

## POPULATION GENETIC INDICES OF VEINED RAPA WHELK GROUPS OF THE BLACK SEA REMOTE REGIONS

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### ABSTRACT

Genetic structure and genetic distance of *Rapana venosa* populations from remote regions of the Black Sea (Odesa Bay and eastern Crimea, Ukraine) were defined. Despite the significant difference in morphometric parameters, the investigated samples of mollusks are genetically very similar, and the distance between them is at the level of local populations. Common features of observed groups of *Rapana* in the northern part of the Black Sea are the unequal occurrence of some alleles and genotypes, a high level of heterozygosity and nonequilibrium in the Hardy-Weinberg equation. The gene flow is the most significant of the evolutionary factors that form the genetic structure of *Rapana venosa* populations.

**ZUSAMMENFASSUNG:** Populationsgenetische Indikatoren von geäderten Rapa-Wellhornschncken Gruppen der abgelegenen Regionen des Schwarzen Meeres.

Genetische Struktur und genetische Distanz von *Rapana venosa* Populationen aus abgelegenen Regionen des Schwarzen Meeres (Bucht von Odesa und Ostküste der Krim, Ukraine) definiert wurden. Trotz des signifikanten Unterschieds in den morphometrischen Parametern sind die untersuchten Exemplare von Schalentieren genetisch sehr ähnlich, und der Abstand zwischen ihnen liegt auf der Ebene lokaler Populationen. Gemeinsame Merkmale der beobachteten Gruppen von *Rapana* des nördlichen Teils des Schwarzen Meeres sind das ungleiche Vorkommen einiger Allele und Genotypen, ein hohes Maß an Heterozygotität und Nichtgleichgewicht in der Hardy-Weinberg-Gleichgewicht. Der Genfluss ist der bedeutendste unter den Evolutionsfaktoren, die genetische Struktur von *Rapana venosa* Populationen bilden.

**REZUMAT:** Indicii genetici ai populației grupurilor de rapană din regiunile îndepărtate ale Mării Negre.

Au fost definite structura genetică și distanța genetică a populațiilor de *Rapana venosa* din regiunile îndepărtate ale Mării Negre (Golful Odesei și estul Crimeei, Ucraina). În ciuda diferenței semnificative a parametrilor morfometrici, probele investigate sunt foarte asemănătoare din punct de vedere genetic, iar distanța dintre ele este la nivelul populațiilor locale. Trăsăturile comune ale grupurilor de *Rapana* observate în partea de nord a Mării Negre sunt apariția inegală a unor alele și genotipuri, un nivel ridicat de heterozigozitate și dezechilibru în ecuația Hardy-Weinberg. Fluxul de gene este cel mai semnificativ dintre factorii evolutivi care formează structura genetică a populațiilor de *Rapana venosa*.

## INTRODUCTION

The veined rapa whelk, the gastropod mollusk (*Rapana venosa*, Valenciennes, 1846) was first discovered in Novorossiysky Bay in 1947. However, it is believed that this species appeared in the Pontic basin earlier, in the last 30-40 years of the last century. In less than two decades, the coastal zone around the entire Black Sea was colonized by this mollusk (Eberzin, 1951; Drapkin, 1953; Stark, 1957; Chukchin, 1961; Băcescu, 1963; Gomoiu, 2005). Currently, *Rapana* is a very dangerous invasive species that attracts the attention of researchers not only in the countries of the Black Sea basin. There are data about these mollusk populations in many areas of the World Ocean: Italy, Great Britain, Netherlands, USA, South America, New Zealand (Mann et al., 2004). All this testifies to the great adaptive capacity of rapa whelk. It should be noted that all *Rapana* populations in the secondary ranges are derived from the Black Sea *Rapana* population, and not from the native range (Chandler et al., 2008).

Various aspects of *Rapana*'s adaptative properties as a species are well studied (Chukchin, 1970; Chukchin, 1984; Zolotarev, 1996; Mann and Harding, 2003; Gaevskaya, 2006; Savini and Occhipinti-Ambrogi, 2006; Shadrin and Afanasova, 2009; Zaika et al., 2010). However, the basics of the considerable adaptability of *Rapana*, in particular the genetic ones are not well-studied.

The elucidation of mechanisms of formation of *Rapana* groups' genetic structure in the Black Sea is necessary to predict the spread of rapa whelk. Such data can elucidate the impact of *Rapana* on existing biocoenosis and justify the nature of protection measures, as well as estimate the opportunities for fishing of rapa whelk and other species.

The priority is given to methods based on DNA analysis using PCR in genetic and population studies. All potential genetic determinants (both active and inactive) are identified by this approach. The results of the "Human Genome" program showed a certain paucity in understanding the genetic information implementation only on the basis of nucleotide sequence determination (Venter et al, 2001; Venter, 2003). The international program "Human Proteome" launched in 2010 testifies the importance of proteomic and transcriptomic research. In this regard, iso-enzyme analysis (as an element of proteome research), which includes only active genetic determinants, seems relevant for studying the structure of populations not only in the genetic but also in the ecological aspect.

In our opinion, the high adaptive abilities of rapa whelk from Black Sea may be due to its significant genetic polymorphism. Mechanisms that ensure a high level of internal genetic diversity of *Rapana venosa* are not well understood. Earlier, we made the assumption that significant genetic heterogeneity and disequilibrium (according to the Hardy-Weinberg equation) of rapa whelk groups in the northwestern part of the Black Sea is associated with a high intensity of migration processes. In our studies it was shown that the different groups of *Rapana* in the northwestern part of the Black Sea from the western coast of the Crimea (Cape Tarhankut) to the island of Zmiinyi can be considered as one population (Toptikov et al., 2017). According to our data, the genetic distance by Nei between the investigated groups of rapa whelks is on average  $0.024 \pm 0.003$ , which corresponds to the difference between local populations (Ayala and Kiger, 1984). The role of significant migration activity was also shown in the study of different age rapa whelk groups living in the same biotope (Toptikov et al., 2019). The great importance of migration in the forming of the flexible genetic structure of Black Sea *Rapana* groups can be related to two main causes: the presence of special distinct currents in the Black Sea (Ivanov and Belokopytov, 2011; Belevich et al., 2013) and long pelagic larval stage of the mollusk (Chukchin, 1970). This, combined with the great fertility of the *Rapana*, ensures the motion of a significant number of such organisms into the open sea, their transfer to long distances and crossings of incomers with local individuals.

It should be noted that in different areas of the northwestern part of the Black Sea, hydrological and hydrochemical conditions are quite similar and differ significantly from other regions of the Black sea (Tamaychuk, 2009; Polonsky et al., 2013; Gazyetov et al., 2015; Artamonov et al., 2017). In this regard, the purpose of this study was to determine the genetic indices and the genetic distance of various groups of *Rapana venosa* from remote regions of the Black Sea, which differ in their hydrological and hydrochemical properties.

### MATERIAL AND METHODS

The *Rapana venosa* samples were collected manually, by a non-selective method, by divers in July-August 2012 in two remote water areas of the Black Sea from solid substrates at a depth of 5-15 m (Fig. 1). The sampling sites were located in Odesa Gulf (Odesa, the northwestern sea area, 46°43'8"N, 30°77'2"E) (23 individuals) and in the Karadagsky Reserve area (the biostation, near Kurortne settlement, northeast aquatorium, 44°90'6"N, 35 19'4"E) (25 individuals). Samples of mollusks were immediately frozen and stored at -28°C until analysis.

Morphometric and age parameters were estimated by Kovtun et al. (2014). For the possibility of mathematical calculation of the mean values of age, the age categories 3+, 4+, etc., adopted in hydrobiology were designated as 3.5, 4.5, etc. The enzymes in the gels were detected according to the current recommendations (Manchenko, 2003). Nephridium and Leiblein gland were used as a source of enzymes. Esterases of EC 3.1.1.-(EST) and DT-diaphorase EC 1.6.99.2 (DT) were determined in the nephridium extract. Acid and alkaline phosphatases of EC 3.1.3.-(ACP i ALP, respectively), NADH oxidases of EC 1.6.99.3 (NOX), alcohol dehydrogenases of EC 1.1.1.1 (ADG),  $\alpha$ -amylases of EC 3.2.1.1 (AMI), ATP-ases EC 3.6.1.-(ATPase) and glutathione peroxidases EC 1.11.1.9 (GPX) were determined in the Leiblein gland.

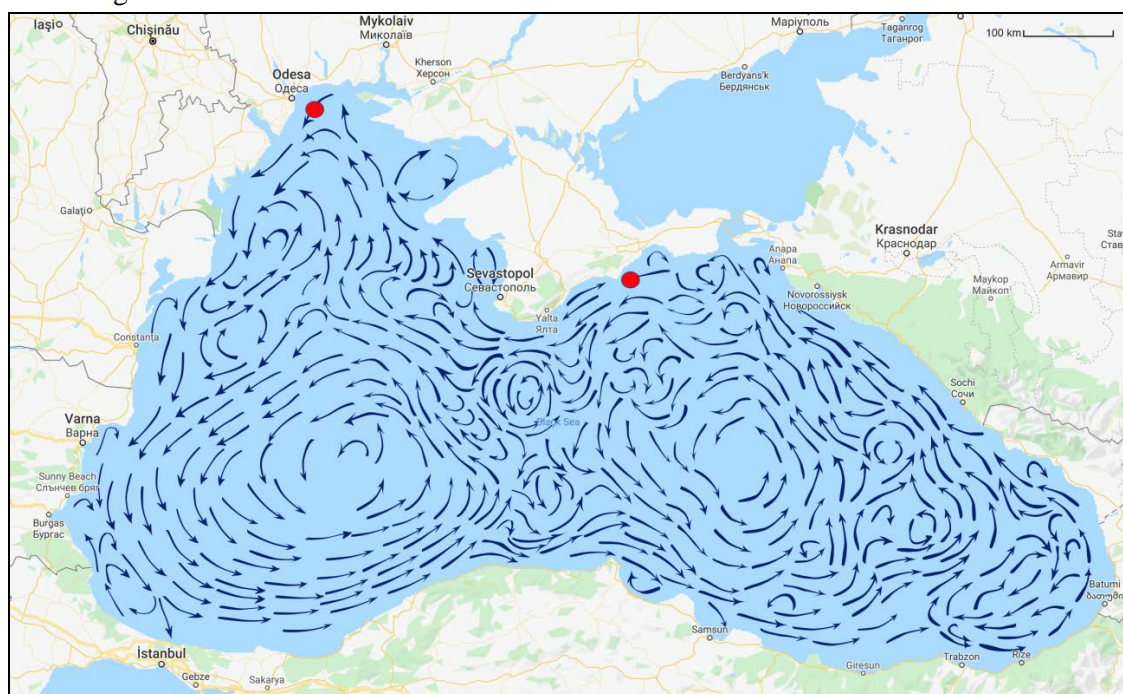


Figure 1: Sampling places (red dots) for collecting stocks of mollusks and patterns of currents in the Black Sea ([http://blacksea-map.ru/map\\_black\\_sea\\_current\\_576665.html](http://blacksea-map.ru/map_black_sea_current_576665.html)).

Analysis of the genetic structure of *Rapana venosa* groups was carried out according to generally accepted approaches and formulas (Wright, 1951, 1978; Nei, 1972; Li, 1976; Ayala, 1982; Ayala and Kiger, 1984; Slatkin, 1985; Altukhov, 2003; Kornienko et al., 2005; Kartavtsev, 2008). The analysis was carried out only on polymorphic loci. This approach was used because it makes possible to clearly see the difference in the level of gene diversity in different populations Pudovkin (1998). The investigated groups of *Rapana* were analyzed as described in a previous work (Toptikov et al., 2019).

## RESULTS AND DISCUSSION

Morphometric indicators of the investigated mollusk groups are presented in table 1. Minor differences in the sexual structure for the studied *Rapana venosa* samples have been established. Taking into account the insignificant (within the limits of statistical inaccuracy) number of females present in the Odesa sample group, it can be assumed that this difference will not affect the general conclusions. Mollusk samples from the two study sites did not differ significantly in age. The average age of rapa whelks from the water area near Karadag was  $3.6 \pm 0.1$ , for mollusks from Odesa Bay it was  $3.9 \pm 0.2$ . Thus, we can assume that the differences found between these groups have no fundamental connection with gender and age.

Table 1: The mean values of the morphometric indicators of *Rapana venosa* from the investigated Black Sea water areas.

Indices	Morphometric indicators								
	Shell height, H, mm	Shell width, W, mm	Total weight, $\Sigma$ , g	Shell weight, $M_s$ , g	Mass of soft body, $M_b$ , g	W/H	$M_b/\Sigma$	Solidity of the shell, $M_s/H$	Coefficient of fatness %
Karadag (n = 25) ( $\sigma^7 = 25$ )									
Average $\pm SE$	<b>67.11</b> $\pm 1.18$	<b>56.55</b> $\pm 1.01$	<b>49.57</b> $\pm 2.76$	<b>34.45</b> $\pm 1.65$	<b>15.12</b> $\pm 1.37$	<b>0.84</b> $\pm 0.01$	<b>0.30</b> $\pm 0.01$	<b>0.51</b> $\pm 0.02$	<b>21.99</b> $\pm 1.49$
Coefficient of variation, %	<b>8.78</b>	<b>8.91</b>	<b>27.88</b>	<b>23.98</b>	<b>45.19</b>	<b>4.86</b>	<b>21.15</b>	<b>17.19</b>	<b>33.78</b>
Odesa (n = 23) ( $\sigma^7 = 21$ , $\sigma^2 = 2$ )									
Average $\pm SE$	<b>75.67</b> $\pm 1.58$	<b>63.23</b> $\pm 1.41$	<b>71.34</b> $\pm 4.69$	<b>42.31</b> $\pm 2.94$	<b>29.03</b> $\pm 1.95$	<b>0.84</b> $\pm 0.01$	<b>0.41</b> $\pm 0.01$	<b>0.55</b> $\pm 0.03$	<b>38.00</b> $\pm 1.97$
Coefficient of variation, %	<b>8.08</b>	<b>8.64</b>	<b>25.45</b>	<b>26.89</b>	<b>25.96</b>	<b>2.46</b>	<b>8.58</b>	<b>19.29</b>	<b>20.11</b>
Reliability of differences between groups by indicators, $p$	<0.001	<0.001	<0.001	0.03	<0.001	not reliably	<0.001	not reliably	<0.001

As can be seen from table 1, between the two groups of mollusks there is a difference in many morphometric parameters of the shell and body and also in degree of variation of these parameters. But, the question arises whether these differences are a consequence of hereditary determination or they are a result of phenotypic plasticity. To clarify the degree of genetic heterogeneity of these two studied groups, an allozyme analysis of their representatives was carried out.

*Rapana* enzyme systems with the most unequivocal interpretation of their electrophoretic distribution by genetic positions were chosen here. In connection with the purpose of this work to elucidate intraspecific (interpopulation) heterogeneity, only polymorphic loci were considered. As a result 19 loci of nine enzymes were selected for testing genotypes (Tab. 2). As can be seen from the data of table 2, the occurrence of alleles in both groups of rapa whelks is almost similar. Each of the groups has insignificant features of the allele's distribution. Thus, in the sample of *Rapana* individuals from the sample of Karadag, the frequency of the alleles "a" of both alkaline phosphatases was almost twice as low compared to the same frequency in Odesa sample. And contrariwise, in case of allele "a" of ATP-ase locus its occurrence was twice as high as in the group of mollusks from the Odesa Bay. From the general features of the occurrence of alleles, we can determine the following: the frequency of alleles "A" of the loci *ALP-1*, *ALP-2*, *ACP-1*, *ACP-2*, *NOX-2*, and *GPX* is approximately twice as high as the alternative alleles of the corresponding loci. In the distribution of different alleles of the ATPase locus, both alleles "b" and "c" predominate in both of the investigated *Rapana* groups.

There were no fundamental differences in the occurrence of different genotypes in the studied groups of rapa whelk (Tab. 2). Thus, the number of homozygotes "AA" by locus *EST-2* and homozygotes by allele "b" of the *ATPase* locus in the group of individuals in the water area near Karadag is lower in comparison with the group of mollusks in the Odesa Bay. A different ratio of homo- and heterozygotes of the "A" allele at loci of alkaline and acid phosphatases and  $\alpha$ -amylases (*AMI-1*) was also observed. In most other cases, the distribution of genotypes was similar in both *Rapana* populations. Certain features inherent in both rapa whelk investigated groups were identified. No homozygous "AA" were found for loci *AMI-3* and *ADG*. The same result – absence of homozygotes "aa" was typical for *GPX* locus. Homozygous genotypes of "AA" loci *EST-6* and *AMI-2* and also genotypes "aa" of loci *AMI-1* and *AMI-2* were found with a low/very low frequency. A very low proportion of the genotypes "aa" and "ac" at the locus *ATPase* was observed.

As can be seen from the obtained data on the gene pool of studied groups of the Black Sea *Rapana*, not only the prevalence of certain alleles and genotypes is characteristic (Tab. 2, where in bold type are reliable deviations from the Hardy-Weinberg equation). A common feature of the genetic structure of rapa whelk is the significant disequilibrium of its groups, as evidenced by the large number of discrepancies in the alleles distribution to the Hardy-Weinberg equation (Tab. 3) On average, a deviation from the Hardy-Weinberg rule was observed for half the loci. Also, deviations in most loci were common for both groups studied.

In addition, the investigated gene pool of rapa whelks had an increased level of available heterozygosity ( $H_O$ , the  $H_E$  is expected heterozygosity) and, accordingly, some excess of heterozygotes (D) (Tab. 3). For most loci (82%), this excess was found, more than half of the loci (63%) were common to both groups. The average excess of heterozygous genotypes was within 15-17%. A high level of heterozygosity is consistent with a very low probability of inbreeding (negative values of the corresponding coefficients  $F'$ ,  $F_{IS}$ ) and a small discrepancy between the effective and predicted number of alleles (Tabs. 3 and 4).

Table 2: The frequency of alleles and distribution of genotypes in the investigated groups of *Rapana venosa*.

Loci	Karadag				Odesa				$\chi^2$							
	Allele A	Actual frequencies of genotypes			Allele A	Actual frequencies of genotypes			Karadag	Odesa						
		AA	Aa	aa		AA	Aa	aa								
EST-2	0.480	0.04	0.88	0.28	0.500	0.17	0.66	0.17	<b>15.42</b>	2.54						
EST-3	0.440	0.16	0.56	0.28	0.457	0.22	0.48	0.30	0.71	0.10						
EST-4	0.500	0.24	0.52	0.24	0.457	0.17	0.57	0.26	0.18	0.70						
EST-5	0.540	0.24	0.60	0.16	0.543	0.09	0.91	0.00	1.39	<b>17.21</b>						
EST-6	0.440	0.08	0.72	0.20	0.413	0.09	0.65	0.26	<b>5.91</b>	3.24						
ALP-1	0.760	0.64	0.24	0.12	0.522	0.26	0.52	0.22	2.30	0.20						
ALP-2	0.800	0.60	0.40	0.00	0.609	0.26	0.70	0.04	2.67	<b>5.55</b>						
ACP-1	0.660	0.52	0.28	0.20	0.587	0.30	0.57	0.13	3.12	0.94						
ACP-2	0.640	0.44	0.40	0.16	0.652	0.35	0.61	0.04	0.38	3.31						
AMI-1	0.500	0.08	0.84	0.08	0.630	0.35	0.57	0.08	<b>12.34</b>	1.45						
AMI-2	0.500	0.04	0.92	0.04	0.478	0.00	0.96	0.04	<b>18.58</b>	<b>20.36</b>						
AMI-3	0.440	0.00	0.88	0.12	0.413	0.00	0.83	0.17	<b>16.37</b>	<b>12.30</b>						
NOX-1	0.380	0.12	0.52	0.36	0.457	0.13	0.65	0.22	0.51	2.71						
NOX-2	0.660	0.32	0.68	0.00	0.674	0.35	0.65	0.00	<b>7.52</b>	<b>6.29</b>						
ADG	0.440	0.00	0.88	0.12	0.435	0.00	0.87	0.13	<b>16.37</b>	<b>14.55</b>						
DT-1	0.580	0.36	0.44	0.20	0.522	0.26	0.52	0.22	0.24	0.23						
DT-2	0.460	0.08	0.76	0.16	0.522	0.17	0.70	0.13	<b>7.66</b>	<b>4.08</b>						
GPX	0.620	0.24	0.76	0.00	0.696	0.39	0.61	0.00	<b>10.27</b>	<b>5.32</b>						
ATPase	Alleles	aa	bb	cc	ab	ac	bc	Alleles	aa	bb	cc	ab	ac	bc	<b>6.49</b>	<b>4.03</b>
	a-0.220							a-0.152								
	b-0.420	0.08	0.08	0.12	0.24	0.04	0.44	b-0.544	0.04	0.26	0.13	0.22	0.00	0.35		
	c-0.360							c-0.304								

Table 3: Heterozygosity at the studied loci of rapa whelk groups from different ranges.

Loci	Areas					
	Karadag			Odesa		
	H <sub>O</sub>	H <sub>E</sub>	D	H <sub>O</sub>	H <sub>E</sub>	D
<i>EST-2</i>	0.88	0.50	-0.38	0.65	0.50	-0.15
<i>EST-3</i>	0.56	0.49	-0.07	0.48	0.50	0.02
<i>EST-4</i>	0.52	0.50	-0.02	0.57	0.50	-0.07
<i>EST-5</i>	0.60	0.50	-0.10	0.91	0.50	-0.41
<i>EST-6</i>	0.72	0.49	-0.23	0.65	0.48	-0.17
<i>ALP-1</i>	0.24	0.37	0.13	0.52	0.50	-0.02
<i>ALP-2</i>	0.40	0.32	-0.08	0.70	0.48	-0.22
<i>ACP-1</i>	0.28	0.45	0.17	0.57	0.49	-0.08
<i>ACP-2</i>	0.40	0.46	0.06	0.61	0.45	-0.16
<i>AMI-1</i>	0.84	0.50	-0.34	0.57	0.47	-0.10
<i>AMI-2</i>	0.92	0.50	-0.42	0.96	0.50	-0.46
<i>AMI-3</i>	0.88	0.49	-0.39	0.83	0.49	-0.34
<i>NOX-1</i>	0.52	0.47	-0.05	0.65	0.50	-0.15
<i>NOX-2</i>	0.68	0.45	-0.23	0.65	0.49	-0.16
<i>ADG</i>	0.88	0.49	-0.39	0.87	0.49	-0.38
<i>DT-1</i>	0.44	0.49	0.05	0.52	0.50	-0.02
<i>DT-2</i>	0.76	0.50	-0.26	0.70	0.50	0.20
<i>GPX</i>	0.76	0.47	-0.29	0.61	0.42	-0.19
<i>ATPase</i>	0.72	0.65	-0.07	0.52	0.59	0.07
	<b>0.63 ± 0.05</b>	<b>0.45 ± 0.01</b>	<b>-0.15</b>	<b>0.66 ± 0.03</b>	<b>0.46 ± 0.02</b>	<b>-0.17</b>
<i>F</i>	<b>0.037</b>					

Table 4 shows the values of  $F'$  (the coefficient of inbreeding of a specific sample),  $F_{IS}$  (the measure of the deviation of the actual and expected genotypes frequencies within individual samples),  $F_{IT}$  (the measure of the deviation of the actual and expected frequencies of genotypes of a specific sample as compared to a generalized set of samples) as well as  $F_{ST}$  (the measure of gene differentiation of the investigated samples) and  $G_{ST}$  (relative gene differentiation between the samples studied). The sign \* is mean, that data were calculated for a single sample relative to a generalized set of samples.

As can be seen from the presented data (Tab. 4), the  $F_{IS}$  and  $F_{IT}$  values are significantly larger than the  $F_{ST}$  level. This indicates the predominance of individual and intra-group variability over the general genetic heterogeneity of rapa whelks of the northern part of the Black Sea.

Table 4: The indicators of genetic variability of *Rapana venosa* groups investigated

Investigated groups	Indicators of genetic diversity and the degree of inbreeding				
	$F'$	$F_{IS}$	$F_{IT}$	$F_{ST}$	$G_{ST}$
Karadag	-0.295	-0.324*	-0.451*	-0.096*	0.020
Odesa	-0.321	-0.294*	-0.451*	-0.121*	
Entire set of samples	-0.450	-0.292	-0.283	0.009	

The question of the affinity of rapa whelk groups in various areas of the Black Sea area is of very high practical importance, it is necessary mainly for scientifically based exploitation of marine resources. The use of various indicators of genetic similarity gave corresponding results (Tab. 5). For comparison, data from other sources are given in table 5.

The Nei' genetic distance between the investigated rapa whelk groups from different areas of the Black Sea was not significant (0.017). This level of genetic distance indicator is characteristic for local populations (Ayala, 1982). The genetic similarity index of Jeffries-Matusita also testifies to the lack of a reliable disagreement between rapa whelk groups from Karadag and Odesa (the threshold of reliability of differences is  $< 0.92$ ). The indicator of genetic distance  $DH_{XY}$  in the calculation of heterozygosity shows the same composition of genotypes in the compared samples, because the obtained value of the indicator is very close to zero. Negative value of the indicator is associated with significant deviations from the Hardy-Weinberg equilibrium (Wolf et al., 2001).

What causes the difference in the frequency and spread of alternative alleles and genotypes is difficult to guess. This may be a consequence of the bottleneck effect due to the migration to the Black Sea of a part of *Rapana* individuals from the original range. We cannot exclude the adaptive value of certain allozymes and the preservation of the corresponding alleles as a result of natural selection as well. The elucidation of this question requires additional specific research.



As is known, significant genetic variability is observed under changing environmental conditions and potentially provides a high level of genetic adaptability (Ayala et al., 1973; Johnson, 1974; Leclair and Phelps, 1994). The northern part of the Black Sea, especially the northwestern part, is characterized by significant seasonal fluctuations in temperature and salinity. Under such conditions, it is possible that the high heterogeneity of Black Sea bream populations will lead to significant adaptation and ensure that this particular type of gastropod will be able to spread in the World Ocean. (Chandler et al., 2008) According to Kholina (2005), one of the reasons for the high level of heterozygosity can be a relatively significant life expectancy, which leads to the presence of overlapping generations and increases the effective genetic population number. This mechanism of heterozygous growth can also work for the type of *Rapana* in the northern Black Sea. Here we want to draw attention once again to the fact that the analysis was performed only on polymorphic loci.

Table 5: The genetic relationship of *Rapana venosa* various groups.

Habitats of the investigated rapa whelk groups	Indicators							Source
	Genetic similarity		Genetic differentiation					
	J	GSI	F <sub>ST</sub>	D	DH <sub>XY</sub>	Φ <sub>ST</sub>	Θ	
Odesa and Karadag, Ukraine	0.983	0.961	0.009	0.017	-0.093	-	-	Present work
Odesa and island Zmeinyi, Ukraine	0.988	-		0.012	-	-	-	Toptikov et al., 2017
Island Zmeinyi and Tarkhankut, Ukraine	0.992	-		0.008	-	-	-	
The Yellow Sea: Qingdao, China Inch'on, S. Korea	-	-		-	-	0.007	-	Chandler et al., 2008
Qingdao, China Mikawa Bay (east Japan, the Pacific coast)	-	-		-	-	0.111	-	
South and Central of the Bo Hai Sea	-	-		-	-	-	0.000	Yang et al., 2008
The southern part of the Bo Hai Sea and the northern coast of the Yellow Sea	-	-		-	-	-	0.067	

It should be noted that the low genetic distance between the *Rapana* groups occurs against the background of a significant difference in morphometric parameters. This combination of relative genetic conservatism with significant phenotypic disagreements is characteristic for species that are able to adapt well to various environmental conditions and rapidly spread in their area. In particular, it is known for example about Eastern Asian species of mice (Mezhzherin, 2001). We suppose that *Rapana venosa* can also be attributed to this kind of species.

Returning to the question posed at the beginning of the paper on the nature of the differences in the morphometric parameters between rapa whelk groups from the different parts of the Black Sea, we can assume the following. Taking into account the insignificant genetic difference of the studied *Rapana* groups, it can be assumed that their morphological features belong to the category of modification variability. So the species *Rapana venosa* is characterized by a broad norm of reaction to environmental conditions. It is known that the rapa whelks linear-mass dimensions strongly depend on ecological, primarily trophic conditions (Bondarev, 2010, 2016; Kovtun et al., 2014). It is the insufficient food base in the Karadag area that determines the lower values of the morphometric parameters of individuals of this region.

A very high level of genetic polymorphism has been found in many other animal species, in particular in marine mollusks (Fujio et al., 1983; Yang et al., 2008; An et al., 2013).

The combination of disequilibrium with an excess of heterozygotes is indeed an infrequent phenomenon. However, it takes place in nature. In the work of some Chinese researchers on the development of microsatellite DNA markers, it was shown that at 10 loci out of 23, there was a deviation from equilibrium, and in three cases an excess of heterozygotes was observed (An et al., 2013). In a different study by the same researchers, there was disequilibrium in 24 out of 57 loci with an excess of heterozygotes in 13 loci (Sun et al., 2014). In addition, disequilibrium with an excess of heterozygotes was shown for the European catfish (*Silurus glanis*) population (Kuciński et al., 2015) and the green lizard (*Lacerta viridis viridis*) (Böhme et al., 2007) and even for the purebred dogs (Shinkarenko et al., 2010).

Significant deviations of the genetic equilibrium at many loci can indicate the presence of pressure of evolutionarily significant factors on the genetic structure of the studied *Rapana* groups. In this connection, the question becomes which of the evolutionary factors determine the features of the gene pool formation of the Black Sea rapa whelk? We are well aware of the complexity of the question posed. On one hand, answering this question is limited by the insufficient base of existing field observations. On the other hand, the use of the known equations of population genetics has its limitations and requires certain conditions to be met. Nevertheless, we hope that our study will allow us to preliminarily assess the role of the environmental factors in the development of the genetic structure of rapa whelk population of northern Black Sea.

Table 6 shows the values of the following indicators:  $N_e/N$  – ratio of effective strength of the group to the total, SE – standard error,  $\delta$ ,  $V_{\delta q}$  – gene drift indicators,  $f'_o - f'_e$  – the difference between the actual (available) and the expected variances. It should be noticed, that the value of the  $N_e/N$  indicator can be significantly greater than one in the case of intercrossing generations, which indicates \* sign.

As can be seen in table 6, the studied groups of rapa whelk have a high effective  $N_e$  value, which is probably due to the overlap in generations in the selected samples. The large value of  $N_e$  and the low level of genetic differentiation of the studied mollusk populations indirectly indicate a low level of genetic drift. This conclusion is supported by calculations of the drift indicators.

Table 6: The value of some evolutionarily significant indicators.

Samples	Indicators				
	$N_e/N$	$N_m$	$\Delta \pm SE$	$V_{\delta q} \pm SE$	$f'_o - f'_e$
Karadag	0.772	-2.8580	$0.109 \pm 0.002$	$0.003 \pm 0.000$	0.00001
Odesa	0.757	-2.3170	$0.116 \pm 0.001$	$0.003 \pm 0.000$	0.00004
Totality	0.765	29.006	$0.080 \pm 0.001$	$0.002 \pm 0.000$	0.00007
The maximum possible value of the indicator	$> 1^*$	$\rightarrow \infty$	0.353	0.125	$\rightarrow 1$

Evaluation of the natural selection role is the most difficult. The genetic heterogeneity of the various groups of rapa whelk taken into study may reflect the existence of selection. As a preliminary solution to this problem, we used the approach proposed in the works of Altukhov (2003). Comparison of the actual and expected variances of groups (Tab. 6) indicates the weak selective environmental pressure on the gene pool as a whole in the observed rapa whelk groups. If  $f'_o < f'_e$ , then there will be an effect of stabilizing selection, with  $f'_o > f'_e$  – a disruptive selection (Altukhov, 2003).

Based on the study of four groups of rapans of the northern part of the Black Sea (this work; Toptikov et al., 2017, 2019) we assume that the main factor determining the characteristics of the *Rapana* groups' genetic structure is the gene flow. The values of the coefficient  $N_m$  (Tab. 6) are much larger than 0.5, which according to Wright (1931) indicates a significant advantage of the gene flow over the drift. According to calculations by Kimura and Maruyama (1971), if the migration rate exceeds four, then the population exists as the singular panmictic structure. However, the migration processes between rapa whelk groups in Karadag and Odesa are not as intense as between these mollusks' groups in the northwestern part of the Black Sea. Thus, according to our data, the value of  $N_m$  for individual groups of the northwestern water area of the Black Sea averaged 17.827 (Toptikov et al., 2017). This discrepancy between the intensity of gene flow in different sea regions is understandable given the distance between these sea regions and the presence of the Black Sea main stream branch from the Crimean Peninsula from north to south (Fig. 1). This water flow can cut off part of rapa whelk larvae from the transference in a western direction.

### CONCLUSIONS

Thus, on the basis of the data obtained, it is possible to establish a definite genetic and significant phenotypic (by morphometric features) heterogeneity of different rapa whelk groups in the northern part of the Black Sea. Despite this, they can be considered local parts of one population. Panmixia is mediated through the migration of *Rapana* larvae in other areas of water. The high intensity of migration processes causes genetic heterogeneity and disequilibrium of the rapa whelk groups in the northern part of the Black Sea. The gene flow has a significant advantage over genetic drift, which does not have a significant effect on the genetic structure formation of rapa whelk groups from the investigated Black Sea water areas. Additional research is needed for further clarification of these problems.

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