

Cytogenetic Characterization of the Zebra Mussel *Dreissena polymorpha* (Pallas) from Miedwie Lake, Poland*

Alicja BOROŃ, Paweł WO NICKI, Lidia SKUZA and Roman ZIELIŃSKI

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Cytogenetic characterization of *D. polymorpha* was carried out using banding techniques such as C-banding, fluorochrome CMA₃ and silver nitrate treatment. The diploid chromosome number of both investigated *D. polymorpha* forms (typical and albinotic) was the same $2n = 32$ ($NF = 56$). The karyotype consisted of 5 pairs of metacentric, 7 pairs of submetacentric and four pairs of subtelo-acrocentric chromosomes. Ag-NORs were located in the telomeric position on the largest subtelo-acrocentric chromosome pair. C banding patterns indicate many sites of constitutive heterochromatin mainly located in the telomeric regions and interstitially in some chromosomes. CMA₃-sites were observed in almost all chromosomes; apart from the Ag-NORs sites, they were located terminally on the chromosome arms and interstitially on three chromosome pairs. Sixteen chromosomes could be counted at the diakinesis stage of meiosis. No differences in banding chromosome patterns were found neither between both analyzed forms of *D. polymorpha* nor between males and females.

Key words: Bivalvia, banding patterns, cytogenetics, *Dreissena*, meiosis.

Alicja BOROŃ, Department of Zoology, Faculty of Biology, Warmia and Mazury University in Olsztyn, Oczapowskiego 5 St., 10-718 Olsztyn, Poland
E-mail: alibo@uwm.edu.pl

Paweł WO NICKI, Faculty of Environment Protection and Fisheries, Warmia and Mazury University in Olsztyn, Oczapowskiego 5, 10-718 Olsztyn, Poland.

Lidia SKUZA, Department of Cell Biology, Faculty of Natural Sciences, Szczeciński University, Szczecin, Poland.

Roman ZIELIŃSKI, Department of Genetics, Faculty of Biology, Warmia and Mazury University in Olsztyn, Plac Łódzki 3, 10-967 Olsztyn, Poland.

Bivalves are represented by approximately 15 000 species. The cytogenetics of 200 of these are restricted to descriptions of the chromosome number and/or chromosome morphology. Banding chromosome patterns are known in very few mussels (mainly species from the genus *Mytilus*) (INSUA *et al.* 1994; MARTÍNEZ-LAGE *et al.* 1994, 1995, 1997; PASANTES *et al.* 1996; MARTÍNEZ-EXPOSITO *et al.* 1997; GONZÁLEZ-TIZÓN *et al.* 2000) and oysters (INSUA & THIRIOT-QUIÉVREUX 1991, 1993; THIRIOT-QUIÉVREUX & INSUA 1992). The paper of THIRIOT-QUIÉVREUX (2002) contains a review of the literature on bivalve chromosomes since 1992.

The freshwater mussel *Dreissena polymorpha* (Pallas) is used as a model species in ecological studies because it plays an important role in the process of biological self-purification of water. Since the last two hundred years the distribution of

the zebra mussel has expanded from the Black, Caspian and Azov Seas to the central and western part of Europe and North America (STAŃCZYKOWSKA 1977; HEBERT *et al.* 1989; STRAYER 1991). *D. polymorpha* inhabits all kinds of water bodies: slow rivers, lakes, ponds, dam reservoirs, including those with heated or brackish water (STAŃCZYKOWSKA *et al.* 1983; LEWANDOWSKI 1991; PIECHOCKI & DYDUCH-FALNIOWSKA 1993).

The zebra mussel belongs to ten species characterized by the highest levels of genetic variability (BOILEAU & HEBERT 1993; ZIELIŃSKI *et al.* 1996, 2000; MARSDEN *et al.* 1996; SOROKA 2002, 2003). This species also possesses great morphological diversity in the color and shape of the shell. The albinotic morphotype with a bright and yellow body and cream shell without the black zigzags has been found in Miedwie Lake (ŚWIERCZYŃSKI 1994).

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This morphotype forms mixed populations with the typical morphotype, the frequency of albinotic forms reach 5-6%. Genetic differences between both *D. polymorpha* forms have not been detected by electrophoresis of enzymes (ZIELIŃSKI *et al.* 1994).

Three papers by GRISHANIN (1987), BARSHIENE (1994) and WO NICKI and BOROŃ (2003), on the karyotype of *D. polymorpha*, have been published so far. In this study a cytogenetical characterization of the zebra mussels from Miedwie Lake was carried out, including the typical and albinotic forms, using conventional staining and banding techniques such as C-banding, fluorochrome CMA₃ and silver nitrate treatment.

Material and Methods

The karyotype analyses were based on 84 and 108 metaphase plates of 10 albinotic and 10 typical forms of zebra mussel, respectively, collected from Lake Miedwie in Poland. Mussels were transported alive in water and then placed in a well aerated aquarium in the laboratory. Mussels were injected with 0.1% colchicine solution using a dose of 0.1-0.3 ml per specimen (with a shell length of 15 to 25 mm) into the foot and then were kept in an aquarium for 12 hours. The gills and/or gonads were dissected, sliced with scissors into small pieces and hypotonized in distilled water for 45-50 min. Fixation was carried out in a solution of methanol and acetic acid (3:1) with three changes of 30 min duration at room temperature. After at least 1.5 hours of fixation the fixed cells were dissociated in a 50% water solution of acetic acid. The cell suspension was dropped onto microscope slides (slides were cleaned with a 1% solution of HCl in ethanol for 30 min) heated to 40-45°C and after several seconds the suspension drop was withdrawn back into the Pasteur pipette. Chromosome slides were stained with 4% Giemsa solution in phosphate buffer at pH 6.8 for 10 min.

The AgNOR staining to detect active NORs was performed according to the method described by HOWELL and BLACK (1980). Chromomycin CMA₃ staining was done according to SOLA *et al.* (1992). Chromosomes were observed under an Olympus BX52 microscope and photographed using a Coolpix 995 camera. Chromosomes were counted and classified according to LEVAN *et al.* (1964).

Results

The chromosome number ranged from 30 to 32 in the sample of 84 metaphase plates of the albinotic morphotype of *D. polymorpha*, although in most (96%) 32 chromosomes were counted. Among

108 metaphase plates of the typical form, 98 had 32 chromosomes, whereas 30 and 31 chromosomes were observed in 7 and 3 plates, respectively.

The diploid chromosome number of both investigated *D. polymorpha* forms (typical and albinotic) was the same $2n = 32$, and the chromosome arm number NF was 56. The karyotype was characterized by relative chromosome length, arm ratios and centromeric position. The karyotype consisted of 5 pairs of metacentric, 7 pairs of submetacentric and four pairs of subtelocentric chromosomes (Fig. 1a-d). The silver-staining technique was performed on 6 animals of each form; 28 metaphases of albinotic and 37 of typical forms were analyzed. The silver staining revealed one pair of NOR-bearing chromosomes. The NOR sites were located on the telomeric regions on the short arm of the largest subtelocentric chromosome pair (pair 13 in Fig. 1e, shown by arrows in Fig. 1f).

The constitutive heterochromatin was studied in 11 animals from 28 metaphase plates of 6 typical individuals, and in 16 metaphase plates of 5 albinotic specimens. C banding revealed the occurrence of many sites of constitutive heterochromatin in the telomeric regions on the chromosome arms (see pair number 2, 4, 5, 6, 8, 9, 10, 13 in Fig. 1d) and interstitially (see pair number 3, 6, 15 in Fig. 1d).

The number and location of CMA₃-positive signals on 23 metaphase plates of typical and 12 of albinotic forms were analyzed. The metaphase plate of the *D. polymorpha* albinotic form after CMA₃ staining is shown in Fig. 1e. The CMA₃-sites were observed on almost all chromosomes; apart from the Ag-NORs sites, they were located terminally on the long or short arms of chromosomes and interstitially on three chromosome pairs (Fig. 1e).

The chromosomes in meiosis are shown in Fig. 1g. At diakinesis individual chromatids were less visible. Sixteen chromosomes could be counted at this stage.

No differences in chromosome banding patterns were found neither between both analyzed forms of *D. polymorpha* nor between males and females.

Discussion

The number and morphology of chromosomes of some mollusc species have been described (NAKAMURA 1985, 1986; THIRIOT-QUIÉVREUX 2002). Among bivalves, the most intensively investigated were mussels (INSUA *et al.* 1994; MARTÍNEZ-LAGE *et al.* 1994, 1995, 1996, 1997; PASANTEZ *et al.* 1996; MARTÍNEZ-EXPOSITO *et al.* 1997; GONZÁLEZ-TIZÓN *et al.* 2000) and oysters (INSUA & THIRIOT-QUIÉVREUX 1991, 1993; THIRIOT-QUIÉVREUX & INSUA 1992) because of

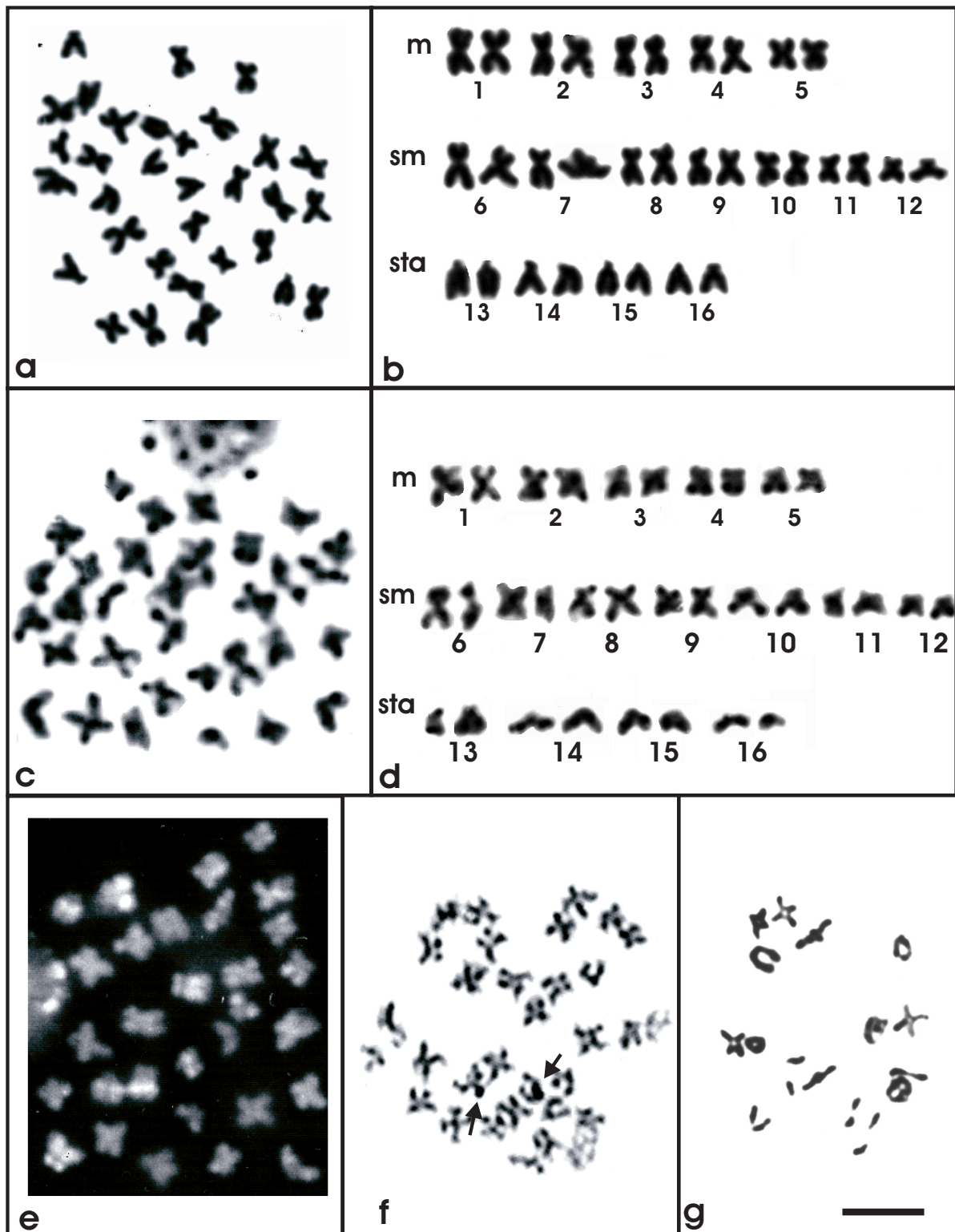


Fig. 1. Metaphase plate (a) and karyotype (b) of the albinotic *D. polymorpha* morphotype; metaphase plate (c) and karyotype (d) of the typical morphotype after C banding; metaphase chromosomes of the typical form stained with chromomycin CMA₃ (e) and metaphase chromosomes of the albinotic form after silver nitrate staining (f); meiotic chromosomes (g) from a gonad of the typical form of *Dreissena polymorpha* from Miedwie Lake. Bar = 5 μm.

their commercial importance. The number of chromosomes of the Mytilidae species varied from 32 to 24, but species of the genus *Mytilus* have $2n = 28$ chromosomes. The karyotypes of 22 species of Ostreidae were composed of 20 chromosomes, except for one species with $2n = 18$.

The presented results revealed that both investigated *D. polymorpha* forms, viz. typical and albinotic, were characterized by the same $2n = 32$ chromosome number and no differences in the investigated banding patterns were detected. The zebra mussel is characterized by high intraspecific genetic diversity detected, for example, by protein electrophoretic patterns (BOILEAU & HEBERT 1993; MARSDEN *et al.* 1996; ZIELIŃSKI *et al.* 1996; SOROKA 2002, 2003). Genetic diversity could be connected and associated with its large dispersal ability. The albinotic morphotype was similar to the typical form in 16 enzymatic loci, the coefficient of genetic similarity was 0.978. The results of genetic studies identified the albinotic form as a phenotype of *D. polymorpha* (POŁOK *et al.* unpublished).

Two papers, by GRISHANIN (1987) and BARSHIENE (1994), included information on the karyotype and one paper on the chromosome banding patterns of *D. polymorpha* (WO NICKI & BOROŃ 2003). 21 to 32 chromosomes were described from 240 metaphase plates from pallium cells (GRISHANIN 1987). The diploid chromosome number $2n = 32$ in 64 metaphases consisted of 20 metacentric and 12 acrocentric chromosomes. The meiotic chromosomes were observed as 16 bivalents, although a few aneuploids were detected. Cytogenetic disturbances such as the occurrence of hypo- and hyperploid and polyploid cells in *D. polymorpha* from a Lithuanian hydrosystem due to ecotoxic factors have been observed (BARSHIENE 1994).

According to the present paper and former results (WO NICKI & BOROŃ 2003) the karyotype of *D. polymorpha* undoubtedly consists of 12 pairs of biarmed chromosomes (meta- and submetacentrics) and 4 pairs of uniarmed chromosomes (subtelocentric and acrocentric) and the number of chromosome arms NF equals 56.

The variable number of chromosomes classified to different categories by different authors is rather typical for molluscs. Difficulties in classification of submetacentric and subtelocentric chromosomes in the genus *Mytilus* reported in some papers have been explained as a consequence of variation in the centromeric index values, which is greater in chromosomes with unequal arms due to the dynamic process of chromosome condensation along the length of each chromosome (MARTÍNEZ-LAGE *et al.* 1996). Oyster karyotypes are usually

composed of metacentric and submetacentric chromosomes, which are difficult to identify (NAKAMURA 1985; INSUA & THIRIOT-QUIÉVREUX 1991; THIRIOT-QUIÉVREUX 2002).

Positive silver staining Ag-NORs have been observed in more than 20 bivalve species since 1992 (THIRIOT-QUIÉVREUX 2002). It seems to be a rule that NORs are located terminally on biarmed chromosomes of bivalves (INSUA *et al.* 1994; MARTÍNEZ-LAGE *et al.* 1995, 1996, 1997; GONZÁLEZ-TIZÓN *et al.* 2000). The NORs sites in karyotypes of species from the family Ostreidae were located in a terminal position on two nonhomologous metacentric chromosomes. The number of active NORs – detected by staining with silver nitrate, revealed from 1 to 3 sites.

A variable number, from one to three, of Ag-NOR chromosomes have been identified within and between individuals of *Ostrea denselamellosa* (INSUA & THIRIOT-QUIÉVREUX 1991).

The number of NOR-bearing chromosomes of three species from the genus *Mytilus* (INSUA *et al.* 1994; MARTÍNEZ-LAGE *et al.* 1995, 1996, 1997; GONZÁLEZ-TIZÓN *et al.* 2000) varied from 2 to 5. They were located in a telomeric position on submeta-subtelocentrics and one was located on the *p* arm of a metacentric chromosome. Intraspecific NOR polymorphism has also been observed (MARTÍNEZ-LAGE *et al.* 1995). Nucleolar organizer regions located in a terminal position on the short arm of one metacentric chromosome pair and of one submetacentric characterized *M. californianus* from the Pacific Canadian coast (MARTÍNEZ-LAGE *et al.* 1997). DNA rich in GC pairs (detected with CMA₃) was located, apart from one species specific chromosome, in the NOR sites.

In the presented zebra mussel karyotype at least 10 pairs with chromomycin signals were detected (Fig. 1e). In the *D. polymorpha* karyotype most of the CMA₃-positive sites did not correspond with NORs. Similar results in zebra mussels from the heated Konin lakes system have been described (WO NICKI & BOROŃ 2003). GC-rich heterochromatin connected to NORs regions was reported as typical for lower vertebrates (SUMNER 1990) and for some invertebrate species such as myriapods and other bivalves (WO NICKI, unpublished). A clear coincidence between C-banding and NORs was detected for *Mytilus* species (MARTÍNEZ-LAGE *et al.* 1995; INSUA *et al.* 1994).

The C-banding analysis presented in this study confirms that *Dreissena polymorpha* possesses small amounts of constitutive heterochromatin, located mainly in the telomeric regions and interstitially, but rarely in the centromeres. The differences in the amount and location of constitutive heterochromatin seem to be a mechanism of karyotype

differentiation among species of the genus *Mytilus* (MARTÍNEZ-LAGE *et al.* 1995, 1996, 1997). Terminally located constitutive heterochromatin was also observed on several chromosomes in the karyotype of *Ostrea denselamellosa*, but apart from these, large centromeric C-bands were always present on four chromosome pairs (INSUA & THIRIOT-QUIÉVREUX 1991).

The zebra mussel *D. polymorpha* inhabiting Miedwie Lake was characterized by a karyotype of $2n = 32$ chromosomes, consisting of 24 banded (meta- and submetacentric) and 8 unibanded (acrocentric) chromosomes. The karyotype contained one unibanded pair of NOR-bearing chromosomes, in contrast to other bivalves. Similarly, as in most molluscs, Ag-NOR sites corresponded to CMA₃-positive signals and to C bands.

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