Proc. Indian Acad. Sci., Vol. 83 B, No. 4, 1976, pp. 139-142

C-banding in male meiotic chromosomes of *Poekilocerus pictus* (Acrididae: Orthoptera)

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MS received 2 December 1975

ABSTRACT

Studies on the C-heterochromatin during the meiosis in the Indian grasshopper *Poekilocerus pictus* has been carried out using the BSG technique presumably for the first time. It is shown that the genome of this grasshopper is endowed with large amounts of repetitive DNA in the centromeric, interstitial and telomeric regions. This Acridid under study differs in having distinct centromeric, interstitial and telomeric blocks in majority of the chromosomes from the British Acridid *Myrmeleotettix maculatus* which has interstitial bands only in the X-chromosome and the B-chromosome but not in the autosomes.

1. INTRODUCTION

A SURVEY of the literature has revealed that a host of investigators have contributed towards our understanding of the distribution of heterochromatin in mammalian chromosomes.[1,2] But very little work has been done on the heterochromatin in insect chromosomes—For example in *Gryllus argentinus*,[3,4] in the British grasshopper *Myrmeleotettis maculatus*,[5] in the desert locust *Schistocerca gregaria*,[6] in *Dichroplus silveiraguidoi*[7] in *Acheta domesticus*.[8] As there is no information available with regard to the heterochromatin pattern in the Indian grasshoppers, this project was undertaken. The present paper deals with the localisation, structure and behaviour of C-heterochromatin during meiosis in the Indian grasshopper *Poekilocerus pictus*.

2. MATERIAL AND METHODS

Males of *Poekilocerus pictus*, a short-horned grasshopper collected from Manasa Gangotri, Mysore (India) formed the material for study whose cytology has been extensively worked out ($\delta 2n = 18 + X$) with 2 pairs of long (L) and 7 pairs of medium (M) chromosomes and the X-chromosome being the longest of the complement. Testes were dissected out from the animals and treated with hypotonic 0·9% sodium citrate solution for

45 minutes. The material was fixed in 1:3 acetic acid/methanol. After three changes of fresh fixative the slides were flame dried.

The staining method used is the barium hydroxide-saline-Giemsa (BSG) technique of Sumner[9] with minor modifications; that is after denaturing in saturated barium hydroxide for 15 minutes at room temperature, briefly rinsed in 0·02N HCl and distilled water, and air dried the slides. After reassociating in 2 × SSC at 60–65° C for 3 hours in a petridish, briefly rinsed giving three changes of distilled water, stained in buffered (pH 6·8) Giemsa, and mounted in DPX.

3. OBSERVATIONS

Using this technique the pattern of C-heterochromatin can be studied from the spermatogonial prophase through the spermatogonial metaphase up to the second meiotic anaphase. Darkly stained heterochromatin blocks can be seen in the gonial prophase chromosomes (figure 2). In the gonial metaphase plates the C-heterochromatin can be clearly seen at the centromeric regions. Along with the C-bands there are also C-positive heterochromatin blocks in the intercalary and terminal regions of the chromosomes. The long X-chromosome has six distinct heterochromatin blocks which has been designated by the authors as "Zebra" chromosome (figures 1 and 3).

During the preleptotene stage (figure 4) many heterochromatin blocks of variable sizes are found distributed all over the nucleus. The spring-like X-chromosome is seen projecting out with the distinct heterochromatin blocks. In leptotene (figure 5) the thread like chromosomes have distinct heterochromatin blocks. The pachytene stage (figure 6) is characterised by the paired heterochromatin blocks at some regions and at other regions fused to give the appearance of single blocks. Sometimes several heterochromatin pairs formed a coalesced mass. The centromeric heterochromatin is extremely conspicuous in the diplotene (figure 7). The demarcation of the heterochromatin blocks is clearly seen in the X-chromosome. Constitutive heterochromatin is faintly detectable at diakinesis. Centromeric and interstitial heterochromatin blocks are visible in anaphase I. Constitutive C-positive heterochromatin blocks are noticed at metaphase II (figure 8) and anaphase II (figure 9) at the centromeric, telomeric and intercalary regions. In all these stages centromeric and telomeric heterochromatin blocks are more conspicuous than the intercalary heterochromatin blocks. The heterochromatin segments have been measured using a dial calipers and the percentage is found to be 64·42 (table).

4. DISCUSSION

With the more commonly used stains such as Hematoxylin or Feulgen, the centromeres and other heterochromatin blocks are not differentially

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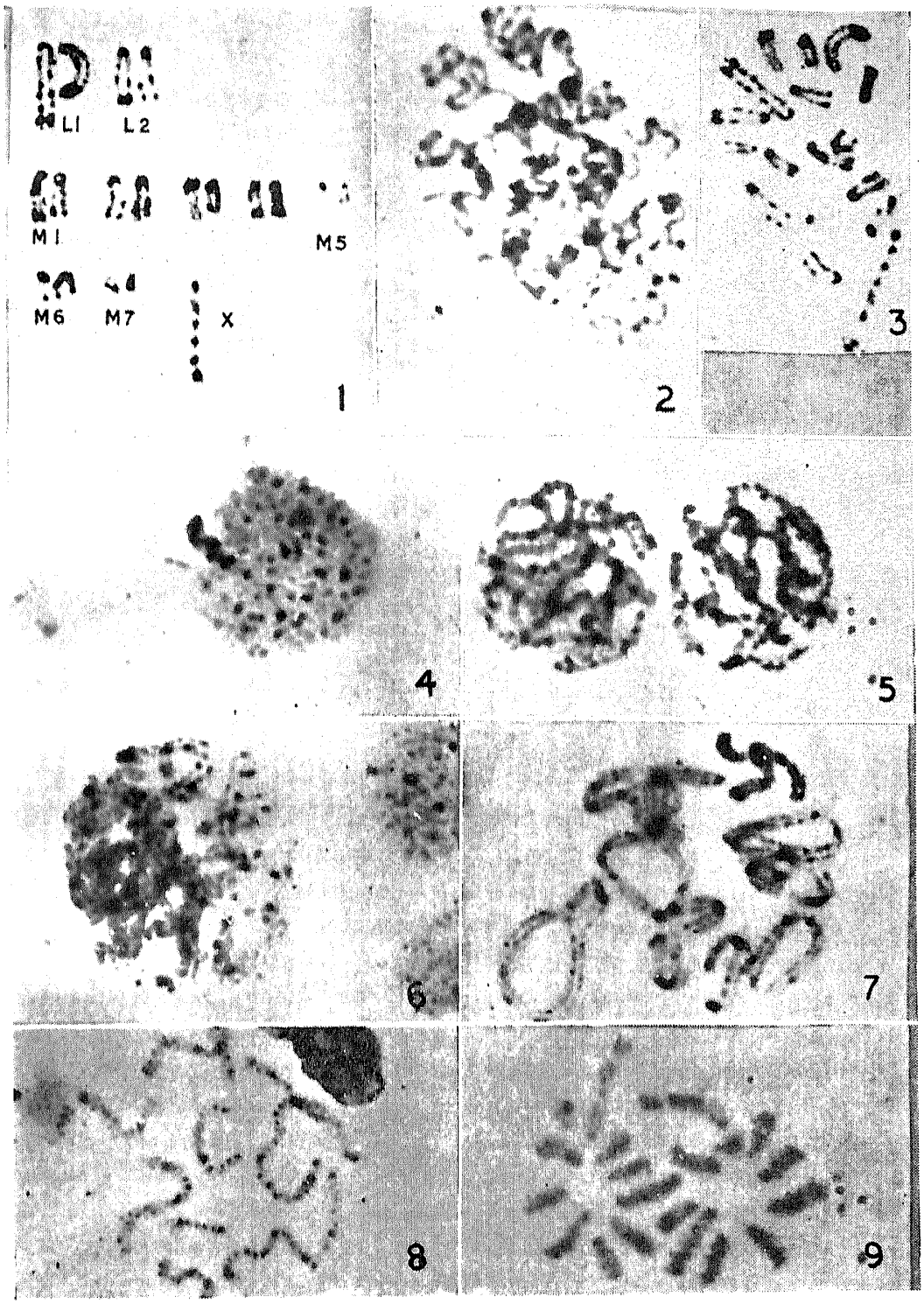

Figures 1–9. Fig. 1. Banded karyotype of *Poekilocerus pictus* constructed from the metaphase plate shown in Fig. 3, × 800. Fig. 2. Spermatogonial prophase showing blocks of heterochromatin, × 800. Fig. 3. Spermatogonial metaphase showing the distribution of constitutive heterochromatin, × 800. Fig. 4. Blocks of heterochromatin in the preleptotene stage, × 800. Fig. 5. Leptotene showing the heterochromatin, × 800. Fig. 6. Heterochromatin in pachytene, × 800. Fig. 7. Diplotene chromosomes differentially stained with Giemsa, × 800. Fig. 8. Metaphase II showing heterochromatin, × 800. Fig. 9. Differential staining of the anaphase II chromosomes, × 800.

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Table 1. Showing the lengths of heterochromatin segments in different chromosomes.

Chromosome number	Total chromosome length (TCL)	Length of the heterochromatin segments
L_1	17·95	15·25
L_2	11·55	7·20
M_1	10·32	6·00
M_2	9·40	7·40
M_3	8·42	3·80
M_4	7·90	5·70
M_5	7·40	2·10
M_6	6·00	2·50
M_7	3·97	2·30
X	23·80	16·50
TOTAL ..	106·71	68·75

Percentage heterochromatin in the genome = 64·42.

stained. So the C-banding technique using Giemsa stain has become very useful for the visualization of the centromeric regions throughout meiosis. Interstitial and telomeric heterochromatin have been described in many species—both among animals (mammals and insects) and plants. Among mammals, Chen and Ruddle in mouse,[10] Bianchi and Ayres in green monkey,[11] among Orthoptera; centromeric and telomeric blocks have been described in *Acridium japonicum*,[12] in *Gryllus argentinus*,[3] in *Dichroplus silveiraguidoi*.[7] Centromeric and interstitial blocks have been described in the X-chromosome and B-chromosome of *Myrmeleotettix maculatus*.[5] In the plant material also the centromeric and interstitial blocks have been observed in the chromosomes of *Scilla sibirica*.[13] In the present material the densely stained regions characterise the presence of as large fraction of the repetitive DNA of the genome. Along with the centromeric heterochromatin there are C-positive telomeric and intercalary block which suggest the occurrence of a high percentage of redundant DNA in *Poekilocerus pictus*. In *Myrmeleotettix maculatus*, Gallagher *et al.*[5] have described the presence of faint interstitial heterochromatin bands only in the two arms of the metacentric B-chromosome and in the X-chromosome but not in the autosomes. But in *Poekilocerus pictus* the telomeric and interstitial heterochromatin blocks are present in majority of the autosomes. and in particular the X-chromosome has distinct heterochromatin blocks.

It has been observed by Arrighi *et al.*[14] that in animals in which the X-chromosome is large like that in the mammal *Microtus agrestis* a large amount of constitutive heterochromatin is present in the sex chromosome. Strangely in the grasshopper *Poekilocerus pictus* studied here the X-chromosome is very large and also contains large amount of heterochromatin. If this view is accepted as a generality, then it is possible that inversions and translocations must have occurred in the karyotypic evolution of this Acridid which gives meaning to the type of occurrence and distribution of C-positive telomeric and interstitial heterochromatin.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. N. V. Aswathanarayana and Mr. K. L. Satya Prakash for the technical advice. One of us (KRK) is grateful to Council of Scientific and Industrial Research for the financial assistance.

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