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# Light and Electron Microscope Study of the Mitotic Apparatus of the Ring-Legged Earwig, Euborellia annulipes (Lucas)

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# A Light and Electron Microscope Study of the Mitotic Apparatus of the Ring-Legged Earwig, *Euborellia annulipes* (Lucas)

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## ABSTRACT

The mitotic apparatus in dividing cells of the ring-legged earwig is composed of the cell center or pole, chromosomal fibers, continuous fibers, and chromosomal and background structure. Parts from cellular elements such as Golgi membranes, endoplasmic reticulum, and nuclear envelope as well as whole mitochondria associate with the spindle microtubules to produce the diffraction pattern of the spindle fibers as seen with the light microscope. The microtubules of the chromosomal fibers attach independently and demonstrate the diffuse nature of the centromere or polycentric condition of the chromosomes.

## INTRODUCTION

The mitotic apparatus has been defined by Mazia and Dan (1952) as "...the ensemble of structures constituting the 'chromatic' and 'achromatic' figures in the classical description of mitosis." In view of the amount of cytological investigation that has been done on insects, surprisingly little electron microscopy has been directed toward study of the mitotic apparatus of animals in this class and of arthropods in general.

The purpose of this study was to determine the ultrastructure of the mitotic apparatus of the ring-legged earwig, *Euborellia annulipes* (Lucas) at metaphase and to identify the cellular elements contributing to the appearance of the spindle fibers as seen with the light microscope.

## MATERIALS AND METHODS

Specimens of *E. annulipes* were obtained from greenhouses on the University of Arkansas campus and farm. Earwigs to be prepared for light microscopy were fixed in cold Flemming's strong solution for 24 hours and dehydrated with ethanol.

The testes were dissected from each insect in 100% ethanol, cleared in xylene and embedded in Paraplast. They were cut into sections 8 microns thick and mounted on slides with Haupt's adhesive. The sections were stained with Heidenhain's hematoxylin and orange G.

Testes to be prepared for electron microscopy were dissected out in cold 2.5% glutaraldehyde buffered to pH 6.0 with 0.1M phosphate buffer (Pease, 1964). The material was rinsed in several changes of buffer for a total of 2 hours prior to secondary fixation by 1% $OsO_4$ in pH 6.0 phosphate buffer for 2 hours. The tissue was rinsed again in buffer before dehydration in a graded ethanol series. Infiltration and embedding in Epon 812 were accomplished according to the procedure outlined by Luft (1961).

Once metaphase cells had been located by the survey method outlined by Trump et al. (1961), sections were cut at 600-1,000 Å with an LKB Ultrotome and were picked up on 300-mesh copper grids. After drying, the sections were double stained with 2% uranyl acetate (Watson, 1958) and alkaline lead citrate (Reynolds, 1963). The cells were viewed and photographed with a Siemens IA electron microscope.

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## RESULTS AND DISCUSSION

*Light Microscopy.* The mitotic apparatus of *E. annulipes* at metaphase as seen with the light microscope is composed of the cell center or pole, chromosomal fibers which attach to the chromosomes and extend toward the cell center, and continuous fibers connecting the two centers. The chromosomes are compact, densely staining masses which show no arms or constrictions. The chromosomal fibers appear to arise from a broad area of the chromosome rather than from a single point (Fig. 1), a condition defined as polycentric or as a diffuse centromere.

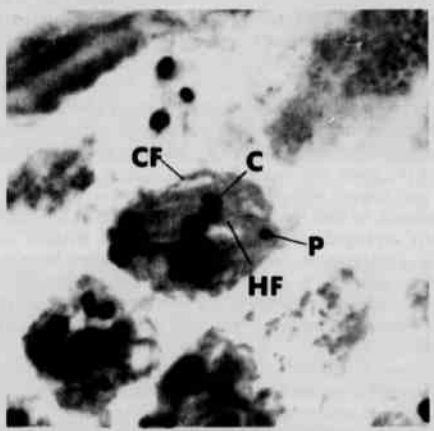


Figure 1. Side view of metaphase. Mitotic apparatus is composed of continuous fibers (CF) and chromosomal fibers (HF) extending from chromosomes (C) to vicinity of cell center or pole (P). 1,600x.

*Electron Microscopy.* Electron microscopy reveals that the cell center at metaphase is composed of a centriole surrounded by a granular halo which is slightly more electron dense than the rest of the background (Fig. 2). The background appears to be the same throughout the cell except that particulate matter is distributed more evenly and agranular tubules and vesicles of the endoplasmic reticulum appear to be more concentrated in the polar region. A few microtubules pass into the halo region but none was observed to contact the microtubules of the

centriole. Furthermore, they are not radially oriented into asters.

Chromosomal fibers are resolved as individual microtubules arising from different sites along the leading edges of the chromosomes (Fig. 3). This finding substantiates the polycentric nature of the chromosomes. As the microtubules converge, they form the fibrous structure seen with the light microscope. Vesicular elements of the cytoplasmic vacuolar system contribute to the density of the fibers.

Continuous microtubules are seen to be present between mitochondria in a longitudinal view of metaphase after dissolution of the nuclear envelope (Fig. 3). Mitochondria, which are distributed around the periphery in prophase activities, are sometimes found among spindle microtubules.

When the microtubules seen in electron micrographs (Figs. 2, 3) are compared in size and number with the fibers seen in the light micrograph (Fig. 1), it is obvious that the spindle microtubules alone could not account for the apparent density of the spindle fibers observed with the light microscope. Portions of components of the cytoplasmic vacuolar system and the mitochondria contribute significantly to the light microscope image but require special treatment and high resolution to be detected as structures separate from the true spindle fibers. The spindle microtubules observed have a 160 Å outer diameter; each wall is 40 Å and the inner diameter is 80 Å. Å.

The spindle of *E. annulipes* is unusually clear under light microscopy. Such clarity of the spindle in dividing cells of some insects and other animals has been reported in numerous cases; however, the spindle is difficult to observe in cells of many organisms. The writers' findings suggest that diffraction of light by parts of cellular components such as the endoplasmic


Figure 2. Section through cell center. Microtubules (Mt) enter halo (H) but do not contact centriole (Ce). Vesicular elements of endoplasmic reticulum are concentrated in this region. 60,000x.

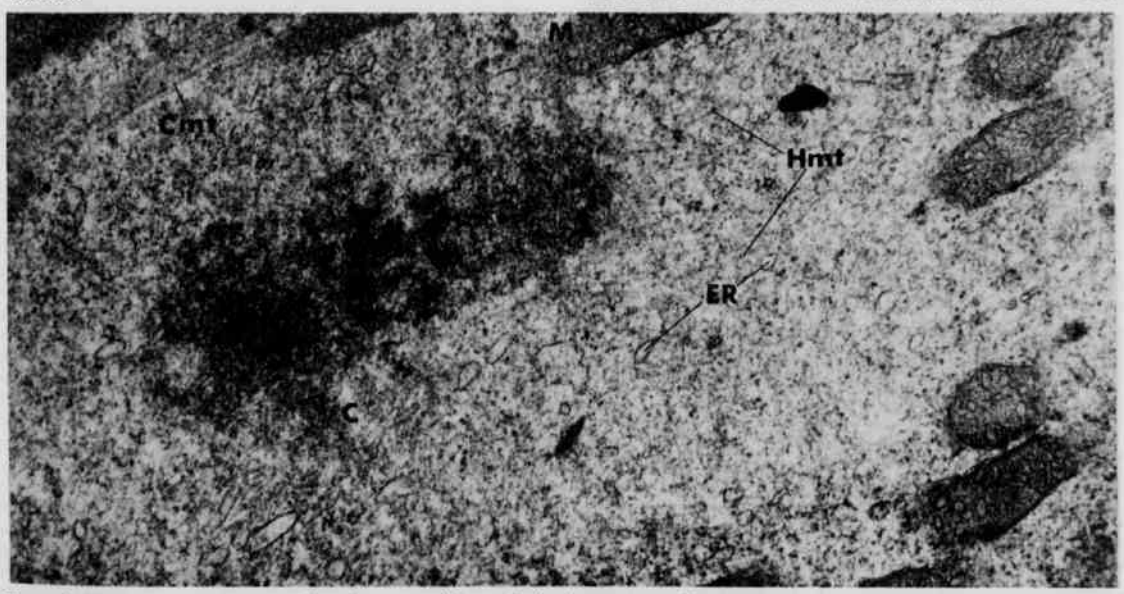


Figure 3. Metaphase chromosome lying close to periphery of mitotic appartus. Chromosomal microtubules (Hmt) arise at different points along chromosome (C). Elements of endoplasmic reticulum (ER) are present in this region and mitochondria (M) commonly are observed between continuous microtubules (Cmt) and chromosomes. 30,000x.

reticulum, nuclear envelope, and the Golgi apparatus as well as whole mitochondria intermingled with the spindle microtubules is responsible to a significant degree for the image of the spindle. Variation in quantity of these vacuolar elements dispersed among the spindle microtubules probably bears an important relationship to the microscopic appearance of the spindle and spindle fibers.

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