dwarfism is caused by a dominant autosomal mutation that also has more drastic effects in homozygotes.

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Spontaneous and gamma ray induced chromosome breakage in *Drosophila melanogaster*.

Woodruff, R.C., and John P. Russell. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403.

Chromosomal rearrangements are more frequent in humans than previously thought. For example, the diploid genome sequence of J. Craig Venter (his company, Celera Genomics, and the Human Genome Sequencing Consortium first sequenced the human genome) contained 292,102 heterozygous insertion/deletion events (1 to 571 base pairs), 559,473 homozygous indels (insertions and deletions of one to 82,711 base pairs), 90 inversions, and numerous duplications (Levy *et al.*, 2007). The rate of new chromosome aberrations in humans is about 4/1000 live births (Sankaranarayanan and Wassom, 2005), with one in 500 humans carrying a new reciprocal translocation (Gajecka *et al*., 2008). In addition, many chromosome rearrangements are associated with human genetic defects and cancer (Strachan and Read, 2004; Lupski 2007; Hastings *et al*., 2009). Hence, it is important to identify spontaneous and induced chromosome breakage events and to estimate their rates in a model organism such as *Drosophila melanogaster*.

It is the objective of this study to measure spontaneous and gamma ray induced X-chromosome breakage events in an F1 assay in *D. melanogaster*. This hyperploidy chromosome breakage assay involves the identification of breakage events that delete segements of the X chromosome in males, which are then recovered as extra chromosomal fragments (hyperploidy) in F1 females. This assay is shown in Figure 1, and is discussed in Auerbach (1962) and Blount and Woodruff (1986). The C(1)DX, *y w f* chromosome is two X chromosomes attached to a single centromere and containing the recessive markers *y* (yellow, yellow body color), *w* (white, white eyes), and *f* (forked, short bristles) (Lindsley and Zimm, 1992); *D. melanogaster* that have two X chromosomes and a Y chromosome are fertile females. In this cross, y^+, w^+ and f^+ denote the wild-type alleles of the three genes; Canton-S is a wild-type stock (containing a y^+ w^+ f^+ X chromosome), and O is a centromere. It should be noted that some exceptional C(1)DX, $y\ w\ f\ /\ y^+\ w^+\ f^+$ triplo-X female progeny occur in older cultures in this breakage assay. These XXX females have grey bodies, red eyes, and long bristles, plus they usually have slightly deformed wings, move slowly, have slow

development, and are sterile (see Lindsley and Zimm, 1992, for more details). Since these triplo-X females do not contain breakage events, they will not be counted in this experiment.

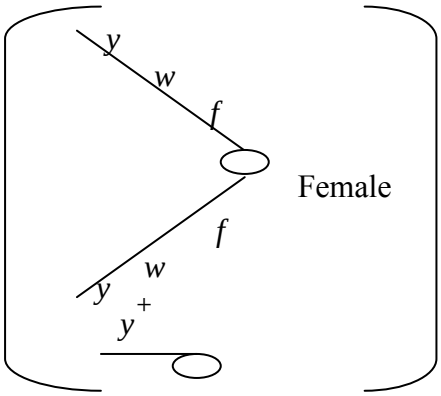
C(1)DX, *y w f*/Y females X Canton-S/Y (y^+ w^+ f^+/Y) males
Untreated (controls) or gamma ray trea

↓

F1 No chromosome breakage — Possible X-Chromosome breakages

C(1)DX, *y w f*/Y females
yellow body color
white eyes
short (forked) bristles

1. C(1)DX, *y w f*/y^+ females
grey body color
white eyes
short (forked) bristles



2. C(1)DX, *y w f*/w^+ females
yellow body color
red eyes
short (forked) bristles

3. C(1)DX, *y w f*/f^+ females
yellow body color
white eyes
long bristles

4. C(1)DX, *y w f*/y^+ w^+ females
grey body color
red eyes
short (forked) bristles

Figure 1. Hyperploidy chromosome breakage assay for X-chromosomes in males that are identified as chromosome fragments in F1 females.

In this study, the spontaneous rate of chromosome breakage will be measured and added to the historical spontaneous rate at Bowling Green State University of two breakage events among 88,161 scored F1 females (0.00002) (74,959 are from Blount and Woodruff, 1986, and 13,202 are from subsequent control runs). In addition, males were treated with 2,010 rads of gamma rays from a cesium source at the University of Toledo Medical Center (treatments were performed by Eddie

Brentlinger, Radiation Safety Officer). This is a positive control, because gamma rays are known to cause chromosome breakage in *Drosophila* and humans (Alexander and Bergendahl, 1962; Ganetzky, 1977; Gubb *et al*., 1984, 1985; Hilliker and Trusis-Coulter, 1987; Ashburner, 1989; Sankaranarayanan and Wassom, 2005).

The chromosome breakage results for this study are shown in Table 1.

Table 1. Rates of chromosome breakage in *Drosophila melanogaster* males.

	Breakage Events	Total F1 Females	Rate
Spontaneous			
a. Historical	2	88,161	0.00002
b. This Study	0	7,613	0
c. Total	2	95,774	0.00002*
Gamma Ray Treated (2,010 rads)	2	1,521	0.0013*

*P < 0.0001

The hyperploidy assay has been used to identify chemical-induced, X-ray-induced, and transposable DNA element-induced chromosome breakage in *D. melanogaster* (Auerbach, 1962; Blount and Woodruff, 1986). In this study, this assay was used to identify gamma ray-induced chromosome breakage. A significant ($P < 0.0001$) increase in chromosome breakage was caused by gamma rays. The two gamma ray-induced breakage events observed in this study were C(1)DX, $y\ w\ f\ /\ y^+\ w^+$ females that had grey body color and red eyes. These X-chromosome fragments, therefore, contained the yellow and white loci and a segment of the chromosome that contained a centromere, *i.e.*, a two-break deficiency occurred that removed a part of the X chromosome between the white locus and the centromere.

The hyperploidy assay could be used in a teaching environment to screen other possible chemical and physical agents for their ability to cause chromosome breakage, including ultraviolet light (see Toth *et al*., 2007) and tobacco products that are mixed into *Drosophila* food. The students could be asked to suggest possible chromosome breakage agents that could be tested using the hyperploidy assay.

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