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Chromosomal polymorphism of *Chironomus plumosus* (Linnaeus, 1758) (Diptera: Chironomidae) from Bulgaria (Pazardjik region) and the Czech Republic (Ceske Budejovice region)

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Abstract. Chromosomal polymorphism of *Chironomus plumosus* (L.) from two geographically isolated populations is studied. Both populations revealed clear differences in their polymorphism. The Czech population is characterized by a low level of chromosome polymorphism. The karyotype of *C. plumosus* in this population differs from that of the Bulgarian population by the monomorphism of arms B, D, E, F and G and a low level of hetero- and homozygous states in arms A and C respectively. Nine sequences which range 9 genotypic combinations were found in this population. The high frequency of genotypic combination pluC1.1 would suggest it has selective value in this population. The studied Bulgarian population revealed a high level of chromosome polymorphism. Thirteen banding sequences and 16 genotypic combinations were observed in the region studied. In arms A, C, D alternative homozygous sequences and heterozygous between them were detected. They are adapted to different environmental components.

Key words: *Chironomus plumosus*, Bulgaria, Czech Republic, chromosomal polymorphism, cytogenetic distance.

Introduction

Natural populations maintain a great degree of chromosomal polymorphism expressed by inversions, translocations and extra chromosomes (White, 1978). Many evolutionary cytogenetists have emphasized the role of chromosomal polymorphisms as cytogenetic markers of population adaptation to different environments and speciation processes (King, 1993; Kaidanov, 1996; Kiknadze et al., 2007). Paracentric inversion polymorphism is remarkably common in many chironomid species (Keyl, 1962; Martin, 1979; Butler et al., 1999; Kiknadze et al., 2007; Petrova et al., 2007). Such inversion polymorphism is common in chironomids owing to heterozygous advantage (Lande, 1984; Dobzhansky, 1990). Very often chromosome inversions have been found to occur in almost all the chromosomes of the chironomid karyotype. However, in some chromosome arms their frequencies far surpass those in others. This tendency is very well seen in *Chironomus plumosus* (Linnaeus, 1758) – a widely distributed species in the Pa-

Table 1. Localities and number of studied individuals of *C. plumosus*.

Country	Type of basins	Localities	Time of collecting	Number of studied specimens
Bulgaria	A channel formed by spring water	Pazardjik (Saraja)VI.	VI.2002	35
			VI.2003	15
			VI.2004	20
Czech Republic	Fishpond "Svetlik"	Ceske Budejovice	1989	25

laearctic and Nearctic. Its larvae can be found in artificial and natural fresh waters, in stagnant and running waters of different depth and contamination level.

Chromosomal polymorphism of this species has been studied extensively in different Palaearctic populations (Keyl, 1962; Pedersen, 1978; Michailova, Fisher, 1986; Michailova, Petrova, 1991; Shobanov, 1994; Petrova et al., 1996, 2007; Butler et al., 1999). Some authors showed a high chromosome polymorphism in *C. plumosus* collected from different habitats (Krastanov, Michailova, 2008) as well as in long term studies (Matena, 1991; Petrova et al., 2007). In addition to inversion polymorphism genomic polymorphism also appeared in Palaearctic populations. It is associated with the presence of additional B chromosomes (Keyl, Hägele, 1971; Michailova, Mettinen, 2000). Another interesting event has been described in this species: considerable polymorphism in the size of centromeric heterochromatin for different populations (Kiknadze, Siirin, 1991; Kiknadze et al., 1991; Michailova, Petrova, 1991; Hankeln et al., 1994).

In this paper the chromosomal polymorphism of *C. plumosus* from two geographically isolated populations (Bulgaria and Czech Republic) is studied. The cytogenetic structure of Bulgarian population has been observed over three years.

Material and methods

The material for these studies has been collected from two geographically isolated regions: in the Pazardjik regions (Bulgaria) and the "Svetlik" fishponds at the periphery of the town of Ceske Budejovice (Czech Republic). The samples in Bulgaria were collected in a channel formed from spring water, from the muddy bottom at about 0.3-0.4 m. The samples from the Czech Republic were taken from the muddy bottom at a depth of 0.4-0.7 m. The locality here is classified as a eutrophic pond. The sampling in Bulgaria was done during a period of three years (2002-2004). The time of collection and sample size are shown in Table 1. A total of 95 IVth instar larvae were analyzed cytogenetically. Acetic acid: alcohol (1:3) fixed, 2% aceto-orcein stained squash preparations of salivary gland chromosomes were analyzed for specific band sequences of all chromosomes.

Mapping of the chromosome arms was done according to Bulter et al (1999). The banding sequences were indicated by the symbol of the species, the symbol of the chromosome arm, and by number of the sequence (for instance: pluA1; pluA2; and others). The homozygous and heterozygous combinations of the banding sequences were shown as pluA1.1; pluA2.2 or as pluA1.2, pluA2.4 (Butler et al., 1999).

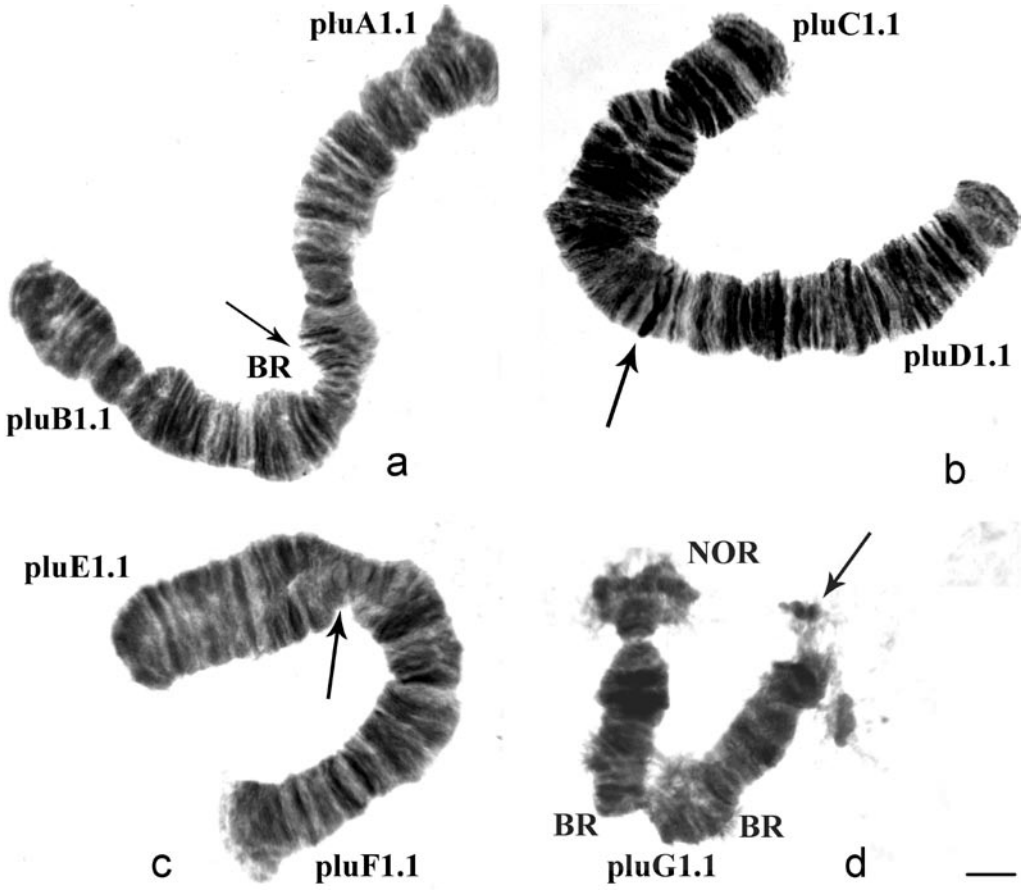

Fig. 1. Polytene chromosomes of Chironomus plumosus L. from the Czech Republic. **a -** chromosome AB (pluA1.1 pluB1.1). **b -** chromosome CD (pluC1.1 pluD.1.1). **c -** chromosome EF (pluE1.1 pluF1.1). **d -** chromosome G (pluG1.1). BR - Balbiani ring; NOR – nucleolar organizer, arrow – the position of centromere region. Bar = 10 μm.

The frequency of heterozygous individuals of different populations was compared by G test (Sokal, Rohlf, 1995) using "Easy - Start" program, version 2.3. For each population the frequencies of band sequences as well as genotypic combinations were calculated. Heterozygous frequencies within each population were tested for agreement with the expectation of Hardy-Weinberg equilibrium using $\chi 2$ test. A probability of $P<0.05$ was taken as significant. The significance of Chi-square is tested by Fischer test (Snedecor, Cochran, 1967).

Results

1. Cytogenetic characteristics

The karyotype of *C. plumosus* (2n=8) consists of three long chromosomes (chromosomes AB and CD – metacentric, chromosome EF – submetacentric) and one short (chromosome G – acrocentric) (Fig.1, a-d). It has three Balbiani rings (BRs): one in chromosome arm B and two in chromosome G. The nucleolar organizer (NOR) is localized also in chromosome G (Fig.1, d). *C. plumosus* belongs to the cytocomplex "thummi" with chromosome arm combinations: AB CD EF G. The centromere

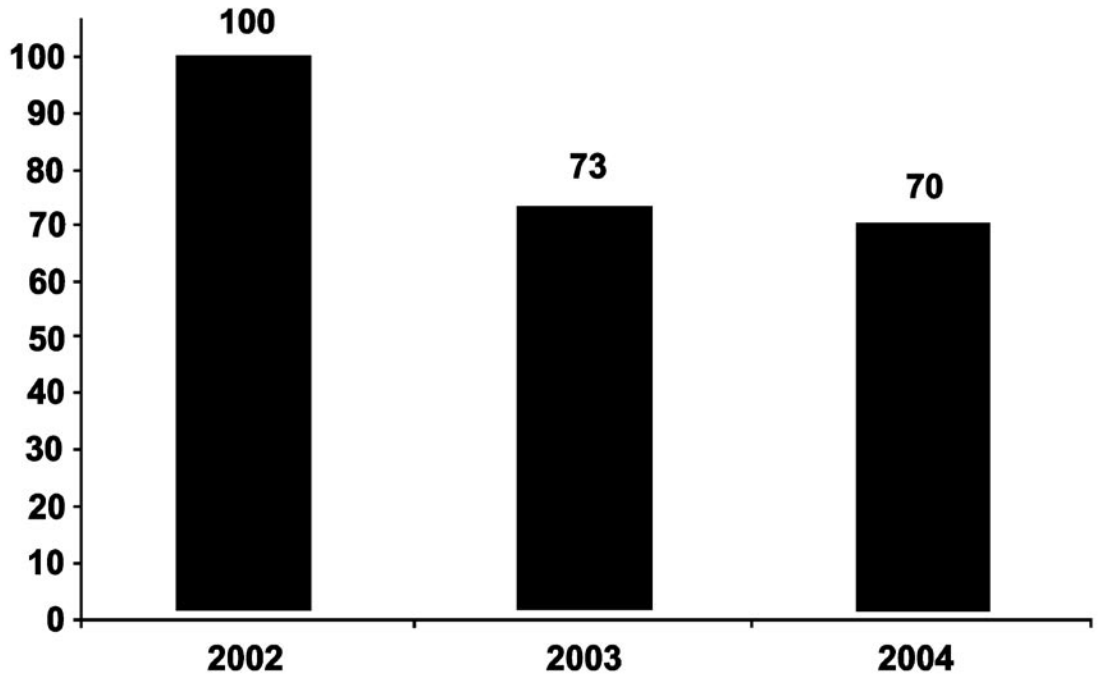

Fig. 2. Frequency of heterozygous larvae in a channel of spring water (Bulgaria, Pazardjik), during three years.

regions of the chromosomes are expressed by thin dark bands in the studied populations.

2. Chromosome polymorphism

Inversion polymorphism in arms A, B, C, D and F was found in Bulgarian population. Czech population is almost monomorphic. The type and frequencies of band sequences and chromosome inversions are given in Table 2 and Table 3 respectively. In Bulgarian population the following genotypic combinations for every arm were observed: 4 for arm A; 2 for arm B; 3 for arm C; 3 for arm D; 2 for arm F; and 1 for arms E and G. In Czech population the genotypic combinations were: 2 for arms A and C; 1 for arms B, D, E, F and G. It is important to emphasize that the observed zygotic frequencies in all chromosomes are in good agreement with Hardy-Weinberg expectation except in arm C of Czech Republic population (Table 3).

Arm A. It had three sequences in the populations studied (Table 2). A considerable prevalence of the pluA1 sequence was found in the Czech population (Table 2, Fig.1, a). The sequences pluA2 and pluA1 occurred almost at the same frequency in the Bulgarian population (Table 2). The sequence pluA3 was found only as a heterozygote at a very low frequency in the Bulgarian population (Table 3).

Arm B. This arm had two sequences (Table 2). The pluB1 sequence predominated and appeared in homo- and heterozygous states (Table 3). The sequence pluB3 occurred at a low frequency in the heterozygous state in the Bulgarian population only (Table 3).

Arm C. There were two alternative sequences only: pluC1 and pluC2 (Table 2) which appeared in homo- and heterozygous states (Table 3). The homozygous genotypic combination pluC1.1 predominated in Ceske Budejovice (Table 3). In the Bulgarian population in three years studied the heterozygous state pluC1.2 dominated.

Table 2. Frequency of band sequences in the polytene chromosomes of *C. plumosus* from water basins in Bulgaria and Czech Republic

Band sequences	Bulgaria			Czech Republic
	2002	2003	2004	1989
pluA1	0.51	0.47	0.50	0.90
pluA2	0.47	0.50	0.50	0.10
pluA3	0.01	0.03	0	0
pluB1	0.99	1	1	1
pluB3	0.01	0	0	0
pluC1	0.66	0.63	0.47	0.96
pluC2	0.34	0.37	0.52	0.04
pluD1	0.29	0.70	0.40	1
pluD2	0.71	0.30	0.60	0
pluE1	1	1	1	1
pluF1	0.99	1	1	1
pluF2	0.01	0	0	0
pluG1	1	1	1	1

Arm D. Two sequences were detected in this arm (Table 2). In the Czech population only the genotypic combination pluD1.1 was observed. In the Bulgarian population the combinations pluD1.1, pluD1.2 and pluD2.2 were found. However, in the three years studied they appeared in different frequencies without significant differences between them (Table 3).

Arm E. This arm was found to be monomorphic, including only one sequence, plu E1 (Table 2 and Table 3).

Arm F. This had two sequences – plu F1 and plu F2. The dominant sequence was plu F1 (Table 2) whereas the sequence plu F2 was observed in the heterozygous state in the Bulgarian population, in one year only (Table 3).

Arm G. This was monomorphic, with only one sequence plu G1 (Table 2 and Table 3).

In all, 13 banding sequences and 16 genotypic combinations were observed in the Bulgarian population. The average number of heterozygous inversions per larva was between 0.7 and 1.8. The frequency of heterokaryotypes in the three studied years was between 68.8% ± 8.2 and 95% ± 4.9. The frequencies of heterozygotes in 2002 were significantly different from those in 2003 ($G = 9.05$, $df = 0.01$) and 2004 ($G = 30.07$, $df = 1$, $P < 0.001$) (Fig.2).

Nine band sequences were observed in the Czech population which produced 9 genotypic combinations. *C. plumosus* from this population was less polymorphic: the frequency of the heterokaryotypes was 20% ± 8. The average number of heterozygous inversions per individual was 0.2.

Discussion

Study of chromosome variability of *C. plumosus* from two geographically isolated populations revealed clear differences in their chromosome polymorphism. The very low level of chromosome polymorphism observed in the Czech population confirms the previous data from another Southern Bohemia Czech population (Matena, 1991) where the heterozygous inversions were 0.44 per individual. In the studied population it was even less, 0.2. Rates of heterozygous inversions from lakes in south Trans Ural region also appeared to be lower (between 0.4 and 0.6) (Philinkova, 2007). Also, the level of chromosomal polymorphism is much lower in peripheral populations of *C. plumosus* (Petrova et al., 1996). In the studied fishpond "Svetlik" the sequence C1 dominated. The same sequence has been established by Matena (1991) in a fish pond in Southern Bohemia. (In the paper by Matena (1991) this sequence was indicated as C2. However, according to the new chromosome nomenclature it corresponds to the sequence C1). The high frequency of this sequence may

Table 3. Types and frequency of chromosome inversions of *C. plumosus* from natural water basins in Bulgaria and Czech Republic. * Significantly deviate from Hardy-Wainberg equilibrium (P < 0.01).

Inversion sequences	Bulgaria						Czech Republic	
	2002		2003		2004		1989	
	Number of specimens	Frequency	Number of specimens	Frequency	Number of specimens	Frequency	Number of specimens	Frequency
pluA1.1	7	0.20	2	0.13	7	0.35	20	0.800
pluA1.2	21	0.60	9	0.60	6	0.30	5	0.200
pluA2.2	6	0.17	3	0.20	7	0.35	0	0
pluA1.3	1	0.03	1	0.07	0	0	0	0
χ^2		1.92		1.22		3.2		0.25
pluB1.1	34	0.97	15	1	20	1	25	1
pluB1.3	1	0.03		0	0	0	0	0
χ^2	0.008		0		0		0	
pluC1.1	15	0.43	5	0.33	4	0.20	24	0.960
pluC1.2	16	0.46	9	0.60	11	0.55	0	0
pluC2.2	4	0.11	1	0.07	5	0.25	1	0.040
χ^2	0.007		0.49		0.03		**6.31***	
pluD1.1	5	0.14	7	0.47	3	0.15	25	1
pluD1.2	10	0.29	7	0.47	10	0.50	0	0
pluD2.2	20	0.57	1	0.06	7	0.35	0	0
χ^2	0.007		0.03		0.04		0	
pluE1.1	35	1	15	1	20	1	25	1
χ^2		0		0		0		0
pluF1.1	34	0.97	15	1	20	1	25	1
pluF1.2	1	0.03	0	0	0	0	0	0
χ^2	0.008		0		0		0	
pluG1.1	35	1	15	1	20	1	25	1
χ^2	0		0		0		0	

indicate its selective value in this population. Pedersen (1978) also showed a high frequency of another sequence (plu B2) in Lake Tystryp-Bave (Denmark), which is characterized by oxygen demand. Pedersen (1984, 1986) has found the B12 heterozygote to be superior to both homozygotes under conditions with an oxygen deficit. In another fishpond in South Bohemia without oxygen deficit at the bottom, significantly fewer B12 heterozygotes compared with HW equilibrium were consistently found in subsequent generations over a period of 7 years (Matena 1991). Moreover, a gradual shift from the prevalence of B2 to B1 sequences was observed during the study. Even more, Petrova et al. (2007) called this sequence "ecological" because it was shown that the increase in frequency of the hetero-

zygous inversions pluB1.2 depended on the concentrations of water-dissolved oxygen. So, the variability of the set and frequencies of inversion sequences might be correlated with hydrobiological parameters of water basins.

On the other hand, the studied Bulgarian population of *C. plumosus* revealed a rather high level of chromosomal polymorphism (the average number of heterozygous inversions per individual was between 0.7 and 1.8 in different years studied). A high level of chromosomal polymorphism was also detected in different Bulgarian fish pools (the average number of heterozygous inversions ranged for 1.2 to 1.5 (Krastanov, Michailova, 2008). These authors established also a high average number of heterozygous inversions per larva in Polish fish pools (1.13). In the studied years of the Bulgarian population a polymorphic system was found in arms A, C and D, manifested by the alternative homozygous sequences and heterozygous between them. Such polymorphic system has been observed in some Siberian (Kiknadze et al., 1987; Butler et al., 1999), German (Krieger-Wolf, Wülker, 1971), as well as in some Bulgarian populations (Krastanov, Michailova, 2008). However, the sequences, involved in polymorphic systems, have different selective values and are adaptive to different environmental components. So, these data confirmed Michailova's, Petrova's (1991) idea that the polymorphic system in *C. plumosus* could be treated as an "adaptive population strategy" depending on the fluctuation of homo and heterokaryotypes. So, chromosomal polymorphism can be maintained by various selective gradients in nature such as climatic, seasonal, anoxia and several others (Lande, 1984).

Also, in the studied Bulgarian population some inversions were found at a low frequency: pluA1.3, pluB1.3 and pluF1.2, detected also in some Russian populations (Butler et al., 1999; Golygina, Kiknadze, 2001; Petrova et al., 2007). According to Ruiz, Wasserman (1999) they can be considered as rare inversions appearing at a low frequency. They can persist for a long time in the populations but do not increase in frequency or spread to a large portion of the species range. Such rearrangements are often associated with exposure to natural or anthropogenic contaminants (Dobzhansky, 1970).

When we compared the band sequences of the studied populations we detected some common banding sequences (pluA1, pluB1, pluC1, pluE1, pluF1, and pluG1) which are called by Gunderina et al. (1999) as Main Common Palaearctic sequences. In spite of that both studied populations are distinguished by their cytogenetic variability. Bulgarian population revealed a high level of chromosomal polymorphism, while Czech population is almost monomorphous. The Czech population is differed by its cytogenetic structure not only from the Bulgarian population but also from other European populations. In several European populations it was established a high level of chromosomal polymorphism: 22 banding sequences with 19 genotypic combinations (Butler et al., 1999). However, also it is important to underline that Gunderina et al. (1999) reported a very low variability of cytogenetic structure in some Siberian populations. So, these data confirmed the idea of Gunderina et al. (1999) and Michailova et al. (2005) that there isn't any geographical gradient of cytogenetic variability. It is quite possible that the observed chromosome variability has some advantage in a specific ecological niche available to a population. The results obtained allow us to consider the chromosomal polymorphism as a genetic mechanism for adaptation to changing environmental conditions.

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