A reinvestigation of the lecanoid chromosome system in *Kerria lacca* (Kerr)

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Abstract

A cytological reinvestigation of somatic cells and of spermatogenesis in the Indian lac insect, *Kerria lacca* (Kerr), confirmed a typical lecanoid chromosome system. There was no A-T rich constitutive heterochromatin in its genome although the facultatively heterochromatised haploid set of chromosomes in male somatic cells fluoresced brightly with Hoechst 33258 or Quinacrine dihydrochloride. The heterochromatisation of the haploid set of chromosomes was not reversed in gut or malpighian tubule cells of the male lac insect as occurred in some other lecanoid species.

Introduction

Different groups of coccids have evolved the most divergent and interesting chromosomal systems, such as the lecanoid, diapsidid, or comstockiella types (Hughes-Schrader, 1948; Brown, 1963; Nur, 1979). The lac insect is an economically important coccid due to its resinous secretion, the lac. Brown (1959) reported on chromosomes in some lac insect species of the genus *Tachardiella* which possess the lecanoid system.

In spite of previous cytological (Tulsyan, 1963; Dikshith, 1964, 1966) and genetic (Chauhan, 1970, 1977) studies, the cytogenetics of the Indian lac insect, *Kerria lacca* (Kerr) is not well understood. Indeed previous studies have provided incomplete and contradictory information on its chromosome system. Thus while Tulsyan (1963) reported an orthodox XX/XO sex-chromosome system, Dikshith (1964, 1966) studied spermatogenesis in *K. lacca* and suggested a lecanoid system like that in mealy bugs (Hughes-Schrader, 1948). On the other hand, Chauhan (1970, 1977) studied the inheritance and expression of certain body colour mutants in *K. lacca* and concluded that as in other lecanoids, the male lac insects breed as haploids, transmitting only the maternal genome. However, in their somatic cells they do not manifest the lecanoid system since both maternally and paternally inherited mutant alleles were found to be expressed in male offspring.

Somatic chromosomes of *K. lacca* have not been studied in detail, and so the present study was undertaken to resolve the chromosome system and the divergent claims about its cytogenetics. The present results confirm that the Indian lac insect has a typical lecanoid chromosome system.

Materials and methods

The lac insects were cultured on potted plants of *Meghania macrophylla* at the Institute's plantation at Namkum, Ranchi. In order to examine the somatic chromosomes, embryos were removed from gravid females, treated with colchicine (1 μg/ml) in 0.67% saline for 1 h and then placed in 1% sodium

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citrate for 10 min at 24°C. Following hypotonic treatment, they were fixed in aceto-methanol (1:3).

To obtain air-dried preparations (Lakhotia and Kumar, 1978) from individual embryos, each embryo was transferred to a drop of 60% acetic acid on a slide prewarmed to about 50°C, and the embryo was teased with fine needles to release the cells. After about 20 sec, drops of aceto-methanol were added around the acetic acid drop; the excess fluid was drained off and the slides allowed to dry in air.

After rinsing in absolute ethanol, redrying and treating with 4% Giemsa stain (5—6 min), the preparations were examined under a light microscope. Some preparations were stained with Hoechst 33258 (5 μg/ml) or quinacrine dihydrochloride (0.5 mg/ml) and examined for the fluorescence patterns of mitotic and interphase nuclei (Lakhotia and Kumar, 1978).

For examining the pattern of heterochromatin in somatic cells, the gut and malpighian tubules from different immature and adult stages were dissected out and were either lightly squashed in 2% aceto-orcein for observation under phase-contrast optics, or were first fixed in aceto-methanol and processed for air-dry preparations as described above for embryos. To study spermatogenesis, testes from late 1st or 2nd instar males were immediately fixed in aceto-methanol. Their air-dried preparations were made as above and treated with Giemsa stain.

Observations

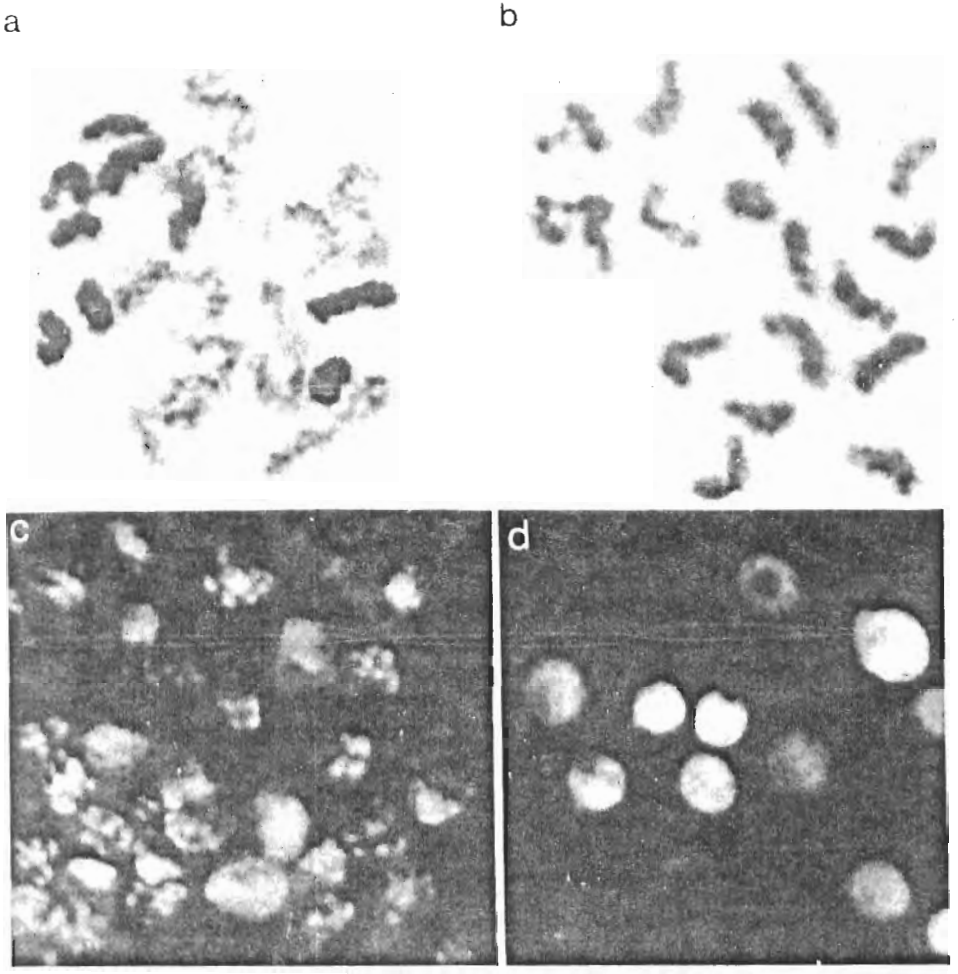
Heterochromatin in somatic cells

Cytologically, the different lac insect embryos were classified into two distinct types: (1) those having a prominent heterochromatic body in nearly all interphase nuclei, and (2) those without heterochromatin in any nucleus. In preparations of type 1 all the metaphase plates had euchromatic and heterochromatic haploid sets of chromosomes (Figure 1a), while in type 2 all the chromosomes were euchromatic (Figure 1b).

In both types, the diploid chromosome number was 18 with no heteromorphic pair. As in other coccids, the chromosomes have a diffuse centromeric region and thus no primary constriction or centromere was identifiable in any chromosome. In many instances, particularly when the chromosomes were less condensed, they had a diffuse banded appearance.

In Hoechst 33258 or quinacrine dihydrochloride stained preparations, the interphase nuclei as well as the metaphase chromosomes from some embryos appeared uniformly dull when fluorescing (Figure 1d), while those in other embryos manifested a more brightly fluorescing mass in interphase nuclei (Figure 1c) and an intensely fluorescing haploid set of chromosomes during mitosis. The other haploid set of chromosomes in these mitotic cells appeared uniformly dull when fluorescing. No other chromosomes or chromosome regions showed brighter fluorescence in either types.

Preparations of gut and malpighian tubules from different individuals of the 1st, 2nd or 3rd instar stages had either all interphase nuclei with a



Figures 1a and **1b** Giemsa stained metaphase chromosomes from male (Figure 1a) and female (Figure 1b) embryos of *Kerria lacca*. Note that one haploid set is darkly stained in the male but not in the female embryo. x 3,000.

Figures 1c and **1d** Hoechst 33258 fluorescence in preparations of male (Figure 1c) and female (Figure 1d) embryos showing a single or several brightly fluorescing regions in Figure 1c but a uniform fluorescence in Figure 1d. x 2,200.

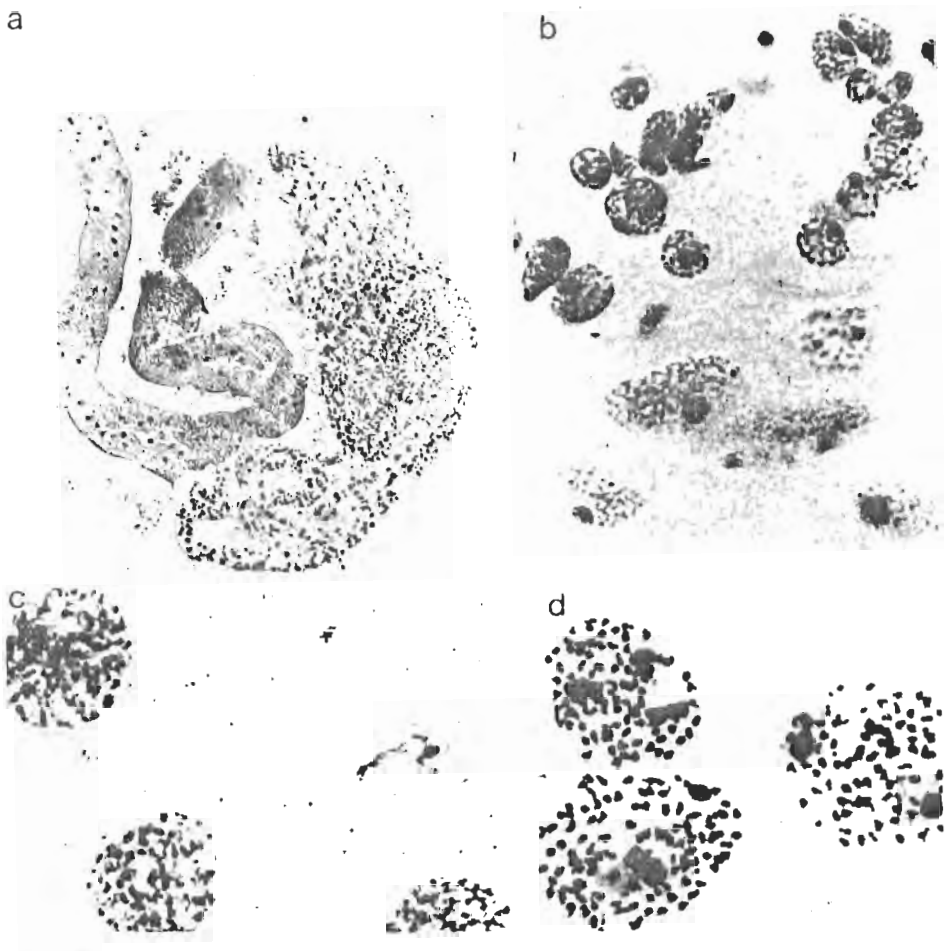


Figure 2a (top, left) Part of the gut and malpighian tubules from a 2nd instar male lac insect (orcein stained light squash); a small region of the same was magnified in **Figure 2b** (top, right) to show the dark staining heterochromatic body in all nuclei. Figure 2a, x 80; Figure 2b, x 1,200.
Figures 2c (bottom, left) and **2d** (bottom, right) Giemsa stained polyploid nuclei from gut region of female (Figure 2c) and male (Figure 2d) lac insects. x 1,200.

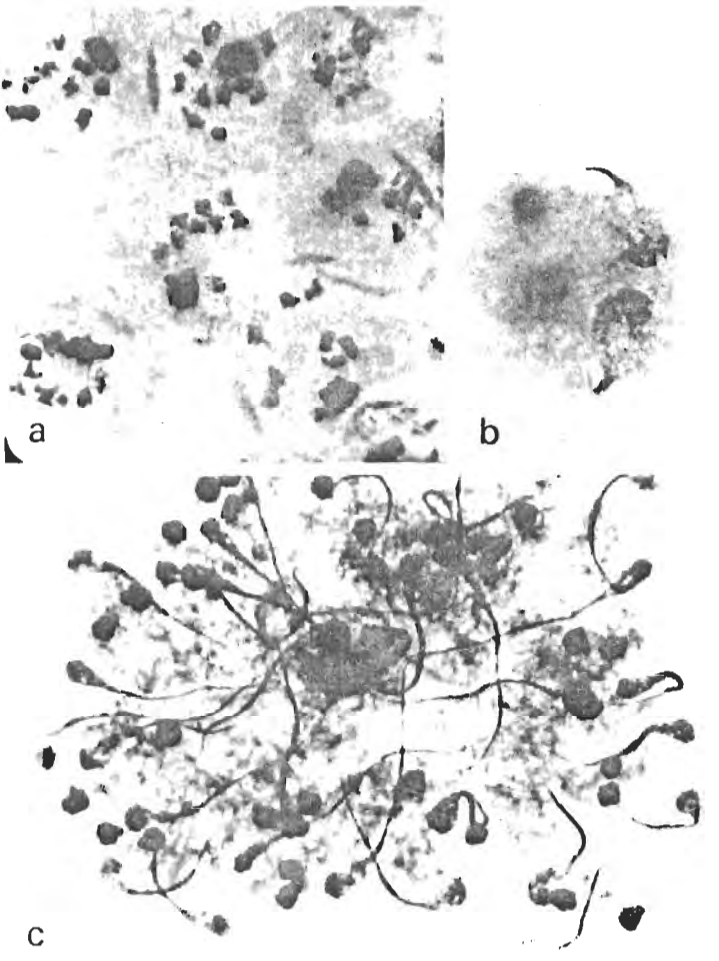


Figure 3 Lecanoid spermatogenesis in *K. lacca*.
Figure 3a Late prophase stages with nine euchromatic chromosomes and a dense cluster of heterochromatic chromosomes.
Figure 3b A tetranucleate cell with two heterochromatic and two euchromatic nuclei which are beginning to grow into spermatozoa.
Figure 3c A bundle of growing spermatozoa specifically associated with the euchromatic nuclei while the dense heterochromatic nuclei remain inactive. x 1,600.

prominent heterochromatic body (Figures 2a,b) or were all without heterochromatin. In 2nd and 3rd instar stages when the sexes can be unambiguously distinguished, the interphase heterochromatin was seen in gut and malphigian tubule nuclei of male individuals only. In view of this correlation, the preparations of gut and malpighian tubules and of embryos were classifiable into male and female depending upon the presence or absence, respectively, of the heterochromatin. Thus, the somatic chromosomes in *K. lacca* follow the lecanoid pattern in terms of heterochromatisation of the haploid set of chromosomes in males but not in females.

Nuclei of various sizes were seen in malpighian tubules and gut from insects of all ages. Some of these appeared distinctly polyploid with a large number of chromosomes (Figures 2c and 2d). It is interesting to note that in males, such polyploid nuclei usually showed two or three heterochromatic bodies (Figure 2d), which appeared smaller relative to the euchromatic chromosomes.

Spermatogenesis

The present observations (Figure 3) show that spermatogenesis in *K. lacca* follows a typical lecanoid pattern (Hughes-Schrader, 1948; Dikshith, 1964). The two haploid sets of chromosomes were found to be distinct from the very early stages of meiosis (Figure 3a) and during the 2nd division anaphase, the hetero- and euchromatic chromosomes were segregated. As in other lecanoids, the four products of meiosis formed a tetranucleate stage with two heterochromatic and two euchromatic nuclei (Figure 3b). At a later stage, sperms were seen to be developing only around the euchromatic nuclei (Figure 3c).

Discussion

The present study confirms that the Indian lac insect displays a typical lecanoid chromosome system as found in other lac insect species by Brown (1959). In somatic cells of males, but not females, one haploid set of chromosomes is consistently heterochromatised. During meiosis, this set is segregated to be subsequently not included in developing sperm and is thus not transmitted to the next generation. Preliminary observations following paternal X-irradiation suggest that the heterochromatised haploid set of chromosomes in male offspring is paternal in origin.

The absence of any more brightly fluorescing region(s) in interphase nuclei and metaphase chromosomes from females stained with Hoechst 33258 or quinacrine dihydrochloride suggests that there is no A-T rich constitutive heterochromatin in *K. lacca*. The brighter fluorescence of the chromocentre in interphase nuclei and of a haploid set of chromosomes in metaphase plates in these male embryos is apparently related to a greater condensation because of their being facultatively heterochromatised (Brown, 1966). Indeed, it is known that more condensed chromosome regions show brighter Hoechst 33258 or quinacrine dihydrochloride fluorescence (Das *et al.*, 1979).

The cytological data may thus appear at variance with Chaunhan's (1970, 1977) conclusion based on genetic data that the paternally derived chromo-

somes are active in somatic cells of male as well as female progeny. The apparent expression of some paternally derived mutant alleles in male offspring led Chauhan (*op. cit*) to suggest 'that the unorthodox genetic system of *K. lacca* differs from the lecanoid system in gene expression'. However, these findings are not necessarily in conflict with the lecanoid system since in mealy bugs also a limited expression of the paternal genome is essential for the normal development of male offspring (Nur, 1967, 1979).

Moreover, earlier studies in mealy bugs (Brown and Nur, 1964; Nur 1967) have documented the reversal of heterochromatisation in several tissues of males. A comparable situation in the lac insects could perhaps explain the expression of paternally derived body colour genes in male offspring (Chauhan, 1970, 1977). However, it remains to be seen that reversal of heterochromatisation does occur in certain cell types of the male lac insect. Our observations in the lac insect show that unlike in mealy bugs (Nur, 1967), there is no reversal of heterochromatisation in malpighian tubules or hind gut cells.

Other cell types have yet to be studied for reversal of heterochromatisation. In this context, it is not known whether the relatively smaller number of heterochromatin bodies in the highly polyploid cells (Figure 2d) is due to under replication of heterochromatic chromosomes during polyploidisation (Nur, 1966) or is due to a partial reversal of heterochromatisation.

Certain genes on the facultatively heterochromatised X-chromosome in somatic cells of female mammals are known to escape inactivation (Marshall Graves, 1983). Since like the mammalian X chromosome, the paternal genome in male lac insects is also facultatively heterochromatised (Brown, 1966), it is possible that as in the former, certain genes may escape inactivation in spite of the apparent heterochromatisation of the entire haploid set contributed paternally. Indeed, these genes could also cause an apparent diploid expression in males as found by Chauhan (1970, 1977). More cytogenetic information is thus needed to understand gene expression in lac insects.

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