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Achiasmatic male meiosis in *Cimex* sp. (Heteroptera, Cimicidae)

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Abstract - Chromosomes and their behaviour in spermatogenesis in *Cimex* species (Heteroptera, Cimicidae) from a bat species *Myotis emarginatus* were studied by using both a conventional staining method and sequence-specific fluorochromes CMA_3 and DAPI. Spermatogonial metaphase revealed 28 autosomes, one large Y chromosome and four small X chromosomes. The Y was almost fully C-band positive as well as most telomeres of autosomes. The same regions appeared to be CMA_3 and DAPI positive, indicating that in heterochromatin GC-rich clusters are dispersed within AT-rich repeats. In meiosis homologous chromosomes in bivalents are physically associated with one or seldom two sites or collochores, i.e. meiosis is achiasmatic. In MI cells chromosomes in bivalents were aligned in parallel, but at early AI bivalents open out and chromosomes move to the poles one telomere foremost. Meiosis is post-reductional for the sex chromosomes.

Key words: achiasmatic male meiosis, C-banding, Cimicidae, fluorochrome sequence-specific staining, Heteroptera, holokinetic chromosomes.

INTRODUCTION

Achiasmatic meiosis is invariably restricted to the heterogametic sex, and is known to occur sporadically among various insect orders, e.g. Mecoptera (ULLERICH 1961), Orthoptera (WHITE 1965a,b), Trichoptera (SUOMALAINEN 1966), Lepidoptera (SUOMALAINEN *et al.* 1973), Diptera (WHITE 1973) and Coleoptera (SERRANO 1981). In Heteroptera, the existence of achiasmatic meiosis has been revealed in five families, belonging to two infraorders: Leptopodomorpha, in family Saldidae (NOKKALA and NOKKALA 1983) and Cimicomorpha, in families Nabidae (NOKKALA and NOKKALA 1984), Miridae (NOKKALA and NOKKALA 1986a), Anthocoridae (NOKKALA and NOKKALA 1986b), and Microphysidae (NOKKALA and GROZEVA 2000). The occurrence of achiasmatic meiosis provides an important cytological marker for the understanding of phylogenetic affinities among and within different taxa.

The family Cimicidae belongs to the infraorder Cimicomorpha and includes ectoparasites of bats, man, and some birds (e.g. USINGER 1966). The karyotype of different *Cimex* species has been extensively examined in different aspects, e.g. the occurrence of multiple sex chromosomes, the behaviour of the chromosomes in experimental hybrids and others (see UESHIMA 1979), but there is only little information on the pattern of male meiosis in Cimicide species.

In the present study, the behaviour of meiotic chromosomes in a population of a *Cimex* species from Bulgaria was studied, paying special attention to the presence or absence of chiasmata.

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MATERIALS AND METHODS

Adults and nymphs of a *Cimex* species were collected in Reserve Ropotamo (Bulgaria, Burgas District, village Primorsko) from a bat species *Myotis emarginatus* in August 2000. Based on morphological characters, the pubescence of the paragenital sinus, the shape of pronatal anterior expansions, Dr J. Pericart suggests these specimens to belong to the *Cimex lectularius - C. columbarius* species group. Specimens were fixed alive in 3:1 ethanol:acetic acid mixture and stored in fixative. Squashes were made in a drop of 45% acetic acid. The cover slips were removed with dry ice method, after which slides were dehydrated in fresh fixative for 15 min, and air-dried. Majority of the slides were stained according to the Feulgen-Giemsa procedure (GROZEVA and NOKKALA 1996). Some of the slides were pretreated for C-banding and stained with GC-specific fluorochrome chromomycin A_3 (CMA_3) and with AT-specific fluorochrome DAPI (4'-6- diamidino-2-phenylindole) following the description of SCHWEIZER (1976) with slight modifications. To improve staining reaction the staining solutions contained 5% methanol and to prevent fading of CMA_3-fluorescence the mounting medium included 1% n-propyl-gallate. After fluorochrome staining, slides were washed twice in 70% ethanol for 30 min and stained with 4% Giemsa for C-banding.

RESULTS

In *Cimex* males, high chromosome number and the highly complicated behaviour of the chromosomes in meiosis make cytological analysis difficult. The spermatogonial metaphase contains 28 autosomes and five sex chromosomes, among them one is extremely large (Fig. 1). At this stage, almost all chromosomes show DAPI positive telomeric blocks. In prophase, the large sex chromosome could be seen in every plate (Fig. 2). This chromosome is almost fully heterochromatic after C-banding, and several large C-positive bands are visible in other chromosomes. This finding is confirmed by DAPI staining (Fig. 3). After chromomycin A_3 (CMA_3) staining, the large sex chromosome shows huge telomeric bright bands and one interstitial band (Fig. 4). When chromosomes condensed out from the diffuse stage, the homologues of bivalents have opened out and in some cases a thin thread-like structure connecting homologous chromosomes could be seen (Fig. 5). The connecting threads represent collochores comparable to those described in *Drosophila melanogaster* male (COOPER 1964) and found later in Miridae species (NOKKALA and NOKKALA 1986a). In metaphase I (MI), the 14 autosomal bivalents and the sex chromosomes form a ring (Fig. 6). Within the bivalents, the homologous chromosomes lie parallel, facing opposite poles. In anaphase I (AI), chromosomes open out and move with one telomere foremost towards the poles (Fig. 7). The chromosomes show telokinetic activity. Second metaphase (MII) plate is radial and in the center of the plate, the sex chromosomes show typical touch and go pairing. In anaphase II (AII), the large sex chromosome goes alone to one pole, and four small sex chromosomes go to the other pole (Fig. 8).

DISCUSSION

There are numerous papers on the behaviour of the chromosomes in both male and female meiosis in different populations of *Cimex lectularius* (see UESHIMA 1979). The standard chromosome formula of these species is $2n=26+X_1X_2Y$ with high polymorphism in the number of X chromosomes, ranging from 2 to 15 in males. The same chromosome formula has been reported for a closely allied species *C. columbarius*. Among other eleven *Cimex* species karyotyped, nine species display 28 autosomes and variable number of sex chromosomes, caused by multiple X chromosomes, and two species display lower chromosome number $2n=10+XY$ and $2n=11+XY$, respectively.

The specimens in the present study showed 14 autosomal bivalents, one very large and four small sex chromosomes. The large sex chromosome is expected to be the Y, as in the genus the multiple X system is prevailing. Thus, the chromosome formula in the males studied is determined as $2n=28+X_1X_2X_3X_4Y$. Evidently, the specimens do not belong to the *Cimex lectularius - C. columbarius* group, characterized by 13 autosomal pairs. It could be a species of the *Cimex* species groups with 14 pairs of autosomes (m. b. a species from *C. pipistrelli* group), but this question needs additional examination.

The karyotype of the species studied here displayed C-heterochromatin localized mainly in the large Y chromosome and in most telomeres of the autosomes. This finding has been confirmed by DAPI staining. A large amount of GC-rich repeats was detected by CMA_3 staining both in the large sex chromosome and the autosomes. The find-

ings of both fluorochrome stainings show that in the heterochromatin there are GC-rich clusters dispersed within AT-rich repeats. The CMA_3 staining does not reliably reveal NOR in this species.

In meiosis, at mid condensation stage, homologous chromosomes in bivalents are physically associated by one or seldom two collochores. In MI, they lie parallel, and homologous chro-

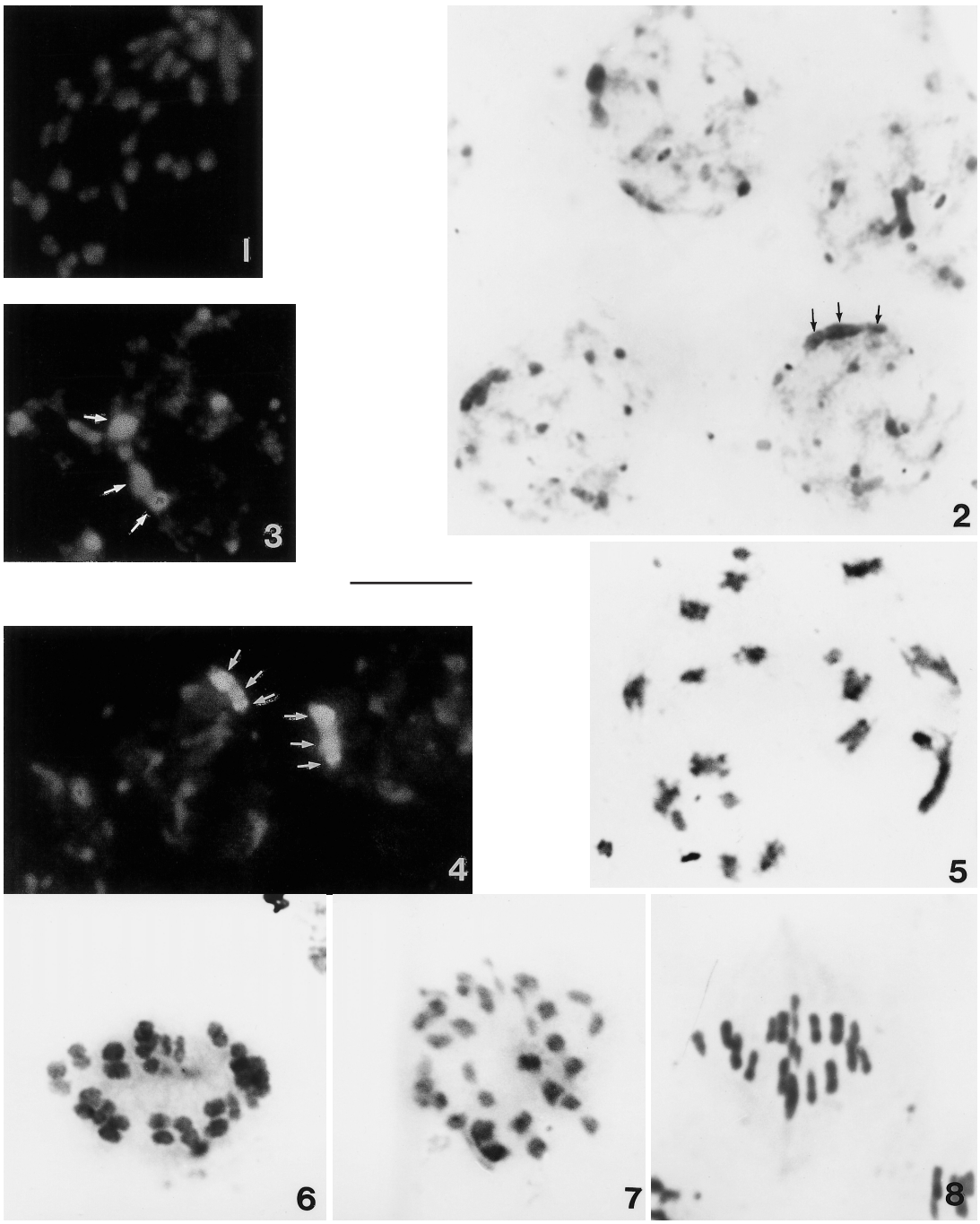

Fig. 1 – Spermatogonial mitotic prometaphase, $2n = 28+X_1X_2X_3X_4Y$. C-banding revealed by DAPI. Fig. 2 – Prophase plates after C-banding, C-positive bands arrowed. Fig. 3 – Prophase plates after DAPI staining, DAPI positive bands arrowed. Fig. 4 – Prophase plates after CMA_3 staining, CMA positive bands arrowed. Fig. 5 – Condensation stage with collochores between homologues in bivalents. Fig. 6 – Metaphase I (MI), homologous chromosomes lie parallel, facing opposite poles. Fig. 7 – Anaphase I (AI), opened out chromosomes with one telomere foremost towards the poles. Fig. 8 – Anaphase II (AII), the large Y and the four small X are going towards opposite poles. Bar 10 μm.

mosomes oriented to opposite poles. In early AI, the bivalents open out and the chromosomes move to the poles with one telomere foremost. The male meiosis is achiasmatic and of collochore type, similar with the male meiosis in Miridae described by NOKKALA and NOKKALA (1986a). UESHIMA (1967) examined details in the spermatogenesis, oogenesis and embryogenesis of *C. lectularius* in laboratory strains, originated from Cairo (Egypt) and Berkeley (USA) populations. He described that the homologous chromosomes of each bivalent in male meiosis lay in parallel, but interpreted erroneously male meiosis as chiasmatic.

In the infraorder Cimicomorpha achiasmatic meiosis has been so far described in four families (see above) and now confirmed for the Cimicidae. The achiasmatic meiosis in Heteroptera, and in Cimicomorpha, must be of old origin, because it has been found in the roots of different branches of the Cimicomorpha, as well as in a primitive out-group for them, Leptopodomorpha. Three patterns of achiasmatic male meiosis in Heteroptera have been found (NOKKALA and GROZEVA 2000). The most common pattern (Saldidae, Microphysidae and Anthocoridae) is characterized by side-by-side aligned homologues in bivalents, and touch and go pairing of sex chromosomes in MII. Meiosis in the Nabidae slightly differs with a distance pairing of sex chromosomes in MII. The third pattern of aschiasmatic male meiosis differs most from the others. The homologous chromosomes in this type are not physically aligned along their length during prophase, but are physically associated in one or two sites via so called collochores. Meiosis of this type has been described in Miridae and now also confirmed in the Cimicidae and, hence, providing additional evidence for a close relationship between these two families.

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