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GENETIC STRUCTURE OF THE CHINESE CLAM *ANODONTA WOODIANA* LEA, 1834 (BIVALVIA: UNIONIDAE) FROM ITS FIRST LOCALITY IN POLAND

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ABSTRACT: To date, the Chinese clam *Anodonta woodiana* Lea, native to the Amur and Yangtze river basins, has been reported from three localities in Poland. Since the early 1990s, the species has massively colonised its first Polish locality, i.e. heated lakes near Konin in central Poland. Since 2002 and 2003, the clam has also been found in, respectively, fish ponds near Sieraków (Greater Poland) and the heated channel of the Dolna Odra power station near Szczecin, NW. Poland. In order to estimate the genetic variability of the first, pioneer populations in Poland, 12 enzymatic loci were analysed using starch gel electrophoresis. *A. woodiana* exhibits a strong genetic variation, as demonstrated by the following parameters of genetic variability for its Polish populations: 66.7–83.3% polymorphic loci, 2.3 alleles on average per locus and 2.6–2.9 per polymorphic locus, an average of 2.53 genotypes per population, and average expected heterozygosity of 0.30–0.35. The results suggest either a massive colonisation by genetically diverse individuals, or a multiple colonisation by founder populations coming from various water bodies. The genetic similarity (Nei) between the populations of *A. woodiana* is high (0.83 to 1.00), which suggests a strong genetic homogeneity of this species in its first locality in Poland.

KEY WORDS: *Anodonta woodiana*, enzymatic loci, genetic variability, population, invasive species

INTRODUCTION

The Chinese clam *Anodonta woodiana* Lea, 1834 was first reported in Poland in 1993 in the system of heated lakes near Konin (PROTASOV et al. 1994, ZDANOWSKI 1994). The species comes from the Far East, from the Amur and Yangtze river basins (KISS 1995, WATTERS 1997). It was brought to Europe mainly from China with introduction of Chinese carps, which had started in 1963 (KISS 1995, WATTERS 1997). *A. woodiana* reached Poland most probably from Hungary in the mid 1980s, together with imported stocking material of silver carp and bighead carp (AFANASIEV et al. 1997, KRASZEWSKI & ZDANOWSKI 2001).

In Poland, *A. woodiana* has been reported from three localities. Since the early 1990s, the species has massively colonised the site where it was first recorded, i.e. heated lakes near Konin in central Poland (PIECHOCKI & RIEDEL 1997, PROTASOV et al. 1997). In 2002, the clam was also found in fish ponds near Sieraków, Greater Poland and, the following year, in the heated channel of the Dolna Odra power station near Szczecin, north-western Poland (DOMAGAŁA et al. 2003).

The species was first reported in Poland in 1993, when specimens representing one or more species of Chinese clams *Anodonta* were found in the system of heated lakes near Konin (PROTASOV et al. 1994, ZDANOWSKI 1994). The specimens exhibited variation in both shell morphology and distribution pattern, which gave rise to an argument whether one or two species of Chinese clam were the case (ZDANOWSKI 1994). The authors who carried out intense hydrobiological and ecological studies in the system of the Konin heated lakes maintained that one less abundant species, *Anodonta woodiana*, inhabited the lakes, while another unidentified *Anodonta* species massively colonised the heated channels (ZDANOWSKI 1994,

AFANASIEV et al. 1997, PROTASOV et al. 1997). Genetic studies with isoenzyme electrophoresis and PCR-RFLP of the mitochondrial cytochrome oxidase subunit I (*COI*) gene revealed that all those *Anodonta* mussels in the Konin heated lakes were conspecific and were actually *Anodonta woodiana* (SOROKA & STACHÓW 2000, SOROKA & ZDANOWSKI 2001). These authors have also demonstrated that the wide morphological variation observed among the bivalves is of a rather environmental character and is **not** a marker of significant genetic differences.

Ecological studies on this newly introduced species in Poland have only just begun and belong to very few ongoing projects in Europe or world-wide (KISS 1992, 1995, PROTASOV et al. 1994, 1997, SOROKA 2000, KRASZEWSKI & ZDANOWSKI 2001). The clam has not been subject to any extensive genetic studies. First attempts involved very small samples of *A. woodiana* and *Anodonta* sp. and thus require continuation (SOROKA 1998). On the other hand, morphometric studies on shells of *A. woodiana* collected from various sites located within the heated lake system have demonstrated their strong habitat-related variability in shape and size (SOROKA & ZDANOWSKI 2001).

The present study was based on enzyme electrophoresis, a method commonly applied in population-genetic studies on wild populations of various plant and animal species. The results of such analyses provide a basis for inferences on ecology, life strategies, and evolutionary histories of particular taxa (SAFRIEL & RITTE 1986, HAMRICK & GODT 1990, WENNE 1993, HOEH et al. 1998). A reduced level of genetic variability (heterozygosity) exhibited by a population suggests that the loss of diversity is a result of genetic drift, the random effect that has occurred in the population (CHAKRABORTY & NEI 1977, PACKER et al. 1991, BERRY 1992).

In China, where *A. woodiana* occurs massively, populations of this species theoretically should not be subject to random events. Genetic drift, however, cannot be ruled out when new populations of the species are established, like those in Poland. It is expected that first founder populations of *A. woodiana* should be characterised by a reduced genetic variability and low genotypic variation in relation to the original populations, due to the founder effect (CARLSON & TEMPLETON 1984). However, the expected reduced level of genetic variability that might have resulted from genetic drift was not observed in the founder populations of the invasive zebra mussel, *Dreissena polymorpha*, either in Europe or in the Great Lakes of North America (HEBERT et al. 1989, GARTON & HAAG 1991, ZAPKUVIENNE 1992, MARSDEN et al. 1996, SOROKA 1999).

The aim of this study was to describe the genetic structure of the first founder populations of *A. woodiana* in Poland and to establish the level of genetic variability of this bivalve. It may exhibit traits characteristic of populations that experience genetic drift. The results discussed in this study are an outcome of the first comprehensive genetic research ever carried out on this species in Poland or world-wide.

MATERIAL AND METHODS

Anodonta woodiana were collected in September 1998 in various places of the component water bodies (lake Licheńskie, the warmest channel, and the man-made initial cooling reservoir) of the cooling system of the "Konin-Pątnów" power station near Konin, central Poland. The sampling sites differed in their temperature and water retention. The mean water temperature in the lake during the summer stagnation period was 26°C and in the reservoir it was 32°C. Water exchange in both water bodies occurred every three days. The mean summer water temperature in the channel was 30°C, and the water flow was $15 m^3 s^{-1}$ (SOROKA & ZDANOWSKI 2001).

Electrophoretic analyses included a total of 29 specimens collected from lake Licheńskie (population 1), 27 from the warmest channel (population 2), and 23 specimens from the initial cooling reservoir (population 3).

The three sampling sites are from 300 m (populations 2 and 3) to 5 km apart (populations 1 and 2, as well as 1 and 3). Despite such short distances, no water exchange between them takes place and, consequently, migration of larvae or adult forms from one site to another is to a large extent restricted, though it cannot be definitely ruled out. The clams from each site were treated as a separate population.

Foot muscle tissue was dissected for the electrophoretic analysis. Variation of seven enzymes, which are listed in Table 1, was analysed with starch-gel electrophoresis which followed standard procedures (PASTEUR et al. 1988, SOLTIS & SOLTIS 1989).

Following genetic interpretation of the resulting images, the results in the form of genotypic records of all the loci were processed statistically using the BIOSYS–1 software package (SWOFFORD & SELANDER 1983). Additionally, the occurrence of individuals with unique genotypes involving seven enzymatic loci was analysed. A multi genotype was considered unique if found only once in the population.

Table 1. Enzyme systems, gels and buffers used in this study

Enzyme system	Number of loci	Abbreviation	E.C. number	Gel and buffer
Aspartate aminotransferase	2	AAT	2.6.1.1	Ashton
Esterase	2 (1 visible locus[a], 1 UV locus[b])	EST	3.1.1.2	Ashton
Superoxide dismutase	2	SOD	1.15.1.1	Ashton
NAD-dependent malate dehydrogenase	2	MDH	1.1.1.37	Morpholine-citrate
Isocitrate dehydrogenase	1	IDH	1.1.1.42	Morpholine-citrate
Glucose-6-phosphate isomerase	1	PGI	5.3.1.9	Lithium-borate
Hexokinase	2	HEX	2.7.1.1	Lithium-borate
	Total = 12			

[a]α-Naphthyl acetate and β-Naphthyl acetate used as substrate
[b]4-methylumbelliferyl acetate used as substrate

RESULTS

The electrophoretic analysis of seven enzymes has demonstrated the presence of 12 enzymatic loci. Loci *Sod2* and *Hex2* were monomorphic in all sites. *Aat2* locus was monomorphic in populations 1 and 2 and *Mdh2* in population 2. Other variable loci contained 2 to 4 alleles each (Table 2).

The percentage of polymorphic loci was high, ranging from 58.3% to 83.3% with polymorphism criterion 95%, and from 66.7% to 83.3% at 99% and with no criterion assumed (Table 2). The highest percentage of polymorphic loci was found in population 3, the lowest – in population 2, in both cases on all the applied criteria.

On average, between 2.6 and 2.9 alleles were found per polymorphic locus and 2.3 alleles at each locus (Table 2). In the analysed sites the alleles were the same. The populations differed in having or lacking single rare alleles.

The values of the coefficient of expected heterozygosity per locus in a population (h), the mean expected heterozygosity per locus in a population (H), and the mean value of the coefficient of expected

Table 2. Number of alleles at 12 loci and descriptive measures of genetic variation for *A. woodiana*

Locus	Number of alleles in population			
	1	2	3	Mean
Aat1	3	3	3	3.0
Aat2	1	1	2	1.3
Est1	2	2	2	2.0
Est2	2	2	2	2.0
Sod1	3	3	3	3.0
Sod2	1	1	1	1.0
Mdh1	3	3	3	3.0
Mdh2	2	1	2	1.7
Idh1	3	4	3	3.3
Pgi1	3	3	3	3.0
Hex1	2	2	2	2.0
Hex2	1	1	1	1.0
Number of specimens	29	27	23	
Mean number of alleles per locus[a] (A_1)	2.25 (0.3)	2.25 (0.3)	2.33 (0.3)	
Mean number of alleles per polymorphic locus (A_2)	2.67	2.88	2.60	
Polymorphic loci (P%)[b]	75.00	66.67	83.33	

[a](± S.E.)
[b]polymorphism criterion 0.99

heterozygosity per locus in a population (Hs) are presented in Table 3. The highest values of expected heterozygosity (h > 0.650) were observed at locus *Idh1* in all populations. High levels of this coefficient were also found at locus *Pgi1* in populations 1 and 3 (with equal values of 0.606). The lowest values of h, except for monomorphic loci (where h = 0.0), were found at loci *Est1* and *Mdh2*. At the remaining loci, the coefficient ranged between 0.159 and 0.585. On average, the highest variability (H) was found, successively, at loci *Idh1*, *Pgi1*, and *Mdh1*, the lowest – at polymorphic loci *Aat2*, *Mdh2*, and *Est1*. Populations 1 and 3 exhibited similar and high levels of Hs (Table 3).

The number of distinguished genotypes in the three populations of *A. woodiana* ranged between 1, at monomorphic loci, and 5. All the populations had similar mean numbers of genotypes per locus, equal or close to 2.5. The highest numbers of genotypes (4 and 5) were found at loci *Aat1* and *Idh1*, for which the mean number of genotypes per locus was equal and amounted to 4.33. Also at locus *Sod1* the number of genotypes was high, 4 in populations 1 and 2 each, and 3 in population 3, with the mean of 3.67. The lowest mean numbers of genotypes were characteristic of loci *Aat2* (1.33) and *Mdh2* (1.67). Loci *Est 2*, *Adh1* and *Hex1* exhibited an equal number of genotypes in all the populations, differing, however, in their frequencies in particular populations. Conversely, at six of the studied loci (*Aat1*, *Aat2*, *Est1*, *Sod1*, *Mdh2*, and *Pgi1*), the same, most common, genotype was found in all the populations (with frequencies ranging from 0.421 to 1.00). Locus *Idh1* was characterised by the highest genotypic variation among the populations.

Eleven genotypes were not found in the three populations, at seven loci. This absence pertained to equal homozygous groups, except locus *Idh1*, where type 14 heterozygote was missing in populations 1 and 3.

The frequency of alleles at each locus enabled calculating the expected genotype distribution using the Hardy-Weinberg equilibrium equation; the hypothesis about a panmictic character of the populations of *A. woodiana* was verified with Chi-square test. The results of this analysis are presented in Table 5, which does not include monomorphic loci. The analysis of the loci in terms of the Hardy-Weinberg principle revealed that 5 loci in each population were not in equilibrium ($p < 0.05$). In all the populations, the lack of the Hardy-Weinberg equilibrium involved loci *Mdh1*, *Idh1*, and *Pgi1*, at which heterozygotes prevailed ($0.675 < D > 0.209$, Table 5). A complete lack of heterozygotes (D = –1.00) was recorded for loci *Aat1*, in population 3, and *Mdh2*, in populations 1 and 3. In 60% cases, the lack of the Hardy-Weinberg equilibrium was due to an excess of heterozygotes, while in 40% cases it resulted from an excess of homozygotes ($D < 0.0$). Loci *Aat1*, *Est1*, and *Hex1* were always in the Hardy-Weinberg equilibrium (Table 5).

A high individual genotypic variation at 12 loci was found in all the populations. In all, 67 unique, i.e. occurring only once, genotypes were found among 79 examined specimens, which represented 84.81% of all genotypes. The percentage of unique genotypes in particular populations ranged between 77.8% and 100% (population 1) (Table 6). Recurring genotypes were only four, and were found in two to four specimens.

The coefficient of genetic similarity (I_N, according to NEI 1978) among the analysed populations of *A. woodiana* at individual polymorphic loci was very high, ranging between 0.986 and 1.000, except for locus *Est2* (Table 7). At this locus, the genetic difference was the highest between populations 1 and 3, as well as between 1 and 2, as expressed by genetic similarity of, respectively, 0.830 and 0.927. Conversely, no gen-

Table 3. The expected heterozygosity (h) in all loci, the mean expected heterozygosity per locus (H) and mean heterozygosity per locus in a population (H_S) in 3 populations of *A. woodiana* (according to NEI 1978)

Locus	Population			Mean (H)
	1	2	3	
Aat1	0.577	0.492	0.572	0.547
Aat2	0.0	0.0	0.159	0.053
Est1	0.236	0.039	0.194	0.156
Est2	0.479	0.475	0.405	0.453
Sod1	0.476	0.444	0.445	0.455
Sod2	0.0	0.0	0.0	0.0
Mdh1	0.568	0.559	0.585	0.571
Mdh2	0.067	0.0	0.159	0.075
Idh1	0.659	0.691	0.661	0.670
Pgi1	0.606	0.543	0.606	0.585
Hex1	0.383	0.252	0.315	0.317
Hex2	0.0	0.0	0.0	0.0
H_S [a]	0.344 (0.08)	0.297 (0.08)	0.350 (0.07)	

[a] (± S.E.)

Table 4. Frequencies of genotypes of the polymorphic loci analysed and number of genotypes in three populations of *Anodonta woodiana*

Locus	Genotype	Population			Number genotypes/locus
		1	2	3	
Aat1	11	0.241	0.370	0.316	
	12	0.517	0.444	0.421	
	13	0.069	0.148	0.105	4.33
	24	0.138	0.037	0.158	
	22	0.034	–	–	
Aat2	11	1.000	1.000	0.913	1.33
	22	–	–	0.087	
Est1	11	0.727	0.960	0.826	
	12	0.272	0.040	0.130	2.33
	22	–	–	0.043	
Est2	11	0.103	0.481	0.565	
	12	0.586	0.259	0.304	3.00
	22	0.310	0.259	0.130	
Sod1	11	0.448	0.593	0.435	
	12	0.138	0.111	0.304	3.67
	13	0.345	0.111	0.261	
	22	0.069	0.185	–	
Mdh1	11	0.172	0.148	0.130	
	12	0.483	0.629	0.391	3.00
	13	0.345	0.222	0.478	
Mdh2	11	0.965	1.000	0.913	1.67
	22	0.034	–	0.087	
Idh1	11	0.103	0.148	0.043	
	12	0.241	0.259	0.391	
	13	0.345	0.037	0.174	4.33
	23	0.310	0.444	0.391	
	14	–	0.111	–	
Pgi1	11	0.034	0.074	–	
	12	0.621	0.815	0.696	2.67
	13	0.345	0.111	0.304	
Hex1	11	0.483	0.704	0.609	2.00
	12	0.517	0.296	0.391	
Number genotypes/polymorphic locus/population		2.9	2.8	2.8	
Number genotypes/locus/population		2.58	2.50	2.50	

Table 5. Chi-square statistics for concordance with the Hardy-Weinberg equilibrium and deficiency or excess (D) of heterozygotes for enzymatic loci in three populations of *Anodonta woodiana* (*p < 0.05)

Locus	Population 1		Population 2		Population 3	
	Ch^2	D	Ch^2	D	Ch^2	D
Aat1	11.513	0.233	7.686	0.257	10.006	0.165
Aat2	–	–	–	–	30.049*	–1.000
Est1	0.448	0.132	0.000	0.000	3.287	–0.341
Est2	1.125	0.204	6.078*	–0.465	1.736	–0.266
Sod1	8.593*	–0.002	14.437*	–0.509	3.250	0.242
Mdh1	13.775*	0.431	14.146*	0.496	12.862*	0.454
Mdh2	57.018*	–1.000	–	–	30.049*	–1.000
Idh1	8.848*	0.336	24.635*	0.209	10.198*	0.416
Pgi1	24.331*	0.565	19.212*	0.675	22.000*	0.615
Hex1	3.256	0.326	0.703	0.152	1.189	0.216

Table 6. Percentage of unique multi genotypes (GU) in three population of *Anodonta woodiana*

Population	Number of individuals	% GU
1	29	100.00
2	27	77.80
3	23	91.30

Table 7. Matrix of coefficients of genetic similarity (above the diagonal) and genetic distance (below) of NEI's (1978) for analysed loci of three populations of *A. woodiana*

Locus	Population	1	2	3
Aat1	1	–	0.986	1
	2	0.014	–	1
	3	0.000	0.000	–
Aat2	1	–	1	0.998
	2	0.000	–	0.998
	3	0.002	0.002	–
Est1	1	–	0.995	1
	2	0.005	–	0.998
	3	0.000	0.002	–
Est2	1	–	0.927	0.830
	2	0.075	–	0.998
	3	0.186	0.002	–
Sod1	1	–	0.993	1
	2	0.007	–	1
	3	0.000	0.000	–
Sod2	1	–	1	1
	2	0.000	–	1
	3	0.000	0.000	–
Mdh1	1	–	1	1
	2	0.000	–	0.992
	3	0.000	0.008	–
Mdh2	1	–	1	1
	2	0.000	–	0.998
	3	0.000	0.002	–
Idh1	1	–	1	1
	2	0.000	–	1
	3	0.000	0.000	–
Pgi1	1	–	1	1
	2	0.000	–	1
	3	0.000	0.000	–
Hex1	1	–	0.995	1
	2	0.005	–	1
	3	0.000	0.000	–
Hex2	1	–	1	1
	2	0.000	–	1
	3	0.000	0.000	–

etic variability was found between the analysed populations at such polymorphic loci as *Idh1* and *Pgi1* (Table 7). Considering all the loci, the highest genetic similarity was observed between populations 2 and 3 (I_N = 1.0); between the remaining populations it was equal and also very high, 0.993. An analogous pattern was found in the coefficient of genetic distance (D_N) for the analysed populations at particular enzymatic loci (Table 7). These were very small, 0.007 to 0.0, for the populations, and 0.186 (locus *Est2*) to 0.0, for the loci.

DISCUSSION

Invasive and introduced species have been an object of intensive genetic studies for a number of years. Such studies are to establish their genetic variability in the central and founder populations and their genetic predispositions to constantly enlarge their distribution range and to colonise diverse habitats. Comprehensive studies on the ecology, developmental biology, and genetics of such species are necessary in order to preserve the biodiversity of ecosystems, to control expansions, and to evaluate positive or negative outcomes of their presence in water bodies (MAY & MARSDEN 1992, SPIDLE et al. 1994, LEE 1999).

Invasive species are most often characterised by a high level of genetic variability, both in their central (parent) and invasive (founder) populations (MAY & MARSDEN 1992, SPIDLE et al. 1994, LEE 1999). RITTE & PASHTAN (1982) explain this on the grounds of natural selection, which maintains a high variability in populations comprising individuals coming from distant areas and from diversified environmental conditions.

Cerithium snails (*C. scarbidum* and *C. caeruleum*), which have successfully colonised the Mediterranean Sea, display a very high level of genetic variation, with the coefficient of expected heterozygosity (H_S) ranging from 0.61 to 0.66 (RITTE & PASHTAN 1982). For the bivalve *Brachidontes variabilis*, the value of this coefficient is 0.62–0.66 (SAFRIEL & RITTE 1986), whereas for *Dreissena polymorpha* it ranges from 0.34 to 0.50 (ZAPKUVIENNE 1992, SPIDLE et al. 1994, MARSDEN et al. 1996, SOROKA 1999) and 0.27–0.44 (MAY & MARSDEN 1992, SPIDLE et al. 1994) in its European and North American populations, respectively. Four *Macoma* species, which are also considered to be inva-

sive organisms, have this coefficient ranging between 0.21 and 0.44 (WENNE 1993). Exceptional in this respect is the parthenogenic New Zealand mudsnail *Potamopyrgus antipodarum*, which was introduced in Northern Europe in the mid 19th century, successfully adapting to the new environment. In its Danish and UK populations, the RAPD revealed the presence of only three genotypes (HAUSER et al. 1992, JACOBSEN et al. 1996). A higher genotypic variation of this species in European populations was found by means of isoenzyme electrophoresis (JACOBSEN et al. 1996).

Expansion capability of these species is primarily due to high fecundity of females. Moreover, bivalves exhibit other characters that facilitate their dynamic spreading. These are: external fertilisation, planktonic (veliger) or parasitic (glochidium) larvae as a developmental stage, ability to survive several days outside water, as well as adult mobility (GRIFFITHS et al. 1991, PIECHOCKI & DYDUCH-FALNIOWSKA 1993, KISS 1995). The parasitic larvae of *Anodonta* allow the clams to spread rapidly on the one hand, but limit their range to areas where hosts for the larvae are available, on the other. However, the larvae of *A. woodiana* do not require particular fishes as host organisms, and can change their hosts depending on the latitude (KISS 1995).

A. woodiana is indigenous to the Far East, where it massively inhabits the basins of the Amur and Yangtze rivers, and the populations of this species theoretically should not be subject to random events. Genetic drift, however, cannot be ruled out when new populations are established, which has been the case in Poland. It is expected that first founder populations of *A. woodiana* should be characterised by a reduced genetic variation in relation to the original populations due to the founder effect (CHAKRABORTY & NEI 1977, CARLSON & TEMPLETON 1984).

The present study revealed a high level of genetic variation within *A. woodiana* populations and at the same time high genetic similarity between the new founder populations of *A. woodiana* in Poland. The parameters of genetic variability for this species are comparable to those for the founder European and North American populations of *Dreissena polymorpha*, for which the estimated coefficient of heterozygosity ranged between 0.27 and 0.50 (MAY & MARSDEN 1992, ZAPKUVIENNE 1992, SPIDLE et al. 1994, MARSDEN et al. 1996, SOROKA 1999). Unfortunately, no literature data exist on the genetic variation of *A. woodiana* within its original distribution range, i.e. the Far East, or in other areas of Europe.

Genetic PCR-RFLP studies carried out so far on the mitochondrial *COI* (cytochrome oxidase subunit I) gene of *A. woodiana* have revealed no variability among the Polish specimens of this bivalve (SOROKA 2005, SOROKA & GRYGIEŃCO-RAŻNIEWSKA 2005). Likewise, sequence analyses of this gene (for female F haplotype) have revealed no variation among the Polish specimens of this species and 6–8% variation between the Polish and Japanese individuals (SOROKA 2005).

The coefficients of genetic similarity and genetic distance (NEI 1978) are commonly used to compare populations, based on allele frequencies. In *A. woodiana*, the observed differences in allele frequencies between the populations were small and related to the presence or absence of rare alleles with frequency not exceeding 0.09. This contributed to the high values of genetic similarity coefficients (I_N) at particular loci and to the low values of genetic variability (D_N, Table 7). Other bivalves exhibit a positive correlation between the level of genetic variability of the populations and the geographical distance between them (BERG et al. 1998, SOROKA 2002). The close location of the populations of *A. woodiana* has a strong effect on the parameters of genetic similarity, allowing a gene flow and, in consequence, poor genetic variation of Polish populations of this species.

The low genetic variation among the first populations of *A. woodiana* in Poland is not surprising. The collected specimens were aged 2 to 6 years (SOROKA 2000) and belonged to young and geographically close populations, which had not had much time to differentiate in terms of genetics.

The observed variability among the Polish populations of *A. woodiana* is very low (D_N ranging between 0.000 and 0.007), compared to other freshwater species of unionids. The published indices of unbiased genetic distance (NEI 1978) among unionid populations average 0.047 (0.000–0.252) for six species (NAGEL et al. 1996, STIVEN & ALDERMAN 1992). The results obtained in this study are comparable to those of three populations of *Anodonta cygnea* which showed little differentiation (mean genetic distance of 0.008; range 0.00–0.012), though over large geographic distances (NAGEL et al. 1996). However, other species of *Anodonta* reported in the same study showed mean values between 0.063 and 0.108 among populations within the same river basin.

Other authors analysing 12 enzyme loci in *Pyganodon grandis* (formerly *Anodonta grandis grandis*) from Colorado reported that the coefficient of genetic variability (NEI 1978) was 0.00–0.07 and 0.055 for five (LIU et al. 1996a) and two populations of this species (LIU et al. 1996b), respectively. A low genetic variation (genetic distance of 0.000 to 0.037) was observed among populations of *Quadrula quadrula* which was strongly correlated with the geographical distance between them (BERG et al. 1998).

The parameters of genetic variability in *A. woodiana* in this study (Tables 2 and 3) are high, compared to other species of freshwater unionids. The genetic structure of seven North-American populations of *Quadrula quadrula* was characterised by 50–70% polymorphic loci, with 1.8–2.6 alleles per locus and average heterozygosity ranging from 0.20 to 0.27 (BERG et

al. 1998). On the other hand, four species of *Utterbackia* were found to have up to 44% polymorphic loci, the maximum number of alleles per locus being 1.6 (HOEH et al. 1998).

The high levels of genetic variability parameters (H_S, P, A_1, A_2, GU; Tables 2, 3, 4, and 6) indicate the mode of colonisation by *A. woodiana*, which most probably was not accompanied by any reduction in the genetic pool of the population. The colonisation may have been accomplished by huge numbers of genetically diverse individuals, or it was a multiple colonisation involving founder populations from various water bodies. Unfortunately, there are no literature data on genetic studies of this species in Europe or in the original Chinese populations. A similar pattern of colonisation, without reduction in the level of genetic variation, was observed in founder, isolated populations of another freshwater bivalve, *D. polymorpha* in North America and Europe (GATRON & HAAG 1991, MARSDEN et al. 1996, SOROKA 1999, ZIELIŃSKI et al. 2000).

Among the Polish populations of *A. woodiana*, the highest genetic variability parameters were found in population 3 (Tables 2 and 3). The population inhabits the initial cooling pond of the power station effluent, where the temperature is the highest, compared to the other habitats of *A. woodiana* habitats, i.e. 32°C in the summer.

Stress factors, such as high temperature, salinity, and elevated trophic level of the water affect genetic variability and genetic structure of wild populations. Comprehensive genetic studies on *D. polymorpha* revealed that populations inhabiting warmer lakes exhibited a higher genetic variability, compared to those living in water bodies with normal water temperature (SOROKA 2003).

The fact that *A. woodiana* colonised the three sites is not accidental. The species prefers habitats with considerable water turbulence and relatively high water temperature, 30–34°C (KRASZEWSKI & ZDANOWSKI 2001). It could be speculated that for this bivalve higher temperature is a natural rather than a stress factor. Despite having the parasitic glochidium larval stage, which enables the clam to spread rapidly, the species has not succeeded in colonising other Polish water bodies, with water temperature typical of our climate.

None of the analysed populations of *A. woodiana* reached the Hardy-Weinberg equilibrium in the case of five different loci, but these were always: *Mdh1*, *Idh1*, and *Pgi1*. For loci *Aat1* and *Mdh1*, a complete deficiency of heterozygotes was observed (excess of homozygotes), whereas an excess of heterozygotes was most commonly found at the remaining loci (60% of loci). The lack of the Hardy-Weinberg equilibrium was observed in many wild populations of *D. polymorpha* in the case of enzymatic loci, which was due to an excess of homo- or heterozygotes (LEWIS et al. 2000, ROSE & ECKROAT 1991, SOROKA 2002), as well as in the case of microsatellite loci, which resulted from an excess of homozygotes (ASTANEI et al. 2005).

Likewise, the lack of the Hardy-Weinberg equilibrium was observed in over 25 species of marine bivalves, resulting from an excess of homozygotes (ZOUROS et al. 1980, SINGH & GREEN 1984, ZOUROS & FOLTZ 1984). There are many hypotheses explaining the phenomenon, the most important being: the presence of null alleles (FOLTZ 1986), Wahlund's spatial effect (TRACY et al. 1975, KOEHN et al. 1976), partial division of a local population into reproductive groups, i.e. the breeding season dependent on the kind of genotype (ZOUROS & FOLTZ 1984) and selection (TRACY et al. 1975).

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