

## Cytological evidence for holocentric chromosomes in *Pieris brassicae* (Pieridae : Lepidoptera)

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Among insects the holocentric chromosomes is widely observed in orders, Hemiptera, Dermaptera (Hughes-Schrader and Schrader, 1961). Although evidence suggested the holocentric nature of lepidopteran chromosomes, it is still a open question as stated by White (1973). Cytogenetic studies making use of *in vitro* injection of colchicine and conventional Giemsa staining have been carried out. Chromosomal preparations were made from brain ganglia and testes by using NaCl-acetic Carnoy-air drying method. The nature of the centromere and orientation in meiosis of *Pieris brassicae* (Pieridae: Lepidoptera) chromosomes were investigated using irradiation as a tool in this study. The late third or early fourth stage of the instar larvae of *Pieris* were irradiated with Cu-X-radiation at a wavelength of 1.5418 Å, V=30 Kvp and I= 10mA for two minutes. The results of the experiments were as follows:

– Metaphase chromosomes showed no distinct primary constriction even after treatment with hypotonic solution (Fig. 1)

– Chiasmata underwent complete terminalization during diplotene/diakinesis (Fig. 2 and 3)

– Chromosome type mutation including fragmentation were noted in the form of minute chromosomes in metaphase I (Fig. 4)

Holocentric chromosomes has well been studied in species of Homoptera (Hughes-Schrader and Schrader,

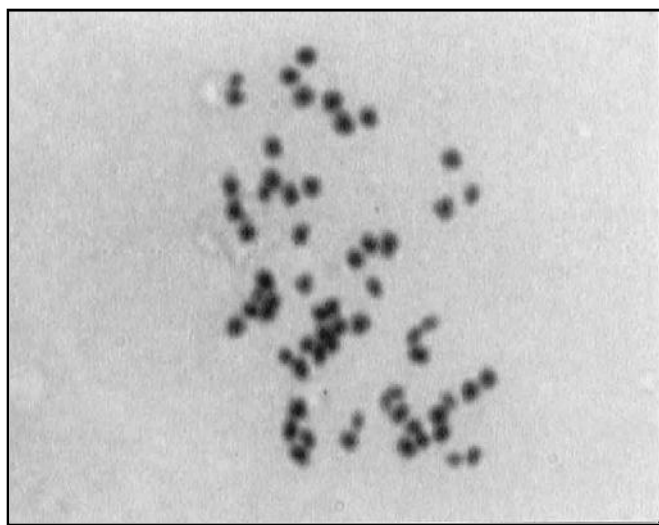


Fig. 1 : Metaphase I

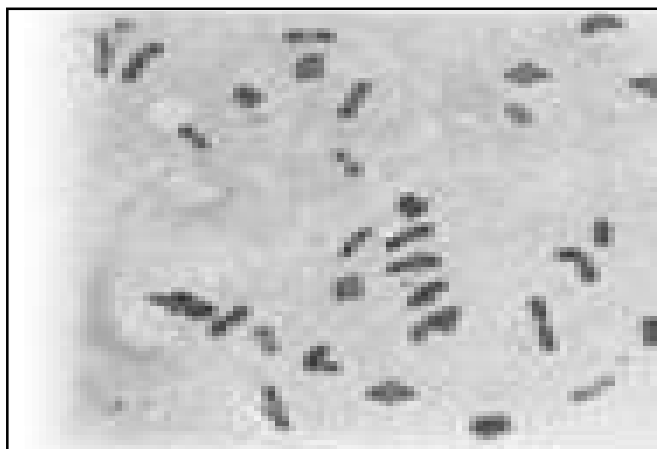


Fig. 2 : Diplotene



Fig. 3 : Diakinesis

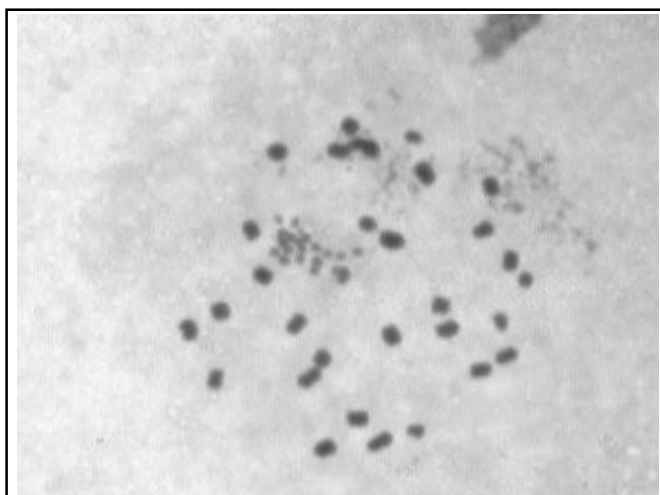


Fig. 4 : Fragmentation

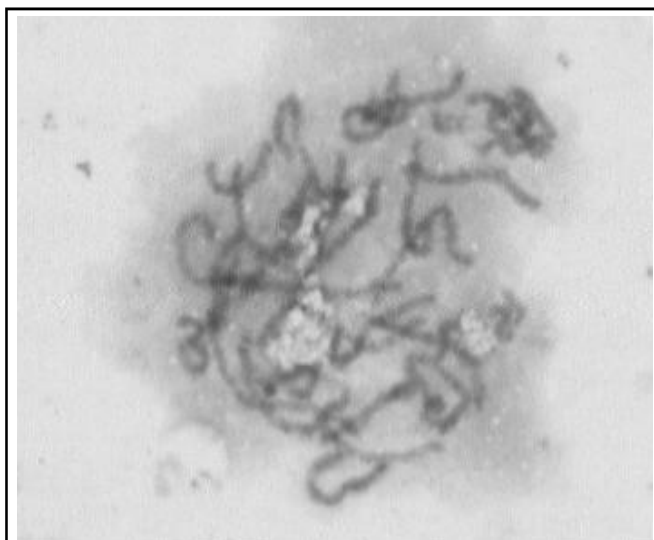


Fig. 5 : Prometaphase

1961) and in plant *Luzula purpurea* (Nordenskiöld, 1962). The nature of holocentric chromosomes is characterized by the attachment site of spindles along their whole length, although it is extremely difficult to demonstrate such diffuse centromeres especially in the tiny chromosomes of lepidopterans. Thus it seems that additional criteria are needed. One is based on the fact that the mitotic and meiotic behaviour of holocentric chromosomes differs somewhat from that of chromosomes with localized centromere (White, 1973). The criteria for the holocentric chromosomes as seen with the light microscope are : (1) No distinct primary constriction appears at metaphase, and at least during somatic mitosis, (2) sister chromatids separate by parallel disjunction at anaphase, (3) at metaphase they orient with their long axes in the equatorial plane, (4) chiasmata undergo complete terminalization until the end of diakinesis and two pairs of chromatids are connected end-to-end by terminal chiasma at metaphase I, and (5) frequently, X-ray induced fragments behave normally. Although the above mentioned characteristics may not be sufficient as evidence for the presence of holocentric chromosomes, it can be said that some of them would give, at least in part, an indication of holocentric nature of chromosomes.

The slides prepared from the irradiated larva showed the presence of one or more fragments in diplotene, diakinesis and metaphase-I stages. No such fragments were found in these stages in the cells of non-irradiated control larvae (Fig. 5 and 6). No fragments were found in the cytoplasm away from the plate at diplotene/diakinesis/metaphase-I stages in the cells examined from irradiated material. Therefore, it appeared that all the fragments had migrated to the plate. It seems reasonable

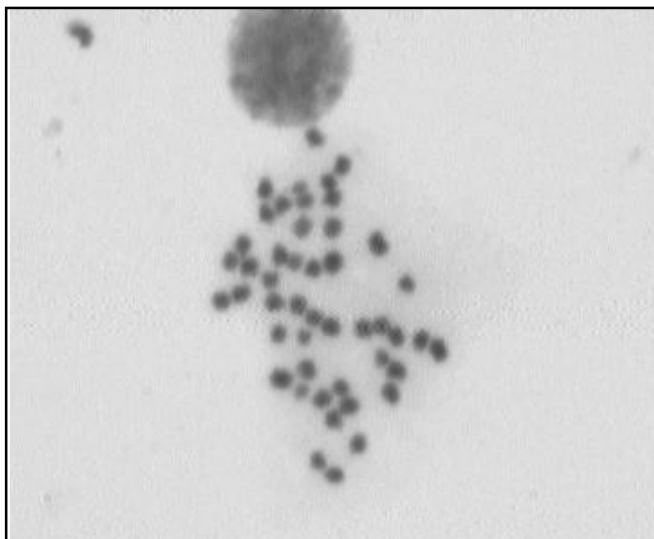


Fig. 6 : Metaphase-I

to assume that, since the fragments showed centromeric activity, the chromosomes must have diffuse type of centromere.

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