Experimental verification that crossing-over events within inversion heterozygotes are eliminated in the gametes of *Drosophila melanogaster* females.

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Early in the development of *Drosophila* genetics, heterozygous autosomal "genes", "genetic factors", or "crossover-suppressors" were isolated that eliminated (or almost eliminated) the recovery of recombinant gametes for some genetic markers in germ cells (Muller, 1916; Sturtevant, 1917, 1921; Ward, 1923; Payne, 1924). Sturtevant (1926) hypothesized that these crossover reducers were inversions and that crossing over leading to recombinant gametes did occur in inversion *homozygotes.* Calvin Bridges (1937; quoted in Sturtevant, 1926) confirmed that the crossover reducers were inversions by cytogenetic analysis of salivary-gland polytene chromosomes.

Why are recombination events in inversion heterozygotes eliminated in the gametes of *Drosophila melanogaster*?

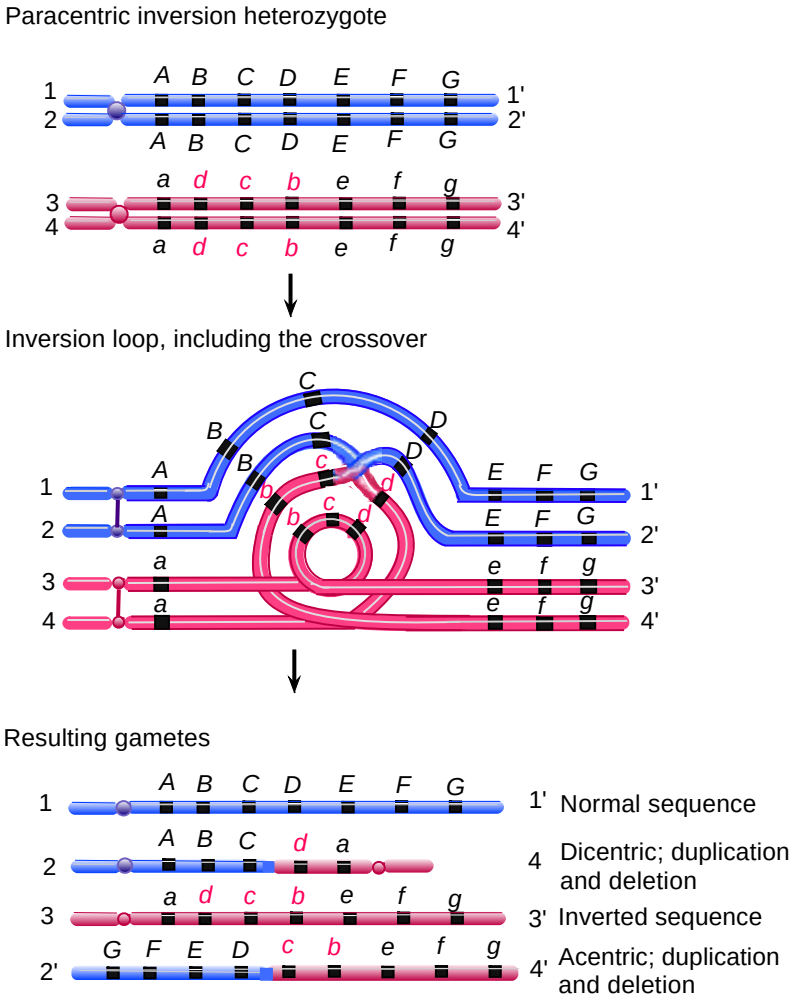


Figure 1. Consequence of crossing-over within a paracentric inversion heterozygote. NCO = non-crossover; SCO = single crossover. (After Klug, William S., Michael R. Cummings, Charlotte A. Spencer, and Michael S. Palladino, 2010, *Essentials of Genetics*, 7th Edition, p. 124. Pearson Education, Inc., Upper Saddle River, NJ).

Recombinant gametes from crossing-over events within inversion heterozygotes have chromosomes with large duplications and deficiencies, plus, in some cases, acentric (no centromere) chromosomes or dicentric (two centromeres) chromosomes (for reviews of this topic see Ashburner, 1989; Kirkpatrick, 2010). These altered chromosomes at meiosis are not included in the egg nucleus of *D. melanogaster*, or early embryos

that contain these chromosome duplications and deficiencies do not survive (Beadle and Sturtevant, 1935; Sturtevant and Beadle, 1936). These chromosome events are shown in Figure 1 for paracentric inversions (centromeres outside the inversions) and in Figure 2 for pericentric inversions (centromeres inside the inversions). In Figure 1, crossing-over events within the paracentric inversion heterozygote give rise to gametes with no or two centromeres and with duplications and deficiencies. In Figure 2, crossover gametes from heterozygous pericentric inversions have the normal number of centromeres, but duplications and deficiencies. The chromosomal changes shown in Figures 1 and 2 have been observed directly by cytology in corn (McClintock, 1933), red trillium (*Trillium erectum*) (Smith, 1935), fungus gnat (*Sciara implatiens*) (Carson, 1946), and *D. melanogaster* (Stone and Thompson, 1935; Hinton and Lucchesi, 1960).

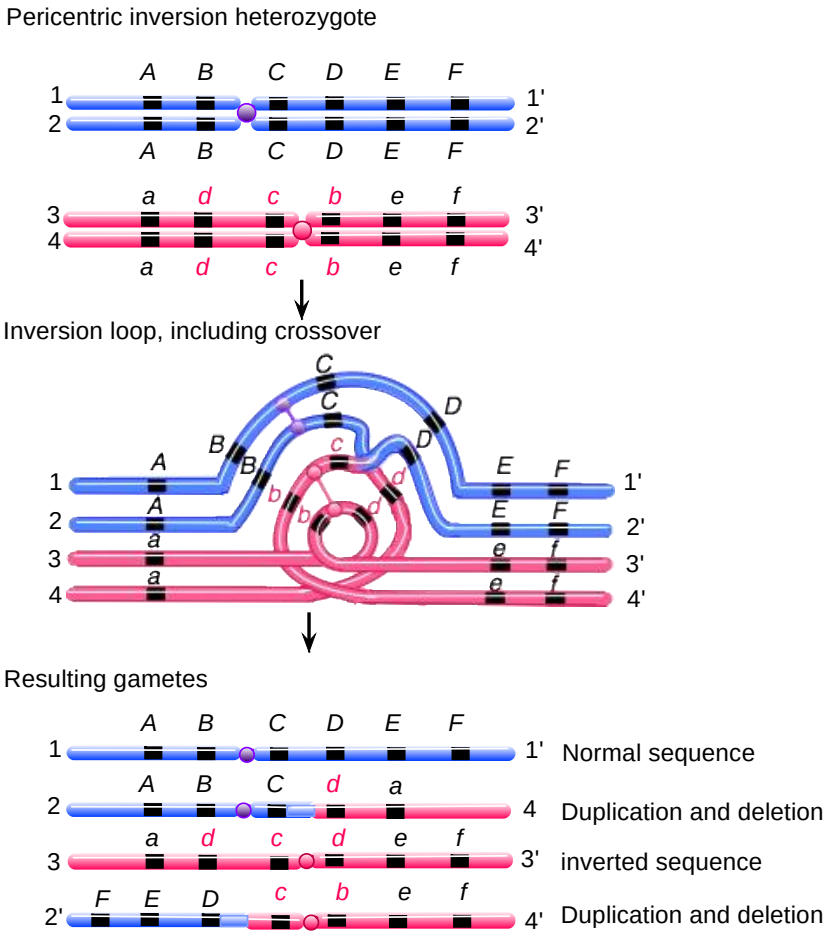


Figure 2. Consequence of crossing-over within a pericentric inversion heterozygote. NCO = non-crossover; SCO = single crossover. (After Klug, *et al.*, 2010, see Figure 1 citation).

In this study we have attempted to confirm genetically the historical observations on the consequence of crossing-over events in inversion heterozygotes by screening for recombination events within an inversion on the right arm of the third chromosome of *D. melanogaster* females. As controls, we also screened for recombination in females homozygous for a structurally normal third chromosome and in males, which are known not to undergo recombination (Morgan, 1912; Sturtevant, 1917; Woodruff and Thompson, 1977).

Screen for possible crossing-over events within In(3R)P / gl^2 e^4 heterozygous females:

To confirm that recombinant gametes are not recovered from crossing-over events within paracentric heterozygotes, we performed the following crosses. In(3R)P is a paracentric inversion with salivary-gland chromosome breakpoints at 89C-D and 96A on the right arm of chromosome three (Lindsley and Zimm, 1992, p. 967). The third-chromosome marker glass-2 (glass eyes, gl^2) is at cytological position 91A1-2 (Lindsley and Zimm, p. 252), and ebony-4 (ebony body color, e^4) is at position 93D2-6 (Lindsley and Zimm, 1992, p.180). Hence gl^2 and e^4 are within the chromosomal

region of the In(3R)P rearrangement (breakpoints 89C-D and 96A). In(3R)Hu, $Antp^{Hu}$ Sp^{spi} / In(3R)P is Indiana University Drosophila Center Stock #3396 and gl^2 e^4 is Indiana University Drosophila Center Stock #507. Sb^{spi} = dominant mutation causing stubble (short) bristles.

In(3R)Hu, $Antp^{Hu}$ Sb^{Spi} / In(3R)P females × gl^2 e^4 / gl^2 e^4 males

In(3R)P / gl^2 e^4 females × gl^2 e^4 / gl^2 e^4 males
(with normal bristles)

Non-recombinants		Recombinants	
gl^2 e^4 / gl^2 e^4 =	glass & ebony	gl^2 + / gl^2 e^4 =	glass
or		or	
+ + / gl^2 e^4 =	wild-type	+ e^4 / gl^2 e^4 =	ebony

From these crosses, we observed one recombinant (+ e^4) and 4,506 non-recombinants, for a map distance of 0.02 (1/4,507 = 0.0002; one map unit is equal to one percent recombination). The one recombinant may have arisen by double recombination or as a gene conversion event (Ashburner, 1989, p. 486). Lindsley and Zimm (1992) give the map distance between *gl* (3-63.1) and *e* (3-70.7) as 7.6 map units. Based on the expected map distance of 7.6, if recombinant gametes from crossing-over in In(3R)P heterozygotes were not eliminated, we should have seen about 343 recombinants and 4,164 non-recombinants instead of one out of 4,507 ($P < 0.0001$). Hence, these results clearly support the early observations that recombinant gametes from crossing-over events within inversion heterozygotes are not included in offspring.

Screen for possible crossing-over events within + + / gl^2 e^4 heterozygous females:

As a control, we also screened for recombination in heterozygous females for gl^2 e^4 and a third chromosome with a normal structure (from the laboratory wild-type stock, Canton-S), by the following crosses.

Canton-S females × gl^2 e^4 / gl^2 e^4 males

+ + / gl^2 e^4 females × gl^2 e^4 / gl^2 e^4 males

Score for non-recombinants and recombinants as shown in the above crosses.

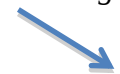
From these crosses, we observed 136 recombinants and 1,840 non-recombinants, for a map distance of 6.9 (136/1,976 = 0.069). In comparison to the expected map distance of 7.6 (150 recombinants and 1,836 non-recombinants), a result of 136 and 1840 for Canton-S / gl^2 e^4 is not

significantly different (P = 0.42) from the expected frequency. Yet, the frequency of recombinants in In(3R)P / gl^2 e^4 females is significantly lower than in + + / gl^2 e^4 females (1/4,508 *vs*. 136/1,976; P < 0.0001). Hence, the lack of recombination in the In(3R)P / gl^2 e^4 females is not due to a crossover suppressor in the gl^2 e^4 third chromosome.

Screen for possible crossing-over events within + + / gl^2 e^4 heterozygous males:

As an additional control we screened for recombination in Canton-S / gl^2 e^4 males that are known not to undergo recombination (Morgan, 1912; Sturtevant, 1917), by use of the following crosses.

Canton-S females × gl^2 e^4 / gl^2 e^4 males



gl^2 e^4 / gl^2 e^4 females × + + / gl^2 e^4 males

Score for non-recombinants and recombinants as shown in the above crosses.

As expected, we observed zero recombinants and 3,742 non-recombinants from the Canton-S / gl^2 e^4 heterozygous males (0/ 3,742 *vs*. 258/3,742, if we recovered the expected 6.9 percent recombination that was observed in females; P < 0.0001).

A summary of the results of this experiment is given in Table 1.

Table 1.

Genotype	Number Recombinants	Number Non-Recombinants	Percent Recombination
In(3R)P / gl^2 e^4 females	1	4,506	0.02[a]
+ + / gl^2 e^4 females	136	1,840	6.9[a,b]
+ + / gl^2 e^4 males	0	3,742	0[b]

[a]P < 0.0001; [b]P < 0.0001

The results from this study clearly support the early observations that gametes from crossing-over events within inversion heterozygotes are not recovered in progeny. Yet, why is this an important observation?

First, recent DNA sequences of homologous chromosomes of individuals have identified many more inversions in each human than previously assumed; for example, 90 inversions in a single diploid genome (Levy *et al*., 2007), some of which are caused by recombination between transposable DNA elements (Hancks and Kazazian, 2012). Hence, inversions and other rearrangements are important in altering the position and expression of genes (position-effect variegation; Spofford, 1976; Dimitri and Pisano, 1989), in some cases causing cancer (Hancks and Kazazian, 2012).

Second, genes within an inversion can evolve together, since they will not be separated by recombination in heterozygotes. This has important evolutionary implications that allow for groups of genes to evolve and interact over time (Hedrick, 2011).

As part of a class discussion, students might be given four examples where there has been

selection for groups of genes within inversions: 1) in *D. melanogaster* for changes in response to global warming (Balanya *et al*., 2006) and body size (Kennington *et al*., 2007); 2) in butterflies for wing color (Joran *et al*., 2011), and 3) in humans for an increase in offspring numbers (Stefansson *et al*., 2005). Yet, the genes have not been identified in the inversions of these four examples. Hence, students might also discuss the two alleles of an odorant-binding protein gene (*Gp-9*) that is located in a large inversion that determines the social structure of fire ants (Krieger and Ross, 2002; Wang *et al*., 2013). For example, the two variants of the *Gp-9* gene determine if ant colonies have one queen or many queens, because of the killing of queens by workers if the queens have the wrong *Gp-9* genotype.

References: Ashburner, M., 1989, Drosophila: *A Laboratory Handbook.* Cold Spring Harbor Laboratory Press, pp. 509-528; Balanya, J. *et al*., 2006, Science 313: 1773-1775; Beadle, G.W., and A.H. Sturtevant 1935, Proc. Natl. Acad. Sci. USA 21: 384-390; Carson, H.L., 1946, Genetics 31: 95-113; Dimitri, P., and C. Pisano 1989, Genetics 122: 793-800; Gillespie, J.H., 2004, *Population Genetics: A Concise Guide*. The Johns Hopkins University Press, Baltimore; Hancks, D.C., and H.H. Kazazian, Jr. 2012, Current Opinion in Genetics and Development 22: 191-203; Hedrick, P.W., 2011, *Genetics of Populations*: Jones and Bartlett Publishers, Sudbury, MA; Hinton, C.W., and J.C. Lucchesi 1960, Genetics 45: 87-94; Joron, M. *et al*., 2011, Nature 477: 203-208; Kennington, W.J., A.A. Hoffman, and L. Partridge 2007, Genetics 177: 459-556; Kirkpatrick, M., 2010, PLoS Biology 8(9): e1000501. doi:10.137/journal.pbio.1000501; Klug, W.S., M.R. Cummings, C.A. Spencer, and M.A. Palladino 2010, *Essentials of Genetics*. Pearson Education, Inc., San Francisco; Krieger, M.J.B., and K.G. Ross 2002, Science 295: 328-332; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York; Lowry, D.B., and J.H. Willis 2010, PLoS Biology 8(9): e1000500. doi: 10.1371/journal.pbio 1000500; McClintock, B., 1933, Z. Zellforsch 19: 192-237; Morgan, T.H., 1912, Science 36: 719-720; Muller, H.J., 1916, Amer. Nat. 50: 103-221; Payne, F., 1924, Genetics 9: 327-342; Smith, S.G., 1935, J. of Genetics 30: 227-232; Spofford, J., 1976, *Genetics and Biology of* Drosophila, Vol. 1C, pp. 995-1018 (Ashburner, M., and E. Novitski, eds.). Academic Press, New York; Stefansson, H. *et al*., 2005, Nature Genetics 37: 129-137; Stone, W., and I. Thomas 1935, Genetica 17: 170-184; Sturtevant, A.H., 1917, Proc. Nat. Acad. Sci. USA 3: 555-558; Sturtevant, A.H., 1921, Proc. Natl. Acad. Sci. USA 7: 235-237; Sturtevant, A.H., 1926, Biologisches Zentralblatt 46: 697-702; Sturtevant, A.H., and G.W. Beadle 1936, Genetics 21: 554-604; Wang, J. *et al*., 2013, Nature 493: 664-668; Ward, L., 1923, Genetics 8: 276-300; Woodruff, R.C., and J.N. Thompson, jr. 1977, Heredity 38: 291-307.

An attempt to identify new recessive sex-linked visible mutations in *Drosophila melanogaster*.

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The first attached-X chromosome in *Drosophila melanogaster*, where two X chromosomes are attached to a single centromere, was isolated by Lillian V. Morgan in 1921 (Morgan, 1922). This compound stock, which is now called C(1)RM (the two X chromosomes are attached in reverse), and the C(1)DX attached X chromosome (the two X chromosomes are attached in tandem), which was isolated by H. J. Muller in 1943 (Muller, 1943), have been used to isolate new visible mutations on