Odonatologica 17(3): 275-280 *September 1, 1988*

GERM-LINE CHROMOSOMES OF TWO SPECIES OF *DAVIDIUS*, WITH SPECIAL REFERENCE TO THE SEX CHROMOSOMES (ANISOPTERA: GOMPHIDAE)

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Received and Accepted May 21, 1988

The female of *D. fujiama* Fraser has a 2n = 24 karyotype, including a pair of X chromosomes which are similar in size and the largest elements in the complement. The males of both *D. fujiama* and *D. m. moiwanus* (Okumura) have the same number of chromosomes, 2n = 23 and n = 12; in each sp. a single X chromosome, which is the largest element, is always observed in the spermatogonial karyotype. At diakinesis and early MI, the X chromosome consisting of a pair of chromatids is easily distinguished from the autosomal bivalents on account of their different morphology. Preliminary examination of C-banded spermatogonial metaphases and spermatocyte complements at diakinesis in *D. fujiama* indicates that discrimination between the X chromosome and the autosomes is easy because of a peculiar distribution pattern of constitutive heterochromatin of the X chromosome. Such chromosomal evidence reveals that *D. fujiama* is characterized by a male heterogametic sex chromosome system of the XO/XX-type. No *m*-chromosome could be encountered in either sp.

INTRODUCTION

Species of *Davidius* are distributed in the Eurasian Continent. In Japan, three endemic species are found (HAMADA & INOUE, 1985) and only one of them, *Davidius nanus*, has cytologically been examined so far (KICHIJŌ, 1939; KIAUTA & KIAUTA, 1982). Therefore, the present study aims to examine the chromosomes of two other Japanese species of *Davidius*. As has generally been known, odonates have a heterogametic sex chromosome system in the male. However, as far as we are aware, observations of the female mitotic and meiotic chromosome complements have rarely been made. In this study, consequently, efforts were made to morphologically identify the sex chromosomes in both

sexes. Some results obtained are outlined below.

MATERIAL AND METHODS

Two males and one female of *Davidius fujiama*, collected in the suburbs of Hirosaki-shi, Aomori-ken and three males of *D. m. moiwanus*, captured at Tsuta, Aomori-ken were used for the study of conventional Giemsa-karyotypes. Two additional males of *D. fujiama*, collected in the suburbs of Hirosaki-shi, were used for the preliminary study of C-banded karyotypes.

Preparation of Giemsa-karyotype. — Testes of one of the two males of *D. fujiama* were first treated with 0.075M KCl for 15 min at room temparature and then stored in Carnoy (3:1) until used. The testis-material was dissociated in 60% acetic acid and suspended in Carnoy. Chromosome slides were prepared by the conventional air-drying method.

Testes of another male of *D. fujiama* and those of the three males of *D. m. moiwanus* were first immersed in 0.025% colchicine in orthopteran Ringer solution for 2.5 h at room temperature. Then, they were crashed with a fine needle in 0.1% collagenase at 37° C. After hypotonic pretreatment (0.075M KCl,, 20-30 min) and subsequent centrifugation (1500 r.p.m., 5 min), the cells harvested were fixed with Carnoy and conventionally air-dried.

Ovarian chromosomal spreads were prepared without colchicine pretreatment in the same way as the collagenase-treated testes.

All slides were stained with Giemsa (4%) for 15 min.

C-banding. — Chromosomal spreads prepared by the conventional air-drying method from collagenase-treated testes were C-banded by the BSG method of Sumner (1972).

OBSERVATIONS

DAVIDUS FUJIAMA FRASER

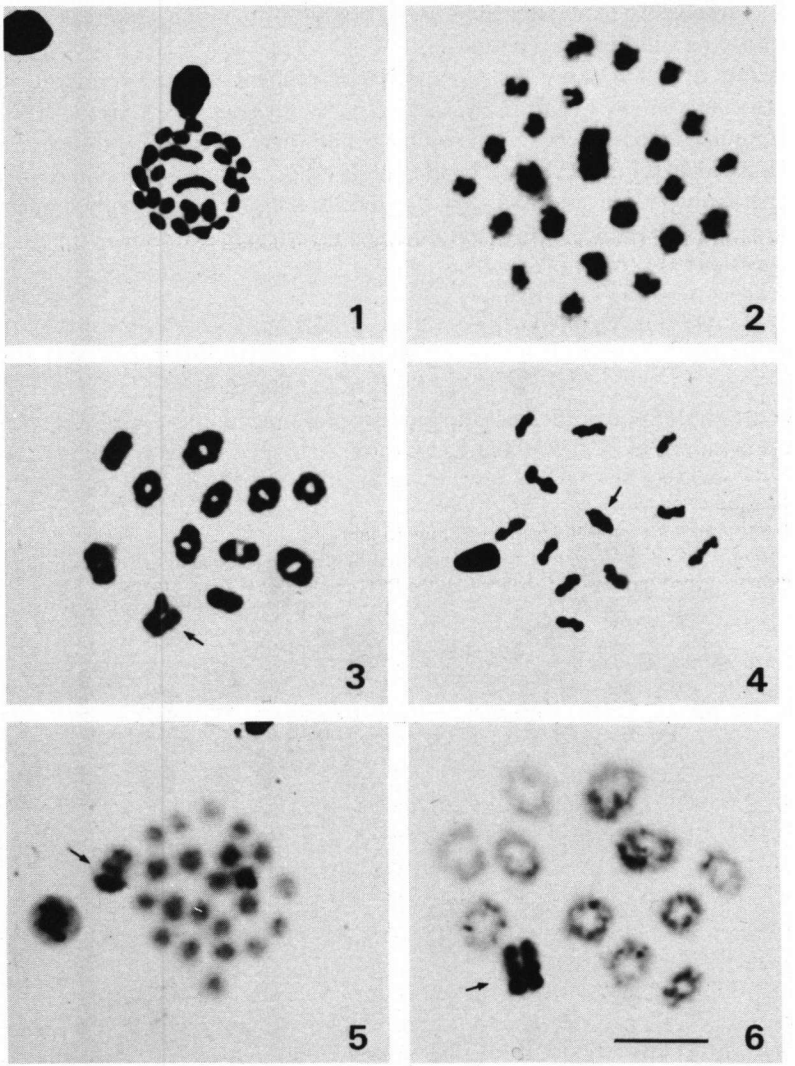
Figures 1-6

In ovarian mitosis, 24 elements were counted in 7 of the 8 spreads examined. As clearly shown in Figure 1, two of them, which are similar in both appearance and size, are distinctly larger (longer) than the remaining 22. They are the largest elements in the complement and therefore conspicuously marked. No *m*-chromosome could be distinguished. Oocyte chromosomes were not encountered in the present female material.

In spermatogonial mitosis, 23 chromosomes were counted with certainty in all of the 18 spreads examined. The complement comprises one marked element which is easily and certainly distinguished from the remaining 22 by its especially large size. This element is always found in unpaired condition. It is therefore identified as an X chromosome, in consideration of the chromosomal condition in the female diploid cells above mentioned. The 22 other elements which are all much smaller than the X are undoubtedly autosomes. No *m*-chromosome could be distinguished (Fig. 2).

Twelve elements are observed in 87 of the 101 spreads examined in the first division of spermatocytes and in 43 of the 52 spreads in the second division.

Especially at diakinesis and early MI, the X chromosome, which consists of a



Figs 1-6. Germ-line chromosomes of *Davidius fujiama* Fraser (X chromosome indicated by arrow; scale bar: 10μm): (1) Oogonial mitosis, 2n=24; — (2) Spermatogonial mitosis, 2n=23; — (3) Early primary spermatocyte metaphase, n=12; — (4) Early secondary spermatocyte anaphase, n=12; — (5) C-banded X chromosome in spermatogonial mitosis; — (6) same, at primary spermatocyte diakinesis.

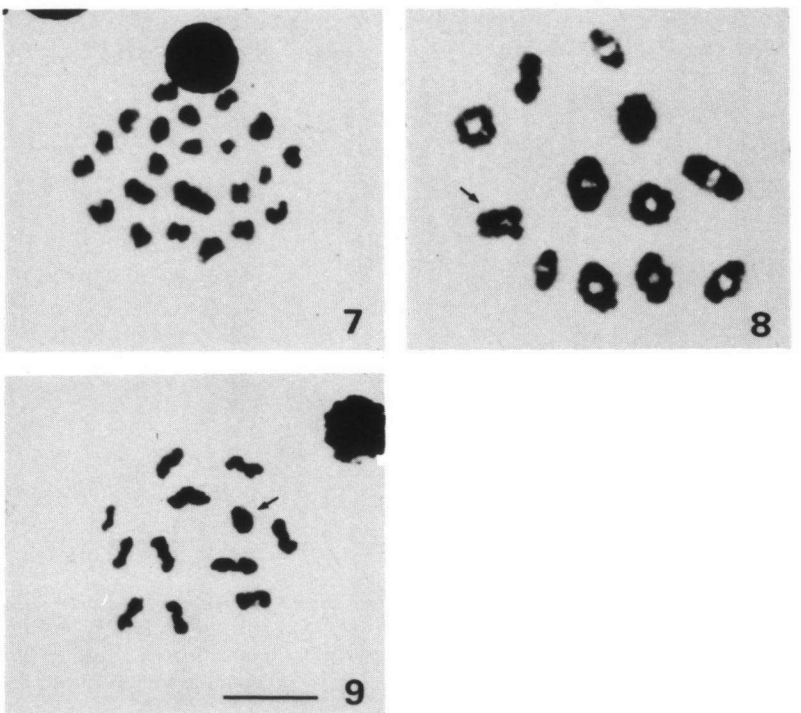
pair of chromatids, can certainly be discerned from each of the autosomal bivalents, because of their structural differences (Fig. 3). Figure 4 shows the haploid complement of the secondary spermatocyte; the autosomes are some-

what dumbbell-shaped and they seem ready to separate, whereas the X chromosome still remains contracted.

Figures 5 and 6 show the C-banded complement in the spermatogonial mitosis and that in the primary spermatocyte division, respectively. The X chromosome is conspicuous by the visible bands; there are three C-positive parts. One is a deeply stained, terminal band and the other two are faintly stained in the spermatogonial X chromosome (Fig. 5); whereas they are more deeply stained in the primary spermatocyte X chromosome (Fig. 6). Analysis of C-band patterns of autosomes is now in progress.

DAVIDIUS MOIWANUS MOIWANUS (OKUMURA)
Figures 7-9

As the above species, the male diploid chromosome number is 23; 23 elements were ascertained in 28 of the 33 spreads examined in spermatogonial mitosis. One



Figs 7-9. Germ-line chromosomes of *Davidius m. moiwanus* (Okumura) (X chromosome indicated by arrow; scale bar: 10 μm): (7) Spermatogonial mitosis, 2n=23; — (8) Early primary spermatocyte metaphase, n=12; — (9) Early secondary spermatocyte anaphase, n=12.

element is marked by its large size and it is always found in unpaired condition (Fig. 7). This suggests that the element is the X chromosome and consequently the others are autosomes. No *m*-chromosome could be distinguished in the present material.

The haploid chromosome number is 12 in the male; 12 elements were ascertained in 128 of the 137 spreads in the first division, as well as in 70 of the 77 spreads in the second division.

At diakinesis and early MI, the X chromosome which consists of a pair of chromatids, is especially dissimilar in shape to each of the autosomal bivalents (Fig. 8), making discrimination between them easy. The X chromosome at metaphase II is represented by a roundish element which, in strong contrast to the autosomes, shows no sign of separation (Fig. 9).

DISCUSSION

Previous studies (KICHIJO, 1939; KIAUTA & KIAUTA, 1982) and our present examination revealed that all the examined taxa of *Davidius* of Japan are consistent in having the same number of chromosomes, 2n=23 and n=12, in males and their karyotypes are uniformly featured by the X chromosome, which is the largest in the complement.

The occurrence of two markedly large chromosomes in the female diploid cells and one in the spermatogonia, as verified in *fujiama,* is considered as chromosomal evidence of the male heterogamety (XO).

The parallel arrangement of a pair of chromatids that is especially visible at diakinesis and early MI (Figs 3, 6 and 8) and lack of evidence of separation at meiosis-II of the X chromosome suggest its postreductional behavior.

Recent advances in the study of the Japanese odonate insects have increased the knowledge of their ecology and distribution; according to HAMADA & INOUE (1985), the genus *Davidius* comprises three taxa in Japan, one, *D. moiwanus,* being represented by three subspecies, *m. moiwanus, m. taruii* Asahina & Inoue and *m. sawanoi* Asahina & Inoue. From a cytotaxonomic standpoint, their karyological relations are very interesting, but the chromosome complements have not been examined in *m. taruii* and *m. sawanoi* as yet.

The banding study of odonate chromosomes has started very recently (FRANKOVIĆ & JUREČIĆ, 1987). Considering the present results, C-banding seems useful for the elucidation of the sex chromosome system of this group of insects. Therefore, a further analysis is desired.

ACKNOWLEDGEMENT

We are very grateful to Prof. B. KIAUTA at the University of Utrecht, the Netherlands, for his critical comments on a draft of this paper.

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