

C-heterochromatin in chromosomes of *Ophiogomphus cecilia cecilia* (Four.) (Anisoptera: Gomphidae) with notes on the sex chromosome origin in the species

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Abstract – The karyotype of *Ophiogomphus cecilia cecilia* ($2n \delta = 23, X0$) was analyzed using C-banding technique. All autosomes possess terminal C-bands. The X-chromosome is the largest element of the set and it consists of heterochromatic region at one of the ends and euchromatic part with three interstitial heterochromatic blocks. Possible ways of the formation of the X-chromosome are considered.

Key words: C-banding, karyotype, Odonata, sex determination.

INTRODUCTION

The dragonflies of the family Gomphidae are one of the best studied cytogenetically among Anisoptera. Together with Polythoridae the Gomphidae are the only dragonfly families with chromosome number $n=12$. In the majority of species studied the mechanism of sex determination is $X0(\delta)/XX(\text{♀})$, and the X-chromosome is one of the smallest elements of the set (KIAUTA 1972). At the same time there are several species with other chromosome numbers and other than $X0/XX$ modes of sex determination (KIAUTA 1969, 1972; TYAGI 1977, 1978; KIAUTA and VAN BRINK 1978). Some species have the sex chromosome which is often the largest or one of the largest elements of the mitotic and meiotic complement (KIAUTA 1969, 1972).

Ophiogomphus cecilia (Four.) is one of those species whose the sex chromosome is the largest element of the karyotype. This species has the modal chromosome set $n=12$ with $X0/XX$ mode of sex determination. Some samples of the subspecies *O. cecilia cecilia* from the Western Siberia have been investigated cytogenetically using C-

banding technique for the better understanding of the origin of the X-chromosome in this species.

MATERIALS AND METHODS

Seven adult males were collected near the Inya River, 60 km to the east from Novosibirsk, in July 1999.

The abdomens of insects were dissected ventrally, the testes being taken out and then being fixed in ethanol-acetic acid mixture (3:1). The fixed material was washed and kept in 70 percent ethanol. Air-dried preparations were made by squashing tissues in 60 percent acetic acid and freezing them on dry ice. C-banding technique (according to SUMNER 1972) was used with some minor modifications.

RESULTS

Several stages of spermatogenetic cycle are observed and discussed. Spermatogonial metaphase consists of 23 chromosomes (Fig. 1), one of them is very large (at least four times longer than the largest autosome pair) and represents the X-chromosome. Other elements are of gradually decreased magnitude. At this stage almost all the autosomes do not show the presence of C-heterochromatin. The smallest autosome pair differs

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from the other autosomes by the presence of the large amount of heterochromatin. The X-chromosome shows the difference of heterochromatin distribution throughout its length. There is a large heterochromatic region at one of the ends of the chromosome (about 2/5 of chromosomal length), the other part being euchromatic.

In pachytene the X-chromosome looks like a round or oval body which is usually at the edge of the cell (Fig. 2). Heterochromatic region occupies one side of the chromosome and does not show the entirely heterochromatic nature, but it looks like heterochromatic with interstitial euchromatic sections. The euchromatic part of the X-chromosome also has intercalary C-bands. All the autosomes possess the terminal C-bands.

On diplotene and diakinesis plates all the autosomes have C-blocks at the ends of each chromatide. The sex chromosome consists of a large inhomogeneous heterochromatic part and euchromatic one with three intercalary C-band (Fig. 3). During these stages the X-chromosome often forms the bent structure, so the euchromatic part of the X is near the heterochromatic one (Fig. 4).

Metaphase I complements consist of 12 elements, one of them is the X. All the autosome bivalents possess a single chiasma that usually occupies interstitial position. All the autosomes on metaphase I complement have well discernible C-blocks at the both ends. The smallest autosome bivalent can be easily distinguished by the presence of large amount of heterochromatin (Fig. 5).

At the first meiotic anaphase the sex element is being divided equationally and simultaneously with the autosomes (Fig. 6). At metaphase II plates the X-chromosome lies at a certain distance (usually in the poleward position) from the dividing autosomes and then it precedes undivided to a pole in the postreductional way (Fig. 7).

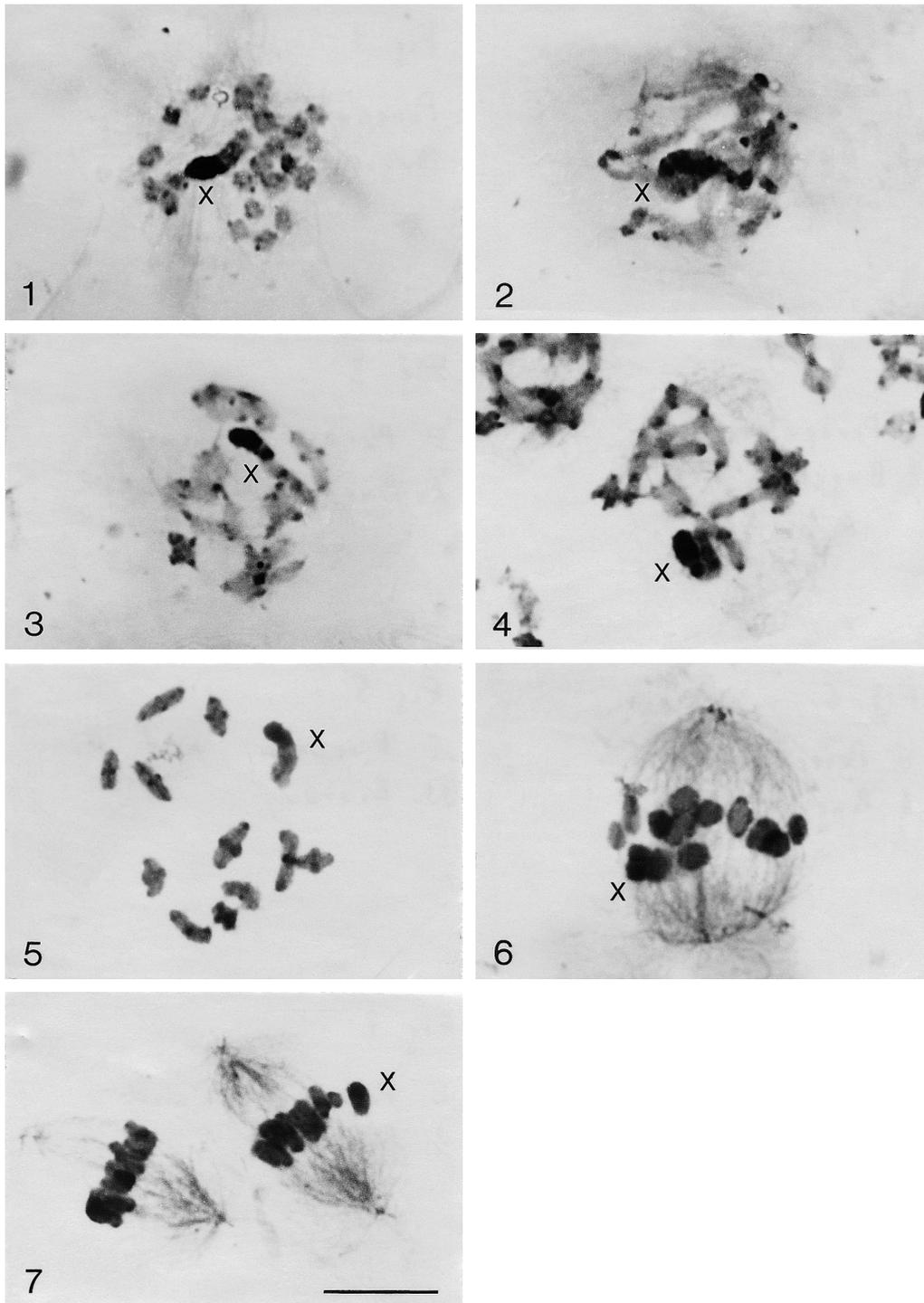
DISCUSSION

The problem of evolution of Odonata karyotype was brightly discussed by KIAUTA (1967, 1969). He proposed that $n=12$ chromosome set of Gomphidae is the secondary set, originated from $n=13$ by the fusion of the chromosomes. According to this hypothesis the process of gradually reducing the chromosome number to $n=12$ and further can go through the fusion of original X with an autosome which results in the formation of the neo-XY mode of sex determination.

Then the neo-Y chromosome originated from an autosome connects with the other autosome giving the neo-X-neo-neo-Y type. Finally, the autosomal part of the neo-X fuses with an autosome (X0 type of sex determination; the X is small, n being reduced by one in comparison with ancestral karyotype). However, this theory seems artificial. There are some species having the neo-XY type, but there are no gomphid species analyzed having the neo-X-neo-neo-Y mode of the sex determination. KIAUTA (1969) assumed the species with extra large X-es to have such type. But during the meiosis the large X-chromosome behaves like ordinary X; the species with real neo-X-neo-neo-Y type of the sex determination form well recognizable sex trivalent in the first meiotic division (GROZEVA and MARYANSKA-NADACHOVSKA 1995; MOLA *et al.* 1999). Moreover, the neo-neo-Y chromosome in these Gomphidae is not also recognized. So, the origin of the extremely large sex chromosome in some gomphids is still uncertain. To answer this questions we have analyzed C-banded karyotype of *O. c. cecilia* from the Novosibirsk Region.

Karyotype and female meiosis of *Ophiogomphus cecilia* were firstly described by OKSALA (1945). Finnish specimens also had $2n \text{♀} = 24$ one pair of which represented the largest X-chromosomes. Previously we have described C-banded karyotype of another subspecies of this dragonfly, *Ophiogomphus cecilia obscura* Bart. from the Sakhalin Island (PEREPELOV *et al.* 1998). Like in the nominative form, the largest X-chromosome is present in the standard $2n \text{♂} = 23$ karyotype. At least eight autosome pairs have the noticeable C-bands, the others do not show any heterochromatic blocks. Also, we were not able to show the differences in staining throughout the X-chromosome, it looks like entirely heterochromatic body.

New data obtained allow us to consider some hypotheses on the formation of the large X in this species. It is evident that the sex chromosome in *O. c. cecilia* represents X0 type of the sex determination. It cannot be the neo-X chromosome as its extremely large size (of at least four times longer than the largest autosomes in the set) requires the presence of the large neo-Y chromosome in the karyotype and of even number of elements in the set. Nevertheless, it is obvious that the X-chromosome is originated from some fusion event. And we suppose that the sex chromosome is originated from the neo-X chromosome which is end-to-end fused with the neo-Y chromosome. Putting in an-



Figs. 1-7 – *Ophiogomphus cecilia cecilia*. C-banding. 1, Spermatogonial metaphase. 2, Pachytene. Note the homologous C-blocks at the heterochromatic and euchromatic parts of the X-chromosome. 3, Dyplotene. Note the intercalary C-blocks at the euchromatic part of the X-chromosome. 4, Diakinesis. 5, Metaphase I. 6, Early anaphase I. 7, Metaphase II. Bar equals 10 μ m.

other way, the X-chromosome consist of three parts, namely the original X chromosome and two autosomal parts representing two chromosomes of

the same autosomal pair. This assumption explains unusual configuration of the sex chromosome in pachytene and diplotene. Here, autosomal parts of

the X conjugate with each other forming round body in pachytene and hook-like structure in diplotene. At the pachytene plates the homologous regions of the X-chromosome show the similar patterns of C-block distribution (Fig. 2). The heterochromatic part of X seems to represent the neo-Y part; the phenomenon of heterochromatinization in neo-XY/neo-XX mode of the sex determination has been discussed previously in other groups of animals (MÜLLER 1914; SAEZ 1963; WHITE 1973; BUGROV and WARCHALOWSKA-SLIWA 1997; BUGROV and GROZEVA 1998). These models were made on the animals with monocentric chromosomes and they assumed that recessive mutations connected with the loss of the function of different genes accumulated in the neo-Y chromosome, because of the absence of crossing-over, resulting in an inactivation and reduction of the neo-Y chromosome. However, the original sex chromosome part and its location in the X-chromosome cannot be distinguished in our material. Another possible way of the occurrence of the extremely large sex chromosome is connected to the amplification of heterochromatin, which has influenced the occurrence of the heterochromatic area at one of the ends of chromosome.

Thus, the usage of C-banding technique has allowed us to show the differences in structural organization throughout the sex chromosome. We assume that the X0 type in this species may originated from the neo-XY type, in which the neo-Y chromosome are fused with the neo-X. Also, the largest X-chromosome may be a result of the amplification of constitutive heterochromatin. The further karyological investigation of the Gomphidae dragonflies seem to provide other patterns of the structural evolution of the sex chromosome determining system as well as the evolution of $n=12$ karyotype in this family.

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