GENETIC STRUCTURE OF THE *LIMECOLA BALTHICA* POPULATION IN THE GULF OF RIGA, BALTIC SEA

Oksana Fokina¹, Dace Grauda¹, Ingrida Puriņa², Ieva Bārda², and Isaak Rashal¹

¹ Institute of Biology, University of Latvia, 1 Jelgavas Str., Rīga, LV-1004, LATVIA

² Latvian Institute of Aquatic Ecology, 4 Voleru Str., Rīga, LV-1007, LATVIA

Corresponding author, izaks.rasals@lu.lv

Contributed by Isaak Rashal

Samples of Limecola balthica with normal and deformed shells were collected from ten sites throughout the Gulf of Riga. Genetic diversity was evaluated by the retrotransposon-based iPBS method. Samples had close mutual genetic distances, which showed that all of them belong to one wider population of the Gulf of Riga. No direct relationship between the activity of retrotransposons and deformation of shells was found.

Key words: retrotransposon-based markers, iPBS, Baltic macoma, genetic distances.

INTRODUCTION

Baltic Sea is the world's largest brackish sea and has restricted water exchange with the North Sea. An increased level of eutrophication and pollution from hazardous substances has caused the Baltic Sea to be classified as one of the most polluted areas of the world (Smolarz and Bradtke, 2011). These multiple anthropogenic as well as climatic factors can lead to irreversible consequences, such as a change in the biodiversity of the Baltic (Ojaveer *et al.*, 2010). The preservation of genetic diversity and improvement of methods for its study is one of the most important tasks nowadays.

The benthic clam *Limecola balthica* is widely distributed over the northern hemisphere, including in the Baltic Sea (Bondsorff and Wenne 1989; Rumohr *et al.*, 1996). As deposit-feeders they are exposed to natural and anthropogenic origin contaminants, and therefore have been used as indicator species to monitor effects of pollution in brackish environments (Griscom *et al.*, 2002; Lehtonen *et al.*, 2003; 2006; Baršiene *et al.*, 2008; Barda *et al.*, 2014). The *L. balthica* shell is usually round or oval with triangular tops, but some of them have visible morphological deformations. Parameters like length, height, width, proportions of posterior side and flexure on it, are often used for the analysis of changes in the environment and their impact on marine ecosystem (Sokolowski *et al.*, 2008; Genelt-Yanovskiy *et al.*, 2017). The reasons for the various changes can be a combi-

nation of several factors. According to Sokolowski *et al.* (2004), the number of deformed shells of *L. balthica* from the Gulf of Gdansk increases with the depth. The concentration of accumulated trace metals (e.g., As, Ag, Cu, and Zn) in the tissues might also cause deformations. The proportion of modified shells can reach up to 65% of the total population size. Over the past decade, various studies have investigated the possible effects of temperature change and predators (Genelt-Yanovskiy *et al.*, 2017), food availability (Beukema *et al.*, 2014), anoxia and hydrogen sulfide concentration at different depths (Sokolowski *et al.*, 2008), acidification (Jansson *et al.*, 2013; Van Colen *et al.*, 2018) and trace metal and other contaminant concentrations (Sokolowski *et al.*, 2004) on the shell growth. There exist several hypotheses to explain the causes of these changes, but the reason for these modifications is still unclear.

Impact of genetic factors on abnormalities of shell shape also is not clear. The mitochondrial DNA *cytochrome oxidase I* gene showed no significant differences between individuals with different shell types (Sokolowski *et al.*, 2008). It is possible that the influence of the changing environment and the activity of retrotransposons are one of the reasons of shell shape modifications.

Inter-PBS (iPBS) is a retrotransposon-based marker method, which was suggested by Kalendar and colleagues (2010). The method is based on the possibility of retrotransposons moving around the genome, leaving their copies in

different places, which may affect genetic diversity of a species and also phenotypes. This technique was shown to have high potential to be effective for genetic analysis in both plant and animal species, without prior knowledge of its genome. It is also suggested that in stress conditions moving of transposons is activated, which can result in increased genetic diversity revealed by iPBS markers.

In this study we used iPBS genetic markers to test for genetic differences between *L. balthica* individuals collected in geographically distant sites of the Gulf of Riga. As well we look for a possible relationship between shell shapes and genetic diversity estimated by the iPBS technique.

MATERIAL AND METHODS

Mussels were collected in the Gulf of Riga during 2012–2014 at ten study sites (Fig. 1). Specimens of *L. balthica* were collected using a Van Veen grab from the r/v “Aranda”. All collected samples were immediately frozen and subsequently stored at –20 °C.

DNA was extracted from *L. balthica* foot tissues by an AnalytikJena kit. The quality of extracted samples was checked on 1.5% agarose gel for 2 hours at 60 V. A spectrophotometer was used for more accurate analysis of type and concentration of contaminants in each sample.

Obtained DNA was found to be unstable, apparently the mollusk’s extracted DNA sample contain enzymes causing DNA fragmentation. Using the iPBS method requires good quality unfragmented DNA. Therefore, it is recommended that *L. balthica* DNA samples be analysed not later than a day after DNA extraction.

Seventeen iPBS primers (Table 1), previously having shown a high level of effectiveness both in animals and plants (Kalendar *et al.*, 2010), were used for preliminary

Fig. 1. *Limecola balthica* collection spots in the Baltic Sea.

Table 1. The list of iPBS-specific primers used in preliminary screening

Primer	Sequence
2076	GCTCCGATGCCA
2079	AGGTGGGCGCCA
2080	CAGACGGCGCCA
2081	GCAACGGCGCCA
2232*	AGAGAGGCTCGGATACCA
2237	CCCCTACCTGGCGTGCCA
2238	ACCTAGTCTCATGATGCCA
2239	ACCTAGGCTCGGATGCCA
2271	GGCTCGGATGCCA
2272	GGCTCAGATGCCA
2273	GCTCATCATGCCA
2375	TCGCATCAACCA
2377	ACGAAGGGACCA
2378	GGTCCTCATCCA
2390	GCAACAACCCCA
2394*	GAGCCTAGGCCA
2415	CATCGTAGGTGGGCGCCA

*primers which were selected for the further analysis

screening to find the most suitable primers with a large number of polymorphic loci. The PCR procedure was as follows. The PCR programme consisted of 32 cycles: first denaturation cycle at 95 °C for 3 minutes, 30 repeated cycles (95 °C for 30 s, 50 °C for 40 s, 68 °C for 1 min), and the last elongated cycle at 72 °C for 5 minutes. The final concentration of reagents in the 25 µl PCR mixture was: 1xDreamTaq Buffer, 200 µM dNTPs, 2.5 U DreamTaq polymerase, 0.06 U Pfu DNA polymerase and 1 µM primer. The PCR products were electrophoresed on 1.7% agarose gel for 3.5 hours at 70 V, and then gel was stained with ethidium bromide.

Experimental data were processed with Popgene software to identify differences among polymorphic loci within and among individuals from different sampling places. Nei's genetic parameters and principal coordinates analysis were calculated using NTSYSpc 2.1 software.

RESULTS

Two of seventeen preliminary tested iPBS-specific primers with the highest numbers of polymorphic loci (2232 and 2394) were chosen for subsequent analysis. Altogether those primers revealed 40 polymorphic loci: 17 loci with primer 2232 and 23 loci with primer 2394, respectively. Percentage of polymorphic loci in each site varied from 53% in site M21 to 85% in M14. From 84 sampled individuals, 39 clams were with some deformation and 45 had normal shells (Table 2).

The most similar samples according Nei's distance were those collected from sampling sites M60 and M62 (genetic distance 0). These places are located at a short geographical distance. However, in other cases, the level of genetic distance between nearby locations, for example, between sites

Table 2. The number of collected samples and genetic characteristics per sampling site

	M01	M07	M11	M14	M21	M25	M60	M62	MV2	MV5	In total
Normal shell	3	4	5	4	6	6	5	4	4	4	45
Deformed shell	5	2	5	5	0	5	7	2	3	5	39
Size of samples	8	6	10	9	6	11	12	6	7	9	84
Number of polymorphic loci	27	27	24	34	21	27	26	28	26	32	40
Percentage of polymorphic loci	68	68	60	85	53	68	65	70	65	80	100

Table 3. Nei's unbiased measures of genetic diversity

Site											
M01	M01										
M07	0.04	M07									
M11	0.05	0.06	M11								
M14	0.02	0.04	0.04	M14							
M21	0.03	0.07	0.05	0.06	M21						
M25	0.03	0.06	0.09	0.05	0.09	M25					
M60	0.03	0.05	0.05	0.03	0.09	0.03	M60				
M62	0.01	0.04	0.03	0.01	0.05	0.04	0.00	M62			
MV2	0.04	0.05	0.09	0.06	0.07	0.07	0.03	0.02	MV2		
MV5	0.02	0.03	0.05	0.03	0.08	0.02	0.02	0.02	0.05	MV5	

MV2 and MV5, was higher than distances between M01 and M14, which were located on opposite sides of the Gulf of Riga (Table 3). Despite the high number of polymorphic loci in each population, the level of Nei's genetic distance among all sites varied only from 0 to 0.09 (Table 3).

Principal coordinates analysis (Fig. 2) showed some genetically separated sites. Site M21 was geographically distant from other sites and also had the highest level of genetic distance from other sites: from 0.05–0.09 (Table 3). Samples of some other sites (M11 and M07) were grouped separately (Fig. 2). Site M11 was closest to the open Baltic Sea, while M07 was in the central part of the gulf. Nevertheless, a general positive correlation between geographical and genetic distance was not observed.

No positive correlation was observed between genetic variability of the used molecular markers and shell shapes. The percentage of polymorphic loci in both groups was similar: 92.5% in deformed shells and 97.5% in samples with a typical shell form (data not shown).

DISCUSSION

According to monitoring data for *L. balthica* in the White Sea (Genelt-Yanovskiy *et al.*, 2017) for the last 20 years, predators have a smaller influence on mollusks than temperature change. A huge number of studies devoted to bivalve mollusks and their adaptation to a changing environment have revealed serious morphological, histological and cytogenetic changes of these creatures (Sokolowski *et al.*, 2004). Despite many positive correlations with depth of the clams and the concentration of heavy metals in tissue, the cause of deformation of the shells has not yet been found as well as the causes of the so often occurring tumors in gill cells of the deformed-shell bivalves (Sokolowski *et al.*, 2004).

All 40 loci revealed by two applied primers were polymorphic. In spite of a large number of alleles, the genetic distance between populations was low and therefore no strong clustering of sampling sites was observed. This showed that samples from the sites probably belong to one wider population of the Gulf of Riga.

Fig. 2. Principal coordinates analysis based on differences of polymorphic loci within and among 10 sites. The sites are displayed in different colours, each sample is visualised as a separate point (NTSYSpc 2.1 software).

A direct relationship between the activity of retrotransposons and deformation of shells in our investigation was not found. It should be noted that the shell deformation has different morphological features, perhaps the reasons for their appearance were some environmental, not genetic causes. Similar data of homogeneous structure were obtained by Sokolowski *et al.*, (2008) using mitochondrial DNA cytochrome oxidase I region of bivalves in the Gulf of Gdansk.

Data on the genetic diversity of bivalves in Gulf of Riga using retrotransposons are published for the first time; this information may help in the conservation and study of biodiversity in the Baltic Sea. For further analyses of the genetic diversity of the species, it would be worthwhile to increase the amount of collected material and widen the number and types of applied molecular markers, including those, which were already successful used in bivalves in some other geographic areas (see, for example, Becquet *et al.*, 2009, Lasota *et al.*, 2018, Šatović *et al.*, 2019).

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BALTIJAS JŪRAS RĪGAS LĪČA *LIMECOLA BALTHICA* POPULĀCIJAS ĢENĒTISKĀ STRUKTŪRA

Limecola balthica paraugi tika ievākti desmit dažādos attālinātos Baltijas jūras Rīgas līča punktos. Starp ievāktajiem indivīdiem bija eksemplāri gan ar tipiskiem, gan arī ar deformētiem gliemežvākiem. DNS molekulārie marķieri tika noteikti ar iPBS metodi, kas pamatojās uz retrotranspozonu mainības analīzi. Noteikts, ka paraugi no dažādām ievākšanas vietām ir ģenētiski tuvi, kas visdrīzāk liecina, ka *L. balthica* Rīgas līcī veido vienu kopīgu populāciju. Saistība starp retrotranspozonu aktivitāti un deformēto gliemežvāku parādīšanos šajā pētījumā nav konstatēta.