THE PHENOTYPIC STRUCTURE OF A *MYTILUS GALLOPROVINCIALIS* LMK POPULATION FROM THE ROMANIAN BLACK SEA SHORE

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Abstract: Among the specimens of *Mytilus galloprovincialis* Lmk. from the Black Sea, function of the color of the ostracum (brown, dark blue or brown with blue stripes), one can differentiate several forms. These colors are genetically determined (Stolbova, Pirkova, Ladyghina, 1996; Scherban, 2000; Shurova, 2001). The present paper analyzes the situation of these phenotypic groups, using specimens sampled at different depths in the area of Agigea dike.

INTRODUCTION

Using the color of the ostracum, the individuals of *Mytilus galloprovincialis* can be divided in three groups: phenotype A – brown valves, phenotype B – dark blue valves and phenotype C – brown valves with radial blue stripes (Fig. 4). Previous studies (Dragoli, 1966; Shurova, Zolotarev, 1990; Pirkova et al., 2000) indicated that the valves color is determined genetically, 2 hypotheses being made:

I. The color is determined by a gene with two codominant alleles. The allele A – ostracum without blue pigment, and allele B – ostracum blue pigmented. The heterozygote (phenotype C) has brown colored valves with blue stripes (Shurova, 2001). It is possible that this is a pleiotropic gene since between the 3 phenotypes there are also physiological and biochemical differences. The protein, RNA and DNA quantity is bigger in the individuals with B phenotype, smaller in those with A and intermediate in phenotype C (Shcherban, 2000). This hypothesis is sustained also by the fact that in optimal conditions the observed frequencies of the three phenotypes corresponds to that expected under Hardy – Weinberg equilibrium. This is the model that we accept in the present paper.

II. The A allele (lack of blue pigment) is dominant and the B allele (blue color of the ostracum) is recessive. The presence or absence of blue stripes is determined by another gene (Pirkova et al., 2000).

The study of the variability of the Black Sea mussel is important for the genetic monitoring and the conservation of its genetic diversity for the mussel culture developing in the last years in the area (Uss, 1986 in Stolbova, Ladyghina, 1994; Zhukovskaya, Kodolova, Logvinenco, 1987).

MATERIALS AND METHODS

In order to see if the 3 phenotypes are in Hardy – Weinberg equilibrium and how they are distributed, we sampled a population of mussels in the area of Agigea dike near the Agigea port. The sampling was done in October 2004 by a scuba diver from a surface of 20 x 20 cm (400 cm^2). A total number of 953 specimens sampled from different depths (0, 2, 4, 6, 8, 10, 12, 14 and 16 m) were analyzed. Only the shells with the length exceeding 25 mm were scored, in smaller ones the color pattern being difficult to asses. Before the analysis one valve for each individual was treated with NaOH 15 % in order to remove the periostracum. For comparing the observed frequencies with that expected under Hardy – Weinberg equilibrium the χ^2 test was used. The heterozygote deficit was calculated using the *D* parameter:

$$D = (H_o - H_c) / H_c$$

were H_o is the number of observed heterozygote individuals and H_c the number of heterozygote individuals expected under Hardy – Weinberg equilibrium. The *D* parameter takes values from 0 (no deficit) to -1 (total deficit).

RESULTS AND DISSCUSIONS

Our results show that the observed frequency of the phenotypes is significantly different from that expected under Hardy – Weinberg equilibrium in four cases: 0 m, 8 m, 12 m and 16 m ($p < 0,05$). The deviation from the expected values is significant for the sample from 0 m ($\chi^2 = 11,76$; $p = 0,0006$), but at 2 m it dramatically diminished ($\chi^2 = 0,71$; $p = 0,4$) growing gradually with the depth. For 8 and 14 m the deviation don’t follow the same pattern being very big at 8 m ($\chi^2 = 13,2886$; $p = 0,0003$) and minimum at 14 m (Fig. 1, Tab. 1, Tab. 2). The heterozygote deficit (*D*)

is biggest at 8 m (D = - 0,37) and smallest at 14 m (D = - 0,02) (Tab. 2). Heterozygote deficit is a frequent phenomenon in mussel populations and it is usually associated with pollution and low salinity. With the pollution the number of heterozygote individuals lowers due to an elevated mortality in larval stage (Mallet et al., 1985 in Shurova, 2001). The area of our investigation is subject to both factors (pollutants from the Agigea port and fresh water from Danube – Black Sea channel).

The different phenotypes are not uniformly distributed on vertical. With the depth the fraction of individuals with A phenotype is rising and that of individuals with B phenotype is lowering (Fig. 2). Between the depth and the frequency of A phenotype a direct linear correlation is observed (R^2 = 0,9247), and between the depth and the frequency of B phenotype an exponential one (R^2 = 0,9561) (Fig. 3). The proportion of individuals with C phenotype is near constant regardless the depth. The same distribution pattern of the three phenotypes was reported by Shurova (2001) for the *M. galloprovincialis* populations from the Ukrainian Black Sea shore.

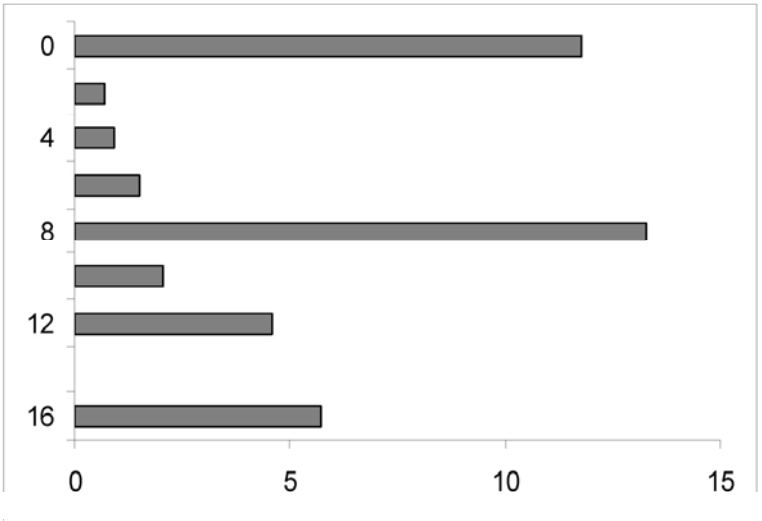

Figure 1. The deviation from the frequencies expected under Hardy – Weinberg equilibrium (expressed as χ^2) ad different depths.

Table 1. The observed and expected frequencies of the phenotypes

depth (m)	Frequency of phenotype A		Frequency of phenotype B		Frequency of phenotype C		Total
	observed	expected	observed	expected	observed	expected	
0	14	6,90	102	94,90	37	51,19	153
2	12	10,156	51	49,16	41	44,69	104
4	34	30,92	59	55,92	77	83,16	170
6	25	21,91	33	29,91	45	51,19	103

8	40	31,19	27	18,19	30	47,63	97
10	25	22,12	15	12,12	27	32,75	67
12	36	31,48	16	11,48	29	38,03	81
14	36	35,59	11	10,59	38	38,82	85
16	49	44,04	14	9,04	30	39,91	93
Total	271		328		354		953

Table 2. The $\chi 2$ statistics (with corresponding p values for $DF = 1$) and D parameter

depth (m)	$\chi 2$	p		D
0 m	11,76	0,0006	(<0,05)	-0,28
2 m	0,71	0,4		-0,08
4 m	0,93	0,33		-0,07
6 m	1,51	0,22		-0,12
8 m	13,29	0,0003	(<0,05)	-0,37
10 m	2,07	0.15		-0,18
12 m	4,57	0,03	(<0,05)	-0,24
14 m	0,04	0,84		-0,02
16 m	5,74	0,017	(<0,05)	-0,25

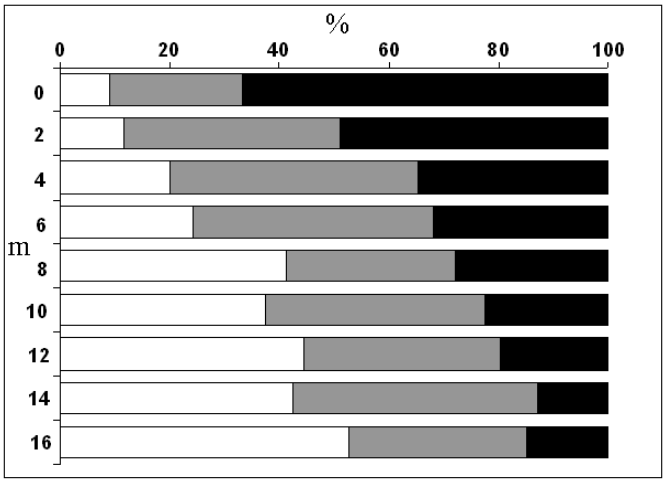

Figure 2. The proportion of the three phenotypes at different depths. A phenotype – white; B phenotype – black, C phenotype –gray

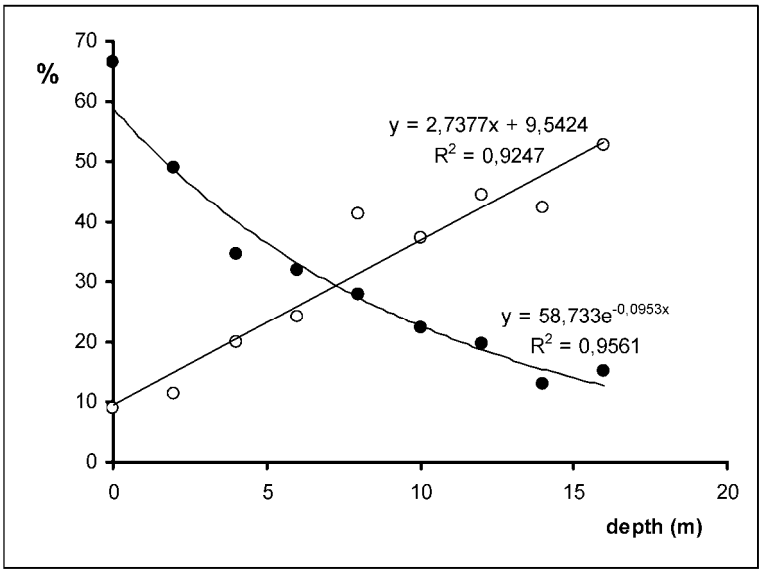

Figure 3. The correlation between the depth and the proportion of A (○) and B (●) phenotypes.

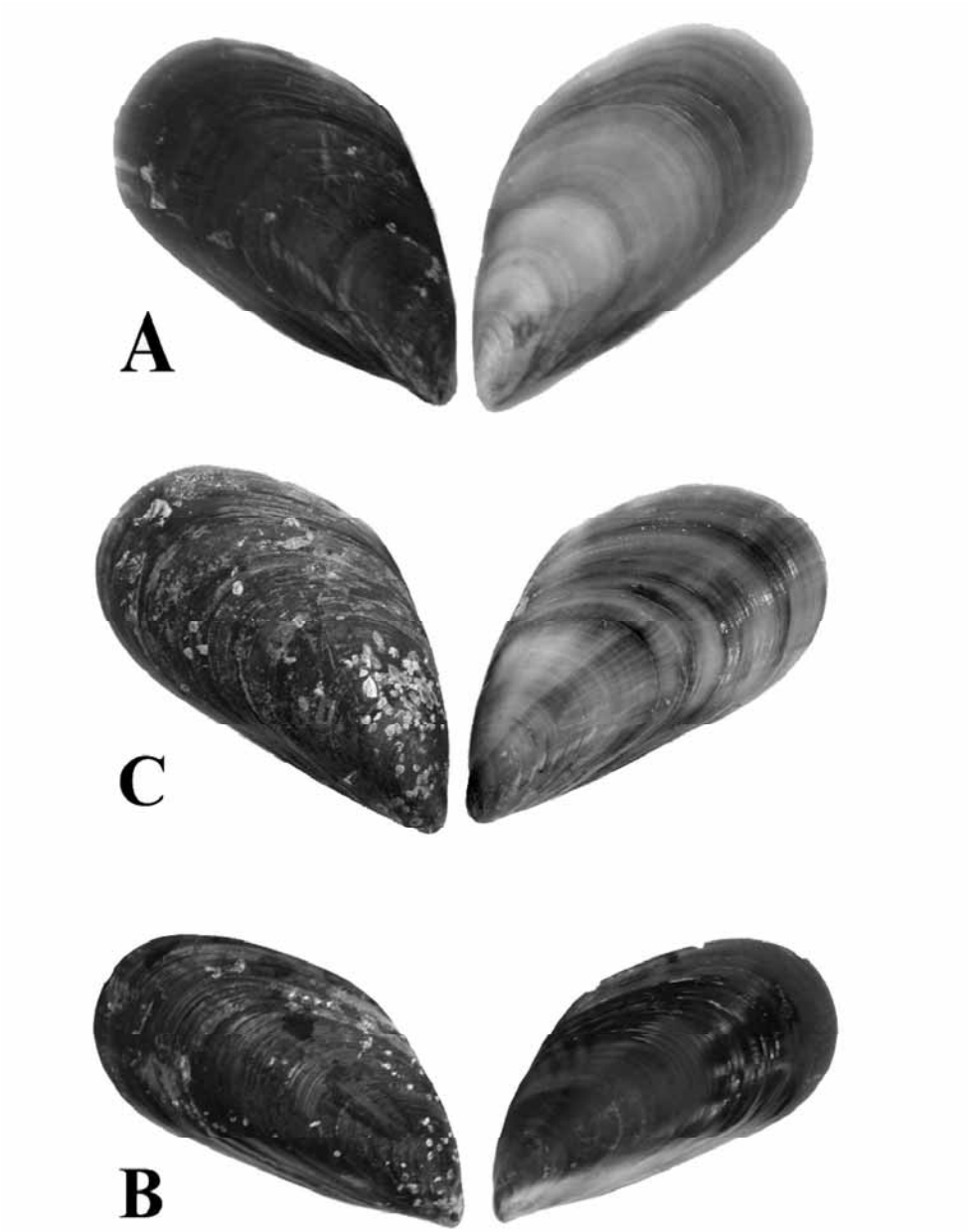

Figure 4. The valves of the three phenotypes (A, B and C) before (left) and after (right) treatment with NaOH solution

CONCLUSIONS

The frequency of different morphs of *M. galloprovincialis* reflects the response of the populations to different environmental conditions. Our data shows that the B phenotype is better adapted to surface conditions and the A phenotype is better adapted to deeper water.

The deviation from the Hardy – Weinberg equilibrium and the heterozygote deficit (*D*) are good indicators for the degree of stress of the population. The significant deviation from the H-W equilibrium and a small *D* parameter in the samples from surface and from 16 m may indicate the pollution of the water surface with low density products and the accumulation of pollutants in the sediment.

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