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THE SEX CHROMATIN BODY AND ITS IMPORTANCE FOR THE DEMONSTRATION OF HETEROCHROMOSOMAL CONDITIONS IN *ORNITHODOROS MOUBATA* (MURRAY, 1877)

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Geigy & Wagner (1957) and Wagner-Jevseenko (1958) studied normal mitoses in squash preparations of metaphase nuclei in the argasid tick *Ornithodoros moubata* during the transition of the oogonia to the primary oocytes and arrived at the diploid chromosome number 20. Morphologically the chromosomes could be differentiated into two main groups: three large, rod-shaped chromosomes, ca. 1·7 μ long, and 17 small, mostly rod- or coccus-shaped structures. In the male, as seen during the division of the spermatogonia, an almost analogous morphology exists; on the whole the chromosomes were only somewhat larger. The three largest chromosomes were 3·1 μ in length. Neither in the female nor in the male was it possible to match the chromosomes into homologous pairs. Geigy & Wagner (1957) could not find distinctive sex chromosomes. Wagner-Jevseenko (1958), therefore, concluded that the hetero-chromosomes are most probably lacking in *Ornithodoros moubata.* This finding agrees with that of Sokolow (1954).

Goroshenko (1962), on the other hand, is of the opinion that the presence of a heteromorphic pair of sex chromosomes in the male *Ornithodoros moubata* can be assumed in view of the marked difference in size and the uneven number of chromosomes (3 large and 17 small). In the female, however, the presence of two similar sex chromosomes is indicated since the difference in size of the chromosomes is not so marked. Goroshenko (1962) could not supply proof in support of his opinion.

The purpose of this investigation was to find out whether there is any sex dimorphism in *Ornithodoros moubata* expressed as a specific chromocentre and in how far the conclusion is justified that this tick species shows heterochromosomes with an XX-complex in the female and an XY-component in the male.

Material and Methods

Adult male and female *Ornithodoros moubata* were collected in huts near Onderstepoort and stored in a climate chamber at 26 to 27°C and 80 per cent R.H. They were attached firmly onto masking tape in a petri dish and dissected under a binocular stereo-microscope. Separate tissue spreads were made from the following organs: ganglion, rectal ampulla, malpighian tubes, tracheae, muscles, mid-gut, accessory sex glands and salivary glands. These were stained by the original Guard-method (Guard, 1959). They were immediately fixed in 96 per cent ethyl alcohol for 10

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minutes and then immersed in 70 per cent ethyl alcohol for 2 minutes. Subsequently the spreads were prestained in Biebrich scarlet (Biebrich-scarlet-water soluble, Dr. K. Hollborn und Söhne—Leipzig, 1·0 gm, phosphotungstic acid 0·3 gm, glacial acetic acid 5·0 ml, 50 per cent ethyl alcohol 100·00 ml) for 2 minutes.

Before counterstaining with Fast Green (Fast Green FCF, Anachemia Ltd., Montreal, 0·5 gm, phosphomolybolic acid 0·3 gm, phosphotungstic acid 0·3 gm, glacial acetic acid 5·0 ml, 50 per cent ethyl alcohol 100·0 ml) for 3½ hours the smears were palced into a 50 per cent ethyl alcohol bath for 2 minutes. After counterstaining the preparations were differentiated in 50 per cent ethyl alcohol for 5 minutes, dehydrated in 70 per cent, 96 per cent and absolute ethyl alcohol for 2 minutes each, cleared in three changes of xylol and mounted in Permount (Fisher Scientific Company, U.S.A.).

To demonstrate the sex chromatin body more distinctly when in its paranucleolar position some of the preparations were placed in 5 N HCl at 37° C for 3 to 5 minutes after fixation before being stained (modified Guard-method, Gothe, 1965).

The preparations were further stained according to the Feulgen-method to indicate the chromosomal origin of the sex chromatin.

The microscopical investigation was undertaken with a Wild-microscope, ocular 10X, oil immersion H 100, 1·25.

Fifteen microscope fields were counted per smear. Only those areas in which the cell nuclei were evenly spread and distinctly stained, were examined.

Observations

With the original Guard-method a sex difference in the nuclear morphology could be observed in the interphase nuclei only; in 93 per cent of the nuclei of the rectal ampulla—malpighian tubes; in 79 per cent of the ganglion nuclei; and in 62 per cent of the accessory sex gland nuclei of the cells investigated. The remaining nuclei in the smears were unsuitable as they were incorrectly spread. The nuclei of the other tissues were also unsuitable, because in them the sex dimorphism was masked either by multiple chromatin granules or by the fact that they did not easily lend themselves to suitable smear techniques due to the inherent characteristics of the nuclei.

The original Guard-method gives differential staining of the sex chromatin body which is rendered prominent, distinct, and varies from blue-red to bright red. Some chromocentres may either appear as indistinct, dark particles or they may stain green. The nucleolus shows up prominently as a dark red mass against its green surroundings. In the modified Guard-method all the nuclear components stain green, except the sex chromatin bodies which are faintly recognized as dark, indistinct granules. In most instances the sex chromatin in the female cell nuclei was found to lie near the nucleolus instead of near the nuclear membrane.

The sex chromatin body in paranucleolar position could be differentiated only by means of the modified Guard-method which causes it to appear as either a round or oval cap on the nucleolus. Without hydrolysis, identification of the sex chromatin mass in this position is difficult. When the nucleolus is excentric, the sex chromatin body often abuts against the nuclear membrane. In cell nuclei with two or more nucleoli only one sex chromatin mass is present. The diameter of this specific chromocentre varies between 0·4 and 1·2 μ (Plates 1 to 6).

The sex chromatin body was Feulgen-positive indicating its chromosomal origin.

In male nuclei the sex chromatin body could not be demonstrated. There were indistinct chromocentres situated near the nucleolus in 7 per cent of the cell nuclei of the malpighian tubes investigated. These are considered as unspecific bodies because of their weak staining affinity and they disappear completely upon hydrolysis

Discussion

The aetiogenesis of the sex chromatin body in the female interphase nucleus has been frequently discussed in the literature and different explanations for it have been advanced. The present tendency is to accept the explanation that the specific chromocentre is formed from a single X-chromosome, which is condensed along its entire length. This positively heteropycnotic X-chromosome, which is genetically almost inactive, can be of either paternal or maternal origin (Lyon, 1961, 1962). Ohno (1963) agrees with Lyon that any X-chromosone in the female somatic interphase nucleus could react euchromatically or heterochromatically and he is of the opinion that the condensation of a single X-chromosome is under autosomal control. A set of autosomes always has one X-chromosome in euchromatic form, while any additional X-chromosomes always appear heteropycnotically. If, however, only one X-chromosome exits, as in the male somatic cells and spermatogonia, it always persists euchromatically.

Ohno's impressions agree with the findings in *Ornithodoros savignyi* (Gothe, 1966) and with the results reported above for *O. moubata*. Because of their weak staining properties, indistinct appearance and disappearance upon hydrolysis, the chromocentres found in 7 per cent of male interphase nuclei of the malpighian tubes should be regarded as unspecific chromatin particles.

Theoretically there should be a sex chromatin body in every female somatic cell nucleus. In *Ornithodoros moubata*, however, this specific chromocentre could be demonstrated only in the interphase nuclei of the rectal ampulla—malpighian tubes (93 per cent), of the ganglion (79 per cent) and of the accessory sex glands (62 per cent). In cell nuclei from other organs its demonstration was impossible due to either the presence of too many chromatin particles or the difficulty of obtaining suitable spreads.

Conclusion

It is thus seen that the results obtained by the original Guard-method indicate that in *Ornithodoros moubata* heterochromosomal conditions are present. This finding is supported by the aetiogenesis of the sex chromatin body as given in the literature (Gothe, 1966), and by acceptance of Ohno's (1963) theory that the specific chromocentre is derived from a single X-chromosome. Furthermore the results obtained in *Ornithodorus savignyi* (Gothe, 1966) and Goroshenko's (1962) views justify the conclusion that the female *Ornithodoros moubata* possesses an XX-complex, is homogametic, while the male presents an XY-complement and is heterogametic.

Summary

In *Ornithodoros moubata* the sex chromatin body could be demonstrated only in the female interphase nuclei of the rectal ampulla, malpighian tubes, ganglion and the accessory sex glands.

These findings allow the conclusion to be drawn that in this species heterochromosomal conditions are present, the female having an XX-complex, while the male has an XY-chromosome complement.

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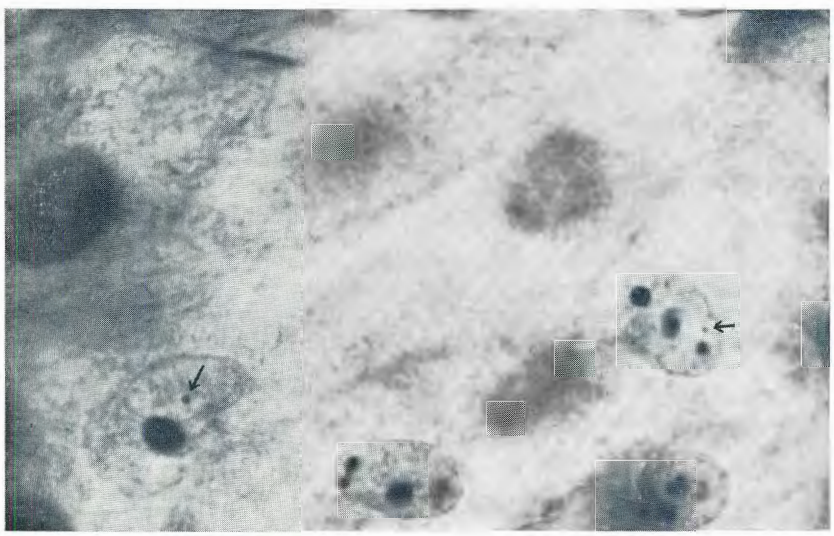

PLATE 1.—Spread of malpighian tubes, showing on the left one sex chromatin near the nucleolus; on the right a nucleus with three nucleoli and one only sex chromatin body
(Magnification 1200×, arrows pointing to sex chromatin bodies)

PLATE 2.—Spread of malpighian tubes, in the left nucleus, the sex chromatin body in paranucleolar position
(Magnification 1200×, arrows pointing to sex chromatin bodies)

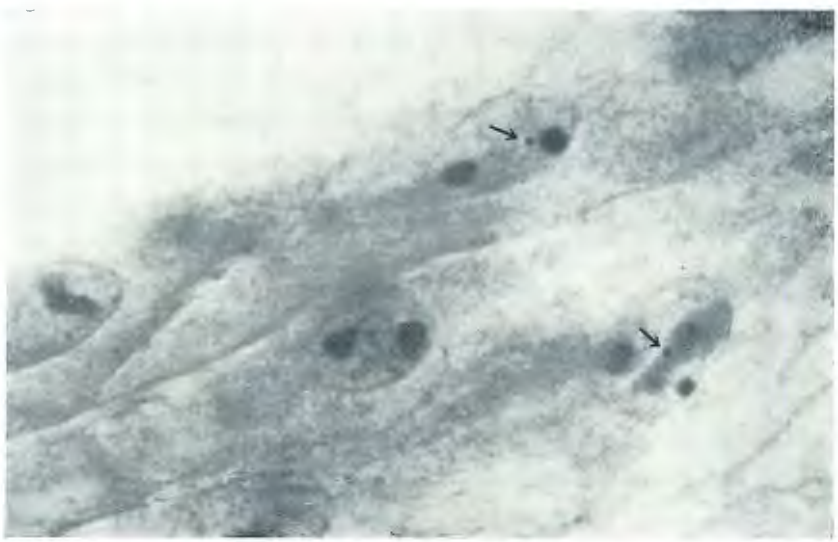

PLATE 3.—Spread of the malpighian tubes, showing the sex chromatin body
(Magnification 1200×, arrows pointing to sex chromatin bodies)

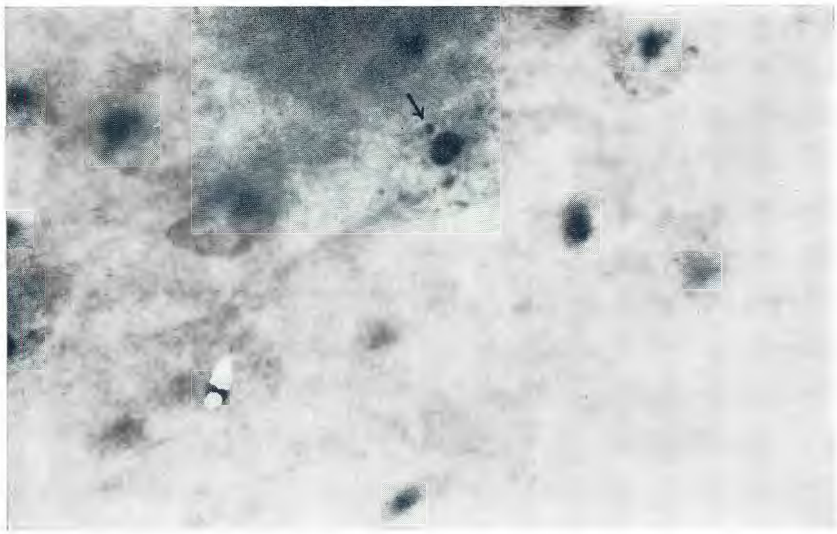

PLATE 4.—Spread of the malpighian tubes, showing the sex chromatin body on the nuclear membrane (Magnification 1200 ×, arrows pointing to sex chromatin bodies)

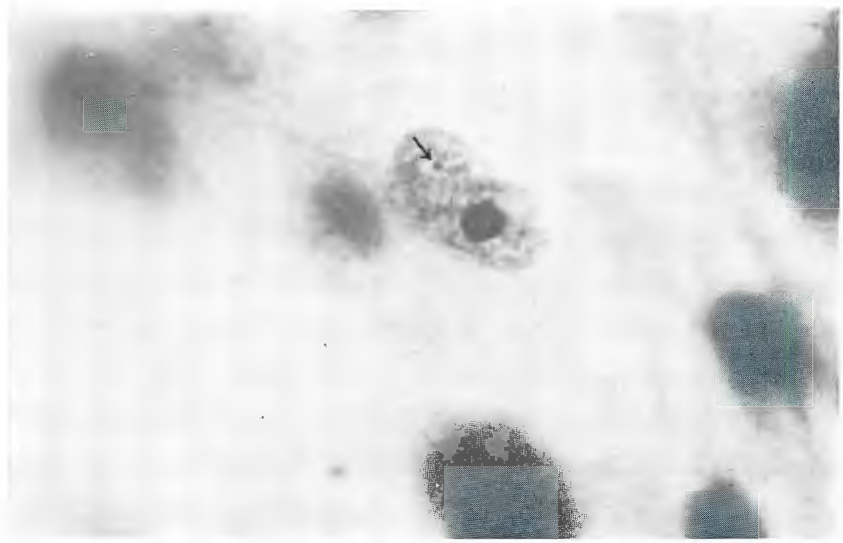

PLATE 5.—Spread of the malpighian tubes, showing the sex chromatin body (Magnification 1200 ×, arrows pointing to sex chromatin bodies)

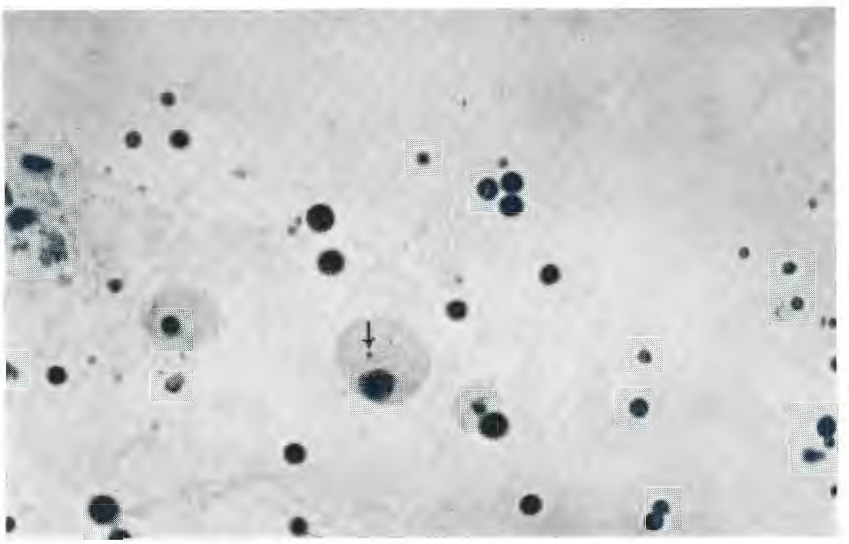

PLATE 6.—Spread of the ganglion, showing the sex chromatin body
(Magnification 1200×, arrow pointing to sex chromatin bodies)

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