PROCEEDINGS OF THE BALKAN SCIENTIFIC CONFERENCE OF BIOLOGY IN PLOVDIV (BULGARIA) FROM 19TH TILL 21ST OF MAY 2005 (EDS B. GRUEV, M. NIKOLOVA AND A. DONEV), 2005 (P. 560–567)

CHROMOSOME POLYMORPHISM IN *CHIRONOMUS BALATONICUS* DEVAI, WÜLKER, SCHOLL (DIPTERA, CHIRONOMIDAE) FROM BOURGAS LAKE

Boris Krastanov*

Institute of Zoology, Bulgarian Academy of Sciences, blv. Tzar Osvoboditel 1, 1000 Sofia, Bulgaria: e-mail: krastanov@zoology.bas.bg

ABSTRACT. Seasonal and year fluctuations of chromosome polymorphism in C. balatonicus from Bourgas Lake were investigated. The chromosome polymorphism features – frequency of larvae with heterozygous inversions and heterozygous inversions per larva showed seasonal dependence: increased in spring and summer and decreased in winter. Statistically significant correlation between seasonal fluctuations of the temperature and frequency of specimens with the heterozygous inversion A12 was also established.
Besides structural chromosome rearrangements high functional activity was found: appearance of new puffs, ectopic pairing between the chromosomes, decondenzation of the telomeres and heterozygous state in BR_2. These structural and functional chromosome aberrations in the light of the impact of abiotic factors on the chironomids karyotype are discussed.

KEY WORDS. Chironomidae, *Chironomus balatonicus,* polytene chromosomes, chromosome aberrations

INTRODUCTION

Chironomus balatonicus DEVAI, WÜLKER, SCHOLL, 1983, is a widely distributed species in Palearctic. This species is characterized by high level of chromosome and genome polymorphism (KIKNADZE *et al.* 1991, BELJANINA, LOGINOVA 1993, MICHAILOVA 1989, 2001, MICHAILOVA, PETROVA 1994, MICHAILOVA, KRASTANOV 2000, MICHAILOVA *et al.* 2002). The chromosome polymorphism is realized by heterozygous and homozygous inversions. Some of these inversions form polymorphous system characterized by transition of one homozygous inversion into another through heterozygotization (MICHAILOVA, PETROVA 1994, PETROVA *et al*. 1994).

The genome polymorphism is expressed with an additional "B" chromosome (PETROVA 1991, MICHAILOVA, PETROVA 1994, GOLYGINA *et al*. 1996).
The purpose of the present study is to clarify chromosomal polymorphism and seasonal and year fluctuation in chromosome variability in *Chironomus balatonicus* from Bourgas Lake.

MATERIAL AND METHODS

The larval material has been collected in the mud from the bottom of Bourgas Lake during the period X.2001-X.2003. The larvae were fixed in a 3:1 mixture of 96% ethanol and glacial acetic acid. Polytene chromosome preparations were prepared by routine aceto-orcein method (MICHAILOVA 1989). The cytotaxonomical differentiation of the larval material has been done according to Michailova (1989) and Kiknadze et al. (1991).
The frequency of aberrations was calculated in percentage. Deviation of heterozygous inversions by Hardy-Weinberg equilibrium was statistically analyzed using χ^2 method. The statistical significance of the heterozygotes frequency between the samples by the different years was tested with G-test. Correlation analysis was used to establish dependence between variation of the chromosome aberrations and the seasonal fluctuation of some abiotic factors. Software programs package "Easy-Stat", version 2.3 (1991) both for "G-test" and "Correlation analysis" were used.
The chemical analysis of superficial water of Bourgas Lake was provided from the Regional Agency of Nature and Water Protection – Bourgas.

RESULTS

Chironomus balatonicus from Bourgas lake shows the same karyological characteristics as the other Palaearctic populations (DEVAI *et al.* 1983, KIKNADZE *et al.* 1991, MICHAILOVA *et al*. 2002.). This species belong to *thummi* complex and has 2n = 8 chromosomes. Chromosomes AB and CD are metacentric, chromosome EF – submetacentric and chromosome G- telocentric. One Balbiani ring BR_1 is localized of chromosome AB (arm B). Chromosome G carries two Balbiani rings (BR_2 and BR_3) and a nucleolus (NOR).
Chironomus balatonicus is the only species identified cytotaxonomicaly in Bourgas lake.
In 98% of studied specimens heterozygous inversions have been established. The average number of heterozygous inversions per larva was 1,5.
In arms B, D and G, the standard banding pattern predominated. In arms A and C, the heterozygotes (A12, C12) were most frequent. In chromosome AB, heterozygous pericentric inversion has been found in 20% of the specimens. The arms E and F were monomorphous. The frequency of heterozygous inversion A12 showed statistically significant values both in 2002 and 2003 year. In 2002 it was more frequent while in 2003 was considerably lower. Significant dependence between seasonal variation of the temperature and frequency of individuals with the same inversion (A12) was established ($P<0.05$), (Fig. 1). The observed heterozygotes and heterozygous inversion per larvae showed also seasonal dependence increased in

spring and summer and decreased in winter ($P<0.05$), (Fig. 2). Annual fluctuations in frequency of heterozygous inversions in arms A and G were also found (Fig. 3). The frequency of the inversion A12 in 2002, was significantly different from those in 2001 and in 2003 years ($P<0.05$, $P<0.001$), while inversion G12, in 2003 year differed from those in 2001 and in 2002 years ($P<0.05$).

High functional activity of the polytene chromosomes was found. In 7% of individuals an active puff near the centromere in arms A and B respectively were detected. In all individuals with the inversion D13 an active puff in the homologue D3 of arm D was also observed. All chromosome arms were involved in ectopic contacts with almost equal frequency (18-20%). With low frequency (13%) in summer larvae only decondenzation of the telomeres of all chromosome arms was observed. In 27% of studied larvae heterozygous BR3 was found.

DISCUSSION

The study of seasonal dynamic of the chromosome polymorphism in *Chironomus balatonicus* from Bourgas Lake showed statistically significant coincidence in the fluctuation of the inversion heterozygotes A12 and seasonal variations of the temperature. Such a dependency was established in *Chironomus balatonicus* for the first time. In the sibling species of *Chironomus balatonicus* – *C. plumosus* LINNAEUS, 1758, connection between frequencies of the heterozygotes B12 and the depth, in which the larvae live, is established. Larvae living in the greatest depth display the highest frequencies of these heterozygotes (PEDESEN 1978). In this species from Novgorod the same inversion is considerably increased in the spring and decreased in autumn and winter (ILYNSKAJA *et al.* 1999). The peak of heterozygosity has been observed in summer and a minimum in spring and autumn. Similar dependency was detected in *C. balatonicus* from Bourgas Lake: the frequency of all larvae with heterozygous inversions and heterozygous inversions per larva were high in spring and summer and lower in winter.

In 2002 the frequency of heterozygous larvae, was higher than those in 2002 and 2003. In this year the frequency of heterozygous inversion A12 was also significantly high and considerably deviated from Hardy-Weinberg equilibrium. It is very important to note that in this year the chemical oxygen demand (COD), exceed the limit of tolerance. The high organic pollution in this year is probably the reason for the observed deviation. High frequency of structural and functional chromosome alterations in stress ecological conditions was also established in other species from genus *Chironomus*. In *C. riparius* MEIGEN, 1804, from regions with increased amounts of heavy metals, are registered different somatic structural aberrations and functional alteration (inductions of novel puffs, telomeric and centromeric decondensations) (MICHAILOVA *et al.* 2001). Ectopic paring between the chromosomes and heterozygous state of BR_2 were observed in *Chironomus balatonicus* from Chernobil (MICHAILOVA, PETROVA 1994) and from Bulgaria in heavy metal polluted biotopes (ILKOVA 2004). The genome reactions of *Chironomus balatonicus* from Bourgas Lake, where the pH, organic and inorganic pollution were upper the standard value are very similar: new puffs, ectopic conjugation between the chromosomes, change of the heterohromatin appearance in the telomeres regions and heterozygous state in BR_2.

It might be possible the anthropogenic pollution in the lake, significantly exceeded the values accepted as standard is the reason for such high structural and functional activity in *Chironomus balatonicus*, but also complex impact both of anthropogenic and abiotic factors of the environment are possible.

ACKNOWLEDGEMENTS

This work was supported by grants B-MU-1104 and grand B-903 from the Ministry of Education and Science of Bulgaria. The author wishes to thank cordially to Prof P. Michailova (Institute of Zoology, BAS) for her advices and valuable suggestions. I wish to thank the Bulgarian Society for the Protection of Birds for the help in collecting of part of studied material and to the Regional Agency of Nature and Water Protection – Bourgas for the information concerning the monitoring of the superficial water of Bourgas Lake.

REFERENCES

AJALA, F., J. KIGER 1987. Modern Genetics. 1031 p. (In Bulgarian).

BELIANINA, C., N. LOGINOVA 1993. Banding pattern in the polytene chromosomes of *Chironomus* species from plumosus group. I. Karyofond of C*hironomus balatonicus*. – *Tsitologia*, 35 (4): 87-91. (In Russian, English summary).

DEVAI, G., W. WULKER, A. SCHOLL 1983. Revision der Gattung *Chironomus* Meigen (Diptera). IX. *C. balatonicus sp. n.* aus dem Flachsee Balaton (Ungarn). – *Acta Zoologica Academiae Scientarum Hungaricae*, 29 (4): 357-374.

GOLYGINA, G., A. ISTOMINA, A. RAKISHEVA, I. KIKNADZE 1996. New banding sequences in the *Chironomus balatonicus* karyofund. – *Tsitologia*, 38 (8): 869-883 (In Russian, English summary).

ILINSKAYA, N., N. PETROVA, I. MATENA. 1999. Inversion polymorphism of *Chironomus plumosus* L. (Diptera, Chironomidae) and it's depending on the type of biotope, season and year.- Genetica, 35, 8, 1061-1070. (Russian, English summary).

ILKOVA, J. 2004. Cromosome variability of species of subfamily Chironominae (Diptera, Chironomidae) from polluted Region near Sofia. *Acta Zoologica Bulgarica,* 56 (1): 15-30.

IVANOV, K., A. SOTIROV, A. ROZDESTVENSKI, D. VODENICHAROV 1964. *Work of the Institute of hydrology and meteorology,* XVI: 241 p. (In Bulgarian).

KEYL, H. G. 1962. Chromosomenevolution bei *Chironomus* Chromosomenumbauten und phylogenetische Beziehungen der Arten. – *Chromosoma*, 13: 464-514.

KIKNADZE, I., A. SHILOVA, I. KERKIS, H. SHOBANOV, N. ZELENZOV, L. GREBENJUK, A. ISTOMINA, V. PRASLOV 1991. Karyotypes and larval morphology of tribus Chironomini, *Atlas*. – *Novosibirsk, Nauka,* 115 p. (In Russian, English summary).

MICHAILOVA, P. 1989. The polytene chromosomes and their significance to the systematic of the family Chironomidae, Diptera, *Acta Zoologica Fennica,* 186, 107p.

MICHAILOVA, P. 2001. Cytogenetic characteristics of species of the *Chironomus plumosus* group (Chironomidae, Diptera) in Finland. – *Cytobios*, 105: 99-114.

MICHAILOVA, P., J. ILKOVA, N. PETROVA, K. WHITE 2001. Rearrangement in the salivary gland chromosomes of *Chironomus riparius* Meig. (Diptera, Chironomidae) following exposure to lead. – *Caryologia*, 4: 349-363.

MICHAILOVA, P., B. KRASTANOV. 2000. Cytotaxonomical Differentiation of *Chironomus plumosus* Group (Diptera: Chironomidae) from Fish Pools near plovdiv, Bulgaria. – *Acta Zoologica Bulgarica*, 52 (1): 29-40.

MICHAILOVA, P., B. KRASTANOV, A. KOWNACKI. 2002. Cytotaxonomical characteristics of Genus *Chironomus* Meigen (Diptera: Chironomidae) from different localities of Poland. Annales zoologici (Warszawa), 52 (2): 215-225.

MICHAILOVA, P., N. PETROVA 1994. Cytogenetic characteristics of *Chironomus balatonicus* Devai, Wulker, Scholl. (Diptera, Chironomidae) from the Chernobyl region. – *Cytobios,* 79: 15-29.

PEDERSEN, B. 1978. Comparisons of the inversion polymorphism in three Danish populations of the midge *Chironomus plumosus* L. (Diptera: Chironomidae). *Hereditas*, 89: 151-162.

PETROVA, N. O. IVANCHENKO, I. KERKIS 1994. Cytogenetic structure of *Chironomus balatonicus* (Diptera, Chironomidae). – *Cytologia*, 36 (5): 469-478 (In Russian, English summary).

PETROVA, N., P. MICHAILOVA 1996. Cytogenetyc monitoring of *Chironomus balatonicus* Devai, Wulker, Scholl (Diptera, Chironomidae) from the Chernobil Region. – *International Journal of Dipterological Research*, **7** (2): 79-86.

Stat-Soft. 1991. „Easy-Stat“. – version 2.3.

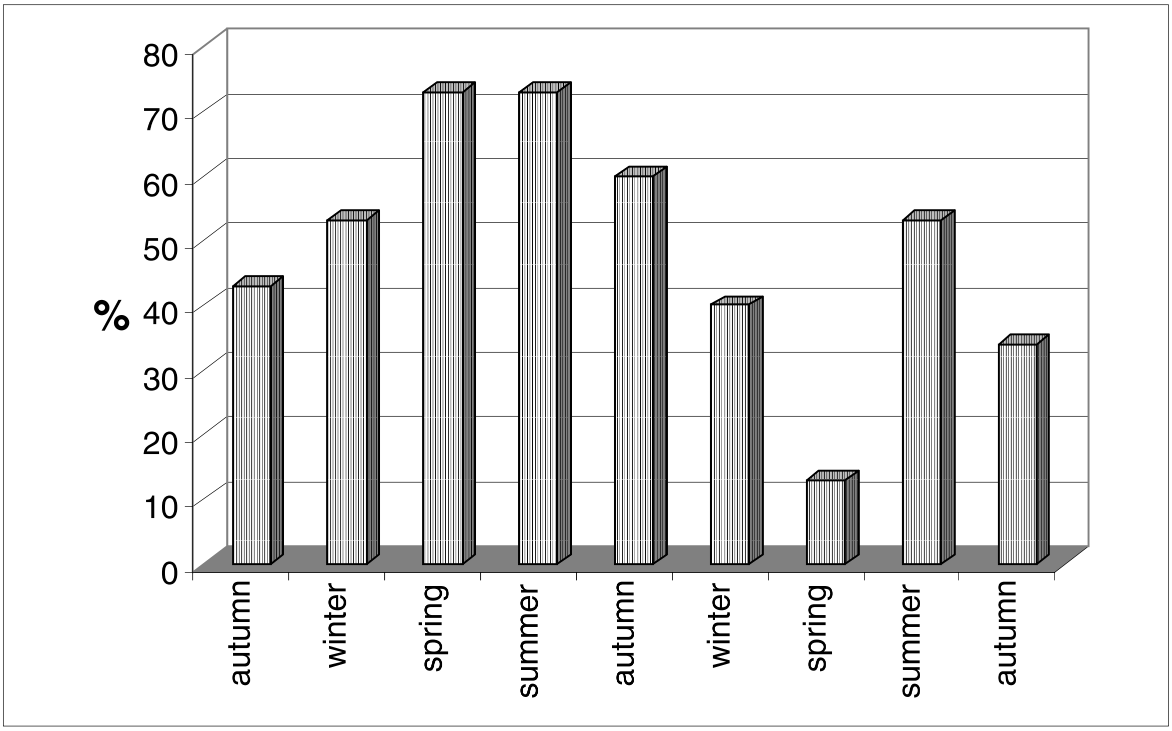

Figure 1. Seasonal fluctuation in frequency of specimens with the heterozygous inversion A12.

Denotes statistically significant values in frequency of heterozygous inversion A12 in spring both in 2002 and 2003 year.

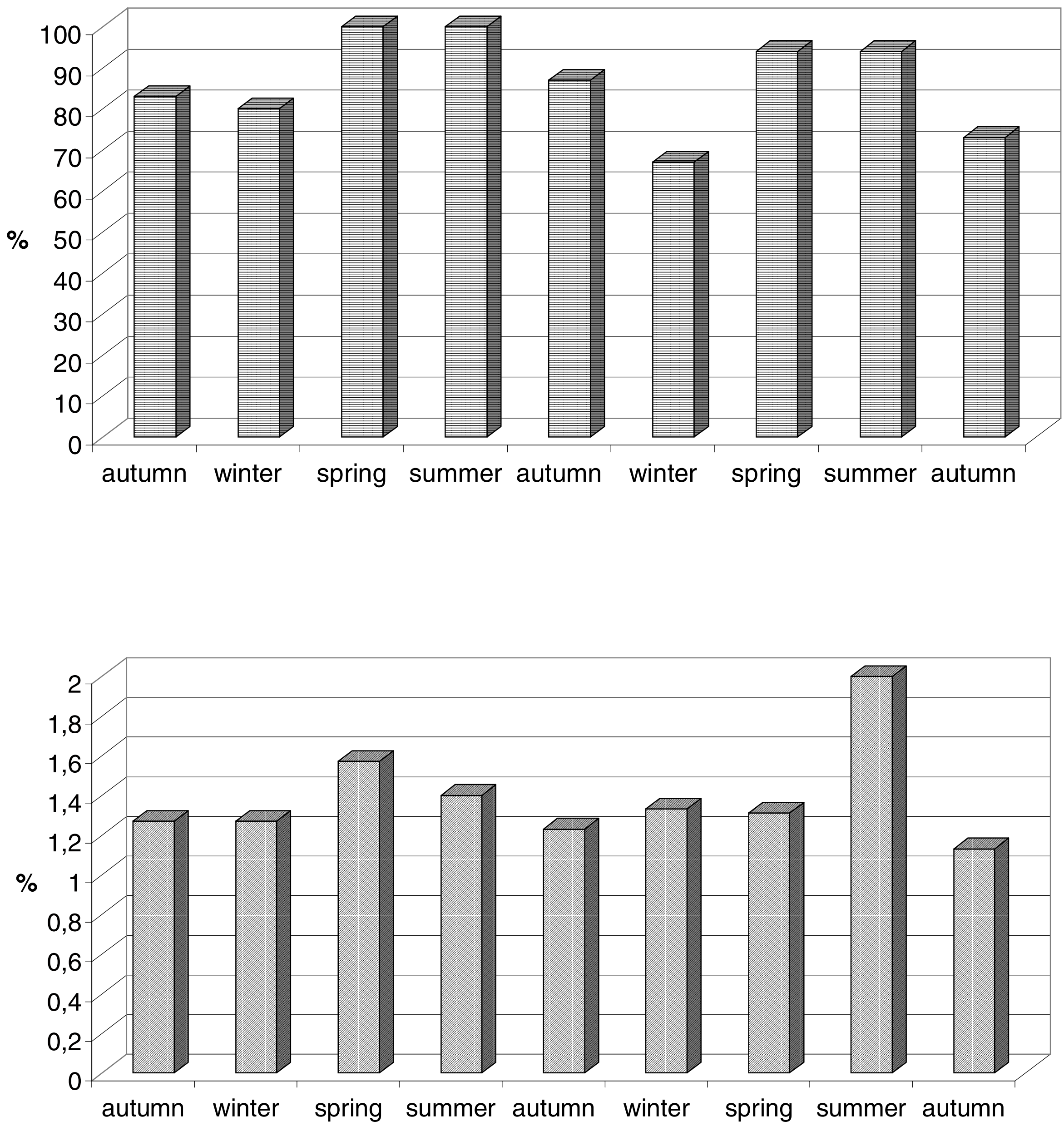

Figure 2. Seasonal alteration in the chromosome polymorphism features:
a) Frequency of larvae wit heterozygous inversions.
b) Heterozygous inversion per larva.

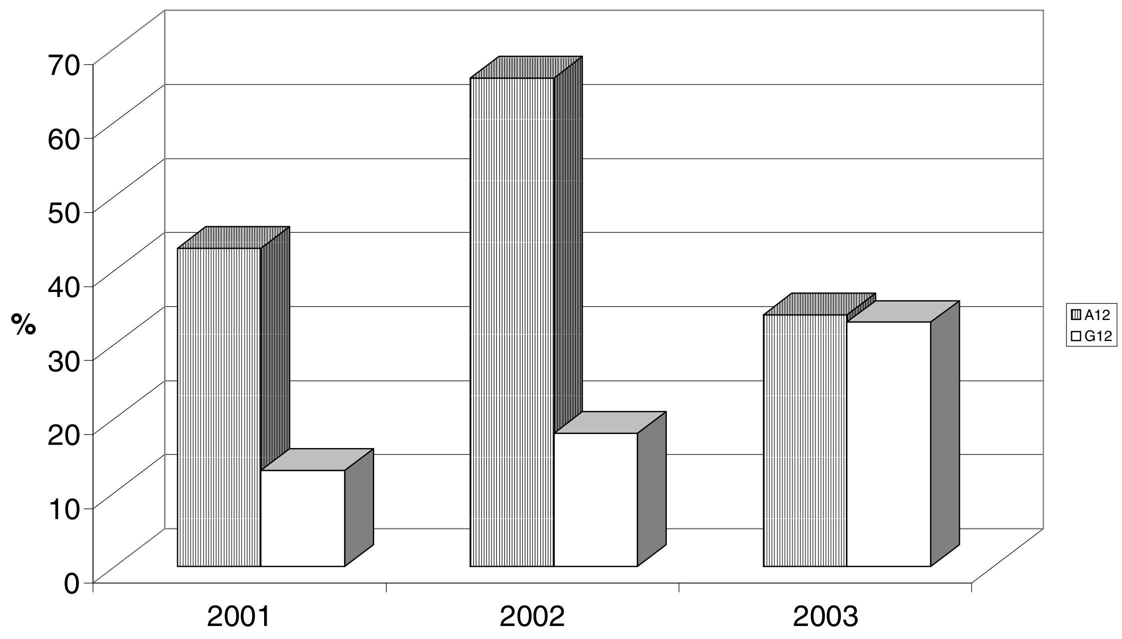

Figure 3. Annual variation in frequency of heterozygous inversions in the arms A and G.