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FIRST DATA ON GENETIC VARIABILITY OF THE SIBERIAN WOOD FROG *RANA AMURENSIS* IN WESTERN SIBERIA AND ITS DIFFERENTIATION FROM THE MOOR FROG *RANA ARVALIS*

The article presents data on genetic variability in populations of two brown frog species: the moor frog *Rana arvalis* and the siberian wood frog *R. amurensis*, in Western Siberia, Russia. Persentage of polymorphic ISSR-PCR-bands in *R. arvalis* was 63–93 %, in *R. amurensis* — 90 %, genetic diversity indices were 0.18–0.20 and 0.31, respectively. The high level of genetic variability in the siberian wood frog is contrary to its low population size, restricted distribution in the study area and the boundary position of the population. Some ISSR-PCR-bands were species-specific, they can be used for fast genotyping and further population genetic studies of the siberian wood and the moor frog in their areas of cohabitation.

❀ **Ключевые слова:** *Rana amurensis*; *Rana arvalis*; polymorphism; ISSR markers; genetic differentiation; cohabitation; West Siberia.

Population genetic structure of *Rana arvalis* Nilsson, 1842 was studied in Europe (Sjogren-Gulve, Berg, 1999; Rafiski, Babik, 2000; Babik et al., 2004; Roček, Šandera 2008; Knopp, Merilä, 2009), and *Rana amurensis* Boulenger, 1886 together with related species of Asian brown frogs — in Asia (Tanaka-Ueno et al., 1998; Kim et al., 2002; Song et al., 2006; Che et al., 2007). There are few works about population genetic structure of the moor frog in Western Siberia (Shapovalov, Zhigileva, 2002; Zhigileva et al., 2014a). Genetic variability of the siberian wood frog in Siberia was not investigated.

In the meantime, the territory of Siberia is interesting in that there are boundary of distribution and contact zone of the Asian and European forms of brown frogs here. The territory of Western Siberia was completely covered with ice during the Pleistocene. Then it was colonized by the moor frog from the west, and by the siberian wood frog — from the east. There was fast settling of vast northern territories from several southern refugia (Veith et al., 2003; Palo et al., 2004). The same Pleistocene history was typical for many animal species of Siberia. This is known in other species, on the border of areal when conspecific partners are rare, the interspecific hybridization probability increases. In Western Siberia there are vast hybridization zone between other Asian and European animal species, for example, in rodents (Abramson et al., 2009), mustelids (Zhigileva et al., 2014b).

The aim of this paper is developing of genetic markers for fast genotyping and further population genetic studies of the siberian wood frog and the moor frog in cohabitation in Western Siberia.

MATERIALS AND METHODS

Frogs were collected during July in 2011–2012 in surroundings of Tobolsk Biological Station of RAS "Missia" in Uvatsky area (58°20′N, 68°25′E), and near the Tyumen city (57°14′N, 65°26′E) in Tyumen region. A total number of 33 individuals of the siberian wood frog and 47 individuals of the moor frog were sampled.

We used the method ISSR-PCR (polymerase chain reaction of inter simple sequences repeats) to compare the genetic profiles of two frog species. ISSR-PCR method identifies polymorphisms between microsatellites sequence and has a high sensitivity for differentiation (Zietjiewicz et al., 1994). Total genomic DNA was extracted from cardiac muscle fixed in 70 % ethanol using the technique of alkaline lysis. Amplification was carried out using 25 μl of reaction mixture containing PCR buffer (0.01 M Tris-HCl, 0.05 M KCl, 0.1 % triton X-100), 4 mM MgCl2, 0.2 mM of each dNTPs, 1 μl of total DNA solution, 2.5 mM of primer and 0.2 unit/μL of Taq-polymerase ("Fermentas"), in the following mode: 94 °C — 7 min; then 94 °C — 30 sec, 52 (56) °C — 45 sec, 72 °C — 2 min (40 cycles); 72 °C — 7 min.

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Table 1

Primers used for study

Primer number	Primer code	The composition of the primer	Primer annealing temperature t °C	Number of bands	
				Total	With good resolution
P1	UBC-808	$(AG)_8C$	52	11	4
P2	UBC-809	$(AG)_8G$	52	10	9
P3	UBC-807	$(AG)_8T$	56	12	6
P4	UBC-818	$(CA)_8G$	56	7	6
P6	UBC-825	$(AC)_8T$	50	6	5
P7	UBC-823	$(TC)_8C$	52	4	2

Six primers were used for ISSR-PCR (table 1). Analysis of ISSR-PCR-fragments was carried out on 2 % agarose gel with using 1X Tris-EDTA-Borate buffer. The sizes of the fragments were determined using 100 *bp* DNA molecular weight markers (fragment length varies from 100 to 1000 *bp*, with step 100 *bp*). Electrophoretic gels were documented using VersaDoc system (Bio-Rad). Electrophoretic results were combined into binary matrices, where the presence of the band in gels was designated as "1" and was considered as a dominant allele; absence of the band was designated as "0" and considered as a recessive allele.

Standard population genetic characteristics — the percentage of polymorphic loci ($P_{95\%}$), observed number of alleles (*na*), effective number of alleles (*ne*), Nei's gene diversity (*h*), Nei's original measures of genetic identity (*I*) and genetic distance (*D*) (Nei, 1972), F-statistics (G_{ST}), were computed using Popgen software (Yeh et al., 1999).

RESULTS AND DISCUSSION

Currently moor frog is very widespread in Western Siberia, it can be found in many habitats, including anthropogenically transformed landscapes. This species is 92–95 % of the abundance of all species of amphibians that inhabit the taiga zone of Western Siberia. In contrast, the siberian wood frog has a narrow spread and uses a limited range of habitats, preferring floodplains. We found a population of siberian wood frog in Uvatsky area where it dwell sympatrically with the moor frog. This allowed us to obtain the first estimates of the genetic variability of the siberian wood frog in Western Siberia and to compare the genetic performance of two species.

We compared the genetic profiles of the siberian wood frog and the moor frog by method of ISSR-PCR. In total, it was studied 50 bands, from them only 32 were used for analysis (table 1, 2). Four (8 %) bands were monomorphic and identical in both species (*P2-5, P2-8, P4-5, P6-4*). Some bands were typical only for *R. arvalis* or *R. amurensis* (Fig. 1–5). Species-specific bands for *R. arvalis* were *P2-2, P3-2,* for *R. amurensis* — *P2-4, P2-6, P6-2*. These bands

Table 2

ISSR-PCR-bands in two frog species

Band number	*R. arvalis*	*R. amurensis*	Band number	*R. arvalis*	*R. amurensis*
P1-1	+	–	*P3-4*	+	+
P1-2	+	+	*P3-5*	+	+
P1-3	+	+	*P3-6*	+	+
P1-4	+	+	*P4-1*	–	+
P2-1	+	–	*P4-2*	–	+
P2-2	+	–	*P4-3*	+	–
P2-3	+	+	*P4-4*	–	+
P2-4	-	+	*P4-5*	+	+
P2-5	+	+	*P4-6*	+	+
P2-6	–	+	*P6-1*	+	–
P2-7	–	+	*P6-2*	–	+
P2-8	+	+	*P6-3*	+	–
P2-9	+	+	*P6-4*	+	+
P3-1	+	–	*P6-5*	+	+
P3-2	+	–	*P7-1*	+	–
P3-3	–	+	*P7-2*	–	+

can be used for species identification of the siberian wood and the moor frog in their areas of cohabitation.

The percentage of polymorphic bands of the siberian wood frog was 90 %, in the moor frog from Uvatsky area — 93 % (table 3). This index, as well as the observed number of alleles per locus were lower in the moor frog from Tyumen, the territory with high levels of anthropogenic

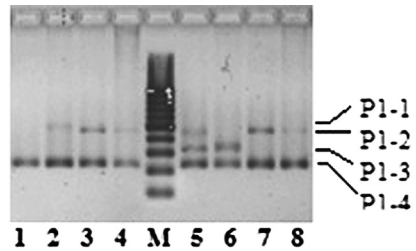

Fig. 1. Elektrophoregram of ISSR-PCR-pattern in frogs of genus *Rana*, with primer *P1*: 1-3, 6-8 — *Rana arvalis*, 4, 5 — *Rana amurensis*, M — DNA ledder 100bp (2 % agarose gel, ethidium bromide, negative)

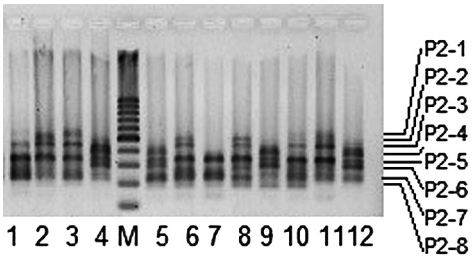

Fig. 2. Elektrophoregram of ISSR-PCR-pattern in frogs of genus *Rana*, with primer *P2*: 1-3, 6-8, 10-11 — *Rana arvalis*, 4, 5, 9, 12 — *Rana amurensis*, M — DNA ledder 100bp (2 % agarose gel, ethidium bromide, negative)

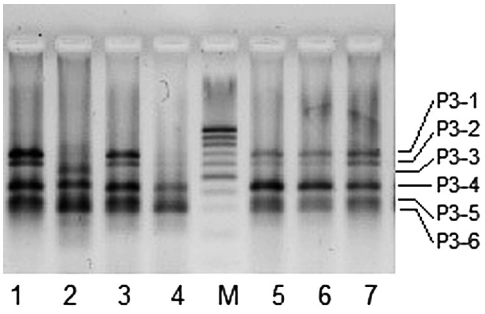

Fig. 3. Elektrophoregram of ISSR-PCR-pattern in frogs of genus *Rana*, with primer *P3*: 1, 3-7 — *Rana arvalis*, 2 — *Rana amurensis*, M — DNA ledder 100bp (2 % agarose gel, ethidium bromide, negative)

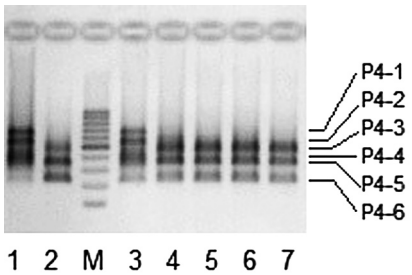

Fig. 4. Elektrophoregram of ISSR-PCR-pattern in frogs of genus *Rana*, with primer *P4*: 2, 4-7 — *Rana arvalis*, 1, 3 — *Rana amurensis*, M — DNA ledder 100bp (2 % agarose gel, ethidium bromide, negative)

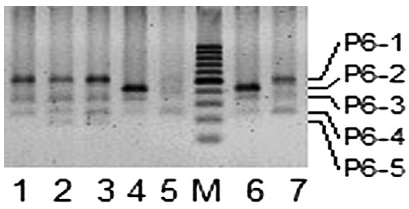

Fig. 5. Elektrophoregram of ISSR-PCR-pattern in frogs of genus *Rana*, with primer *P6*: 1-3, 5, 7 — *Rana arvalis*, 4, 6 — *Rana amurensis*, M — DNA ledder 100bp (2 % agarose gel, ethidium bromide, negative)

transformation. Reducing of the number of alleles and measures of polymorphism are typical for frog populations dwelling anthropogenically transformed landscapes (Hitchings, Beebee 1997). It can be due to genetic drift in small isolated populations of amphibian populations in the fragmentation of habitats.

Measures of genetic diversity of the siberian wood frog population in Uvatsky area were higher, compared to the moor frog population (table 3). This result was unexpected because the first species is relatively rare and has limited distribution. It was shown, that the value of genetic polymorphism of frogs is determined by the effective population size and gene flow to a greater degree than by the climatic and geographical factors (Sjogren-Gulve, Berg, 1999). Therefore, we should expect that the genetic diversity of the siberian wood frog would be lower in the study area of Western Siberia, as in the northwestern periphery of the area. High level of genetic variability of studied population shows its value for conservation of the species biodiversity.

Nei's genetic identity (*I*) among moor frog populations was 0.829, distance (*D*) — 0.187. These indices between species were 0.782 and 0.246, respectively. The G_{ST} value as the measures of genetic subdivision was 0.20, this means that 80 % of the genetic variability is accounted for intrapopulation one.

Despite the fact that the siberian wood frog and moor frog use the same spawning pond, they do not hybridize with each other. We found no hybrid individuals among the

Table 3

Levels of genetic variability of frog's populations according to ISSR data

Summary of genic variation statistics	*R. arvalis* (n = 47)		*R. amurensis* (n = 33)
	Tyumen (n = 19)	Uvatsky (n = 28)	
The percentage of polymorphic loci	63.3	93.3	90.0
Observed number of alleles (*na*)	1.6	1.9	1.9
Effective number of alleles (*ne*)	1.4	1.3	1.5
Index of Nei's gene diversity (*h*)	0.202	0.176	0.311

specimens studied. Different periods of spawning, as well as a different number of chromosomes confirm the fact of interbreeding inability. The siberian wood frog and the moor frogs belong to different groups of brown frogs on the genome size (Litvinchuk et al., 2008). There are 24 chromosomes in moor frog and 26 chromosomes — in siberian wood frog (Kim et al., 2002). While there are hybridogeneous species complex in the green frogs (*Rana esculenta* complex) (Lada et al., 1995), high level of genetic differentiation of species (Kim et al., 2002; Che et al., 2007), subspecies (Song et al., 2006; Litvinchuk et al., 2008) and populations (Palo et al., 2004; Zhang et al., 2010) is observed in brown frogs.

These two species differ not only genetically, but ecologically too. They differ in demographic characteristics and require different conservation strategies (Ishchenko, 1996). Siberian wood frog and moor frog had different sex-age composition of populations and population dynamics. There was an almost equal sex ratio in the population of the moor frog, where juveniles were 63 %. Proportion of males in a population of the siberian wood frog was three times more than females; most of them (82 %) were adults. The siberian wood frog population size was greater in 2011, but the moor frog population was more numerous than siberian wood frog in 2012. These differences were due to different seasonal dynamics, and can testify to the interspecies competition within the same biotope.

CONCLUSION

Level of genetic variability of Western Siberian moor frog populations is high, not lower, than in populations of Europe. Siberian wood frog also has a high level of genetic variation, which is contrary to its low population size, restricted distribution in the study area and the boundary position of the population. High level of genetic differentiation as well as reproductive isolation of the two studied frog species, even when they use the same spawning pond were detected. Some ISSR-PCR-bands were typical only for *R. arvalis* or *R. amurensis*. These species-specific bands can be used for fast genotyping and further population genetic studies of the siberian wood and the moor frog in their areas of cohabitation.

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ПЕРВЫЕ СВЕДЕНИЯ О ГЕНЕТИЧЕСКОЙ ИЗМЕНЧИВОСТИ СИБИРСКОЙ ЛЯГУШКИ *RANA AMURENSIS* В ЗАПАДНОЙ СИБИРИ И ЕЕ ДИФФЕРЕНЦИАЦИЯ ОТ ОСТРОМОРДОЙ ЛЯГУШКИ *RANA ARVALIS*

Жигилева О.Н., Кирина И.Ю.

❀ **РЕЗЮМЕ:** Представлены данные о генетической изменчивости популяций двух видов бурых лягушек: остромордой *Rana arvalis* и сибирской *R. amurensis*, в Западной Сибири. Доля ISSR-PCR-бэндов у *R. arvalis* составила 63,3–93,3 %, у *R. amurensis* — 90 %, индексы генетического разнообразия — 0,18–0,20 и 0,31 соответственно. Высокие показатели генетической изменчивости сибирской лягушки противоречат ее низкой численности, ограниченному распространению на исследуемой территории и краевому положению популяции. Некоторые ISSR-PCR-фрагменты были видоспецифичными, они могут быть использованы для быстрого генотипирования и дальнейших популяционно-генетических исследований сибирской и остромордой лягушек в местах их совместного обитания.

❀ **КЛЮЧЕВЫЕ СЛОВА:** *Rana amurensis*; *Rana arvalis*; полиморфизм; ISSR-маркеры; совместное обитание; генетическая дифференциация; Западная Сибирь.

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