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Research Article

Air Dry Technique for the Preparation of Meiotic Chromosomes from the Testes of Silkworm *Bombyx mori* L.

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ABSTRACT

One strain of mulberry silkworm Bombyx mori L., namely NB_4D_2 at the age of the fifth instar, was selected as an experimental insect. During the fifth instar, testes were used to prepare meiotic chromosomes. The air dry technique for preparing meiotic chromosomes was developed and standardised. Both temporary and permanent micro slides showed clear different stages of meiosis. The different meiotic stages were observed under 1000x magnification and photographed using conventional photographic techniques.

Keywords: *Silkworm, Testes, Gonial Metaphase, Meiotic Chromosomes.*

INTRODUCTION

The silkworm, *Bombyx mori* L., though found to be economically important, still appears to be an untapped insect in cytological aspects. The reason attributed to the lack of information in this line is mainly due to the presence of a large number of smaller chromosomes. The silkworm, *Bombyx mori* L., has a haploid number of 28 and a diploid number of 56 chromosomes (Chowdhury, 1965). The Lepidopteran chromosomes are very small, globular and numerous, enabling their determination possible by their orientation only during cell division. As in other Lepidoptera, the silkworm chromosomes are oval in shape (Chowdhury, 1965) except in prophase I stages, which renders the identification of a single chromosome almost very difficult. Even the sex chromosomes can hardly be distinguished from autosomes. Kawaguchi (Kawaguchi, 1928) demonstrated that n = 28 in *Bombyx mori* and n = 27 in *Bombyx Mandarina* using orthodox paraffin sectioning technique. Murakami and Imai (Murakami & Imai, 1973) obtained metaphase chromosome complements by employing the improved squash method as applied in ants (Imai & Kubota, 1972, & Imai, 1974).

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In 1974, Imai and Kubota (Imai & Kubota, 1972); and Imai (Imai, 1974) have investigated the holocentric nature of silkworm chromosomes in both *Bombyx mori* and *Bombyx Mandarina*. Though several cytological techniques are currently available for the preparation of silkworm chromosomes using larval as well as embryonic cells at different stages of development (Tazima, 1938, Murakami & Imai, 1974, Traut, 1976, Ito, 1977, Lakshnmikumari & Jayaprakash & Ananthanarayana, 1994, Mahesha, 1997, & Mahesha & Honnaiah, 2002), this paper presents a rapid air dry technique to prepare the meiotic chromosomes for laboratory work.

MATERIALS AND METHODS

A silkworm strain, NB_4D_2, a tropical bivoltine, was selected as an experimental system in the present investigation. Disease free layings of the silkworm strain were obtained from the germplasm of the Department of Studies in Sericultural Sciences, University of Mysore, Mysore, and the silkworm rearing was conducted in the laboratory as described by Krishnaswami (Krishnaswami, 1978). Male larvae were dissected out and the testes were dipped immediately in freshly prepared 0.92% Potassium chloride (KCl) solution. Just before treatment with hypotonic solution, the cells from the testes were squeezed out by pressing with grooved forceps. After treating for 20min in hypotonic solution at 37°C, the cells were centrifuged at about 3000 rpm for 5min and the supernatant was discarded. The pellet obtained was dispersed in fresh hypotonic solution and incubated at 37° C for another 25 min. After incubation, the pellet was fixed in freshly prepared fixative (acetic acid: methanol, 1: 3; v/v). After 20 minutes, centrifuged at 1000 rpm for 10 min and supernatant was discarded. Then, the pellet was re-suspended in the required quantity of fixative and 4-5 drops were dropped over a clean slide from a distance of ~30 cm, air dried and stained for 45 min in 12% Giemsa stain in Sorenson's phosphate buffer (pH 6.8). The slides were then washed in distilled water, dried on a slide warmer and mounted in DPX with cove glass. Slides were observed under 1000x magnification and microphotography was carried out with Leitz photomicroscope equipped with 35mm Leica camera. Photography was made on 35mm Nova 125 ASA black and white film. The negatives were processed in a small day light developing tank, following the conventional photographic procedures. The positive prints were made on Sterling Kodabromide printing paper of different grades (normal, soft, hard and glossy) depending on the contrast needed.

RESULTS AND DISCUSSION

This technique has proved highly useful in the preparation of well spread and well stained silkworm meiotic chromosomes (figure 1. A - I). Both temporary and permanent preparations of micro slides showed clear different meiotic stages of meiosis in addition gonial metaphase. This technique can also be conveniently used for the application of different banding techniques and also to study the effect of different mutagens on chromosome structure as studied by Mahesha (Mahesha, 1997); Mahesha and Honnaiah (Mahesha & Honnaiah, 2002). The preparation however showed that the chromosome number is 2n = 56 as reported by Tazima (Tazima, 1938) and Tanaka (Tanaka, 1953) or twenty eight bivalents. Further, gonial metaphase stages also showed that the 2n chromosome number is 56.

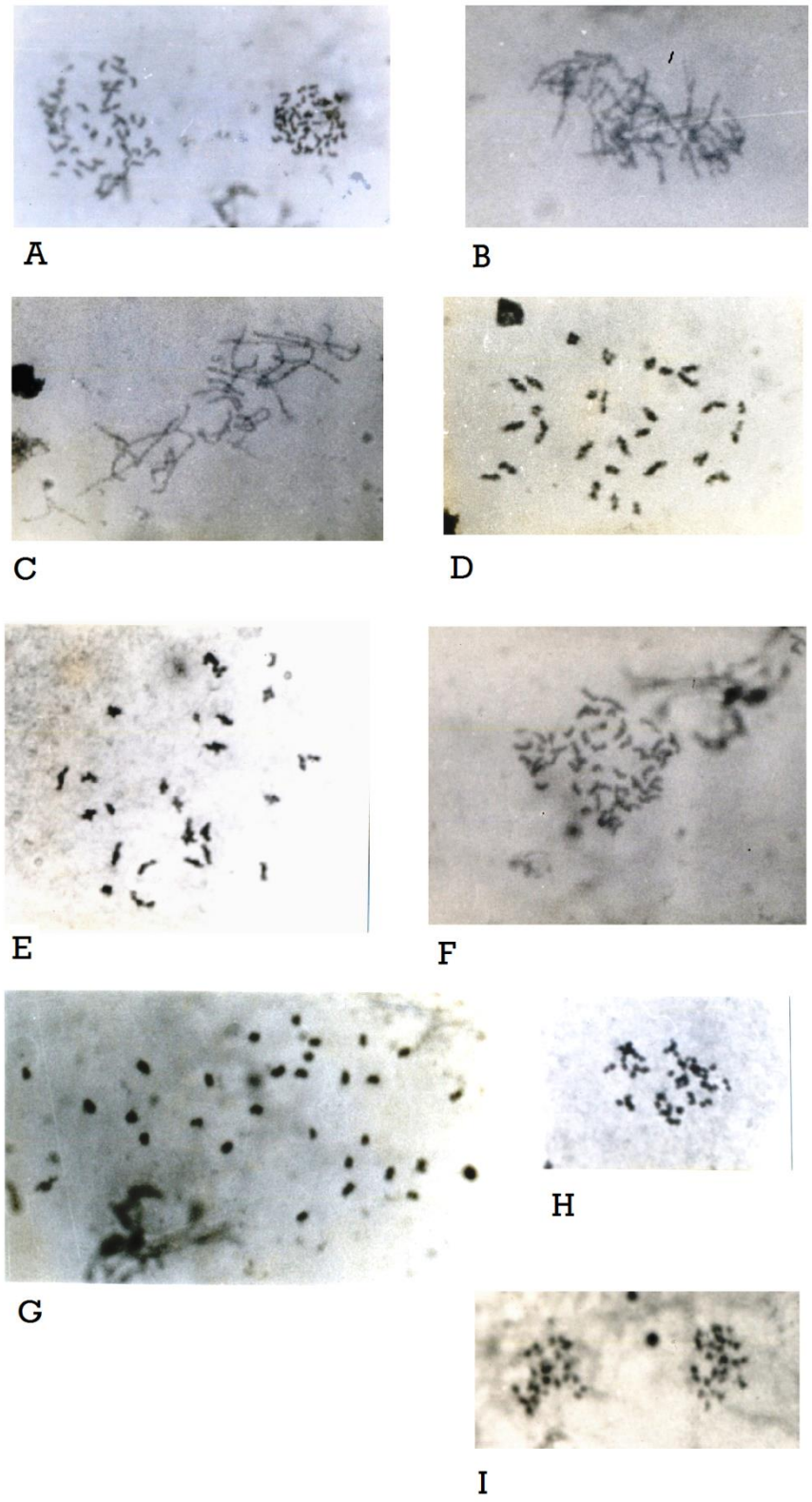

Figure 1. Meiotic Chromosomes in Silkworm
A. Gonial Metaphase (left - early, right - late), B. Zygotene (late)
C. Pachytene, E. Diakinesis, F. Diakinesis (late), G. Metaphase 1,
H. Anaphase 1, I. Metaphase 2.

CONCLUSION

The air dry technique for the preparation of meiotic chromosomes from the testes of silkworm *Bombyx mori* L., is very useful for regular laboratory work, application of banding techniques and to study the chromosomal aberrations with the influence of mutagens.

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The author declares no conflict of interest.

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Both authors contributed equally to establishing the topic of the research and design experiment.

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