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### Chromosomal Fragments Transmitted through Three Generations in *Oncopeltus* (Hemiptera)

**Abstract.** *Chromosomal fragments and translocations induced by x-rays in the sperm of adult milkweed bugs, Oncopeltus fasciatus (Dallas), were detected in the meiotic cells of  $F_1$ ,  $F_2$ , and  $F_3$  males and caused high levels of sterility in untreated progeny. The persistence of these fragments through numerous generations of cells confirmed the holokinetic nature of the milkweed bug chromosomes.*

Since the majority of plants and animals possess chromosomes with a single centromere or kinetochore, these species usually lose any chromosomal fragments at the time of cell division because there is no spindle fiber attachment. In some plant and animal species with holokinetic chro-

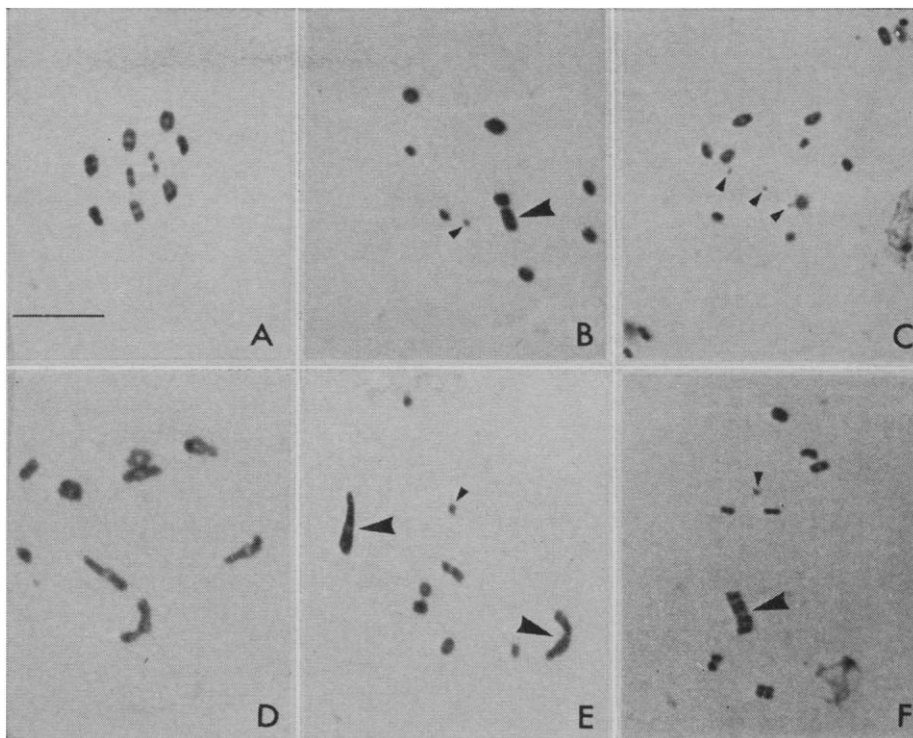


Fig. 1. Chromosomes in the primary spermatocyte of *Oncopeltus*. All magnifications about the same; line in (A) represents 10  $\mu\text{m}$ . (A and D) Normal complement of chromosomes in control males—seven paired bivalents plus X and Y; (B and E) chromosomes in testes of same  $F_1$  male; and (C and F) chromosomes in testes of an  $F_2$  male. Small arrows indicate fragments; large arrows, a heterozygous translocation complex.

mosomes (1, 2) chromosome fragments have been observed to persist for several cell generations (1, 3), presumably because all fragments are capable of orientation and anaphase movement during cell division. The present report describes the induction of chromosomal fragments and reciprocal translocations in the sperm of adult milkweed bugs, *Oncopeltus fasciatus* (Dallas), the transmission of these fragments and translocations through three outcrossed generations, and the consequent effects on the fertility of the progeny.

Adult males, 3 to 5 days old, were irradiated with 9 kiloroentgens of x-rays and placed in individual cages with one untreated female. Eggs were collected from each of 29 pairs and scored for hatchability. Then the  $F_1$  generation was reared to the adult stage and sexed, and the individual males were outcrossed to virgin untreated females as before. The procedure was repeated until  $F_2$  and  $F_3$  progeny were produced. These repeated outcrossings to untreated females assured that all progeny would contain a normal complement of chromosomes from the female and the fragmented or rearranged set from the

male. Also, after a sufficient number of eggs had been collected from each pair, the male was removed, and the testes were dissected, fixed in 45 percent acetic acid, and squashed on a microscope slide. Material on the slide was frozen on a block of Dry Ice, the coverslip was removed, and the testes squash was stained by the Feulgen reaction. Although numerous lines were studied, each originating from a single  $F_1$  male and many exhibiting the persistent transmission of chromosomal fragments and translocations, the data from only a single line are presented. The cytology and fertility of all lines will be reported elsewhere.

One  $F_1$  male derived from an irradiated sperm and an untreated egg had a fertility of 5.8 percent. The extent of chromosomal fragmentation and rearrangement in this male is shown in Fig. 1, B and E, and can be compared with the normal meiotic configurations shown in Fig. 1, A and D. Most sperm produced by this male contained chromosomal duplications or deficiencies, or both, that rendered the sperm incapable of supporting embryonic development. However, three male and eight female  $F_2$  progeny were produced. One of the males was only 2.1

percent fertile and contained the chromosomal fragments and rearrangements shown in Fig. 1, C and F. The other two  $F_2$  males (25.5 and 35.1 percent fertile) had chromosomal fragments or rearrangements that were less drastic but caused a reduction in their fertility. Also, the fertility of some of the eight daughters was affected (range 0.8 to 72.5 percent fertile), and presumably they represent the range of the various types of viable gametes produced by the  $F_1$  male parent (Fig. 1, B and E). Similar types of chromosome aberrations and a consequent reduction in fertility were found in some of the  $F_3$  males.

The persistence of the chromosomal fragments through the multitude of mitotic and meiotic cell divisions that occur in three insect generations is strong evidence for the holokinetic nature of the chromosomes of this species. Furthermore, the transmission of fragments through meiotic divisions demonstrates that the kinetochore activity is not restricted to a limited region of the chromosomes during meiosis as previously suggested by Heizer (4).

The drastic reduction of fertility of the progeny bearing fragmented and translocated chromosomal complements adds further evidence that inherited partial sterility observed in the Lepidoptera (5), which also have holokinetic chromosomes, is based on the continued transmission of aberrant chromosomal complements. These data provide cytogenetic support for the potential application of inherited partial sterility as an innovation in insect control (6).

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## Circadian Periodicity of Bone Marrow Mitotic Activity and Reticulocyte Counts in Rats and Mice

**Abstract.** Mitotic proliferation in the bone marrow of female rats and mice kept under standardized conditions with light from 6:00 a.m. to 6:00 p.m. exhibited a significant circadian periodicity with the greatest activity occurring from 6:00 a.m. to 12:00 noon. The reticulocyte levels in peripheral blood were highest at 8:00 a.m.

Circadian periodicity occurs in a number of hematological parameters such as the mitotic activity in human bone marrow (1), circulating lymphocyte and eosinophil counts in man (2) and mice (3), and plasma iron levels (4). Spontaneous variations of this type in intact normal animals have been found to be of large magnitude and must be considered in assessing experimental results (5). In this study, a circadian periodicity was demonstrated in the mitotic proliferation of the bone marrow and in the numbers of circulating reticulocyte levels in rats and mice.

Female Sprague-Dawley rats (190 to 210 g) and female white Swiss mice (20 to 25 g) were kept for 7 to 10 days under standardized conditions consisting of exposure to light from 6:00 a.m. to 6:00 p.m., isolation in individual

cages, maintenance of room temperature at 70°F (21°C), and protection from disturbances except for daily feeding and watering. All animals had free access to Purina Laboratory Chow.

To demonstrate the circadian variation in the mitotic activity in bone marrow, groups of ten rats and ten mice were given colchicine intraperitoneally (1.0 and 1.2 mg/kg of body weight, respectively) 4 hours before bone marrow samples were taken at intervals throughout the day and night. This was done in order to arrest mitoses in the metaphase during these 4-hour periods. Each animal was anesthetized with ether and rapidly exsanguinated. A femur was split and bone marrow was removed. Smears were prepared, fixed in methyl alcohol, and stained with Giemsa's stain. Two to three thousand cells were counted per animal, and the

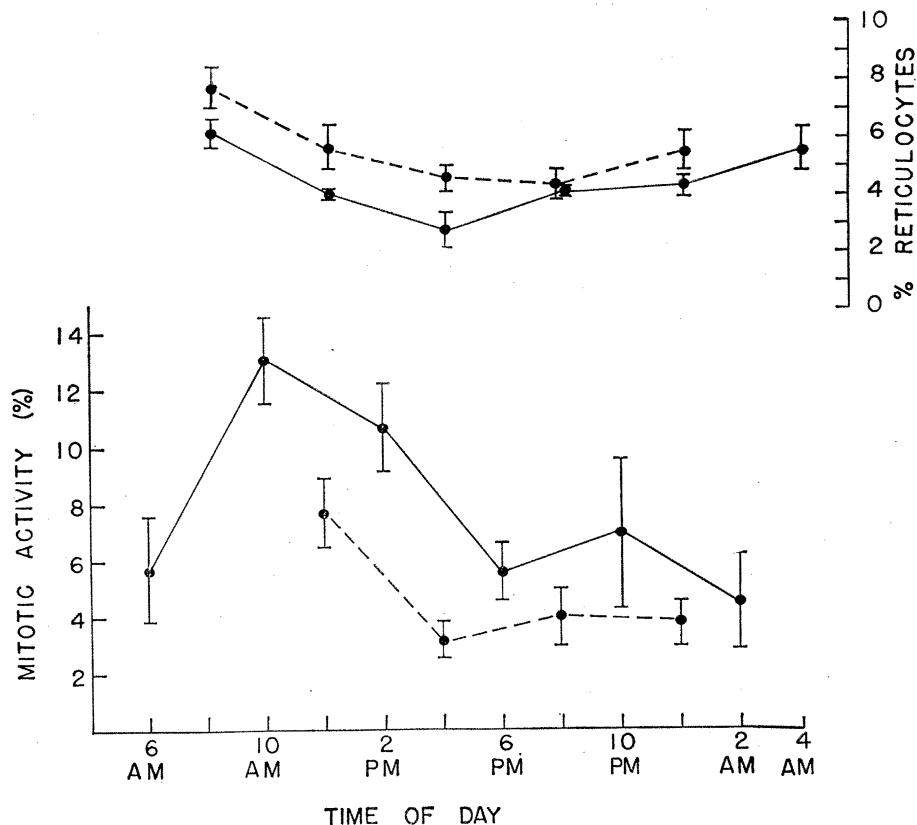


Fig. 1. Circadian variation in the percentage of reticulocytes in the peripheral blood (upper portion) and in the frequency of mitoses (lower portion) of rats (solid lines) and mice (dashed lines). Animals were maintained in light from 6 a.m. to 6 p.m., alternating with darkness. Vertical lines represent standard errors of the means.