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Karyotype, C- and G-band Patterns and DNA content of *Callimenus* (=*Bradyporus*) *macrogaster macrogaster*

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Abstract

Chromosomes and detailed karyotype information (the number, shape, relative length, arm ratio, centromeric index) of *Callimenus* (=*Bradyporus*) *macrogaster macrogaster* Lef. (Orthoptera: Tettigonioidea, Bradyporini) of Turkey belonging to the subfamily Bradyporinae are described. The diploid number of chromosomes was found to be 2n ♂= 23, with 2 metacentric pairs, 2 submetacentric pairs, 6 acrocentric pairs. The X chromosome is metacentric. This species has a XX ♀/ X0 ♂ sex determining mechanism. The basic karyotype is complemented by a description of C- and G-banding patterns. The C-banding pattern in spermatogonial metaphase was characterized by the presence of paracentromeric C-bands in all chromosomes, and a distal C-band in chromosome 5. The G-banding pattern was complex. The 2C nuclear DNA content was found to be 10.26 ± 0.16 picograms by microspectrophotometry.

Keywords: Callimenus (=*Bradyporus*) *macrogaster macrogaster*, Orthoptera, cricket, chromosomes, karyotype, C- and G- banding, DNA content

Introduction

The genus *Bradyporini* belongs to the subfamily Bradyporinae. The karyotypes (only the chromosome number and sex determination) of eight species of *Bradyporini* have been studied (Georgevitch, 1933; Fernandez-Piqueras et al., 1982, 1983; Sentis et al., 1988; Warchalowska-Sliwa, 1998). The chromosome number in the species of this genus ranges from 2n♂ = 25 (Georgevitch, 1933) to 29 (Fernandez-Piqueras *et al*., 1982; Sentis *et al*., 1988).

Callimenus macrogaster macrogaster belongs to the genus *Bradyporini* having a wide geographical range in Balkan area and Turkey (Lodos, 1991). In Turkey, it is found at a considerable range of altitudes above 2200m. Hitherto, no karyological studies in this species have been done.

In the present work the karyotype of *C. macrogaster* was characterized by cytotaxonomical methods, including chromosome number, morphology, and C- and G-banding patterns and their relation to the other known karyotypes of *Bradyporini* is discussed. Additionally, the DNA content of *C. macrogaster* was calculated. The present results form the first part of a cytological survey of the *Bradyporini* and provide a basis for further population investigations and comparetive studies of interspecies or intraspecies of the *Bradyporini*.

Materials and Methods

Five adult males and six females of *Callimenus* (=*Bradyporus*) *macrogaster macrogaster* Lef. were collected from Manisa (The Spil Mountain) on Turkey in June 1999.

The males were injected with 0.1-0.2 ml of 0.1 % colchicine. After 1.5-3 hrs the testes were fixed in ethanol-acetic acid mixture (3:1). Tissues were then minced gently in 50 % acetic acid to prepare a cell suspension. A drop of the cell suspension was placed on heated clean glass slides. The cell left on the slide were dried and then stained for 20 min in 2 % Giemsa solution.

C-banding was performed using the method described by Sumner (1972). G-banding was induced appliying technique of Hillis et al.(1996) with minor modifications. Air-dried, preparations were digested for 25-30 seconds in a 0.02 trypsin solution, rinsed in cold PBS (0.15 M NaCl-0.05 M $NaHPO_4$, pH 7.4) for 10 minutes, stained with 5 % Giemsa (pH 6.8), washed twice in distilled water, dried and then embedded in Enthellan (Merck, www.merck.com).

The preparations were examined under a light microscope with 100X magnification and the metaphasic mitotic plates (10 plates for each male individual) with clear and well-distributed chromosomes were photographed. Chromosomes were classified according to Levan et al. (1964).

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For Feulgen cytophotometric estimation of 2C nuclear DNA content the fixed testes were hydrolysed in 5 N HCl for 30 minutes at room temperature and stained in Feulgen solution for one hour. The stained testes were washed in three changes of SO_2 water (5 gm SO_2, 5 ml HCl in 100 ml distilled water) for 10 minutes each. Darkly stained testes were squashed in a drop of 45 % glaciel acetic acid. Cytophotometric measurement of 2C spermatid DNA was made using a Reichert-Zetopan microspectrophometer, at a wavelength of 550 nm. On average, 35, 2C spermatid nuclei were measured in each of three replicates in this species. Chick erythrocyte nuclei were used as a control. Each 2C chick erythrocyte nucleus contains 2.88 pg of DNA (Mirsky and Ris, 1951).

Results

The analysed individuals of the species *C. macrogaster* were found to have a chromosome number of 2n ♂= 23 (22 A+ X) (FN= 32), and the basic Orthopteran sex determining mechanism, XX ♀/ X0 ♂. At the spermatogonial metaphase of this species two pairs (1, 2) of metacentric, two pairs (3, 4) of submetacentric, and six pairs (5-11) of acrocentric autosomes were visible. The metacentric X chromosome is the second largest chromosome (Figs. 1 & 2). The chromosome length, relative length, centromeric index, arm ratio and chromosome morphology were measured from 10 metaphasal plates and are presented in Table 1.

The C-banding pattern in the spermatogonial metaphase was characterized by the presence of paracentromeric C-bands in all chromosomes. Additionally, a distal C-band is present in chromosome 5 (Fig. 3).

The karyotype obtained after staining the chromosomes for G-bands is shown on Figure 4. The centromeric areas of all chromosomes were G-band positive. In chromosome 1 and X, there were dark bands and light interbands in the short and long arms. Other chromosomes had bands in the their long arms. Additionally, a wide G-band was present in chromosome 6.

The 2C nuclear DNA content of *C. macrogaster* was found to be 10.26 ± 0.16 picograms.

Discussion

Few chromosomal studies on the longhorned grasshoppers have been done in general and of species from Turkey in particular. In the Tettigoniidae, the 2n ♂ chromosome number ranges from 20 to 35 (Warchalowska-Sliwa, 1998) and they exhibit a certain degree of conservatism of karyotypes.

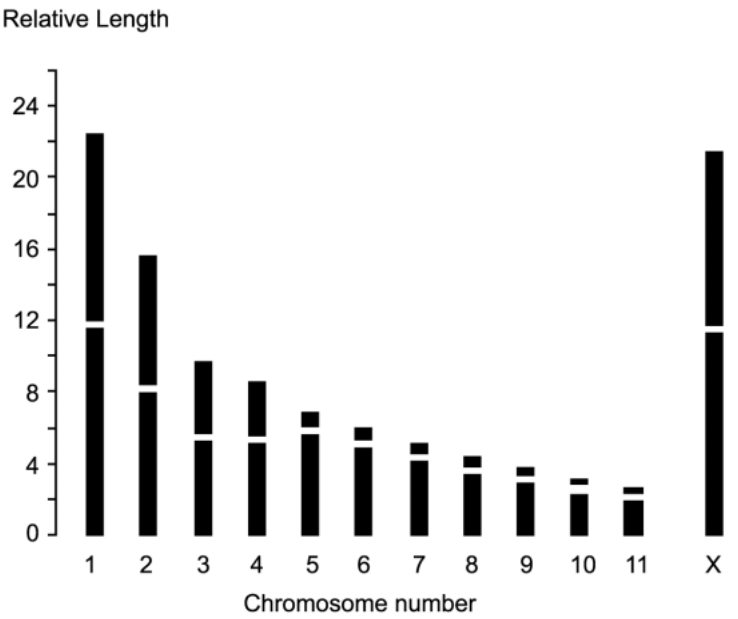


Figure 2. Idiogram of *Callimenus macrogaster*

In the subfamily Bradyporinae, 29 karyotypes (including chromosomal races) of 25 species (out of 12 genera), distributed in the South Palearctic area, have been so far described (Warchalowska-Sliwa, 1998). Three species of *Zichyini* are characterized by 2n ♂= 31, which is considered as the basic karyotype (Warchalowska-Sliwa and Bugrov, 1996). Bradyporinae show karyotypes ranging from 31 to 22 with an X0 or neo-XY sex determination mechanism, the latter occuring only in two species, *Callicrania seoanei* and *Pycnogaster cucullata.*

The chromosome numbers of species of *Bradyporini* range from 25 to 29 (Warchalowska-Sliwa, 1998). *Pycnogaster finotii*, *P. graellsii*, *P. inermis*, *P. sanchezgamezi* and *P. cucullata* have 29 chromosomes (♂) (L_1 metacentric; M_2-S_{14}, X acrocentric) (Fernandez-Piqueras et al., 1982; Sentis et al., 1988). *Bradyporus dasypus* and *Callimenus oniscus* have 27 chromosomes (♂) (L_1-L_2, X metacentric; M_3-S_{13} acrocentric and L_1, X metacentric; L_2 submetacentric; M_3-S_{13} acrocentric, respectively) (Warchalowska-Sliwa, 1998). *Bradyporus macrogaster pancici* has 25 or 29 chromosomes (♂) (Georgevitch, 1933), but the chromosome morphology of this species is not known.

As shown here, the chromosome complement of *C.*

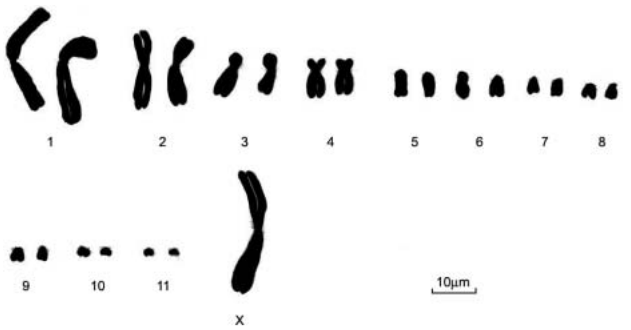


Figure 1. Male karyotype of *Callimenus macrogaster*

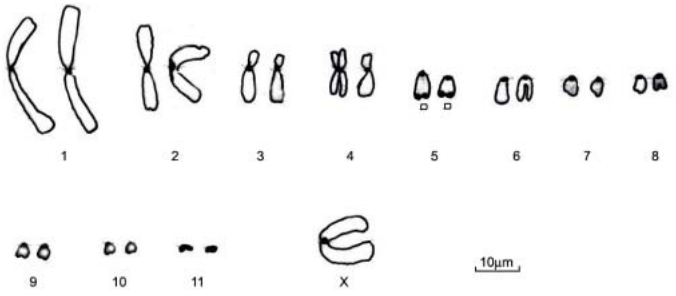


Figure 3. C-banding pattern of *Callimenus macrogaster*, (□) indicates distal C-band

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Table 1. Morphometric characteristics of the chromosomes of *C. macrogaster* based on 10 mitosis

Pair No	Chromosome length (μm) Mean± S. E.	Relative length (% of 2n set)	Centromeric index Mean± S. E.	Arm ratio Mean±S. E.	Chromosome morphology
1	13. 58 ± 0.58	21.90 ± 0.67	46.08 ± 1.83	1.17 ± 0.09	m
2	9. 32 ± 0.41	15.03 ± 0.26	44.44 ± 1.41	1.24 ± 0.07	m
3	5. 67 ± 0.25	9.14 ± 0.20	36.06 ± 2.37	1.79 ± 0.19	sm
4	5. 00 ± 0. 24	8.06 ± 0.17	29.72 ± 3.92	2.46 ± 0.40	sm
5	3. 48 ± 0. 25	5.61 ± 0.25	-	∞	a
6	2. 99 ± 0. 16	4.82 ± 0.10	-	∞	a
7	2. 51 ± 0. 16	4.04 ± 0.13	-	∞	a
8	2. 11 ± 0.10	3.40 ± 0.12	-	∞	a
9	1. 77 ± 0. 10	2.85 ± 0.13	-	∞	a
10	1. 42 ± 0.08	2.29 ± 0.07	-	∞	a
11	1. 18 ± 0. 18	1.90 ± 0.09	-	∞	a
X	12. 96 ± 0. 85	20.90 ± 0.65	43.44 ± 2.30	1.31± 0.11	m
T. C. L.	61.99				

m: metacentric, sm: submetacentric, a: acrocentric; T: C. L.: Total Chromosome Length.

macrogaster consists of the standard karyotype with 2n = 23 chromosomes (two pairs and X chromosome metacentric; two pairs submetacentric; six pairs acrocentric) chromosomes in the male. The lower chromosome numbers 2n ♂ = 27, 25, and 23 in this genus were formed owing to complex translocations (Fernandez-Piqueras et al., 1983; Warchalowska-Sliwa and Bugrov, 1996).

The sex determination XX ♀ and X0 ♂ in *C. macrogaster* was observed. However, Fernandez-Piqueras et al. (1982, 1983) recorded a neo-XY sex chromosome mechanism in *P. cucullata* (2n=26+XY).

The application of banding procedures in cytogenetic studies facilitates the understanding of various problems. In Orthoptera, the C-banding technique and Ag-staining of the nucleolus organizer region (NOR) is normally used in comparative studies of populations, races and species. As described here, paracentromeric C-bands are uniformly present in the long and medium sized chromosomes of *C. macrogaster*. Additionally, in this species pair 5 has a distal C-band. However, paracentromeric, distal and interstitial C-positive bands were demostrated in a few species of Tettigonioidea (Fernandez-Piqueras et al. 1984; Navas-

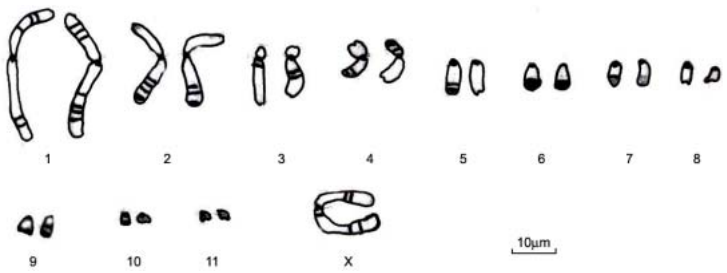


Figure 4. G-banding pattern of *Callimenus macrogaster*.

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Castillo et al. 1986; Warchalowska- Sliwa and Maryanska-Nadachowska, 1992).

Although the G-band technique does not yield G-bands like those in mammalian chromosomes, it provides considerable information about the nature of many chromosomal regions in *C. macrogaster*. As can be seen, the intensity of staining of the G-bands were different and their localization very diversified (Fig. 4). The identification of the particular pairs of chromosomes after G-banding was therefore difficult. In pairs 5, 7, 9, 10 were weakly staining bands in some other pairs they were easily stained (pairs 2, 3, 6, 8, 11), while in still other pairs mixed bands were visible. Dark G bands correspond to chromatin containing AT-rich DNA sequences, while light interbands reflect GC-rich DNA sequences. Moreover, it seems clear that nonhistone proteins and their interactions with DNA are involved in the production of G-bands (Camacho et al. 1991).

DNA content is now known from direct measurements for close to a thousand species of animals and plants and from indirect measurements, such as calculations from chromosome or nuclear size, for many additional species. These amounts in free-living organisms range from 0.007 pg. for an average bacterium to 100 pg. or more for the haploid amount of DNA in some plants and salamaders (Gosalvez et al. 1980).

The first relationship between DNA content and the evolutionary process comes from Mirsky and Ris's works (1951). They suggest three relationships between DNA content and evolution: i) there might be an increase in DNA content, going from the lower invertebrates to the higher invertebrates; ii) related organisms, such as members of the same family, tend to have similar amounts of DNA; iii) evolution of the land vertebrates may have been accompanied by a decrease in amounts of DNA.

In Orthoptera, such measurements have revealed some evolutionary paths of great interest: Fox (1970), and Willmore and Brown (1975), established values for the DNA content in different species. John and Hewitt (1966) found that significant differences in DNA content exist both between species within the same chromosome group, and between member species of the 2n=17 and 2n=23 groups. In the present work, we found that the 2C nuclear DNA content of *C. macrogaster* was 10.26 ± 0.16 picograms. The existing data concerning the 2C nuclear DNA content of Orthoptera in Turkey are absent. This necessitates further investigations, the work presented here being the initiation of such studies.

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