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Banding chromosome patterns of zebra mussel *Dreissena polymorpha* (Pallas) from the heated Konin lakes system (Poland)

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Abstract - The chromosome complement of freshwater mussel *Dreissena polymorpha* was investigated using Giemsa, Ag-NOR and chromomycin A_3 staining. The diploid chromosome number of this species is $2n$=32 and the arm number (NF) = 56. Nucleolar organizer region (NOR) was found on one chromosome pair and it was connected to GC rich chromatin as visualized by CMA_3 staining. Additional GC-rich bands enabled to characterize zebra mussel karyotype with details. The paper presents unknown cytogenetic characteristics of this invasive mussel.

Key words: cytogenetics; Bivalvia; NOR; chromomycine A_3.

INTRODUCTION

The zebra mussel *Dreissena polymorpha*, Pallas 1771, is a widely distributed species whose distribution area has gradually extended (STANCZYKOWSKA 1977). During the Ice Age this species became almost completely extinct and had distribution range limited to the coasts of the Black and Caspian Seas. Over the last 200 years from these area *D. polymorpha* started to expand again (PIECHOCKI and DYDUCH-FALNIOWSKA 1993). The zebra mussel is an important species in the benthic and periphyton communities (STANCZYKOWSKA *et al.* 1988) and plays a key role in the process of the biological self-purification of water in lakes and channels (PROTASOV *et al.* 1994; PROTASOV *et al.* 1997). Recently, zebra mussel has been used for genotoxicity testing of different chemicals and environmental pollutants by micronucleus test (MERSCH *et al.* 1996; MERSCH and BEAUVAIS 1997; PAVLICA 2000). Scarce cytogenetic data inclined us to characterize the karyotype of this species by means of Giemsa, silver (Ag-NOR) and chromomycin A_3 (CMA_3) stainings.

MATERIALS AND METHODS

Twenty six specimens of zebra mussel (15 males and 11 females, distinguished according to gonad view) from the Patnowskie Lake (Konin Lakes – central Poland) were studied. Mussels were injected by 0.1% colchicine solution (0.1 ml per specimen with the shell of 15-25 mm in length) into foot or gonad and have been kept in aquarium for 3-6 hours. The gills were dissected and homogenized in the glass homogenizer in distilled water and then hypotonized for 45 minutes. The cell suspension was fixed by solution of methanol and acetic acid 3:1 for 5 minutes and than centrifuged for 10 minutes at 1000 rpm. Chromosome preparations were made after three centrifugations followed by fixative changes. Chromosome slides were stained with 5% of Giemsa solution in distilled water for 20 minutes.

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Chromomycin CMA_3 staining was done according to SOLA *et al.* (1992) and the AgNOR staining has made according to method described by HOWELL and BLACK (1980). The described results concern only the individuals from were obtained at least 10 metaphase plates good for analyses.

Chromosomes were observed by Nikon Optiphot-2 microscope and photographed by Coolpix 995 camera. Chromosomes were counted and classified according to LEVAN *et al.* (1964).

RESULTS AND DISCUSSION

Diploid chromosome number of *Dreissena polymorpha* was $2n = 32$ and the chromosome arm number was NF = 56. The chromosome number of *Dreissena polymorpha* described in present paper is the same as found by GRISHANIN (1987). Whereas GRISHANIN (1987) described the morphology of karyotype as 20 biarmed and 12 uniarmed chromosomes we found that zebra mussel karyotype consisted of 10 pairs of metacentric, two pairs of submetacentric and four pairs of subtelo-acrocentric chromosomes (Fig. 1). The differences in the chromosomal categories can be caused by an interpopulation polymorphism or by better chromosome morphology obtained using the splash technique proposed in present paper.

Zebra mussel has one pair of NOR-bearing chromosomes. It was confirmed by Ag-staining of NORs and the number of nucleoli per interphase cells (Fig. 2).

The silver staining revealed one pair of NOR-bearing chromosomes with Ag-NOR located on telomeric position on the short arm of the largest subtelo-acrocentric chromosome pair (number 13) (Fig. 2). The number of active (detected by silver staining) nucleoli per nucleus of interphase cells varied from 1 to 2.

Most of the bivalve molluscs investigated so far had more than one chromosome pair with silver NOR (INSUA and THIRIOT-QUIEVREUX 1993; INSUA *et al.*1994; THIRIOT-QUIEVREUX and INSUA 1992). Variability in the number of NORs per cell was observed in all studied populations of species

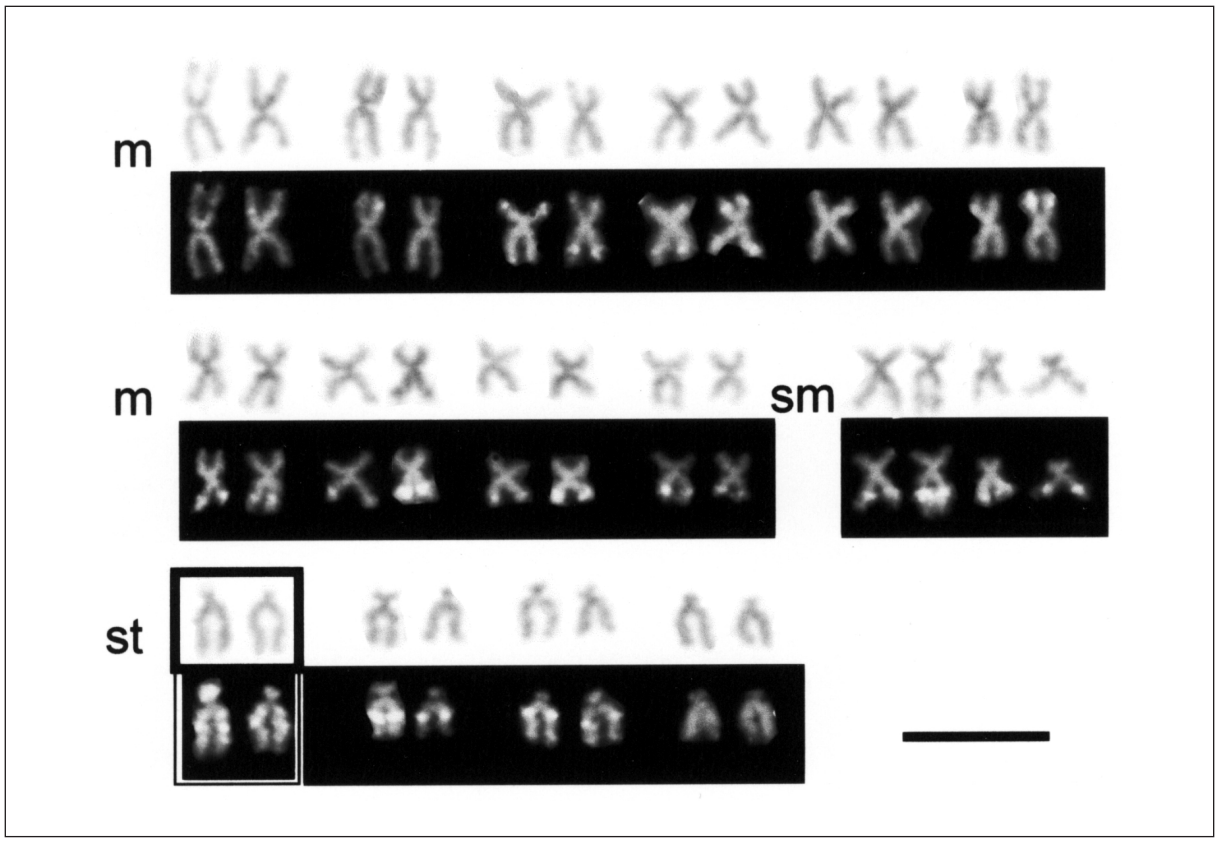

Fig. 1 – The *Dreissena polymorpha* karyotype stained by Giemsa (upper rows) and sequentially by chromomycine CMA_3 (lower rows). NOR-bearing pair is framed. Abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric chromosomes. Scale bar 5 μm.

from the genus *Mytilus*. The number of NOR-bearing chromosomes described in the genus *Mytilus* varied from one to six (INSUA *et al.* 1994; MARTINEZ-LAGE *et al.* 1995; 1996; 1997; GONZALEZ-TIZON *et al.* 2000).

The chromomycine A_3 staining showed a specific banding pattern on *D. polymorpha* chromosomes. Apart from the Ag-NOR sites positive CMA_3 signals were found on almost all other chromosomes, beside the pairs 1, 5 and 16 (Fig. 2B). The largest subtelocentric NOR-bearing chromosome pair showed characteristic CMA_3 pattern with a large positive bands in the NOR site and three other bands along the long chromosome arm (Fig. 1 and Fig. 3). Based on frequent CMA_3 bands and sequential staining with silver nitrate and Giemsa, the detailed karyotype of *Dreissena polymorpha* was constructed (Fig. 3).

No differences in banding chromosome patterns of males and females have been found.

The presence of CMA_3-positive bands on most chromosomes (including NORs) obtained in *Dreissena polymorpha* suggests that the chromatin organization of this species differs from the chromatin organization of other mussels. In the chromosome sets of the species from the genus *Mytilus* CMA_3-positive signals were observed only in NOR sites (MARTINEZ-LAGE *et al.* 1994;1997). In two species of Unionidae (*Anodonta woodiana and A. anatina*) the only positive CMA_3 signals also corresponded with Ag-NOR (WOZNICKI, unpublished). Chromomycine A_3 binds to GC-rich heterochromatin which is associated to NORs in lower vertebrates and probably other groups of organisms (SCHMID 1982; AMEMIYA and GOLD 1986). The results of the present paper suggest the

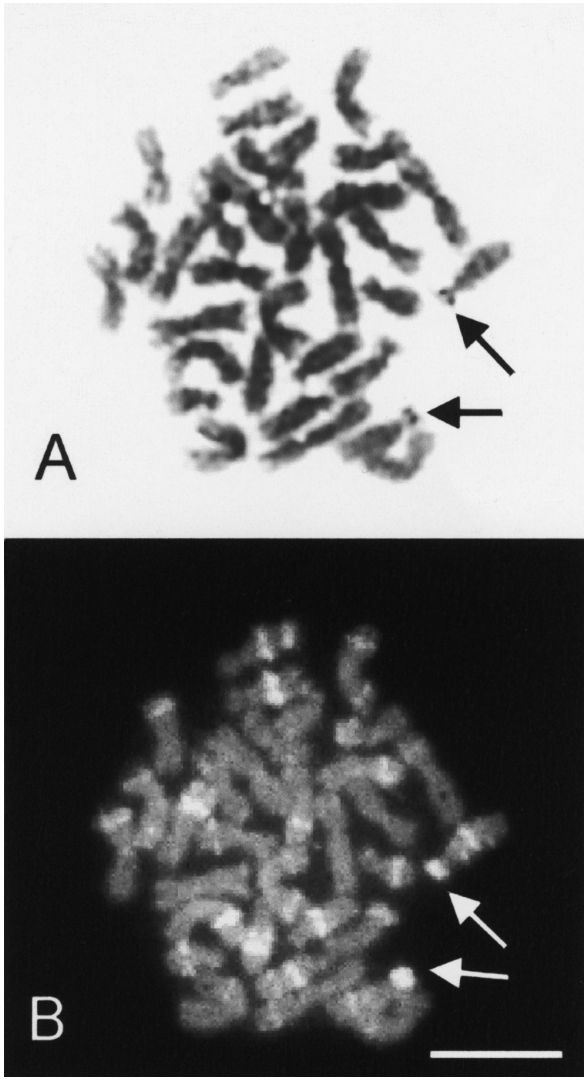

Fig. 2 – Metaphase plates of *Dreissena polymorpha.* A. Stained sequentially by silver nitrate and B. by chromomycine A_3. Arrows indicate the NOR-sites. Scale bar 5 μm.

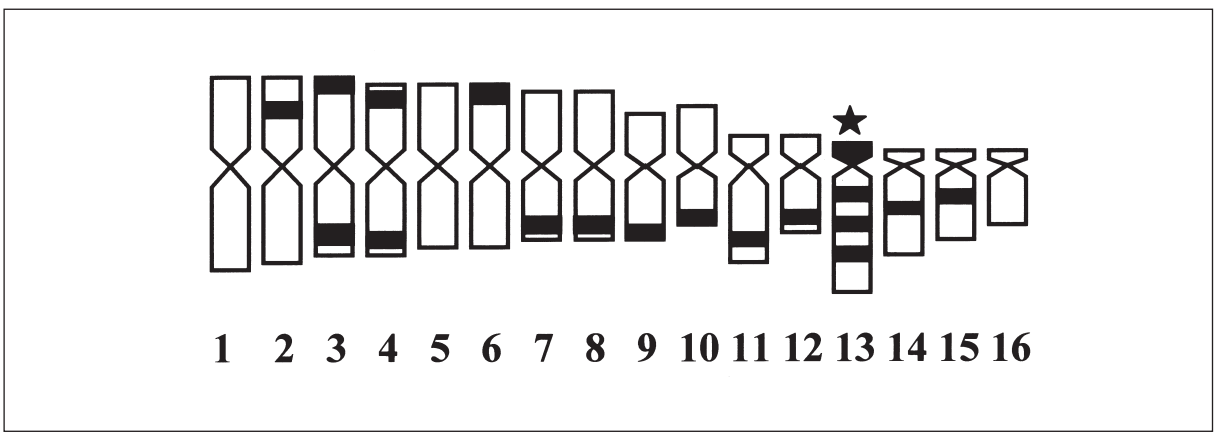

Fig. 3 – The idiogram of banding pattern of *Dreissena polymorpha*; black bands show the location of CMA_3-positive signals, asterisk shows the NOR site (Ag and CMA_3-positive).

compartmentalization of the zebra mussel genome, and especially association of GC-rich heterochromatin with ribosomal DNA sequences (the short arm of 13th chromosome pair).

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