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THE GENETIC STRUCTURE OF THE UNISEX HYBRID *RANA ESCULENTA* COMPLEX POPULATIONS IN THE TRANSCARPATHIANS LOWLAND

Biochemical genic marking, ploidy and sexual structure analysis of green frogs populations of from the Transcarpathians lowland have shown that these places are occupying by the unisex populations consisting exclusively from allodiploid females, including in their own genome an insignificant share of a lake frog genic variety. The phenomenon is discussed in connection with a problem of unisex populations reproduction. The assumption is put forward, that in Transcarpathians hybrid populations hybrids reproduction occurs by parthenogenesis.

The West Palearctic green frogs of *Rana esculenta* L., 1758 complex populations are the perspective model for evolutionary genetic researches. Such type hybridization does not meet more at any terrestrial vertebral taxon in the Northern Eurasia. The reason of this is the completely unique structure and a reproduction way of hybrids which are allodiploids, and in some cases - allotriploids, transmitting to the subsequent generations of frogs, as a rule, a genome only one of parental species, whereas a genome of the second is eliminated during the gametogenesis period [1-3]. The partial genetic autonomy of hybrids and a their genetic structure constancy have got the basis for giving by it the taxonomic status [4-6]. Special interest is attracted by the hybrid populations and specimens heterogeneity on an area, and also ambiguity of their reproduction mechanisms in the populations different geographically. On the most part of an area, particularly on Ukraine territories [7-9], hybrids are a component of parental species populations and their reproduction in generations is carried out only by back-crossings with one of parental species that is caused by low survival rate of F_2 hybrids. In the Central Europe, contrarily, there are populations which on 90 % will consist of hybrids and, hence, should even in part be reproduced by the homotypic hybrid crossings [10-12]. In the Ukraine territory, the region where existence of such populations is possible, is the Transcarpathians lowland, that is a part of the Pannonian basin. In this region particularly the tendency is observed to formation of hybrid populations with the insignificant parental species contents and thus majority of hybrids are the females [12]. However, all these researches were carried out without frogs genetic marking; the hybrids identification was realized by morphological attributes only which given't 100 % diagnostics [13], that is why results on populations structure demand the strict genetic analysis. The purpose of the our research was to realize the genetic structure analysis of populations of the green frogs complex occupying a Transcarpathians lowland.

Material and methods. A samples set used as a material was collected in the lowland part of southern and western Uzhgorod suburbs. The basic material has been taken from the isolated pool which is located near Mynai (the southern Uzhgorod suburb). The pool (Mynai-1) about 1000 m² was formed in 1989 on a place of a foundation ditch, on coast was over with a sedge. The pool's depth does not exceed 1 m. *Elodea canadensis* is a prevailing kind of aquatic vegetation. The nearest water-current (the irrigation channel connected with Latorytsa river) is located in 50 m. Now the channel was completely over and has dried up. In 300 m from a foundation ditch on a place of the former channel the bog by the area no more than 20 m² and depth up to 0,5 m was kept (it is designated as Mynai-2). This place of detection of green frogs is the nearest to the investigated pool Mynai-1and it the represents a small bog, overgrown by a cane. Volume of the sample taken in May from Mynai-1 is 49 individuals. In the autumn sample of the juveniles has been taken repeatedly from the same pool (33 animals). From Mynai-2 sample has been taken in volume of 22 individuals (6 adults and 16 juveniles) in the first decade of October. In addition to these samples it has been caught 21 more animals (sample "Peresh") in the autumn on plain in neighboring woods.

Table 1.

Distribution of genotypes of diagnostic loci at green frogs in Transcarpathians lowland

Locus	Genotypes	Samples				
		Mynai-1 (summer)	Mynai-1 (autumn)	Mynai-2 (autumn)	Peresh (autumn)	Airport (summer)
Ldh-B	Ls/77	36	16	17	17	-
	Ls/Rf	1	0	0	2	1
	Lf/77	10	15	5	0	-
	Lf/Rf	2	2	0	2	-
	77/Rf	-	-	-	-	4
	Rf/Rf	-	-	-	-	2
Aat-1	L/R	49	-	-	-	1
	R/R	-	-	-	-	6
Aat-2	L/R	49	-	-	-	1
	R/R	-	-	-	-	6
Es-5	L/R	49	-	-	-	1
	R/R	-	-	-	-	6
Es-1	L/Rs	11	-	-	-	-
	L/Rf	29	-	-	-	-
Me-1	L/R1	-	-	-	-	1
	L/R2	49	-	-	-	-
	R1/R1	-	-	-	-	5
	R2/R2	-	-	-	-	1

Besides as the control is taken one more sample (7 animals) over the beginning of the Uzhgorod western vicinities foothills from recently dug deep foundation ditch near to the city airport (sample "Airport").

The sexual belonging was defined only at adults individuals by opening. Genetic identification of the frogs caught in the spring was carried out by the analysis of genotypes on a number of loci (Ldh-B, sAat, mAat, Es-1, Es-5, Me-1), having fixing alleles, diagnostic for lake and pool frogs. In the autumn as diagnostic locus Ldh-B is used only. The ploidy has been defined by the caryotype analysis on the preparations prepared on standard technique for amphibians [14].

Results of researches. *Genic marking*. In result of the multilocus genic marking carried out in the samples collected in the spring, it is established that at frogs from Mynai-1 all the individuals were hybrids of the first generation, and in area of the city airport 6 individuals of the lake frogs and 1 hybrid have been caught (tab. 1). The precise conformity has allowed of hybrid genotypes on different loci in autumn samples will be limited only to Ldh-B analysis. As a result of genic marking it is established, that all frogs from Mynai-1 (juveniles), Mynai-2 and Peresh were hybrids (tab. 1).

The genic frequencies comparison of polymorphic loci at lake frogs from the Airport area and a part the hybrids genome, introduced from the lake frog, shows them highly authentic distinctions. So, at 3 hybrids only allele Ldh-B^{Rf} is marked that makes 0,03 ± 0,05, whereas in a frogs population from the airport its frequency is much higher and equal 0,61 ± 0,13 ($t = 4,46$; $p < 0,001$). The authentic tendency of alternative alleles prevalence is observed and on Me-1 locus. So, at Mynai hybrids the allele Me-1^{R2} is fixed whereas at frogs from the Airport the allele Me-1^{R1} prevails with the frequency 0,85. Such facts serve not only the proof of that the lake frogs now living near Uzhgorod, are not ancestral for hybrids, but also pauperization of a genic variety confirmation at hybrids on a lake frog genome, that is precisely shown on two (Ldh-B, Me-1) by the most polymorphic at R. ridibunda to loci [15-16].

Comparison of allelic frequencies of Ldh-B locus in Mynai-1 population at the adults caught in the spring, and the juveniles caught in the autumn, has shown the genic frequencies stability regarding a lake frog genome and the pool frog genome obvious changes (tab. 2). So, frequency of individuals carriers Lf allele increases from 0,24 ± 0,06 up to 0,51 ± 0,09 ($t = 2,45$; $p <0,01$). The causes of frequency change can be: reproduction selectivity of different hybrid forms of or crossings of hybrids with pool frog, distinguished by allelic frequencies.

The caryotypes analysis. In all the 44 adults frogs (16 in the spring and 28 in the autumn) investigated a diploid set of chromosomes 2n = 26 is found out [2].

Sexual structure. All the 58 adult hybrids were appeared females (Mynai-1 - 49 animals, Mynai-2 - 6, Peresh - 3). The gonads condition corresponded to reproduction seasons. Anomalies of genitals development are not marked.

Discussion of the received data. All the green frogs without exception extracted on the Transcarpathians plain are identified as hybrids genetically on the investigated diagnostic loci (tab. 3). Exception is the Airport area where the plain borders on foothills. The result is confirmed for different seasons (spring and autumn), age structure of populations (juveniles, adults) and different biotopes (lake, a bog, a wood). Only in foothills in the city airport area lake frogs have been found out who, however, sharply differ on allelic structure from the ancestral lake frogs who have given rise to hybrid populations on plain.

All the adult frogs from plain analyzed appeared females, and with gonads normally advanced.

All of 44 individuals investigated caryologically appeared to be diploid and had 26 chromosomes that confirms amphihaploid hybrids structure.

Table 2.

Comparison of allelic frequencies of Ldh-B locus at juveniles and adult frogs from habitat Mynai-1

Frogs	L_s	L_f	77	R_f	n
Adults	0,378	0,122	0,469	0,031	49
Juveniles	0,242	0,258	0,470	0,030	33

Thus, as a result of the complex analysis it is proved that in Transcarpathians lowland, in particular in Uzhgorod vicinities, a green frogs populations will consist from diploid hybrid females exclusively. To some extent given result coincides with the materials of other researchers received for adjoining to Transcarpathians regions of Hungary, Slovakia and Poland where in hybrid populations prevail females and diploids though in the north, on Poland plains, the triploids share is high. Whereas in Transcarpathians lowland in Uzhgorod area we deal with the diploid hybrid populations consisting exclusively from females, that puts sharply a question on their reproduction mechanisms. It is necessary to emphasize, however, that we cannot exclude completely the males and triploids presence in the investigated region, though their share, by number of the hybrid individuals investigated for today, will be no more than 5 % from individuals in a population.

Detection of such unique hybrid frogs settlement puts a question: how these unisex isolated populations are reproduced. In our opinion, three variants are possible to explain the unisex hybrid populations phenomenon.

1. Green frogs reproduction in Mynai-1 and Mynai-2 body-waters does not occur in general, and populations are formed due to hybrids migrations, for example from Carpathian mountains foothills where both parental species live and a hybridization occurs constantly. This explanation is unacceptable because of the following circumstances. First, it is not clear, why hybrids only go down on plain. Second, available field supervision confirm the fact of green frogs reproduction on plain. So, in pool Mynai-1 green frogs tadpoles of last stages have been found out in the summer, and in the autumn on its coast the juveniles have been caught.

Table 3.
Specific structure of Transcarpathian green frogs populations

Genetic form	Mynai-1	Mynai-2	Peresh	Airport
Pool frogs	-	-	-	-
Hybrids	82	22	21	1
Lake frogs	-	-	-	6

2. One of parental species (the pool frog) comes in body-waters in the spring only for the period of spawning, and then leaves to live on land or in other body-waters. As is known [17], the semi-terrestrial mode of life is attributed to the pool frog who carries out in water the reproduction period from April till June, and then passes to a ground way of life practically. However all investigated green frogs caught in the autumn on land in a wood (Peresh, 21 animals), appeared to be hybrids. It is necessary to pay attention as well to that circumstance, that the inheritance mechanism at hybrids from Danube basin is non-uniform. In delta of Danube genome of R. ridibunda is eliminated [18]; in Yugoslavia territory a situation return – hybrids inherit same a genome [12]. Proceeding from this circumstance, it is possible to expect in Transcarpathians the mixed populations of hybrids with a lake frog, and the last are strictly adhered to body-waters and consequently them are very easy to find out. It is necessary to emphasize also that the Transcarpathian lake frogs caught by us in the city airport area had other genic pool (tab. 1), than the lake frogs generating hybrids in Mynai-1. Thus, the probability of the second variant of summer unisex populations formation is rather insignificant.

3. Hybrids reproduction occurs by parthenogenesis without any males participation. This way of reproduction is known at reptiles, in the form of gynogenesis it meets at fishes, but it is unknown at Anura amphibians though it is known at Caudata [19]. By the way, the parthenogenetic reproduction hypothesis at hybrids of green frogs already expressed, but in the gynogenesis form [20, 21], though has not received the practical development. It is important to emphasize that circumstance, that the allodiploid populations (including green frogs), that are steadily keeping in a number of generations, are impossible by definition of that they produce gametes of one of parental species and, hence, in populations will be again individuals of parental species in any case. Except for that at homotypic hybrids of green frogs crossings [22, 23], the progeny does not survive practically. Therefore steady existence of hybrid populations is probably only in case of them allotriploidy or at least hybrids allodi- and allotriplod mixes when one of sexes is triploid, that takes place in Poland, as it is considered [6].

Thus, at a modern level of our knowledge, there is no unequivocal and clear explanation of mechanisms of allodiploid unisex populations maintenance except for parthenogenesis. However the fact of parthenogenesis at frogs till now is not known.

SUMMARY. The biochemical genetic marking, ploidy and sexual structure analysis of the green frogs populations from the Transcarpathians lowlands have demonstrated that this region is inhabited by the unisexual populations composed of the allodiploid females only. They includes in their genome the small part of the marsh frog genic diversity. This phenomenon is discussed in relation to the unisexual populations reproduction problem. The assumption is proposed that in the Transcarpathians hybrid populations the hybrids are reproduced by the parthenogenesis.

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