessive visible markers, a dominant visible, and an unknown recessive lethal [9]. This chromosome, *ClB*, was an essential tool in the work that led to his Nobel prize in 1946. *In(1)dl-49*, a second X chromosome inversion discovered by Muller, inverts the middle third of the X, and although both chromosomes allowed infrequent proximal crossover events, *In(1)dl-49* did not allow frequent double crossovers inside of the inversion as *ClB* did. Additional X chromosome inversions were generated by Muller using X-irradiation, including several whole-arm inversions that placed centric heterochromatin near the distal *scute* gene [10].

Because it was obvious that single inversions did not fully suppress exchange over the entire X, efforts were undertaken to build a more effective balancer. By the 1950s, a balancer combining *In(1)dl-49* and a *scute* inversion existed and was known as *First Multiple 1 (FM1)* [11]. With two inversions, this chromosome functioned well as a balancer [12]. Further X-irradiation resulted in additional inversions, which prevented the proximal exchanges that could occur on *FM1* [13,14]. One of the new balancers was *FM7*, the most commonly used X chromosome balancer today [15].

On Chromosome 2, Ward [16] was the first to describe two naturally occurring paracentric inversions that are the progenitors of nearly all second chromosome balancers used today. She was studying the *Curly* mutation isolated from a wild population and noted that when it was heterozygous, reductions in exchange occurred on both chromosome arms. Similar to the early X chromosome balancers, *In(2L)Cy + In(2R)Cy* was not an effective balancer for all second chromosome regions and was subjected to X-irradiation to introduce new inversions. A whole-chromosome inversion resulted in *Second Multiple 1 (SM1)* [11], with subsequent irradiation of *SM1* resulting in *SM5*, which carried additional inversions and a large duplication [17]. Separate irradiation of *In(2L)Cy + In(2R)Cy* created a new balancer known as *Curly of Oster (CyO)* [18]. Single exchange events between *SM1* and *CyO* later produced *SM6* [19].

As noted above, third chromosome balancers also began as simple inversions that were combined and irradiated to produce more complex and effective balancers. X-irradiation of a third chromosome carrying two inversions, *In(3LR)sep* and *In(3R)C*, generated three new inversions to create *Third Multiple 3 (TM3)* [20]. A different chromosome carrying three existing inversions, *In(3L)P*, *In(3LR)P88*, and *In(3R)C* was irradiated to create *TM6*; subsequent exchange of the left arm of *TM6* with another existing inversion, *In(3LR)HR33* (which also carried the three-breakpoint inversion *In(3R)Hu*), yielded *TM6B* [21–23].

The creation of balancers required a sophisticated understanding of chromosome manipulation and stands as a testament to the genius of midcentury *Drosophila* geneticists. In contrast, balancing mutations on the small fourth chromosome is simple. It does not undergo meiotic recombination and therefore does not need a multiply inverted chromosome to suppress exchange. Any fourth chromosome carrying a recessive lethal or sterile mutation can effectively act as a fourth chromosome balancer, although usually a recessive lethal mutation with a dominant visible phenotype, such as *eyeless<sup>D</sup>*, is used.

## Balancers in other species

Any chromosome carrying at least one inversion and a closely linked recessive lethal or sterile mutation can function as a balancer for specific chromosomal regions. Increasing the number of inversions and rearrangements allows a chromosome to function as a balancer for more regions, and dominant visible markers assist in following the balancer in crosses, but they are not absolutely necessary. Because crossovers are suppressed in the vicinity of any heterozygous

aberration breakpoint, translocations, transpositions, and duplications can also be used as balancers—though these uses are rare in *Drosophila melanogaster*.

Inversions have been identified and studied in several *Drosophila* species in addition to *D. melanogaster*. For example, several inversions have been described on the third chromosome of *D. pseudoobscura*, including some that overlap [24]. Inversions have also been well described for many other species, and stocks with either inversions or translocations for at least *D. pseudoobscura*, *D. simulans*, and *D. virilis* are available at the National Drosophila Species Stock Center (<http://blogs.cornell.edu/drosophila/>).

Outside *Drosophila*, chromosomes acting as balancers can be found in a handful of organisms. For example, in *Caenorhabditis elegans*, chromosomes carrying translocations, duplications, or inversions function as balancers [25–30]. Together, these chromosomes cover most, if not all, of the *C. elegans* genome, but unlike *D. melanogaster*, the majority of the *C. elegans* balancers suppress recombination over only small chromosomal regions near their breakpoints. Inversions that suppress exchange, some with recessive lethal mutations and some with dominant visible markers, also exist in *Mus musculus*, but they are available for only a small portion of the genome [31,32].

## The hidden secrets of balancer chromosomes

Despite the pervasiveness of balancers in *D. melanogaster* research, the precise positions of the inversion breakpoints on the most commonly used balancers were only recently elucidated by whole-genome sequencing [33]. Forty-four of the 48 breakpoints on the most commonly used X, second, and third chromosome balancers were mapped using a combination of short-read Illumina sequencing and mate-pair sequencing. Subsequently, Ghavi-Helm and colleagues [34] used chromatin confirmation capture to estimate the positions of the four remaining breakpoints.

Although other balancers do exist in *D. melanogaster*, the balancers that have been sequenced are found in over 95% of the more than 40,000 stocks carrying at least one balancer at the Bloomington Drosophila Stock Center (<https://bdsc.indiana.edu/stocks/stockdata.html>). Furthermore, many of the inversions present on the balancers that were sequenced are also present on balancers that have not been sequenced.

Identifying the genomic position of each breakpoint yielded some surprising findings. Thirty-one breakpoints directly bisect a protein-coding gene, including the 65D breakpoint on *TM3* that bisects all transcripts of the highly conserved tumor suppressor gene *p53* [35]. Other genes have altered expression not because they are directly disrupted, but because of their proximity to a breakpoint. For example, the *light* gene, which encodes a cellular trafficking protein, is likely misexpressed on *SM5* due to abnormal juxtaposition of euchromatic and heterochromatic regions at the 40F inversion breakpoint [36]. Whole-genome sequencing also determined that 117 protein-coding genes are present in a large duplication carried by *SM5* (Fig 2).

Sequencing has shown that some balancers have been incorrectly labeled in stocks, and it is not difficult to see how that might happen. Most second chromosome balancers, for example, are marked with only one easy-to-identify dominant visible marker, *Curly*, leaving no simple way to determine if a stock carries *CyO*, *SM1*, *SM5*, or *SM6*. Indeed, in one study, out of 22 second chromosome balancers sequenced, four were mislabeled [36]. This should be a concern to researchers studying a mutant allele near a breakpoint or within a region poorly balanced by a particular balancer, such as the 42A to 58A segment of *SM1*. Fortunately, recessive markers can help distinguish balancers, and PCR primers are now available for 40 of the 44 breakpoints sequenced [35–37].

Another source of gene disruption on balancer chromosomes are SNPs and indels. Because each balancer was created one time and then distributed to the *Drosophila* community, any mutations on the original chromosome would be spread to all stocks containing that balancer. Because X-irradiation and ethyl methanesulfonate (EMS) mutagenesis were used to induce rearrangements and add visible markers, the number of mutations is probably higher on balancers. Indeed, sequencing a panel of balancers with common origins revealed many shared deleterious alleles, such as nonsense and splice-site variants. A panel of second chromosome balancers, for example, revealed 35 nonsense and 62 likely deleterious splice-site mutations shared among the all the stocks sequenced, as well as 8,898 missense variants whose impacts are unclear but may affect protein function [36]. Because balancers cannot easily replace deleterious alleles by crossing over, they are likely to accumulate unique mutations over time. One sequenced *SM5* balancer, for example, was found to have one nonsense and 24 missense variants that were not found on the other four *SM5* balancers sequenced [36].

In addition to the accumulation of SNPs and indels, sequencing revealed that balancers diverge in sequence as the result of rare double crossovers within inverted segments. For example, multiple X chromosome balancers had tracts of unique sequence within the 8.5-Mb *In(1)dl-49* inversion, which lies in the middle one-third of the X chromosome. These novel tracts were introduced from their structurally normal-sequence homologs. All of these double crossovers replaced a female-sterile allele of the *singed* gene with a normal allele, which may have provided the new balancers with a competitive advantage in stock populations. Although crossovers between effective balancers and their homologs are rare, gene conversions appear to occur at rates similar to or higher than normal [1], providing a mechanism by which shorter tracts of new sequence can be introduced.

### What balancers can teach us about crossover suppression by breakpoints

The distance over which inversion breakpoints suppress exchange is unknown and has been challenging to study using traditional marker-based approaches. Whole-genome sequencing of balancers has helped us chip away at this question. Because a large region at the distal end of left arm of *TM3* can be exchanged with normal-sequence homologs by single crossovers, historical recombination events have been preserved in this interval. Sequencing several *TM3* stocks revealed exchange events as close as 2 Mb from the distalmost inversion breakpoint, providing the first direct observation of the closest distance to a breakpoint a crossover can form in the face of crossover suppression [35].

In a subsequent study [1], crossovers were seen approximately 1 Mb from balancer breakpoints, but, interestingly, gene conversions were observed evenly distributed along the chromosomes and in close proximity to the breakpoints. This tells us that breakpoints have no effect on the placement of double-strand breaks or their repair into gene conversions. Considering data showing that gene conversions do not respond to interference or to the inhibition of meiotic recombination seen near centric heterochromatin, known as the “centromere effect” [33], it appears that inversion breakpoints may suppress exchange through a mechanism similar to interference or the centromere effect.

### The future of balancers

The coming years will likely see precise changes made to balancers using new tools, such as CRISPR and transgene technologies, either to make structural modifications to enhance their function or to introduce alternative markers. Indeed, GFP-expressing transgenes have been added to balancers to speed up screening in mutagenesis experiments by, for example,

allowing for the easy identification of balancer heterozygotes that might be selected for or against [38–40]. Multiply rearranged chromosomes will likely be created for other species of *Drosophila*, allowing the maintenance of deleterious alleles and experiments involving complex crosses. Balancer chromosomes have a rich history and have been key to the development of *D. melanogaster* as a prominent model organism, and it is clear they will be essential to the *Drosophila* community for many years to come.

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