C-heterochromatin Variation in *Deracantha onos* (Pall.) (Deracanthini, Bradyporinae, Tettigoniidae, Orthoptera)

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C-banding patterns have been demonstrated in a few species of Tettigoniidae (Fernandez-Piqueras *et al.* 1983, 1984, Navas-Castillo *et al.* 1986, Warchałowska-Śliwa and Maryńska-Nadachowska 1992, 1995a, b, Warchałowska-Śliwa *et al.* 1993, 1994, 1995). Nevertheless, they have revealed that considerable intra- and inter-specific differences exist in the location and amount of constitutive heterochromatic regions.

So far, only the chromosome numbers of the tribe Deracanthini (sensu Čejchan 1967) of three species: *Deracantha onos* (= *Callimenus onos*) $2n=29$, $NF=31$, *Deracanthina deracanthoides*, and *Zychya baranovi* $2n=31$, $NF=31$ have been described (Ju 1930, Sergeev and Bugrov 1988).

The present work contains detailed information concerning the C-heterochromatin patterns in chromosomes of *Deracantha onos*.

Material and methods

Six males of *Deracantha onos* (Pallas 1772) were collected from two localities in Russia; 1) Transbaikalia, Chita region, near the village Olovyannaya and 2) Far East Siberia, Amur region, near Blagoveschensk (July 1982).

Four out of six males were injected with 0.1% colchicine for 1.5 hr. 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 45% acetic acid and freezing them in dry ice. The C-banding examination was carried out according to Sumner (1972) with slight modifications.

Results

The karyotype of *Deracantha onos* consists of $2n=29$ (XO) chromosomes. The autosomes can be divided into three size groups; one long (L_1), six medium (M_2 – M_7), and seven short (S_8 – S_{14}). Among fourteen autosomes, pair L_1 is metacentric, while the others are acrocentric. The size of the acrocentric X chromosome is intermediate between that of the L and M autosomes (Fig. 1).

At diplotene/diakinesis in L_1 bivalent from 4 to 6 chiasmata, in M_2 two or sporadically one, in the remaining medium size one or sporadically two chiasmata, and in small bivalents only one chiasma was observed. The mean frequency of chiasmata is $\bar{x}=19.66$ (± 0.7).

This species is characterized by the occurrence of a large amount of C-band positive chromatin visible in early prophase (Fig. 2); they can be classified according to their location

Fig. 1. C-banded karyotype. The X chromosome with interstitial C-bands (arrows). Bar equals 10 μ m.

Fig. 2. Early prophase with positive chromatin. Bar equals 10 μ m.

within the chromosomes as follows: paracentromeric, interstitial, and distal.

The C-banding pattern in bushcrickets from two localities is characterized by the presence of paracentromeric C-bands in all autosomes and in the X chromosome (Fig. 1).

The interstitial C-bands are present in M_2 chromosomes in individuals of both localities. C-heterochromatin variation for these autosomes is shown in Fig. 3a–f. In most of the individuals (found in three out of six individuals) M_2 chromosomes carry isomorphic C-blocks (I-variant) (Fig. 3a, b). The II- and III-variants possess heteromorphic C-bands of different size. Two males carry

the II-variant of the M_2 , where one of the homologues shows a larger block than the other (Fig. 3c, d). The III-variant (found in one male) carries in one of the homologues a particularly large band (Fig. 3e, f). In diakinesis/MI the M_2 heteromorphic bivalent possesses two chiasmata in proximal + distal or interstitial + interstitial position, or one chiasma in a distal one (Fig. 4a). At anaphase I of this bivalent more often separated reductionally, than equationally (Fig. 4b, c) owing to the formation of either one or two chiasmata.

Additionally, two thin interstitial C-bands were observed in all individuals in the X chromosome (Fig. 1).

Distal thin C-heterochromatin in the M_3 , M_5 , and M_7 chromosomes was observed. The S_8 and S_9 chromosomes carry large distal C-blocks, whereas a small euchromatic part at the end of these chromosomes is present.

One male was mosaic for the heteromorphic distal C-band in M_6 chromosomes (Figs. 1, 4d).

In all males the heteromorphic S_{10} show two variants of distal C-bands; I—one of the homologues carrying a larger block than the other (Figs. 1, 5a), II—one of homologues possesses a large block, whereas in the other one a small C-band was observed (Fig. 5b).

The two or three Ag-stained areas of the NORs continued in the meiotic prophase up to the pachytene stage (Fig. 6a). In diplotene/diakinesis the M_2 and one small size bivalents

Fig. 3a-f. C-heterochromatin variation for M_2 chromosomes (arrows). (a) and (b) I-variant in (a) mitotic metaphase and (b) metaphase I. (c) and (d) II-variant in (c) mitotic metaphase and (d) diplotene. (e) and (f) III-variant in (e) mitotic metaphase and (f) diplotene. Bar equals $10\mu\text{m}$.

Fig. 4a-d. (a) M_2 heteromorphous bivalent with different position of chiasmata. (b) Anaphase I, note in equational and (c) reductional division of heteromorphous M_2 bivalent (arrows). (d) Heteromorphous distal C-bands in M_6 chromosomes (arrows). Bar equals $10\mu\text{m}$.

preserved nucleolar remnants. Sometimes, in the X chromosome small NOR is visible, and in most of the cells the X possesses many little dots (Fig. 6b).

Discussion

One of the most frequent categories of chromosome differences in Orthoptera involve change in the amount and locality of constitutive heterochromatin. This is evident in terms of the variation in the C-banded material. Particular C-bands are present or absent, and show

Fig. 5a, b. Heteromorphic distal C-bands in S_{10} chromosomes (arrows) (a) I-variant and (b) II-variant. Bar equals $10\ \mu\text{m}$.

Fig. 6a, b. Silver stained (a) pachytene and (b) diakinesis showing the location of the NORs (arrows). Bar equals $10\ \mu\text{m}$.

variation in size. In most cases in Orthoptera variation of the C-heterochromatin in both homologous chromosome involve the shorter member of complements (Hewitt 1979, Navas-Castillo *et al.* 1986).

Hitherto, variation for the heterochromatin C-bands in Bradyporinae has been found in the genus *Baetica* (Fernandez-Piqueras *et al.* 1984, Navas-Castillo *et al.* 1986, Sentis *et al.* 1986), and in the *Steropleurus martorelli* complex (Fernandez-Piqueras *et al.* 1983).

The present study analysed individuals of *Deracantha onos* from two localities which are very far and isolated from one another. Males in both localities showed three types of M_2 , two types of M_6 , and S_{10} chromosomes depending on the C-banding pattern. The C-heterochromatin variation in the common type is related to the species *Baetica ustulata* endemic in the Iberian Peninsula. This species, belonging to the tribe Ehippigerini, consists of $2n=25$ chromosomes, where both pairs of L autosomes are metacentric while the remainder, including the X, are acrocentric. In L_2 chromosomes there are interstitial C-bands in the long arms, which coincide with active NOR. Additionally, in this species extensive variation has been described in heterochromatin existing for the C-blocks in M_3 , M_4 , and M_5 chromosomes (Fernandez-Piqueras *et al.* 1984, Navas-Castillo *et al.* 1986, Sentis *et al.* 1986). Between *Baetica ustulata* and *Deracantha onos* there are some similarities and differences within the location and size of C-heterochromatin: 1) These species are characterized by different chromosome number and by their morphology. On the one hand, there are the same numbers of small size autosomes, and on the other, differences between the number of large and medium chromosomes occurred. The supposed origin of metacentric L_2 chromosomes was connected with mutual translocation of the largest of medium size chromosomes. 2) The analysis of

C-banding patterns of different species of Tettigonidae, and the above-mentioned species reveals that the largest and medium size chromosomes possess small in size paracentromeric C-bands. 3) The M_2 chromosomes in *D. onos* with heterochromatin variation in interstitial position and connected with it active NOR, could correspond with the long arm with interstitial C-band of L_2 in *B. ustulata*. 4) In both species, with similar medium size autosomes, distal C-bands were present. However, the M_6 chromosomes in *D. onos* was characterized by heteromorphic thin distal C-bands, but M_5 in *B. ustulata* possesses some types of variation in the size of C-heterochromatin. 5) Unlike *B. ustulata*, *D. onos* possesses more C-heterochromatin in small size chromosomes, especially in S_8 , S_9 and S_{10} . Thus differences in size and location of C-heterochromatin in the two species generally occur in small size chromosomes. It could be said that the distribution and amount of C-heterochromatin in these species show a parallel tendency. Among all species of Tettigoniidae described so far in terms of C-band, distribution, amount, as well as variation of C-heterochromatin are the most similar in the above-mentioned species. Hence, the cytogenetical data presented here, confirm in a certain way the uniting of the tribes Deracantini and Ephippigerini in one higher taxon, by some systematists (Čejchan 1967, Gorochoy 1988).

Summary

The Mongolian-Siberian species *Deracantha onos* ($2n=29$, $NF=31$) is characterized by an extensive amount of C-heterochromatin occurring on the M_2 chromosomes as a large interstitial C-block and on M_6 , S_8 , S_9 , and S_{10} chromosomes as large distal C-bands.

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