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CONSTITUTIVE HETEROCHROMATIN IN CHROMOSOMES OF SOME AESHNIDAE, WITH NOTES ON THE FORMATION OF THE neo-XY/neo-XX MODE OF SEX DETERMINATION IN *AESHNA* (ANISOPTERA)

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C-stained ♂ karyotypes of *Aeshna crenata* (2n♂ = 27; X0), *A. grandis* (2n♂ = 26; neo-XY), *A. juncea* (2n♂ = 26; neo-XY), *A. nigroflava* (2n♂ = 27; X0) and *Anax imperator* (2n♂ = 27; X0) from W Siberia, N Caucasus, Russian Far East and Hokkaido (Japan) are figured and analyzed.

INTRODUCTION

Dragonflies of the family Aeshnidae are one of the most popular objects in Odonata cytogenetics. Up to now approximately 60 species have been cytogenetically analyzed (MOLA et al., 1999). More than 70% of these have a male diploid set consisting of 27 chromosomes with the X0/XX mode of sex determination. There are also reductions of chromosome numbers down to 2n♂ = 25, 21, 19, 16, 14 via autosomal fusions in some species. One of the variants of structural karyotype transformation within the family is explained by fusion of original sex chromosome and one of the autosomes of the set. Such fusion leads to the neo-XY/neo-XX mode of sex determination and a reduction in chromosome number. It was from the Aeshnidae family that such a mode of odonate sex determination was first described (FUCHSÓWNA & SAWCZYNSKA, 1928; MAKALOWSKAJA, 1940; OKSALA, 1943). Moreover, the presence of heteromorphic sex chromosomes is the reason

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for the frequent use of *Aeshna* species as the model objects in examination of meiosis in species possessing holokinetic chromosomes (OKSALA, 1943; KIAUTA, 1969; MOLA, 1995).

This work describes C-banded karyotypes of six species from western Siberia, the northern Caucasus, Kunashiri Island (Southern Kurile Islands) and Hokkaido (Japan). Notes on the constitutive heterochromatin distribution in these species are given for the first time.

MATERIAL AND METHODS

Locality and number of individuals analyzed are as follows:

Aeshna crenata: 3 ♂ from Novosibirsk region, small ponds near the Inya river, 60 km E from Novosibirsk, 13-VII-1999; 2 ♂ from the Zyryanka river, 25 km S from Novosibirsk, 30-VII-1999.

A. grandis: 5 ♂ from the Zyryanka river, 30-VII-1999; 3 ♂ from the Inya river area (Kuriya lake) in Novosibirsk, 12-VIII-1999.

A. juncea: 4 ♂ from the Inya river, 60 km E from Novosibirsk, 13-VII-1999.

A. nigroflava: 1 ♂ from Kunashiri Island, Southern Kurile Islands, 17 km S from Yuzhno-Kurilsk, 5--VIII-1998; 1 ♂ from Hokkaido Island, Naganuma-city area, 4-VIII-1999.

Anax imperator: 6 ♂ from Kabardino-Balkaria Republik, Nalchik river, 10 km NE from Nalchik, 11-VIII-1997.

Males were caught at flight. The testes were excised, incubated in 0.9% sodium citrate, fixed in ethanol-acetic acid (3:1) and kept in 70% ethanol. Air-dried preparations were made by squashing tissues in 60% acetic acid and freezing them on dry ice. For induction of C-bands the hot barium hydroxide denaturation technique (according to SUMNER, 1972) was used with minor modifications.

RESULTS

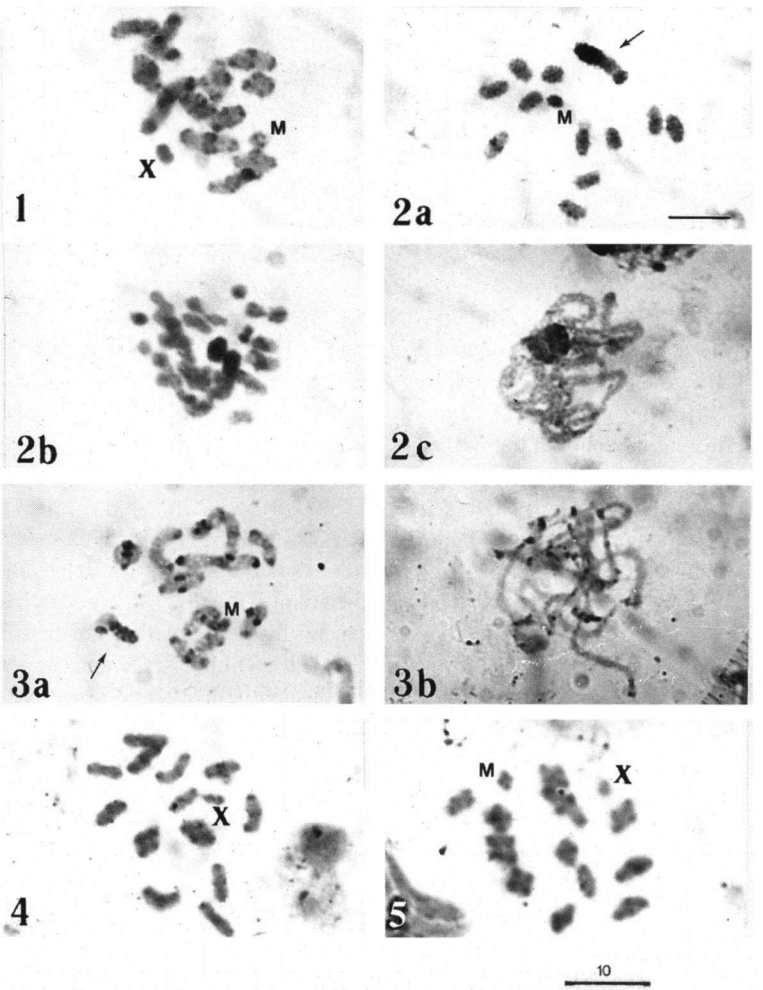
AESHNA CRENATA Hagen, 1856 (2n♂ = 27, X0, *m*)

Metaphase I complements consist of 14 elements with the X0/XX mode of sex determination (Fig. 1). An *m*-bivalent is present and X is the second smallest element of the set. All bivalents present a single chiasma, subterminally located. The same karyotype features in *A. crenata* were previously established by OKSALA (1943, 1952) in the Finnish specimens. All autosomes, including the *m*-pair, have the blocks of constitutive heterochromatin at both ends of each chromatid. These blocks are very small but well recognizable in all autosome pairs except for the largest bivalent that possesses asymmetric C-bands. The X appears entirely heterochromatic.

AESHNA GRANDIS (Linnaeus, 1758) (2n♂ = 26, neo-XY, *m*)

There are 13 elements in metaphase I, including *m* and the neo-X/neo-Y bivalent (Fig. 2a). The neo-XY bivalent is the largest of the set. All autosome bivalents possess a single subterminal chiasma. In contrast with all previous data (see FUCHSÓWNA & SAWCZYNSKA, 1928; MAKALOWSKAJA, 1940; OKSALA, 1943, 1945; KIAUTA, 1967, 1969, 1971) that show the facultative character of X--autosome fusion and the presence of primary and secondary complements in the

same specimen, all our individuals have only the neo-XY mode of sex determination. Noticeable blocks of constitutive heterochromatin are present at the largest autosome bivalent only. These are not symmetric and the subterminal chiasma tends to be located near the larger C-block. The neo-XY bivalent is heteromorphic and can be easily distinguished from all other chromosomes. Both the autosomal part of the neo-X chromosome (X_L-arm) and the neo-Y have a very large amount of constitutive



Figs 1-5. Constitutive heterochromatin in the Aeshnidae male chromosomes: (1) *Aeshna crenata*, metaphase I; – (2) *A. grandis*, metaphase I (a), spermatogonial metaphase (b), and pachytene (c); – (3) *A. juncea*, metaphase I (a), and pachytene (b); – (4) *A. nigroflava*, metaphase I; – (5) *Anax imperator*, metaphase I. – [Arrow indicates the neo-XY bivalent; bar equals 10 μm]

heterochromatin, which is well recognized during spermatogonial metaphase (Fig. 2b) and pachytene (Fig. 2c) stages. The C-bands at the original X-part (X_R-arm) of the neo-X are located in terminal position.

AESHNA JUNCEA (Linnaeus, 1758) (2n♂ = 26, neo-XY, *m*)

There are 13 elements in metaphase I complements (Fig. 3a). The neo-XY is one of the largest elements of the set. Usually a single subterminal chiasma occurs per bivalent, but there are some cells with two chiasmata at one of the large bivalents. Like in *A. grandis*, all our individuals have only the neo-XY mode of sex determination. Large and well noticeable blocks of C-heterochromatin are present in all chromosomes. The X_R-arm of the neo-X possesses large terminal C--heterochromatin blocks, the X_L-arm of the neo-X and the neo-Y chromosome are not entirely heterochromatic, they have large C-blocks separated by small euchromatic regions. In contrast with *A. grandis*, no heterochromatic bodies were seen at pachytene stage (Fig. 3b).

AESHNA NIGROFLAVA Martin, 1908 (2n♂ = 27, X0)

Metaphase I plates consist of 14 elements (Fig. 4). The X is the smallest element of the set. The same karyotype features were previously evidenced by KATATANI (1987). All the autosomes have small terminal C-blocks, no constitutive heterochromatin occurs in the X.

ANAX IMPERATOR Leach, 1815 (2n♂ = 27, X0, *m*)

Its karyotype was previously studied by KIAUTA (1965, 1969). This species has the modal chromosome number n♂ = 14 with *m* and the X0/XX sex determination. Our individuals have the same karyotype morphology (Fig. 5). The X and *m*-bivalent can be easily distinguished from all other elements by their size: they are the smallest of the set, the X is smaller than the *m*-bivalent. The asymmetric C-bands are present in the largest bivalent. The blocks of C-heterochromatin in other bivalents are much smaller and not easily distinguished.

DISCUSSION

So far characters of constitutive heterochromatin distribution have been established for *Aeshna viridis* Eversm. (PEREPELOV et al., 1998). In this species, characterised by 2n♂ = 26 with *m* and the neo-XY/neo-XX mode of sex determination, noticeable C-bands were found in the largest autosome bivalent only. The sex bivalent was shown to be heterogeneous and consists of a heterochromatic part connected with a euchromatic one, with terminal C-block. In the present work, *A. grandis*, closely related to the above species, was studied. Both of those possess a very similar karyotype 2n♂ = 26, neo-XY, *m*. The C-heterochromatin distribution in these species is also identical. Another pair of closely related species, *A. crenata* and *A. nigroflava*,

have the male karyotypes consisting of 27 chromosomes with the X0/XX mode of sex determination. They can be easily separated by the presence of *m*-chromosomes in *A. crenata*. The modal karyotype, with 27 chromosomes, including *m* and the X0/XX, occurs also in *Anax imperator*. The features of C-heterochromatin distribution in these species are very similar. As a rule, only a small amount of constitutive heterochromatin is present in the karyotype. The large and well recognizable C-bands occur in the largest autosome bivalent. Other bivalents possess much smaller blocks, which are often invisible or hardly recognizable. Such a case is common in insects with holokinetic chromosomes and was firstly postulated for such a type of chromosome organization in general (BLACKMAN, 1985). Chromosome sets with a small amount of C-heterochromatin were found also the Coenagrionidae, Lestidae, Platycnemididae and Gomphidae (PRASAD & THOMAS, 1992; E. Perepelov & A.G. Bugrov, unpublished). In this case, *A. juncea* is in a unique position within the Aeshnidae, by having large and well recognizable C-blocks in all the autosomes.

The neo-XY system originated through the fusion of the original X with one of the autosomes; it is particularly frequent in *Aeshna*. According to MOLA & PAPESCHI (1994), 28% of the species studied have such mode of sex determination while in the Order only 5.4% of the species have the neo-system. In many *Aeshna* species, the heteromorphism of the sex bivalent is easily recognized, a fact that is unusual in other odonate genera. The distribution of constitutive heterochromatin within the neo-XY bivalent is also heteromorphic. As a rule, metaphase I bivalents have the heterochromatic part (X_L-arm) connected with the euchromatic one (X_R--arm) with a terminally located block of C-heterochromatin. New data obtained lead us to suggest the heterochromatic part to represent the original autosome pair and the euchromatic one to be the original X chromosome. Some evidence supports such conclusion: there are two heterochromatic bodies in spermatogonial metaphase complements in *A. grandis* (Fig. 2b), one of them is the neo-Y and the other the autosomal part of the neo-X. At the pachytene stage, two heteropycnotic bodies are also observed (Fig. 2c). They are not entirely heterochromatic and have euchromatic nature, with large C-blocks. Subsequently, in condensed metaphase I chromosomes, these blocks get fused and the sex bivalent is seen as a heteromorphic bivalent with a euchromatic part and a dark, entirely heterochromatic one. In contrast, *A. juncea* does not show any heterochromatic body until metaphase I. The autosomal part of the neo-XY has euchromatic nature during diplotene and diakinesis and only condensed metaphase I sex bivalents seem to have some interstitial C-bands in their autosomal parts (Fig. 3a).

So, the formation of the neo-XY/neo-XX mode of sex determination is accompanied by heterochromatinization of the neo-Y and the autosomal part of neo-X chromosome. In *A. juncea*, probably, there is an initial stage of such heterochromatinization. C-heterochromatin in the sex bivalent is visible in the condensed metaphase I plates only. Moreover, even a condensed sex bivalent shows

the presence of euchromatic regions between heterochromatic ones. During metaphase I, *A. grandis* and *A. viridis* have an entirely heterochromatic X_L-arm of the sex bivalent (PEREPELOV et al., 1998).

The phenomenon of heterochromatinization in the neo-XY/neo-XX system has been discussed previously in other invertebrate and vertebrate groups (MULLER, 1914; SAEZ, 1963; WHITE, 1973; BUGROV & WARCHALOWSKA-SLIWA, 1997; BUGROV & GROZEVA, 1998). But in these only the process of heterochromatinization of neo-Y chromosomes was described. Models were made on the animals with monocentric chromosomes and they assumed that recessive mutations, connected with loss of function of different genes, accumulated in the neo-Y chromosome. Due to the absence of crossing-over, this leads to an inactivation and reduction of the neo-Y chromosome. Our results show, however, that the process of heterochromatinization takes place in both the neo-Y chromosome and the autosomal part of the neo-X chromosome.

It seems that analysis of mitotic complements in Aeshnidae may provide new data on the evolution of the neo-XY sex determination.

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