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Genetic characteristics of the *Dreissena polymorpha* population in Latvia (Lake Rāzna) as part of the European population

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Abstract: The zebra mussel *Dreissena polymorpha* is widely distributed in Europe. The expansion of zebra mussels has a negative impact on the native biota of lakes or rivers. Studies of the genetic structure of populations allow the identification of the current state of this invasive species in Latvia as a part of the European population. Despite the increasing importance of microsatellites in studies of population genetics, there is a lack of such data on *D. polymorpha* in Latvia. The present study investigates the genetic population structure of zebra mussels using six microsatellite loci as follows: *DpolA6, Dpo260, Dpo272, Dpo101, Dpo221* and *Dpo04* from Lake Rāzna. The microsatellite loci in the investigated population have a high polymorphism and number of alleles. Allelic diversity at all described loci was high, ranging from 4 to 20 alleles per locus. The mean observed heterozygosity was 0.58 and the mean expected heterozygosity was 0.70. A high genetic diversity enables species to adapt to changing environments.

Keywords: population genetics, *Dreissena polymorpha*, microsatellites, heterozygosity, Lake Razna

INTRODUCTION

The zebra mussel *Dreissena polymorpha* (Pallas, 1771) is a successful invasive bivalve native to the brackish and freshwater systems of the Ponto-Caspian regions. [1] It was introduced accidentally by human activity in European areas [1,2]. The first invasion of the species *D. polymorpha* in Latvia was in the Gulf of Riga in the mid-1800s. [3]. Mussels have a negative impact on the biota that inhabit the pelagic zones of lakes or rivers. Because of their high fecundity and ability to settle on almost any solid substratum, zebra mussels usually outcompete the resident species and cause severe damage to waterworks [4].

Lake Rāzna is the second largest lake as regards water surface area and the first in terms of water volume in Latvia. Lake Rāzna is part of the historical lake region of Latvia, which contains one-fifth of the country's freshwater resources and 90% of freshwater fish species [5]. It is a moderately deep eutrophic lake with an area of 5,756.4 ha and a maximum depth of 17 m. In the last 60 years, the benthic fauna of Lake Razna has changed significantly. The emergence of *Dreissena*, which has not been observed before, has been noted and a comparison of zoobenthos data from 2016 with data from the 1950s demonstrates the reduction of mussel species diversity in the lake. In the 1950s, 17 snail species and 7 mussel species were found in the zoobenthos of Lake Rāzna (zebra mussels were not found). In 2016, 4 snail species and 3 mussel species were found, including the zebra mussel. The biomass of zebra mussels in Lake Rāzna reached 1 kg/m^2 in 2016 [6]. *D. polymorpha* has spread throughout Lake Rāzna, negatively impacting the biota. The main aim of this study was to examine the population genetic structure of the invasive species *D. polymorpha* in Latvia by microsatellite markers. Genetic diversity is one of the basic factors influencing the establishment and spread of invasive species as well as the evolution of invasiveness [7-9]. Understanding the population genetic structures in invasive species should reveal the dispersion capacity and invasion routes of *D. polymorpha*, which would help in the establishment of management strategies to control the invasive potential of this species [10].

Microsatellites or simple sequence repeats (SSRs) are one of the most informative types of molecular markers due to their high variability as the consequence of their high mutation rates, and the ability to resolve population structure even among closely related populations [11]. These markers have also proven to be useful for the genetic characterization of invasive species where genetic diversity is reduced, as well as to infer the sources and pathways of the introduced populations in aquatic ecosystems [12]. In Latvia, microsatellites have been used to investigate other aquatic organisms [13]. *D. polymorpha* genetic monitoring can provide crucial information on the genetic diversity, connectivity, fitness and viability of the population [14].

The population genetic structure of *D. polymorpha* was studied for the first time in Latvia. The main parameters of the population genetic structure of the zebra mussel are presented. Our results should be useful in monitoring and possible control of the expansion of this invader of the Baltic lakeland in the future.

MATERIALS AND METHODS

Sample collection

Mussels were collected from Lake Rāzna (Rezeknes district, Kaunatas, Mākoņkalna, Čornajas administrative territory (56°21'N; 27°27'E)) in Latvia at a depth of 2 m from different localities. After collection, the samples were placed in 96% ethanol and stored at -80°C.

DNA extraction

Genomic DNA was extracted from whole body tissue using Qiagen the DNeasy kit (Qiagen, Inc.; Valencia, CA, USA) according to the manufacturer's instructions. DNA was quantified spectrophotometrically (Shimadzu BioSpec-Nano) at A260/A280 and A260/A230 (~1.8 and ~1.9, respectively) [15]. DNA quality was checked by agarose gel electrophoresis (1.5%) in tris-borate-EDTA (TBE) buffer, and the gels were stained with ethidium bromide (0.5 μg/mL). The size of the DNA fragments was determined by comparison with DNA ladders of known size (100 bp DNA Ladder Plus, MBI Fermentas, Thermo-Fisher Scientific, Waltham, MA, USA). The extracted DNA was stored at -20°C. For subsequent molecular analysis, the DNA was diluted to a final concentration of 20 ng/μL.

PCR amplification

Extracted genomic DNA was used as a template for DNA amplification using the polymerase chain reaction (PCR). Six microsatellite loci were amplified (*DpolA6, Dpo260, Dpo272, Dpo101, Dpo221, Dpo04*) [16]. For genotyping, the PCR products were obtained with fluorescently-marked primers labeled individually on the 5' end with one of the following dyes: TAMRA, HEX, FAM, and examined on an ABI 310 automated analyzer using Genescan ROX 500 (Applied Biosystems, Thermo Fisher Scientific, Preston Ct, Bedford, MA, USA). Microsatellite amplification was performed in an ABI 9700 thermocycler. The PCR mixture was contained in a final volume of 10 μL containing 100 ng of the DNA sample, 10× Taq Buffer with KCl, 1.5 mM MgCl, 2 mM dNTP mix, 0.06 U/μL Taq DNA polymerase and 0.4 μmol/μL of each primer. The PCR thermal cycling program had an initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 30 s, annealing at locus-specific temperature (Supplementary Table S1) for 30 s, and extension at 72°C for 60 s, followed by a 7-min final extension at 72°C and cooling at 4°C. Both positive and negative controls were used during PCR amplification.

Statistical analysis

The allele fragments were made using the GeneMapper 3.7 software (Applied Biosystems, Foster City, CA, USA). The allele number in the locus, its frequency, alleles in the population, observed and expected heterozygosity, and the level in the polymorph locus were measured by GeneAlex 6.41 software [17]. The Micro-Checker 2.2.3. program was used to check the data for typographic errors, to identify the null allele and genotyping errors, short allele dominance (large allele dropout) and the scoring of the stutter peaks. The program estimates the frequency of null alleles, and it can adjust the allele and genotype frequencies of the amplified alleles, permitting their use in further population genetic analysis [18]. The computer program Bottleneck 1.2.02. [19] was used to detect the bottleneck effect on the studied population.

RESULTS

Based on our study, all 6 loci were polymorphic in the population of *D. polymorpha* in Lake Rāzna. Details of the analyzed microsatellite loci are shown in Table 1. Allele size ranges were 246-363 bp for *DpolA6*, 159-286 bp for *Dpo101*, 224-296 bp for *Dpo260*, 140-172 bp for *Dpo272*, 243-318bp for *Dpo04* and 60-183 bp for *Dpo221*. We observed that the longest allele 246-363 bp was in locus *DpolA6*, and the shortest allele 60-183 bp in locus *Dpo221*. The number of alleles on each microsatellite locus was variable. The greatest number of alleles (13) was found in locus *Dpo260* and the minimum (3) in locus *Dpo04*.

The abundance and frequency of the alleles in the investigated loci are shown in Fig. 1. The frequencies of the alleles in the investigated loci were very different. The most variable locus was *Dpo260* (Fig.1F) with the most common alleles 240 and 244 with frequencies of 0.12 and 0.16, respectively. The most common alleles were 144 (frequency 0.5) in locus *Dpo272* (Fig. 1A), 243 and 249 in locus *Dpo04* (frequencies 0.5 and 0.4) (Fig.1D), 318 and 363 in locus *DpolA6* (frequencies 0.4 and 0.25) (Fig.1 B), 81 in locus *Dpo221* (frequency 0.6) (Fig.1E), 243 in locus *Dpo101* (frequency 0.3) (Fig.1C). Allele frequencies followed an L-shaped distribution, i.e., no bias of allele frequencies toward mean values was observed in the population, as expected in a non-bottlenecked population at mutation-drift equilibrium. The level of allelic diversity and heterozygosity exhibited in invasive populations was characteristic of the source population and would not have been detected in the case of a severe bottleneck.

According to MICROCHECKER, putative null alleles were detected in 3 of the 6 loci (*DpolA6, Dpo101, Dpo260*). Microsatellite genotypic data showed no typographic errors or large allele dropout. We found a significant deviation from the Hardy-Weinberg equilibrium (HWE) for the zebra mussel population. Significant departures from the HWE were observed in 4 of the 6 loci after sequential Bonferroni

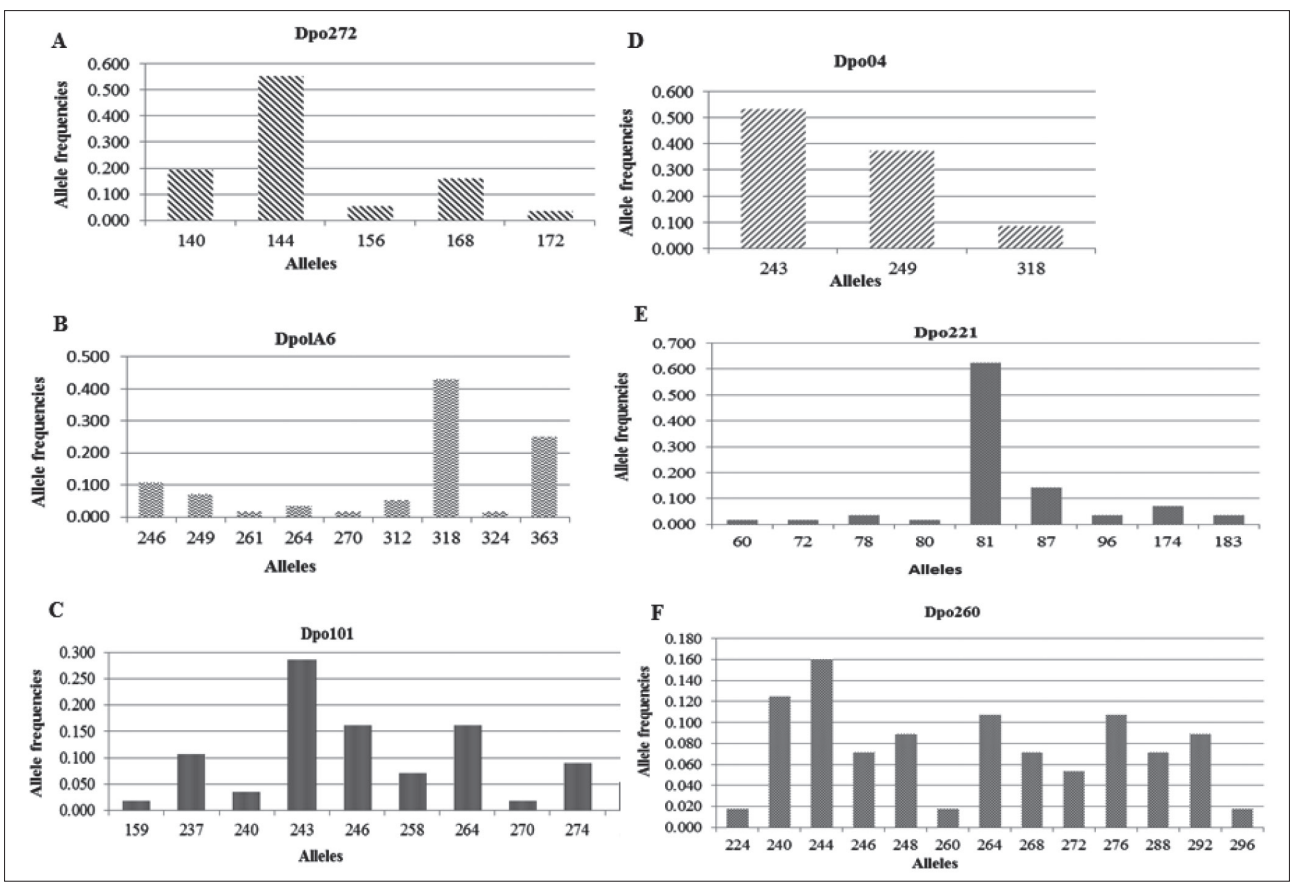

Fig. 1. The common abundance of alleles in microsatellite loci *Dpo272* (**A**), *DpolA6* (**B**), *Dpo101* (**C**), *Dpo04* (**D**), *Dpo221* (**E**), *Dpo260* (**F**).

correction (Table 1). The high polymorphism recorded was reflected in the high expected heterozygosity (He) values between 0.56 in locus *Dpo04* and 0.90 in loci *Dpo260* (Table 1). The actual observed values (Ho) were lower (0.46-0.71) than those expected for 4 loci. The maximum Ho value was at locus *Dpo272* (0.71), and the minimum value was at locus *Dpo260* (0.46). The average observed heterozygosity was less than the expected heterozygosity.

The individual fixation index (FIS) points to a reduction in heterozygosity due to non-random mating and is a measure of the deviation of genotypic frequencies from the HWE in subpopulations in terms of deficiency or excess of heterozygotes. When FIS>0, there is a deficiency of heterozygous individuals (inbreeding); with FIS<0 there is an excess of heterozygotes (unrelated mating), and FIS=0 indicates random pairing. The highest calculated values of the FIS coefficient were shown for the loci *DpolA6, Dpo101, Dpo260* and *Dpo221*, with an average value for 6 loci +0.138 (Table 1).

Table 1. Characteristics of microsatellite loci isolated from the zebra mussel *Dreissena polymorpha*.

Locus	Size (bp)	N_A	Heterozygosity			P
			H_{obs}	H_{exp}	F_{IS}	
DpolA6	246-363	9	0.500	0.732	0.317	***
Dpo101	159-286	10	0.643	0.837	0.232	ns
Dpo260	224-296	13	0.464	0.901	0.484	***
Dpo272	140-172	5	0.714	0.625	-0.143	ns
Dpo04	243-318	3	0.643	0.564	-0.139	***
Dpo221	60-183	9	0.536	0.579	0.075	***
	Mean	8.2	0.583	0.706	0.138	

Locus designations, size ranges of the alleles; numbers of alleles (N_A), observed heterozygosity (Ho), expected heterozygosity (He), Fixation Index (Fis); * P<0.05, ** P<0.01, *** P<0.001; ns – not significant difference.

DISCUSSION

The zebra mussel began expanding its range throughout Europe over 200 years ago [20]. Genetic studies of populations of *D. polymorpha* may help predict possible new areas of invasion, which would allow for the implementation of preventative management to mitigate accidental introduction. All studies on zebra mussels to date report high levels of genetic variability, irrespective of marker type or whether samples come from native or invasive populations [16,21].

The allele size range in the studied population from Latvia was similar to that in the study of 12 zebra populations from the Great Lakes in North America [22] and from Lake Mead and Lake Erie [23]. In our study, the number (3-13) and frequencies of the alleles at each microsatellite locus were different. Authors have shown that the highest number of alleles at a locus was in the American populations (13-21), followed by the European (11-19) and the Irish (6-17) [22]. A decrease in allelic richness could lead to a reduction in the population's potential to adapt to future environmental changes since this diversity is the raw material for evolution by natural selection. Founder events are known to decrease the genetic diversity of a population and are often followed by a demographic expansion. Allelic richness is more sensitive than heterozygosity to founder events that are followed by expansions since allelic richness does not consider the abundance of the alleles but only their presence (a rare allele that is lost in a founder event will probably not affect heterozygosity much, but the loss does reduce allelic richness). Genetic drift or the "founder effect" is often also followed by a loss of alleles.

The present study shows high levels of variability in the Lake Rāzna population; the observed heterozygosity was Ho 0.58 and in Poland it was 0.61, in the Great Lakes 0.59-0.78, in the Netherlands 0.67, in Ukraine 0.80 and in Spain (0.69). The Ho values for the established Latvian population are within the range observed for both native and well-established alien populations although the mean numbers of alleles at the locus are significantly lower.

In our study, a deficit of heterozygotes was recorded for four loci (*DpolA6*, *Dpo101*, *Dpo260*, *Dpo272*). At the same time, the observed and expected heterozygosity at all loci differed insignificantly. The decrease in the observed heterozygosity can induce a decrease in the average fitness of individuals and thus this measure has clear ecological consequences. Other authors [23] also reported a deficit of heterozygotes in *D. polymorpha* populations based on microsatellite loci and concluded that it was most likely caused by null alleles, while other studies [19,22] did not report deviations from the HWE. Disequilibrium may be caused by the presence of null alleles (as indicated by the micro-checker). A null allele is any allele that when amplified on a microsatellite is not visible after

staining or electrophoretic separation [24,25]. The presence of null alleles is an important element of loss of heterozygosity as compared to expected heterozygosity as observed in the present study of the Lake Rāzna population. The reason for the presence of null alleles observed by analysis of the microsatellite markers is thought to be the result of mutation, insertion and deletion [26,27].

Several factors such as inbreeding and the Wahlund effect could account for the observed heterozygote deficits; in other sources [22], 11 microsatellite loci were used to differentiate among 6 Eurasian populations of zebra mussels, including 4 invasive and 2 native populations, which all had high genetic diversity (Ho of 0.70 and He of 0.50.) A deficit of heterozygotes was not reported. In North America, previous genetic studies concluded that the invasion originated from high numbers of founding individuals and/or multiple colonization events from several European locations that resulted in high gene diversity within populations [6].

Due to the loss of genetic variability resulting from a high level of inbreeding within and among populations that can be driven by random genetic drift, populations are declining [28-30]. However, our sampling area in Latvia was too restricted to yield significant conclusions and an answer to this question.

CONCLUSIONS

The genetic diversity of a Latvian population of zebra mussel (Lake Rāzna) as part of the European population was studied. The *Ho* value for the Latvian population is within the range observed for both native and well-established alien populations, although the mean numbers of alleles at the locus are significantly lower. However, there was no evidence that this population has undergone a recent bottleneck event. Significant departures from the HWE in the form of heterozygote deficits were recorded for 3 loci. Of the various factors that contributed to the deficits in the population, e.g., null alleles, inbreeding, selection and the Wahlund effect, null alleles were the most likely cause. High gene diversity values and the lack of recent bottleneck signals supports the hypothesis that this first invasion was affected by a large set of individuals.

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Data availability: All data underlying the reported findings have been provided as part of the submitted article and are available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Morozova%20and%20Shkute_8368_Data%20Report.pdf

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Microsatellite primers: description and amplification conditions.

Name	Repeat motif	Label	PCR conditions		GenBank accession number
			T_a (^{0}C)	Number of cycles	
DpolA6	$[ATT]_3ACT[ATT]_2ATC[ATT]_8[ACT]_3[ATT]_3$	FAM	55	35	AF317427
Dpo04	$(AAC)7AC(AAC)_4$	TMR	52	35	GU213457
Dpo101	$[TGA]_{14}$	HEX	56	35	GU213458
Dpo221	$[TGA]_{25}$	TMR	50	35	GU213461
Dpo260	$[TAGA]_{35}$	FAM	52	35	GU213462
Dpo272	$[AACT]_8$	HEX	50	35	GU213463