

Inheritance of Parental Genomes by a Hybrid Form *Rana “esculenta”* (Amphibia, Ranidae)

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Abstract—In this study, quantitative analysis of paternal genome inheritance by a hybrid form *Rana “esculenta”* (= *Rana esculenta* L., 1758 × *Rana ridibunda* Pall., 1881) (Amphibia, Ranidae) was examined. The hybrid form examined was characterized by a polymodal mode of inheritance (genome of any of the parental species can be inherited). The absence of correlation between the proportion of normal gametes and either sex or ploidity of the producer was demonstrated. The gametes produced could be both haploid and diploid (hybrid or homozygous). The mechanism of allopolyploid reproduction is discussed.

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INTRODUCTION

The present study was focused on characteristics of the mode of inheritance specific to hybrid frog *Rana “esculenta”* (Amphibia, Ranidae), which was made based on quantitative analysis of the genotypes of gametes produced by hybrid animals. Hybrid frog *Rana “esculenta”* is one of the well-known forms of hybrid origin. This frog is characterized by unusual mode of reproduction. The first studies analyzing this phenomenon, showed that *Rana “esculenta”* individuals produced the gametes containing only the lake frog genome (LR → R) [1, 2]. Analogous mode of reproduction (called as hemiclonal) was earlier described in interspecific fish hybrids of the genus *Poeciliopsis* [3]. Thus, hybrid frog *Rana “esculenta”* can be reproduced through backcrossing to that parental species, whose genome is not inherited. However, more detailed analysis of the mode of inheritance specific to hybrid *Rana “esculenta”* frogs showed that it had some specific features. First, in some parts of the species range, hybrid form inherits exclusively of another parental species, the pool frog (LR → L) [4]. Second, in some populations the hybrids produce more than one class of gametes of different genotype and/or ploidity [5]. Finally, in many natural populations contain allotriploids, which are not reproductively isolated from parental species [6]. At the same time, allopolyploids of the genus *Poeciliopsis* are characterized by gynogenetic reproduction [7]. For these reasons, the mode of reproduction typical of hybridogenetic frog *Rana “esculenta”* cannot be considered as hemiclonal in traditional interpretation of this term.

In this study, quantitative analysis of the inheritance of parental genomes by hybrid frog *Rana “esculenta”* was performed.

MATERIALS AND METHODS

Data source. The data on gamete genotypes (shown in Table 1) were taken from the literature and obtained in the present study (for the methods, see below).

Determination of gamete genotypes was performed by means of electrophoretic analysis of germ cell suspension (for males) or individuals eggs (for females). Frogs were caught in 1992 through 2005. Standard methods of sample preparation and electrophoresis were used [4, 8].

Sample preparation. Live animals were transported to the laboratory, where they were anesthetized with ethylic ether of 3-aminobenzoic acid and killed. Gonads were removed. To examine mature egg mass from the ovaries by means of electrophoresis, individual eggs were isolated under a binocular microscope with the help of preparation needles. Then, the eggs were separated from the tissue fluid by placing on a piece of filter paper for 10 to 20 s. After that, each egg was placed into 1–2-ml tube. The tubes were added with two to three drops of 10% sucrose solution stained by addition of bromphenol blue. The tubes were closed and frozen for 12 h. After thawing, each egg was squashed in the tube, and the tubes were shaken several times for more complete mixing of the egg content with the sucrose solution. To analyze male germ cells, testes were washed in distilled water, dried on filter paper, and squashed between two slide plates.

Table 1. Numbers of hybrid frogs *Rana "esculenta"* with different modes of parental genome inheritance

Sex	Soma	Gamete genotypes								n	N	Method	Literature source
		R	L	RR	LL	LR	L + R	L ≥ R	Oth.				
1. Western Europe													
1.1. Germany													
♀ ♀	LR	3	0	0	0	1	0	0	1	5	9	1, 2, 6	[5, 6, 9, 16, 17]
	LLR	0	2	0	1	0	0	0	0	3			
	LRR	1	0	0	0	0	0	0	0	1			
♂ ♂	LR	2	16	0	0	0	6	2	0	26			
	LLR	0	12	0	2	0	1	10	0	25			
	LRR	17	0	0	0	0	0	0	0	17			
1.2. Denmark													
♀ ♀	LR	1	0	0	0	5	0	0	1	7	3	3	[15]
	LLR	0	0	0	0	0	0	0	1	1			
♂ ♂	LR	0	0	0	0	0	0	0	1	1			
	LLR	1	4	0	0	0	0	0	0	5			
	LRR	1	0	0	0	0	0	0	0	1			
1.3. Poland													
♀ ♀	LR	9	0	0	0	5	2	0	9	25	6	1, 5	[9, 16–19]
	LRR	9	0	0	0	0	0	0	1	10			
♂ ♂	LR	12	1	0	0	0	1	0	1	15			
	LLR	0	1	0	0	0	0	0	0	1			
	LRR	10	0	0	0	1	0	0	1	12			
1.4. Switzerland													
♀ ♀	LR	7	0	0	0	0	0	0	0	7	3	2, 5	[2, 19, 20]
♂ ♂	LR	2	0	0	0	0	0	0	0	2			
1.5. Yugoslavia													
♀ ♀	LR	32	0	0	0	0	0	0	0	32	2	5	[21]
♂ ♂	LR	2	0	0	0	0	0	0	0	2			
1.6. Austria													
♀ ♀	LR	47	0	0	0	0	2	0	0	49	1	1, 2, 4	[19, 22–24]
1.7. Hungary													
♂ ♂	LLR	0	0	0	55	0	0	0	0	55	1	2, 4	[24]
2. Eastern Europe													
2.1. Ukraine													
2.1.1. Danube River basin													
2.1.1.1. Transcarpatia													
♀ ♀	LR	35	0	0	0	0	0	0	0	35	6	5	Personal data
2.1.1.2. Mouth of the Danube River													
♀ ♀	LR	0	8	0	0	0	0	0	0	8	3	5	"
♂ ♂	LR	0	21	0	0	0	0	0	0	21			
2.1.2. Dnieper River basin													
♀ ♀	LR	15	0	0	0	1	0	0	0	16	4	2, 5	"
♂ ♂	LR	32	0	0	0	3	3	0	0	38			
2.1.3. Don River basin													
♂ ♂	LR	1	5	0	0	1	3	0	0	10	1	5	"
	LRR	1	0	0	0	0	0	0	0	1			

Table 1. (Contd.)

z	Soma	Gamete genotypes								n	N	Method	Literature source
		R	L	RR	LL	LR	L + R	L ≧ R	Oth.				
Ukraine, overall													
♀ ♀	LR	50	8	0	0	1	0	0	0	59	14	2, 5	
♂ ♂	LR	33	26	0	0	4	6	0	0	69			
	LRR	1	0	0	0	0	0	0	0	1			
2.2. Belarus													
♂ ♂	LR	3	0	0	0	0	0	2	0	5	1	6	[16]
2.3. Latvia													
♂ ♂	LR	29	0	0	0	0	0	0	0	29	2	6	[25, 26]
2.4. Russia													
♂ ♂	LR	2	11	0	0	0	3	0	0	16	2	6	[17]
Europe, overall													
♀ ♀	LR	149	8	0	0	12	4	0	11	184	44		
	LLR	0	2	0	1	0	0	0	1	4			
	LRR	10	0	0	0	0	0	0	1	11			
♂ ♂	LR	86	54	0	0	4	18	4	2	168			
	LLR	1	17	0	57	0	1	10	0	86			
	LRR	29	0	0	0	1	0	0	1	31			
Total		275	81	0	58	17	23	14	16	484			

Note: n – sample size, N – number of populations tested, Oth. – other, other genotypes of gametes; Methods of gamete genotype identification: 1, progeny morphometric analysis; 2, electrophoretic analysis of the progeny using biochemical gene markers; 3, electrophoretic analysis of the progeny using DNA minisatellites; 4, karyological analysis of gametes; 5, electrophoretic analysis of gametes using diagnostic enzymes; 6, analysis of gametes with the help of DNA cytophotometry.

Squashed testes were washed off into filter cone with 2 ml of distilled water. Filtering was performed using macroporous filter paper. The filtrate obtained (it should be turbid, which is the marker of successful sample preparation) was centrifuged for 5 min at 1000 rpm. Supernatant with the help of syringe was placed in a clean tube and centrifuged for 15 min at 5000 rpm. After removal of supernatant, the sediment was added with two to three drops of 10% sucrose, stained by addition of bromphenol blue. Closed tubes were frozen for 12 h. After thawing, the tube was shaken to obtain homogenous turbid suspension.

Gamete classification. The following gamete profiles were considered as normal: a) only haploid gametes of one genotypic class (L or R); b) diploid homozygous gametes of one genotypic class (LL or RR). In the profiles of abnormal gametes four of these were distinguished, including nonreduced diploid gametes (LR); a mixture of two classes of haploid gametes in comparable ratios (L + R), and those with numerical prevalence of one of the classes (L ≧ R); and others (three or more gamete classes, a mixture of diploid gametes, etc.). In all cases not the frequencies of different gamete types were calculated, but the frequencies of individuals, characterized by normal or abnormal gametogenesis.

RESULTS

Correctness of Gametogenesis in Hybrid Form Rana “esculenta”

The proportion of the animals producing normal gametes is independent from sex and ploidity and constitutes from 84% in LR allodiploids to 93% in LRR allotriploids (Figs. 1, 2; Table 2).

Species Affiliation of the Genome Inherited

Among the diploid *Rana “esculenta”* frogs, 67% were the animals inheriting only the lake frog genome (LR → R). The proportion of the animals inheriting exclusively the genome of the second parent (LR → L) was 18% (Tables 1 and 2, Fig. 1). In terms of species affiliation of the genome inherited, triploid *Rana “esculenta”* frogs were different. The LLR triploids almost exclusively inherited the genome of pool frog (86% of the individuals); only about 1% of LLR individuals produced normal gametes with R genotype. None of the LRR triploids inherited the genome of pool frog (Table 2, Fig. 2).

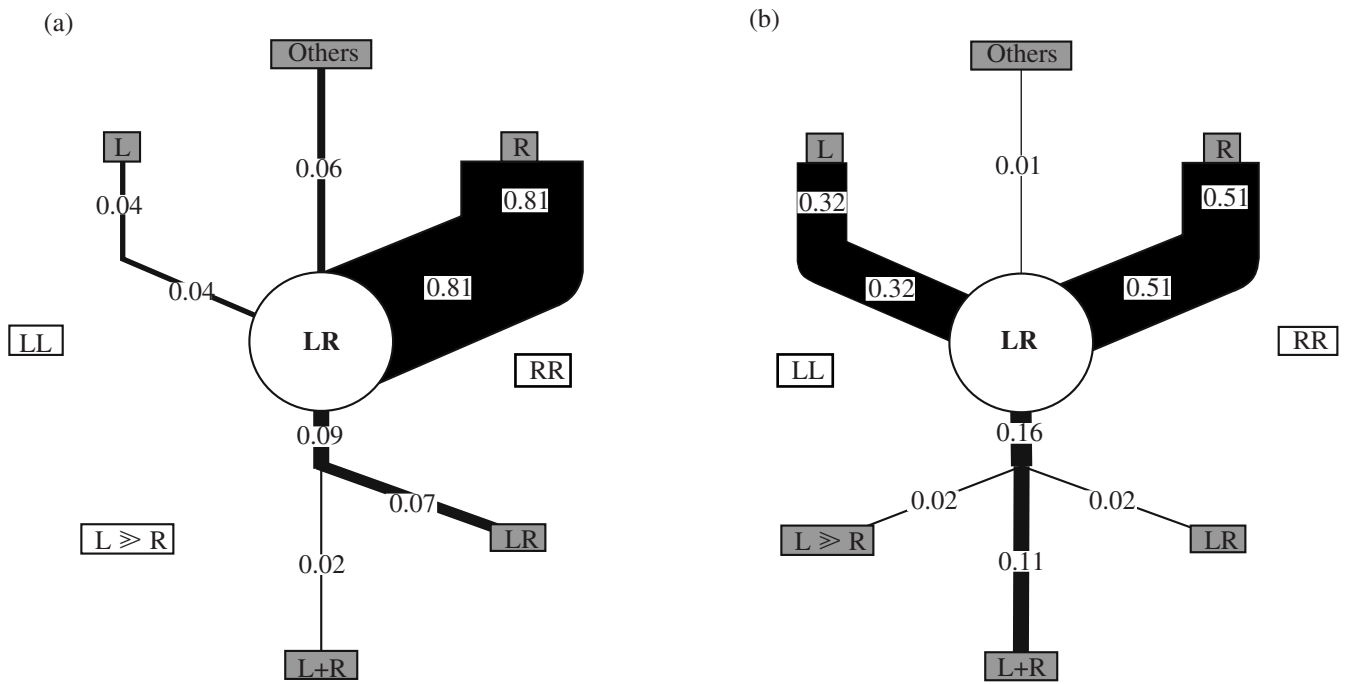


Fig. 1. Distribution of allodiploid females (a) and males (b) in accordance with the mode of inheritance of paternal genomes.

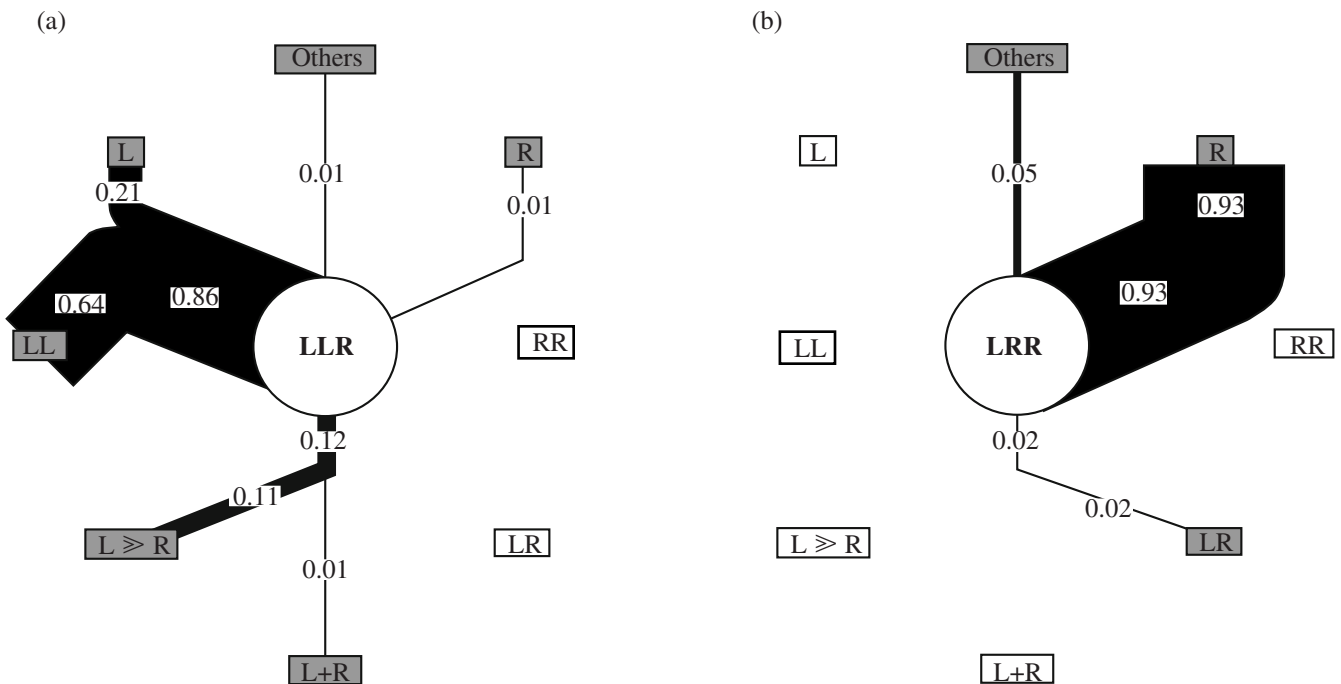


Fig. 2. Distribution of LLR (a) and LRR (b) allotriploids in accordance with the modes of inheritance of parental genomes.

Inheritance in Allodiploid Rana "esculenta" Frogs

All normal gametes produced by allodiploids had a haploid genotype (Table 2). Among allodiploids, the proportion of individuals inheriting the genome of lake frog was greatly variable: from 13% (Russia) and 16%

(Germany) to 53% (Poland) and 65% (Ukraine). The data obtained for other countries indicated that inheritance of only lake frog genome was observed. On the territory of Ukraine, Dnieper River basin, inheritance of only the lake-frog genome ($LR \rightarrow R$) was

Table 2. Frequencies of hybrid *Rana "esculenta"* frogs producing normal gametes

Soma	Sex	Genotypes of gametes produced								
		R		L		LL		Total		<i>n</i>
LR	♀ ♀	0.810	0.029	0.043	0.015	–	–	0.853	0.026	184
	♂ ♂	0.512	0.039	0.321	0.036	–	–	0.833	0.029	168
	<i>F</i>	36.46***		54.18***		–		0.26		
LLR	♀ ♀	–	–	0.5	0.3	0.3	0.2	0.8	0.2	4
	♂ ♂	0.01	0.01	0.20	0.04	0.66	0.05	0.87	0.04	86
	<i>F</i>	0.18		1.61		2.80		0.38		
LRR	♀ ♀	0.91	0.09	–	–	–	–	0.91	0.09	11
	♂ ♂	0.94	0.04	–	–	–	–	0.94	0.04	31
	<i>F</i>	0.08		–		–		0.08		
Total										
2 <i>n</i> + 3 <i>n</i>	♀ ♀	0.799	0.028	0.050	0.015	0.005	0.005	0.854	0.025	199
	♂ ♂	0.407	0.029	0.249	0.026	0.200	0.024	0.856	0.021	285
LR	♀ ♀ + ♂ ♂	0.668	0.025	0.176	0.020	–	–	0.844	0.019	352
LLR		0.01	0.01	0.21	0.04	0.64	0.05	0.87	0.046	90
LRR		0.93	0.04	–	–	–	–	0.93	0.04	42
<i>F</i>	LR-LLR	207.49***		0.56		248.99***		0.30		
	LR-LRR	17.75**		28.15**		0.00		2.77		
	LLR-LRR	163.48***		26.11**		99.48***		1.22		
Overall		0.568	0.023	0.167	0.017	0.120	0.015	0.855	0.016	484

***P* < 0.01; *** *P* < 0.001.

observed. Hybrid frogs from the Danube River basin are heterogeneous in this respect. In the populations from Transcarpatian lowlands, inheritance of only lake frog genome was observed (LR → R), while in the populations from the mouth of the Danube River only pool frog genome was inherited (LR → L). In Ukrainian population from the Don River basin (Severskii Donets) the proportion of hybrids inheriting only the lake frog genome was 10%. Relative to this character, Ukrainian population was similar to Russian popula-

tion the belongings of the populations mentioned to one drainage area.

The gamete profiles produced by diploid hybrid frogs demonstrated statistically significant sex differences. Diploid females of *Rana "esculenta"* (Fig. 1a) inherited predominantly the lake frog genome (81%). Among the diploid males of *Rana "esculenta"* (Fig. 1b), inheritance of the LR → R type was statistically significantly lower (51% of the animals) (Table 2, *P* < 0.001).

Inheritance in Allotriploid Rana "esculenta"

No correlation between the genotypes of gametes produced by allotriploid frogs and sex was observed (Table 2). The LLR triploids from Hungary (one population examined) produced diploid gametes with only LL genotype. In all other populations, normal gametes produced by the LLR triploids practically in all cases had haploid L genotype (Fig. 2a).

All normal gametes produced by the LRR triploids (Fig. 2b) had R genotype. Interestingly, gametes produced by two classes of triploids were different. Specifically, the LLR animals produced two classes of normal gametes (L and LL), while LRR animals produced normal gametes of only one class (R).

DISCUSSION

Species Affiliation of the Genome Inherited

The data presented in this study give the impression that allodiploid females more often inherit the genome of lake frog than allodiploid males. This illusory difference is caused by summation of the data from different population samples, characterized by different sex structures. Within-population analysis showed that only in one sample obtained from Oder population (Germany) females (one individual) inherited the genome of lake frog, while males (two individuals) inherited the genome of pool frog [9]. No such differences were observed in all other samples.

Modes of Reproduction of Hybrid Forms

Currently available data point to the existence of two pathways for the mechanisms of reproduction of hybrid forms. The differences involve such traits as sexual structure of hybrid forms, species affiliation of the genome inherited, and reproductive features of allotriploids.

The first mechanism is realized in *Poeciliopsis* fish [10] and in the genus *Bacillus* [11]. In these cases hybrid individuals are represented exclusively by females, and always inherit maternal genome. Furthermore, the inheritance is strictly unimodal (the genome of the same species is always inherited). Allodiploids are reproduced through backcrossing to the males of that parental species, whose genome is not inherited. Allotriploids are reproduced either gynogenetically, or parthenogenetically [7, 12]. Thus, in this case, allodiploids and allotriploids are reciprocally reproductively isolated.

The second mechanism is realized in the fish of the genus *Tropidophoxinellus* [13], as well as in the green frogs of the genus *Rana*. In this case, hybrids can be both females and males. Genome of any of parental species can be inherited by hybrids (the mode of inheritance is polymodal). Hybrid individuals can produce both haploid and diploid gametes. Furthermore, diploid gametes can carry two genomes of one species, or have hybrid genotype. Finally, allotriploids produce valid

gametes, and are not reproductively isolated from allodiploids.

Origin and Reproduction of Rana "esculenta" Allotriploids

Currently, the available data evidence that *Rana "esculenta"* allodiploids do not produce diploid homozygous gametes (LL or RR). For this reason, allotriploids can arise only among the progeny of diploid hybrids, producing abnormal gametes with genotype LR. Combination of the later with haploid gametes provides the formation of both triploid genotypes (LR + L → LLR, LR + R → LRR). This mechanism of allotriploid development was confirmed for the genus *Poeciliopsis* [7]. In case of homotypic cross between two hybrids, producing diploid gametes, the appearance of both symmetric (LR + LR → LLRR) and asymmetric (LL + LR → LLLR) allotetraploids is possible. These allotetraploids with low frequencies can be really found in natural populations [14, 15]. Thus, allotriploid *Rana "esculenta"* individuals serve as markers of abnormal gametogenesis in this hybrid form.

Theoretically, two ways for reproduction of triploids with genotype LLR (L + LR → LLR or LL + R → LLR) are possible. The second way, as it was already mentioned above, is known for the only population of ER type from Hungary. In this population, allotriploids (100% ♂♂ LLR) are reproduced through crosses with lake frog females. All progeny of these crosses has paternal genotype LLR and consists of only males. Thus, this population system is stable.

It is suggested that reproduction of LRR triploids requires another mechanism. Actually, the LRR triploids produce no diploid gametes with RR genotype. Reproduction of such allotriploids is possible using the only way (LR + R ? LRR). It can be expected that in the populations, where allodiploids produce only haploid gametes, no LRR allotriploids can appear, or be reproduced. It can be suggested that LLR allotriploids have more chances for successful reproduction than LRR allotriploids. This suggestion agrees with the data available. As can be seen from Table 1, the ratio between the population numbers of LLR and LRR allotriploids was almost 2 : 1.

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