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A Chromosome Morphology of Meiotic Prophase in Grasshopper Spermatocytes, *Chorthippus bicolor* (Acrididae : Orthoptera)

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Abstract : The meiotic chromosomes of *Chorthippus bicolor* were studied from squashed preparations of testicular follicles. Seventeen chromosomes were karyotypically analysed in spermatogonial cells. In the course of observations with spermatocytes in early prophase an interesting feature of a partial heterochromatic bivalent has been seen. This characteristic feature on a bivalent constantly appeared through early prophase, from zygotene to diplotene stages. From the pachytene karyogram, the bivalent was identified as the telocentric element of No. 6. The condensed segment localized on one distal thirds of the No. 6 bivalent and the chromosome tended to associate with heterochromatic X element through prophase stages. The significance of the peculiar type of bivalent has not been defined yet.

Introduction

In the history of animal cytogenetics, insects contributed enormously to the development of chromosome research as a superb test object. Consequently there have been very large number of publications on insect chromosome cytology, especially on spermatocyte chromosomes of grasshoppers, Acrididae (Makino 1956).

Excellent visibility of meiotic processes in grasshopper testes provided us many interesting aspects that had been studied and analysed cytologically at the level revealed by ordinary microscopy (White 1973). However, observations of which previously have done focused mostly on the stages of diplotene and diakinesis with special attention to the chiasma formation.

Among the Japanese Acrididae, *Chorthippus* species offers an advantageous material for observing meiotic prophase chromosomes due to their lowest number of the chromosomes. In the course of the present observations with a common species we analysed pachytene chromosomes which gave us an interesting features in elongated chromosome condition.

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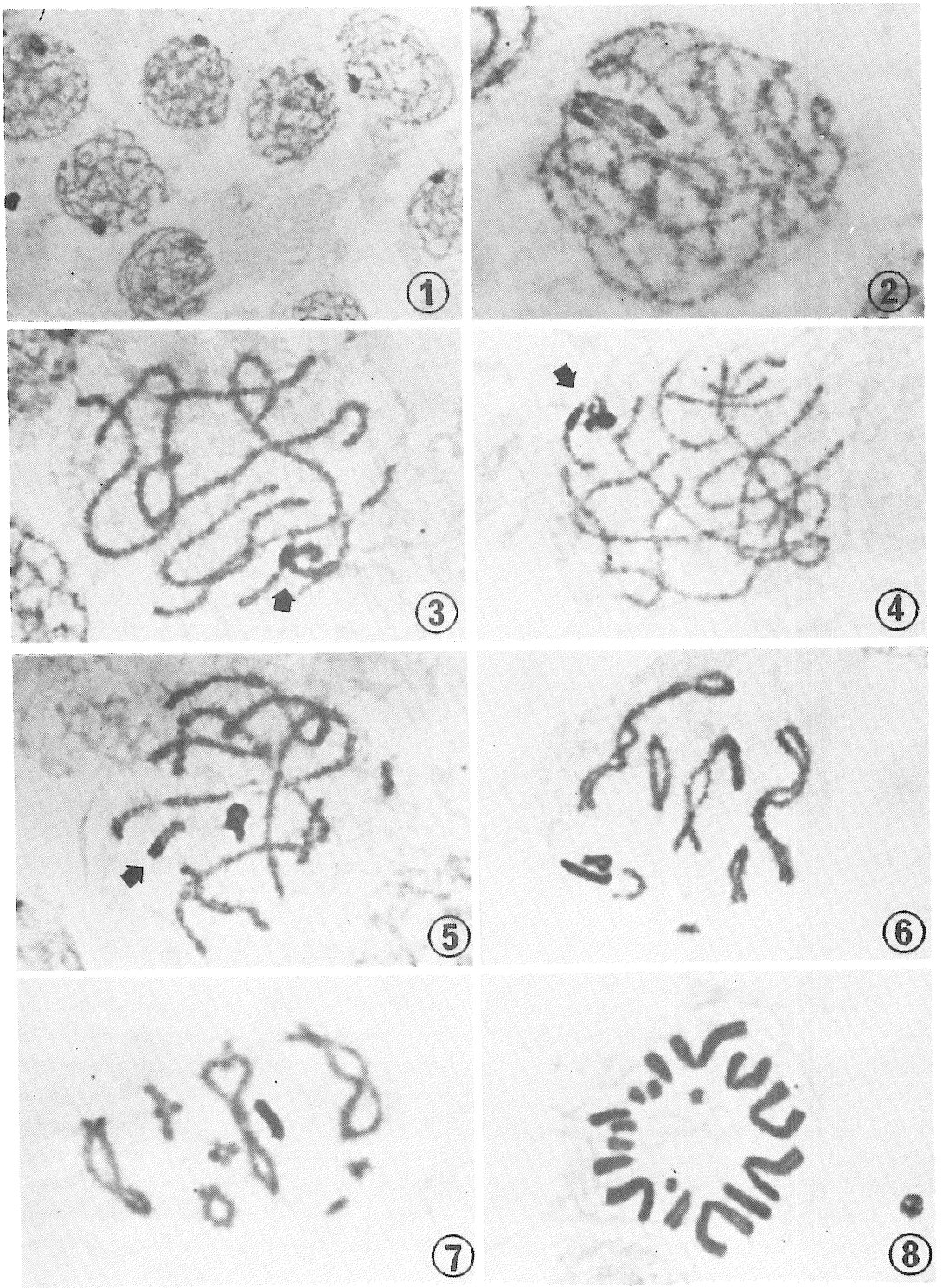
Materials and Methods

Male grasshoppers of *Chorthippus bicolor* Charpentier were collected at about 800 meters elevation at Mt. Daisen, Tottori-ken. Testis follicles were isolated from animals immediately after collection, cleaned fat body, and fixed in ethanol acetic acid (a 3 : 1 mixture). Fixed materials were stocked in the refrigerator at -20°C until making squash preparation. Fresh materials were also directly squashed in acetic orcein after hypotonic pretreatment. The stock materials were squashed in 45% acetic acid. Some satisfactory slides were made permanent by the dry-ice method of Conger and Fairchild (1953) ; the cover glasses were removed after freezing on a dry ice and dipped into the absolute ethanol, dried in air and stained with carbol fuchsin (Carr and Walker 1961).

Almost complete series of male meiosis stages was secured from a single individual collected in the end of June. Diploid chromosome count was also possible in spermatogonial cells without colchicine treatment.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5
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8

Observations

The diploid number of the chromosomes was 17 in male (Fig. 8), which coincides with earlier investigations (Momma 1943, Inoue 1973). The karyotype consisted of 8 pairs of autosomes and a X element ; a pair of large submetacentrics, two pairs of metacentric elements and five smaller pairs of telocentrics. The sex chromosome was distinguished by the largest size among telocentric elements (Fig. 9). The morphological definition, which was obtained from the squash preparation of spermatogonial cells without colchicine treatment, indicated a slight dissimilarity to the Inoue's description (1973) on the largest three pairs of autosomes.

In well-dispersed pachytene nuclei the precise identification of each bivalent was possible, which permitted to establish the pachytene karyotype (Figs. 10 & 11). The chromomere arrangements on each bivalent at zygotene and pachytene were seen. But it was hardly possible to demonstrate a regularity of the arrangements in the karyograms by the ordinary staining.

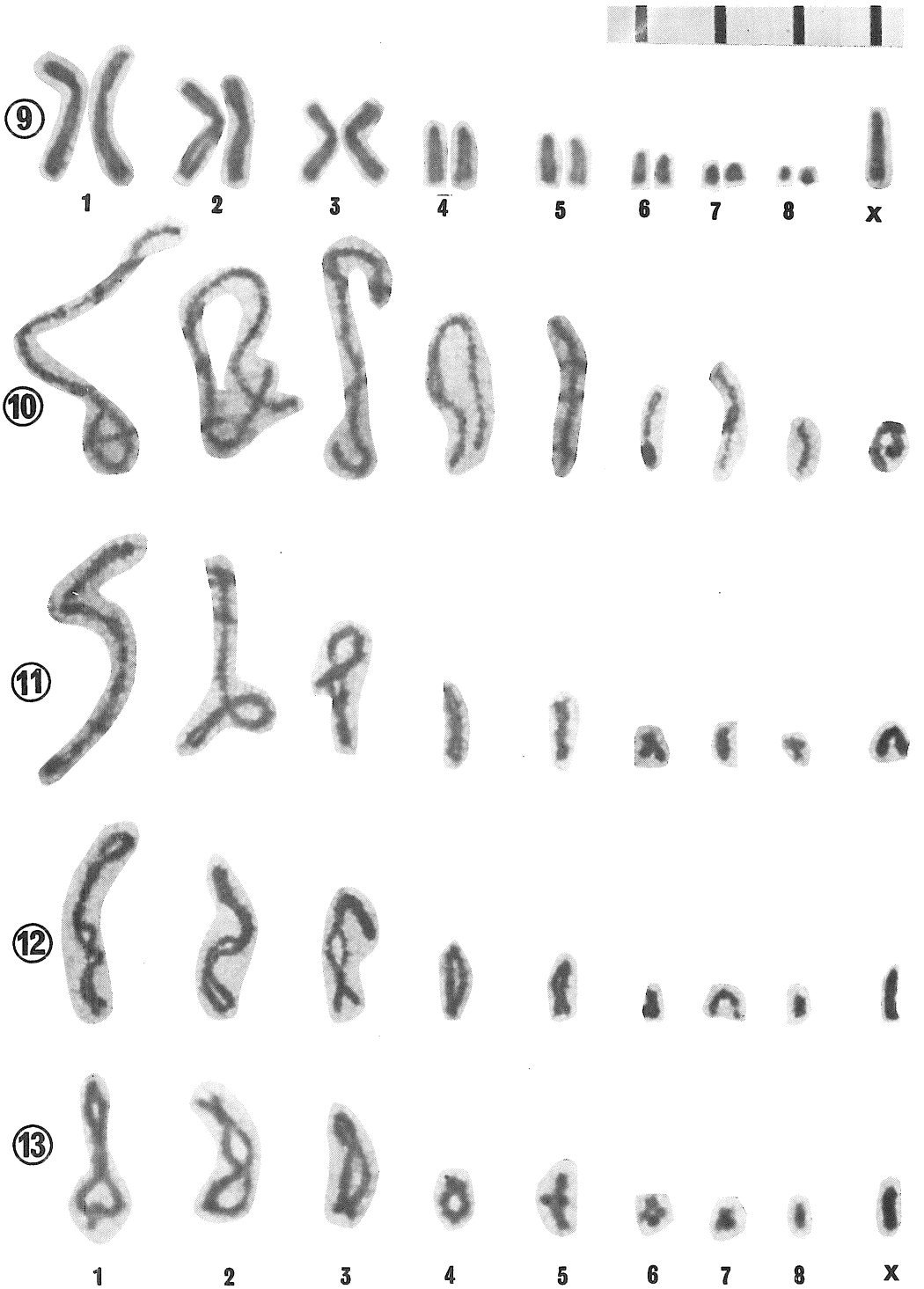
Careful observations determined the number of chiasmata in each bivalent at diplotene (Figs. 7 & 13). Three to five chiasmata were counted in the three largest elements numbered 1 to 3. The bivalent No. 4 usually had two chiasmata ; one is of proximal and another is distal chiasma. Elements Nos. 5 to 8 carried a single chiasma.

An interesting feature has been seen at early meiotic prophase of spermatocytes. Beside heavily stained structure of the X chromosome, so-called heteropycnotic X, a partially condensed bivalent has been found. The characteristic feature on a bivalent constantly appeared through early prophase and has clearly shown in the pachytene stage. From the pachytene karyogram the bivalent was identified as the telocentric element of No. 6 (Fig. 10) ; the one distal thirds segment of the element presented obvious heterochromatic feature (Figs. 4 & 5). In contrast, leptotene and interkinetic nuclei showed only single condensed mass of heteropycnosis which was a common feature of meiotic prophase (Figs. 1 & 2). This partial heterochromatic bivalent tended to associate with heteropycnotic X through early prophase (Figs. 3 & 4). And the bivalent located mostly adjacent to X element at diplotene nuclei (Fig. 6). Cells of late diplotene at which the opening-out of bivalents would indicate that the condensed segment on the bivalent located more distal part of chromosome arm than the chiasma loci (Fig. 13).

In the anaphase separation at the first meiotic division, the partial heterochromatic bivalent separated in the regular manner and there was no significant difference in the movement at the subsequent stages among other bivalents.

Figures 1 to 7. Meiotic prophase nuclei from grasshopper spermatocytes, *Chorthippus bicolor*. The microscopical preparation was made by the squash technique and carbol fuchsin stain. Upper left scale on the photo plate indicates the magnification of Fig. 1 and upper left one for Figs. 2 to 8. One section of the scale corresponds to 10 μm. Fig. 1. Leptotene nuclei. Chromosomes are very long slender threads and a heavily condensed mass is seen in each cell. Fig. 2. Zygotene nucleus. Some chromosome threads are visibly double, indicating homologous chromosomes become closely approximated side-by-side. Heterochromatic chromosome appeared as a condensed mass is identifiable. Figs. 3 to 5. Pachytene nuclei. Eight bivalents are counted, heterochromatic X shows heavily stained and a partial heterochromatic bivalent (arrow) located beside X chromosome. Fig. 6. Early diplotene cell. Chiasmata become visible and contracted feature of all bivalents is characteristic at this stage. Fig. 7. A side view of late diplotene cell. A partial heterochromatic segment on the bivalent No. 6 is still identifiable.

Figure 8. A polar view of spermatogonial metaphase chromosomes (2n=16+X).


⑨
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⑬
1 2 3 4 5 6 7 8 X

Discussion

There were three larger bivalents bearing a heavily stained segment seen at early diplotene stage. They may be referred to a kind of the partial heterochromatic bivalents. However their feature disappeared in pachytene or earlier stages except for the bivalent No. 6. Therefore we would emphasize the peculiarity of bivalent No. 6 which was consistently observed through meiotic prophase.

It is a generally known fact that the sex-chromosome mechanism in all the Japanese species of family Acrididae is characterized by male heterogamety of XO/XX system. The sex element in spermatocytes has remarkable feature of condensed stain through meiotic prophase. No other sex chromosome which is translocated to the autosome has been found in the species. Therefore a heterochromatic autosome undoubtedly recognized neither neo-X nor neo-Y chromosome which were constituted by the fusion of a sex chromosome and an autosome (*c. f.* White 1973).

However, more detailed comparison of male and female karyograms in meiotic prophase cells are required to appearance whether there is sex difference in exsistence of the characteristic bivalent or not.

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Figure 9. Male Karyotype constructed from the spermatogonial metaphase cell, consists of 8 pairs of autosomes and a single sex element.

Figures 10 and 11. Pachytene karyograms. The bivalents are arranged in order of decreasing length. The heterochromatic segment appears on the distal arm of bivalent No. 6. Fig. 10 constructed from the nucleus shown in Fig. 3.

Figures 12 and 13. Diplotene karyograms constructed from nuclei shown in Figs. 6 and 7, respectively. More contraction occurred in three larger elements than smaller bivalents. The stage characterized by the opening out of the paired homologues to form loops and nodes. Nodes represent chiasmata. The scale on upper right of the plate indicates the magnification of Figs. 9 to 13. One section corresponds to 10 micra.