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GENOMES DIVERSITY IN OOCYTES OF HYBRID WATER FROGS *PELOPHYLAX ESCULENTUS* (ANURA: RANIDAE) IN HEMICLONAL POPULATION SYSTEMS FROM UKRAINE

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Pelophylax esculentus (genome denoted as LR) is an interspecies hybrid of pool frog *Pelophylax lessonae* (genome LL) and marsh frog *Pelophylax ridibundus* (genome RR). Typically, hybrids' reproduction occurs due to clonal transmission of one of the parental genomes. Hybrids usually co-exist with one or both parental species in so-called hemiclonal population systems (HPS). Hybrids can produce gametes of both parental species either separately or simultaneously (mixture of L and R gametes). Some hybrids also produce diploid gametes of different genomic compositions, a mixture of haploid and diploid gametes, as well as gametes with partially recombinant genomes. The study aimed to identify types of gametes produced by female *P. esculentus* from Ukrainian HPSs by analyzing allozymes of LDH-1 from their oocytes. Especially we focused on two types of HPSs from the Siversky Donets river basin, where female gametogenesis was never studied thoroughly. Nineteen female frogs (sixteen *P. esculentus* and three *P. ridibundus* as a control) from six different localities were analyzed. Identification of LDH-1 allozymes was carried out based on their migration rate to anode. Identification of enzyme activity was performed with the kinetic UV method. We showed that hybrid females from R-E-HPS can transmit the R genome in their oocytes and confirmed the assumption that triploid females from R-Epf-HPS produce oocytes with L genomes. Frogs from R-E-Ep-HPS and L-E-R-HPS were able to produce oocytes with either L or R genomes, which confirmed previous studies on these types of population systems. Measuring LDH activity showed that the activity of the R-allozyme was about 1.6-fold higher than L-allozyme. No difference in allozymes activity was shown for different types of hemiclonal population systems.

Key words: *LDH-1, edible frog, oogenesis, HPS, Siversky Donets river basin.*

Hemiclonal inheritance is a type of reproduction of interspecies hybrids implying transmission of one or both parental genomes into gametes without recombination. European water frogs *Pelophylax esculentus* are the example of animals with hemiclonal reproduction mode [7]. *Pelophylax esculentus,* edible frog (Linnaeus, 1758; denoted as "E") is a hybrid of pool frog *Pelophylax lessonae* (Camerano, 1882; denoted as "L") and marsh frog *Pelophylax ridibundus* (Pallas, 1771; denoted as "R") [1]. Reproduction of hybrids occurs hemiclonally (i.e., without recombination): gametes possess the genome of one of the parental species. Typically, during gametogenesis in diploid hybrids, one of the parental genomes is eliminated from cells before meiosis; the other genome doubles (endoreduplication) and is transmitted to the gamete clonally [11, 12, 15, 21].

Biosystems in which hybrid frogs reproduce can be described as hemiclonal population systems (HPS) [16]. The composition of HPSs is always connected to peculiarities of hybrid *P. esculentus* gametogenesis [15]. Hybrids can produce gametes of both parental species separately or simultaneously, they can also produce diploid gametes of different genomic composition, a mixture of haploid and diploid gametes, as well as gametes with partially recombinant genomes [3, 8, 9, 23].

In populations belonging to certain species, individuals transmit gametes with genomes of the same species to their offspring. However, the situation is much more complicated with HPSs of water frogs. Gametes of hybrid individuals belong either to one parent species or to another, or a mixture of gametes of both species is produced simultaneously. An individual with genomes of different species forms gametes that do not reflect its genome composition, but belong to one or another parental species. Why do such unusual systems not disappear? What mechanism maintains their stability over time? We do not yet have a complete answer to this question. In order to find it, it is necessary to collect data on the composition of various HPS and the gametogenesis of the hybrids reproduced in them. It is clear that the research on the nature of gametogenesis should be carried out in HPS of different types and different regions. This work is devoted to the study of the oogenesis of hybrid frogs from different regions of Ukraine with the focus on Siversky Donets river basin.

In the Siverskyi Donets river basin, both diploid and triploid hybrids, *P. esculentus,* and only one parental species, *P. ridibundus,* are found and a high diversity of HPS types was recorded [e.g., 6, 16, 19], which became the basis for describing the Siverskyi Donets center of waters frogs diversity in this area [17]. We denote the types of HPSs by the set of frogs' forms it consists of [16]; the symbols used are explained in the caption to Figure 1

There are various methods for determining the genotypes of both somatic and germ cells [7]. The analysis of protein markers is one of the most convenient methods for studying oocytes – large cells with high protein concentrations. Locus *ldh-b* (LDH-H subunit) is polymorphic in both parental species *P. ridibundus* and *P. lessonae*. *P. ridibundus* have two types of alleles: R^f and R^s, which encode allozymes with fast and slow migration rate to anode, respectively. For *P. lessonae* three alleles were found: L^f, L^s and L^i (with migration rate, intermediate between those typical for R^f and R^s) [22]. Oocytes of *P. ridibundus* are characterized by the presence of allele R^f, and *P. lessonae* – L^s. Therefore, in oocytes of hybrids one or both of corresponding allozymes can be found [13].

LDH is one of the key enzymes of glucose metabolism, which is in high importance for amphibians who undergo wintering and suffer from hypoxia during this period. It was shown for *P. esculentus* frogs from Siversky Donets river basin that triploids have lower activity of LDH compared to diploids [5]. But the difference in activity between L and R forms of LDH was not studied for this region.

The aim of this work was to determine the genomes transmitted by hybrid females from different types of hemiclonal population systems and to compare the activity of LDH allozymes in individuals from different HPSs and with different genomes.

MATERIALS AND METHODS

We collected 19 female frogs in four localities of Siverskyi Donets river basin and two localities of Dnipro River basin (Fig. 1) in summer seasons of 2016 and 2017. Species and sex identification was carried out by morphological features. For each individual we identified ploidy by measuring erythrocytes length [4, 14].

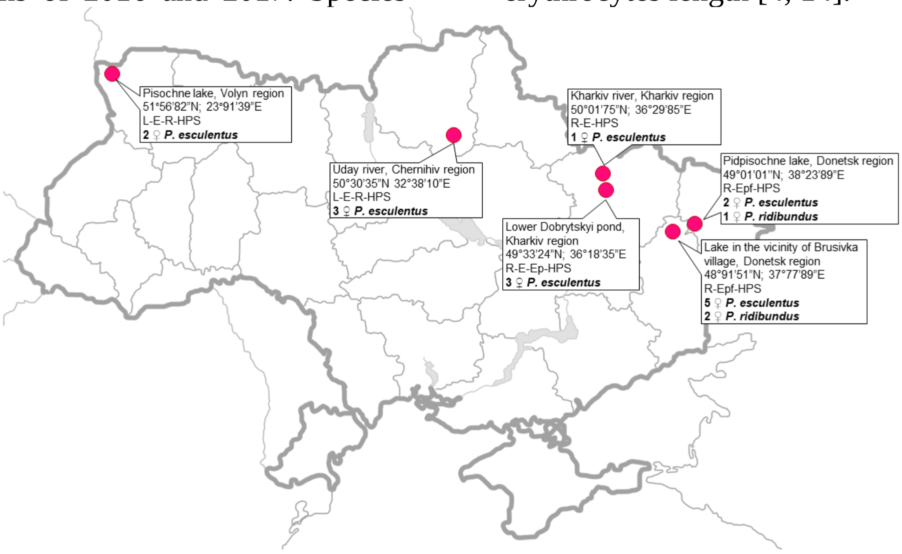

Fig. 1. Localities, types of hemiclonal population systems, and number and species of studied frogs. HPS – hemiclonal population system, R – *P. ridibundus*, E – *P. esculentus*, L – *P. lessonae*, p – polyploids, f – females only. HPSs types described in [10] and [18]. Background map – "Map of Ukraine political simple blank" by Sven Teschke, CC BY-SA 3.0.

We sacrificed frogs using ETAC and dissected ovaries with mature oocytes. Tissues were stored at -20°C. We homogenized 100 µg of oocytes in 400 µl of water glycerol solution (1 : 3), centrifuged it for 5 minutes at 13,000 rpm, and then measured the total amount of protein (280 nm and 205 nm). Electrophoresis was performed in tris-glycine polyacrylamide gel (SDS page, 8%) with tris-borate electrode buffer (TEB, pH 8.3). Specific staining of LDH allozymes was performed by enzymatic reaction with calcium lactate for 1.5 hours at 37°C. The staining mixture (50 ml TEB) contained 80 mg of calcium lactate, 10 mg of NAD, 5 mg of nitrosine tetrazolium chloride and 5 mg of phenazine methosulfate. Identification of allozymes was carried out based on their migration rate to anode (R allozymes migrate faster than L). Three *P. ridibundus* females were used as a control. Identification of enzyme activity was performed with the kinetic UV method [5]. For each frog, we measured only activity once immediately after the sample had been thawed. We used nonparametric tests to compare LDH allozymes and individuals from different HPSs for protein activity.

RESULTS

Among the 16 analyzed *P. esculentus* 11 females had L-allozymes of LDH, therefore were considered to have L genome in their eggs. Other females (five *P. esculentus* and three *P. ridibundus*) had R-allozyme and transmitted R genome (Table 2).

Table 1.

Summarized data on all studied female *Pelophylax esculentus.*

* – females with underdeveloped ovaries. HPS – hemiclonal population system, R – *P. ridibundus*, E – *P. esculentus*, L – *P. lessonae*, p – polyploids, f – females only

№	Locality	HPS type	Taxonomy	Ploidy	Genome in oocytes	LDH activity, µkat/ml × g
1	Pidpesochne lake	R-Epf	*P. ridibundus*	2n	R	2.44
2			*P. esculentus*	3n	L	1.30
3			*P. esculentus*	3n	L	1.40
4	Lake in Brusivka	R-Epf	*P. ridibundus*	2n	R	1.79
5			*P. ridibundus**	2n	R	1.31*
6			*P. esculentus*	3n	L	1.11
7			*P. esculentus*	3n	L	1.66
8			*P. esculentus*	3n	L	1.31
9			*P. esculentus*	3n	L	2.76
10			*P. esculentus*	3n	L	2.83
11	Kharkiv river	R-E	*P. esculentus*	2n	R	3.18
12	Lower Dobrytskyi pond	R-E-Ep	*P. esculentus*	2n	L	1.03
13			*P. esculentus**	3n	R	1.55*
14			*P. esculentus*	3n	R	2.46
15	Pisochne lake	L-E-R	*P. esculentus*	2n	R	3.16
16			*P. esculentus*	2n	L	1.36
17	Uday river	L-E-R	*P. esculentus*	2n	L	2.36
18			*P. esculentus*	2n	R	3.4
19			*P. esculentus*	2n	L	1.32

Results of measuring LDH activity are summarized in Table 1. The activity of R-allozyme was about 1.6-fold higher than L-allozyme ($p = 0.0475$, Fig. 2). Two *P. esculentus* females from R-E-Ep-

HPS and R-Epf-HPS transmitting R genome had notably lower LDH activity (p = 0.0455). These females did not overwinter and had visually noticeable underdeveloped oocytes, which may explain the lower activity of their LDH.

No significant differences in LDH activity were found between four different hemiclonal population systems (p = 0.1116 and p = 0.7063 for R and L genomes, respectively).

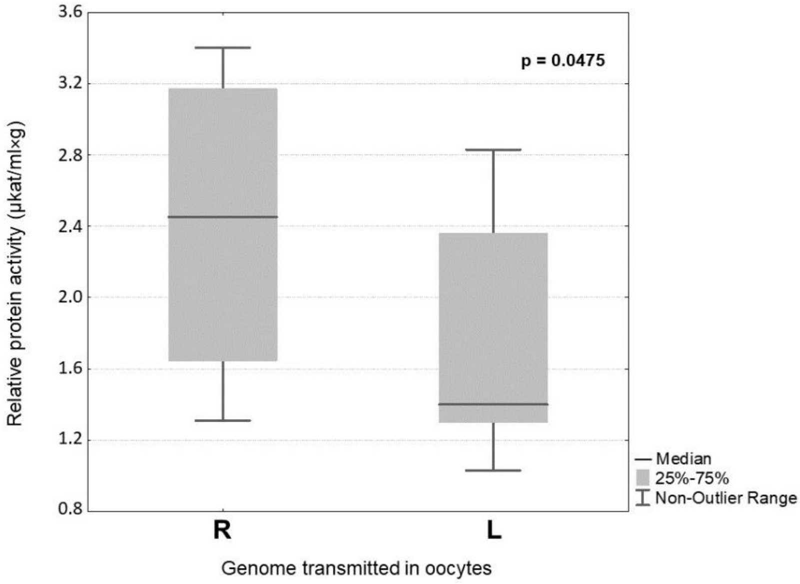

Fig. 2. Activity of LDH-1 in oocytes of frogs transmitting genomes R and L.

DISCUSSION

Our results show that female hybrid frogs *P. esculentus* are able to produce gametes with genomes of either one or another parental species. L-E-R-HPSs are widespread in the Dnipro River basin [19]. The fact that some hybrids can transmit the L genome and others – R, is not unexpected and is consistent with the previous studied on such types of population systems [8].

R-Epf-HPSs described in [10] consist of *P. ridibundus* of both sexes transmitting R genome and it was hypothesized that triploid female *P. esculentus* should transmit clonal genome L. Our results confirm the proposed hypothesis: three female *P. ridibundus* had R-allozyme of LDH, and therefore transmit R genome, while seven triploid female *P. esculentus* have L-allozyme and produce gametes with L genome.

The gametogenesis of hybrid females from R-E-HPSs in the Kharkiv region has not been studied so far. By analogy with L-E-HPSs, we should expect to find gametes with L genome. However, males in R-E-HPSs can produce sperm cells with both L and R genomes [2]. The results of the analysis on oocytes of one female from R-E-HPS showed the presence of R-allozyme in oocytes. One individual is not enough to draw conclusions about the nature of inheritance in R-E-HPS, however, this result suggests that such systems are much more complicated compared to L-E-HPS, and need further investigation.

The difference in activity of R and L allozymes of LDH can be explained by the difference in lifestyle of individuals with RR and LL genotypes [15]. *P. ridibundus* are more related to the aquatic environment, which is why they are more likely to suffer from hypoxia compared to *P. lessonae*, and require more active oxygen metabolism. *P. ridibundus* winters at the bottom of waterbodies and often suffers from frostbites, while *P. lessonae* winters on land, in cavities under tree roots.

CONCLUSION

Our results show that hybrid *P. esculentus* females from different types of hemiclonal population systems can transmit either one or another parental genome in their oocytes.

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References

1. Heppich, S., Tunner, H. G., & Greilhuber, J. (1982) Premeiotic chromosome doubling after genome elimination during spermatogenesis of the species hybrid *Rana esculenta*. Theoretical and Applied Genetics,61(2):101- 104.https://doi.org/10.1007/BF 00273874
2. Mikulicek P., Kotlik P. (2001) Two water frog populations from western Slovakia consisting of diploid females and diploid and triploid males of the hybridogenetic hybrid *Rana esculenta* (Anura, Ranidae). Zoosystematics and Evolution 77(1): 59-64.
3. Ogielska-Nowak M. (1978) DNA content in erythrocyte nuclei of diploid and triploid green frog hybrid of *Rana esculenta* L. complex. Zool. Pol 27: 109-115.
4. Plötner J. (2005) Die westpaläarktischen Wasserfrösche: von Märtyrern der Wissenschaft zur biologischen Sensation. Bielefeld: Laurenti.
5. Shabanov D., Vladymyrova M., Leonov A., Biriuk O., Kravchenko M., Mair Q., Melesko O., Newman J., Usova O., Zholtkevych G. (2020) Simulation as a Method for Asymptotic System Behavior Identification (e.g. Water Frog Hemiclonal Population Systems). In: Information and Communication Technologies in Education, Research, and Industrial Applications. Eds.: V. Ermolayev, F. Mallet, V. Yakovyna, H.C. Mayr, A. Spivakovsky, editors. Cham: Springer International Publishing, p. 392–414. (Communications in Computer and Information Science). https://doi.org/10.1007/978-3-030-39459-2_18
6. Shabanov, D.A., O.V. Korshunov, Kravchenko M.O. (2009) Which of the water frogs inhabit Kharkiv oblast? Perspectives on terminology and nomenclature. V. N. Karazin Kharkiv National University. Biology and valeology 11: 116-125. (in Ukrainian)
7. Shabanov D.A. (2015) Evolutionary ecology of population systems of green frogs' hybridogenetic complex (*Pelophylax esculentus* complex) from Left Bank forest-steppes of Ukraine. Dissertation for a degree of Doctor of Biological Sciences by specialty 03.00.16 – Ecology. Oles' Gonchar Dnipropetrovsk National University. Dnipropetrovsk. (in Russian)
8. Suriadna, N. M., Mykytynets, G. I., Pupiņš, M., & Gasso, V. Y. (2020) Population systems of Eurasian water frogs (*Pelophylax*) in the south of Ukraine. Biosystems Diversity 28(2): 154-162.
9. Suryadna N. N. (2010) New records of triploids of Pelophylax esculentus (Linnaeus, 1758) in eastern Ukraine. Curr Stud Herpetol. Saratov. 10: 128-131.
10. Tunner H. G., Heppich S. (1981) Premeiotic genome exclusion during oogenesis in the common edible frog, *Rana esculenta*. Naturwissenschaften. 68(4): 207–208.
11. Uzzell T., Berger L. (1975) Electrophoretic phenoptypes of *Rana ridibunda*, *Rana lessonae*, and their hybridogenetic associate *Rana esculenta*. Proceedings of the Academy of Natural Sciences of Philadelphia. 127(2): 13-24.
12. Vinogradov, A. E., Borkin, L. J., Günther, R., & Rosanov, J. M. (1990). Genome elimination in diploid and triploid *Rana esculenta* males: cytological evidence from DNA flow cytometry. Genome, 33(5): 619-627. https://doi.org/10.1139/g90-092

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РІЗНОМАНІТТЯ ГЕНОМІВ В ООЦИТАХ ГІБРИДНИХ ЗЕЛЕНИХ ЖАБ *PELOPHYLAX ESCULENTUS* (ANURA: RANIDAE) В ГЕМІКЛОНАЛЬНИХ ПОПУЛЯЦІЙНИХ СИСТЕМАХ В УКРАЇНІ

Федорова А., Шабанов Д.

Pelophylax esculentus (геном позначається як LR) є міжвидовим гібридом ставкової жаби *Pelophylax lessonae* (геном LL) і озерної жаби *Pelophylax ridibundus* (геном RR). Розмноження гібридів в типовому випадку відбувається за рахунок клональної передачі одного з батьківських геномів. Гібриди зазвичай співіснують з одним або обома батьківськими видами в так званих геміклональних популяційних системах (ГПС). Гібриди можуть продукувати гамети обох батьківських видів окремо або одночасно (суміш гамет L і R). Деякі гібриди також утворюють диплоїдні гамети різного геномного складу, суміш гаплоїдних і диплоїдних гамет, а також гамети з частково рекомбінантними геномами. Метою дослідження було ідентифікувати типи гамет, які продукують самки *P. esculentus* з українських ГПС шляхом аналізу алозимів ЛДГ-1 з їх ооцитів. Особливу увагу ми зосередили на двох типах ГПС із басейну річки Сіверський Донець, де гаметогенез самиць детально не вивчався. Було проаналізовано 19 самок жаб (шістнадцять *P. esculentus* і три *P. ridibundus* в якості контролю) із шести різних місцевостей. Ідентифікацію алозимів ЛДГ-1 проводили за швидкістю їх міграції до аноду. Ідентифікацію активності ферментів проводили кінетичним УФ-методом. Ми показали, що гібридні самки з R-E-HPS можуть передавати геном R у своїх ооцитах і підтвердили припущення, що триплоїдні самки з R-Epf-HPS продукують ооцити з геномами L. Жаби з R-E-Ep-HPS і L-E-R-HPS продукували ооцити з геномами L або R, що підтвердило попередні дослідження цих типів популяційних систем. Вимірювання активності ЛДГ показало, що активність R-аллозиму приблизно в 1,6 разів вища, ніж L-аллозиму. Не виявлено відмінностей в активності алозимів для різних типів геміклональних популяційних систем.

Ключові слова: *Pelophylax esculentus, геміклональна популяційна система, гібриди, LDH-1*