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MASS OCCURRENCE OF POLYPLOID GREEN FROGS (*Rana esculenta* COMPLEX) IN EASTERN UKRAINE

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In eastern Ukraine, the *Rana esculenta* complex consists of three species: *R. lessonae*, *R. ridibunda*, and hybrid *R. esculenta*. The first one was rare, whereas two latter frog taxa were very common. Based on DNA flow cytometry, mass occurrence of the triploidy in *Rana esculenta* has been revealed in 14 localities of Kharkov, Donetsk, and Lugansk Provinces. One hybrid specimen from Kharkov Province was tetraploid. All polyploids were recorded along the middle part of Seversky Donets River (above 450 km). Triploids comprised two groups with different genome composition (LLR and LRR), and were found in three types of population systems (E, R–E, and L–E–R). Geographic distribution of polyploidy in European green frogs is briefly outlined. Different methods of ploidy level identification are discussed. The chromosome count and nuclear DNA cytometry provide the most reliable data.

Keywords: hybridogenetic frogs, triploidy, tetraploidy, *Rana esculenta* complex, DNA flow cytometry, Ukraine.

INTRODUCTION

Three taxa of European green frogs (*Rana esculenta* complex) are distributed in eastern Europe. These are two "normal" species – the lake frog, *Rana ridibunda* Pallas, 1771, and the pool frog, *R. lessonae* Camerano, 1882, as well as the edible frog, *R. esculenta* Linnaeus, 1758, which arose as a result of hybridization between the former two parental species. The complex exhibits an unusual mode of speciation, characterized by complicated genetic mechanisms, involving hybridization, so-called hemiclonal (or meroclonal) inheritance, polyploidy, unisexual and bisexual population systems of various kinds (e.g., Uzzell et al., 1977, 1980; Borkin et al., 1987; Vinogradov et al., 1988, 1990, 1991; Graf and Polls Pelaz, 1989a; Berger, 1990; Bucci et al., 1990; Günther, 1990; Tunner and Heppich-Tunner, 1991; Hotz et al., 1992; Caune and Borkin, 1993; Lada, 1995; Ragghianti et al., 1995). *Rana esculenta* is considered to be a *klepton* (in Greek: thief), a special category of taxa of the species group, the character of which does not coincide with that of the biological species (Dubois and Günther, 1982; Dubois, 1990; Günther, 1991).

Although hybridogenetic frogs (*R. esculenta*) are widely known across temperate Europe from France in the west to Volga River in the east (Günther, 1997; Borkin et al., 2003a, 2003b), natural triploidy has been recorded in many populations distributed in western and central parts of Europe only (e.g., Günther, 1970, 1975a; Knudsen and Scheel, 1975; Ebendal and Uzzell, 1982; Regnier and Neveu, 1986; Blommers-Schlösser, 1990; Plötner and Klinkhardt, 1992; Tunner and Heppich-Tunner, 1992; Berger and Berger, 1994; Mikulíček and Kotlík, 2001; Ogielska et al., 2001; Rybacki and Berger, 2001).

During two last decades, we examined by means of DNA flow cytometry numerous samples of green frogs from the territory of the former Soviet Union (Fig. 1). However, no record of triploidy in wild populations has

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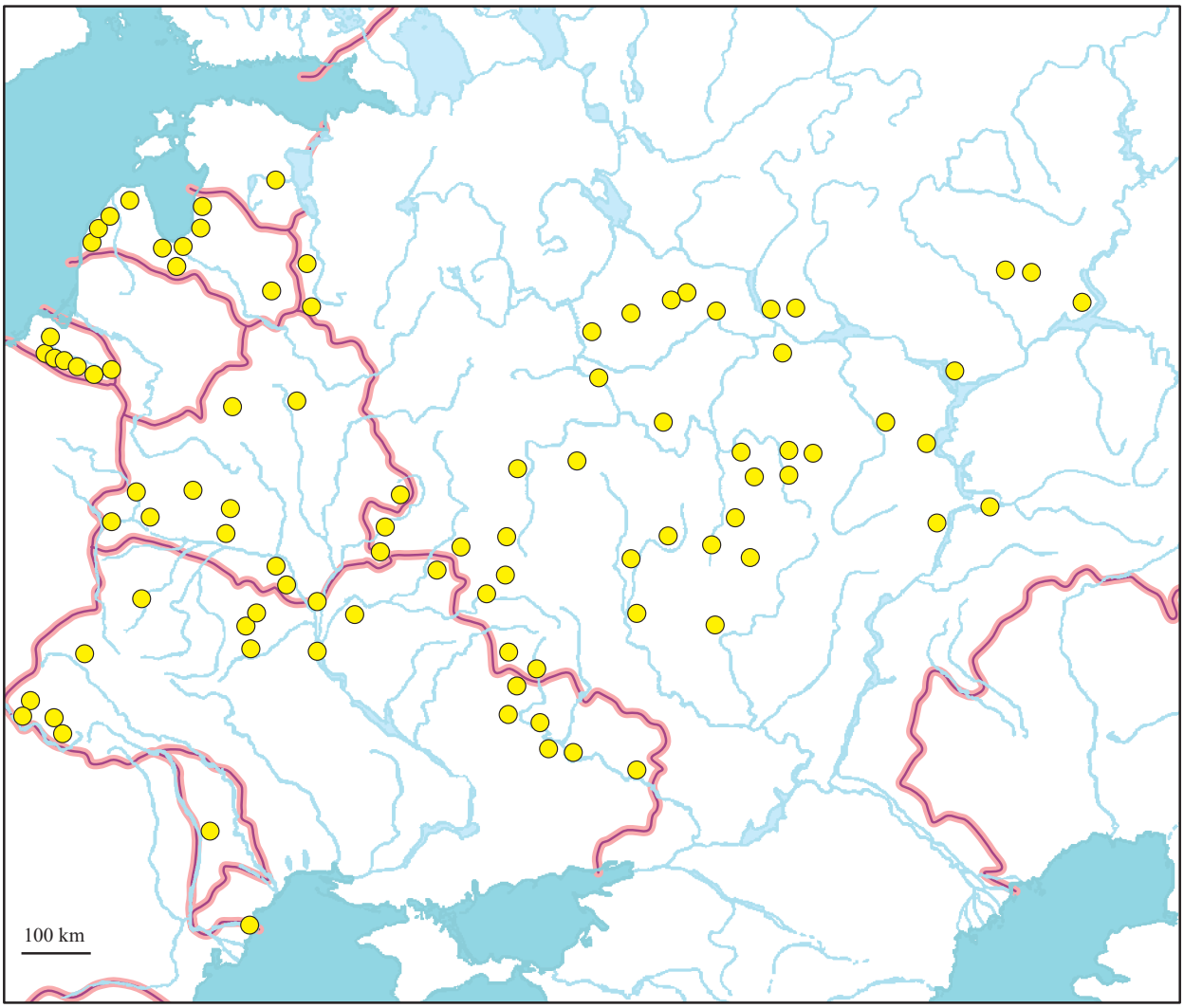

Fig. 1. The distribution of hybrid *Rana esculenta* in European part of the former Soviet Union (based on genome size data).

not yet been published (Borkin et al., 1987; Vinogradov et al., 1988, 1990, 1991; Caune and Borkin, 1993; Lada et al., 1995; Borkin et al., 2003a, 2003b). An enigmatic tetraploid frog only was evidenced by karyotyping from Latvia (Borkin et al., 1979). An occasional triploid, taken by S. N. Litvinchuk from Kaliningrad Province, the westernmost enclave of Russia situated between Poland and Lithuania, has been mentioned by Borkin (2001).

In this contribution, we present the results of our study on green frogs of eastern Ukraine, obtained mainly on the basis of DNA flow cytometry. The aim of the paper is to give the evidence of mass occurrence of various kinds of polyploid frogs (*R. esculenta*) in local populations.

MATERIAL AND METHODS

Since 1989, a total of 551 green frogs were collected in eastern Ukraine and examined by DNA flow cytometry. In 1989 we studied 11 specimens from Lopan River, Kharkov City, presented by A. M. Rudik. In June 1995 we sampled 24 frogs from three other localities of Kharkov Province (Zmiyov and Kharkov Districts). In June 1996 we examined 43 specimens from two localities of Zmiyov and Zachepilovka Districts, Kharkov Province, Ukraine.

In 2002 – 2004 our sampling has been more extensive, and 473 frogs were taken from 38 localities of Kharkov, Donetsk, and Lugansk Provinces (Fig. 2, Table 1). The majority of specimens were collected at the “juvenile” stage, which covered both froglets before

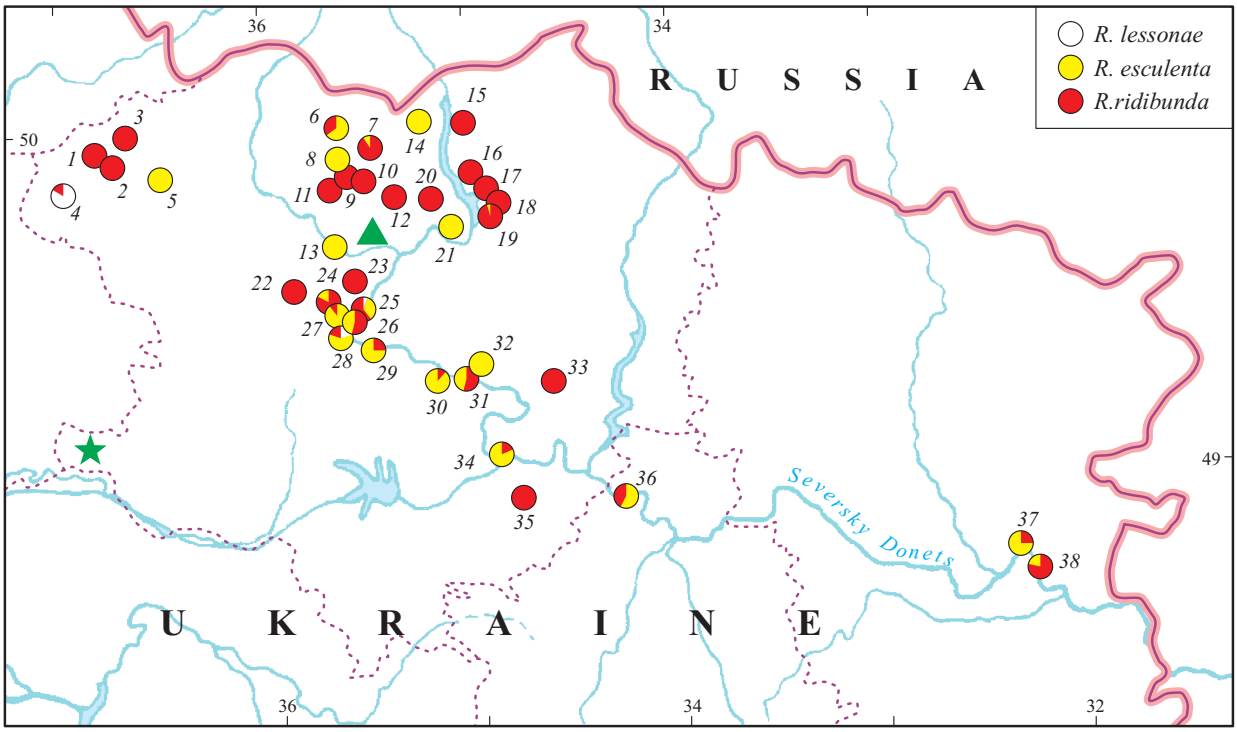

Fig. 2. The distribution of members of the *Rana esculenta* complex in eastern Ukraine (based on genome size data). The triangle marks the Kharkov City sample with *R. ridibunda* studied in 1989. The asterisk denominates the Russkii Orchik sample with three frog species studied in 1996.

TABLE 1. Genome Size Variation (in picograms) in Green Frogs collected from Eastern Ukraine in 2002 – 2004

No.	Locality	Latitude (N)	Longitude (E)	Type	*N*	Mean ± SD	Range	CV, %
			Kharkov Province					
1	Kozievka	50°08′	35°13′	RR	1j	16.10		
2	Gorodnoe	50°06′	35°15′	RR	16j	16.11 ± 0.04	16.03 – 16.18	0.3
3	Gubarevka	50°10′	35°21′	RR	1f + 10j	16.15 ± 0.06	16.05 – 16.27	0.4
4	Mikhailovskoe	50°01′	35°05′	LL	3j	14.13 ± 0.06	14.09 – 14.20	0.4
				LL?	1j	13.68		
				RR	1j	16.19		
5	Sharovka	50°02′	35°28′	LR	3j	15.01 ± 0.05	14.96 – 15.04	0.3
6	Liptsy	50°13′	36°23′	LR	6m	14.89 ± 0.03	14.86 – 14.92	0.2
				RR	3m	16.06 ± 0.07	15.99 – 16.14	0.5
7	Murom	49°24′	37°11′	LR	1j	14.94		
				RR	9j	16.06 ± 0.09	15.88 – 16.15	0.6
8	Russkaya Lozovaya	50°10′	36°19′	LR	7j	14.97 ± 0.05	14.88 – 15.04	0.3
9	Lesnoe	50°06′	36°16′	RR	1j	16.18		
10	Tsirkuny	50°06′	36°27′	RR	2j	16.22	16.20 – 16.25	
11	Pyatikhatka	50°05′	36°17′	RR	2j	15.98	15.98 – 15.98	
12	Ol’khovka	49°58′	36°31′	RR	1f	16.12		
13	Bezlyudovka	49°52′	36°17′	LR	11j	15.02 ± 0.07	14.93 – 15.12	0.5
14	Izbitskoe	50°12′	36°44′	LR	2f + 4j	15.01 ± 0.04	14.95 – 15.06	0.3
				LLR	1m + 1f + 7j	21.88 ± 0.08	21.73 – 22.01	0.4
15	Verkhnyaya Pisarevka	50°11′	36°51′	RR	1m + 5j	16.15 ± 0.04	16.10 – 16.20	0.2
16	Pershotravnevoe	50°00′	36°55′	RR	1m + 9j	16.18 ± 0.05	16.12 – 16.26	0.3
17	Martovoe	49°56′	36°58′	RR	1f + 6j	16.08 ± 0.06	15.97 – 16.18	0.4
18	Artemovka	49°56′	37°00′	RR	8j	16.28 ± 0.05	16.21 – 16.35	0.3
19	Pechenegi	49°53′	37°00′	LR	1j	14.93		
				RR	2m + 2f + 12j	16.20 ± 0.07	16.05 – 16.31	0.4

TABLE 1 (continued)

No.	Locality	Latitude (N)	Longitude (E)	Type	*N*	Mean ± SD	Range	CV, %
20	Pyatnitskoe	49°56′	36°48′	RR	3j	16.17 ± 0.05	16.11 – 16.21	0.3
21	Kitsevka	49°52′	36°49′	LR	1j	15.10		
22	Merefa	49°48′	36°05′	LR	7j	14.96 ± 0.10	14.85 – 15.14	0.6
23	Levkovka	49°44′	36°19′	RR	4j	16.23 ± 0.03	16.20 – 16.26	0.2
24	Zhadanovka	50°13′	36°23′	LR	2j	14.98	14.95 – 15.01	
				LLR	1f	21.90		
				RR	15j	16.06 ± 0.09	15.86 – 16.23	0.6
25	Biostantsiya	49°37′	36°20′	LL	1j	14.01		
				LR	1m + 9j	14.99 ± 0.09	14.83 – 15.09	0.6
				LR?	2j	15.40	15.37 – 15.44	
				LRx	1j	22.36		
				LRR	2j	22.81	22.71 – 22.91	
				RR	11j	16.10 ± 0.04	16.04 – 16.17	0.3
26	Gaidary	49°39′	36°16′	LR	8m + 1f + 40j	14.98 ± 0.07	14.72 – 15.09	0.5
				LR?	1j	15.27		
				LLR	3j	21.76 ± 0.13	21.61 – 21.87	0.6
				LRR	1f + 14j	23.04 ± 0.17	22.65 – 23.25	0.7
				RR	3f + 54j	16.13 ± 0.07	15.80 – 16.26	0.5
27	Gomolshanskii	49°34′	36°16′	LR	10j	14.96 ± 0.04	14.90 – 15.03	0.3
				LLR	4j	21.76 ± 0.09	21.64 – 21.84	0.4
				LRR	9j	22.91 ± 0.12	22.64 – 23.02	0.5
				LLRR?	1j	29.58		
				RR	3j	16.17 ± 0.04	16.12 – 16.20	0.3
28	Velikaya Gomolsha	49°33′	36°14′	LL	1j	13.97		
				LR	3j	14.96 ± 0.08	14.90 – 15.04	0.5
				LLR	1f	21.67		
				RR	1j	15.99		
29	Sukhaya Gomolsha	49°32′	36°20′	LR	2m + 3f	14.97 ± 0.09	14.88 – 15.09	0.6
				LRR	1j	22.96		
				RR	2j	16.07	16.02 – 16.12	
30	Kreidyanka	49°26′	36°48′	LR	5m + 8j	14.91 ± 0.10	14.79 – 15.15	0.7
				LR?	1f	15.44		
				LLR	2f	21.61	21.61 – 21.62	
				LRR	2m + 1j	22.80 ± 0.12	22.73 – 22.93	0.5
				RR	1f + 1j	15.95	15.90 – 16.00	
31	Chervonaya Gusarovka	49°25′	36°52′	LR	4j	14.91 ± 0.07	14.80 – 14.97	0.5
				LLR	1j	21.54		
				LRR	2j	22.75	22.72 – 22.79	
				RR	9j	15.96 ± 0.10	15.74 – 16.07	0.6
32	Ol'khovatka	49°25′	36°53′	LR	5m + 1f + 3j	14.84 ± 0.05	14.76 – 14.91	0.3
				LLR	2m + 1f + 6j	21.56 ± 0.06	21.46 – 21.64	0.3
				LRR	2f + 2j	22.76 ± 0.09	22.68 – 22.85	0.4
33	Veseloe	49°24′	37°11′	RR	1f	16.11		
34	Chervony Shakhter	49°11′	37°02′	LR	1f + 2j	14.96 ± 0.02	14.93 – 14.97	0.1
				LLR	2f + 3j	21.87 ± 0.10	21.75 – 22.03	0.5
				LRR	1m + 4j	23.00 ± 0.04	22.96 – 23.05	0.2
				RR	3j	16.15 ± 0.04	16.10 – 16.18	0.3
35	Petropol'e	49°05′	37°08′	RR	1f	16.01		
				Donetsk Province				
36	Svyatogorsk	49°03′	37°33′	LR	3j	14.99 ± 0.02	14.97 – 15.01	0.1
				LLR	4f + 2j	21.91 ± 0.09	21.76 – 21.99	0.4
				RR	1f + 4j	16.18 ± 0.05	16.11 – 16.21	0.3
				Lugansk Province				
37	Novokondrashovka	48°41′	39°28′	LLR	3j	21.78 ± 0.02	21.76 – 21.80	0.1
				RR	1j	16.22		
38	Stanichno-Luganskoe	48°37′	39°32′	LLR	2j	21.85	21.83 – 21.87	
				RR	8j	16.11 ± 0.12	15.90 – 16.30	0.7

Note. j, juveniles (postmetamorphosed and subadult frogs); f, adult females; m, adult males.

Fig. 3. Iskov Pond in the environs of the settlement Gaidary. The habitat of diploid and triploid *Rana esculenta* as well as *R. ridibunda*. Photo by Alexey V. Korshunov.

the first hibernation and subadults. Sex of adults, determined by dissection, and the age composition (juveniles or adults) of samples are represented in Table 1.

The frog catching by G. A. Lada has been selective in 1995 – 1996 and 2003 because he paid his attention to hybrids, and a few representatives of parental species were taken as a control and an evidence for their existence at a locality. However, Kharkov collectors sampled animals randomly, without any preference.

The amount of DNA per nucleus (genome size, in picograms, pg) was measured by DNA flow cytometry. The detals of the technique have been published previously (e.g., Vinogradov et al., 1990; Rosanov and Vinogradov, 1998; Borkin et al., 2001).

Population systems were identified by us using field observations and genome size data as well as, in a few cases, museum collections. Detailed analysis will be published elsewhere.

The species genome structure in frogs and the species composition in population systems are marked by the initial letter of species Latin names, namely: L is *lessonae*, R is *ridibunda*, and E is *esculenta*.

Frog samples are deposited in the collection of the Department of Herpetology, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia, as well as in collection of the Museum of Nature, Kharkov National University, Kharkov, Ukraine.

The geographic names are given according to the map of Kharkov Province (Anonym, 1992).

RESULTS

Species Genome Composition in Hybrids

According to genome size data, eleven water frogs of the first sample (1989) from Kharkov City (Fig. 2: marked by triangular) proved to belong to *R. ridibunda*.

Twenty four specimens taken from three localities in Kharkov Province (1995) were identified as *R. esculenta*. Five and 15 hybrids were collected from a pond in Koryakov Yar and from Iskov Pond (Fig. 3), respectively; both sites are situated in the environs of the settlement Gaidary (Fig. 2: the locality *26*). Four frogs were captured in ponds of Ol'khovaya Balka Point, situated between villages Borshchevaya and Russkaya Lozovaya (Fig. 2, *8*). All hybrids were diploid.

Another sample from Iskov Pond examined in 1996, contained *R. esculenta* and one male *R. ridibunda*, as it was evidenced by DNA flow cytometry. Among 36 hy-

brids, we distinguished two groups: 35 diploids and one triploid (RRL).

Animals collected in 1996 from Russkii Orchik Point (Fig. 2, marked by asterisk), Zachepilovka District, contained representatives of three green frog species, namely: *R. ridibunda* (1), *R. lessonae* (1), and *R. esculenta* (4 diploids).

Thus, our preliminary studies made in 1989–1996 obviously indicated the occurrence of three members of the *Rana esculenta* complex in Kharkov Province, with numerous hybrids, including one triploid. These data did not included in Tables 1 – 4, since the nuclear DNA content was measured by DNA flow cytometry, with the use of different dye in comparison with our subsequent treatment, and because of different periods of sampling.

In 2002 – 2004 based on significantly more extensive sampling in three provinces of eastern Ukraine (473 specimens), we revealed six groups of animals expressed by different peaks on the DNA histogram (Fig. 4). We identified them as three members of the *R. esculenta* complex, namely: two parental species *R. lessonae* and *R. ridibunda*, and their hybrid *R. esculenta* (Tables 1–4).

The parental species were represented mostly by *R. ridibunda* (220 individuals or 46%), which had genome size values ranging from 15.74 to 16.35 pg. Only five specimens (or 1%) were reliably allocated to *R. lessonae*: their amount of nuclear DNA varied between 13.97 and 14.20 pg. Furthermore, one specimen from Mikhailovskoe (Fig. 2, *4*), most likely, belonging to the same species, had lower amount of nuclear DNA in comparison with other *R. lessonae* (Tables 1 and 2). The ranges of genome size variation in both species did not overlap (Fig. 4, Table 2), and differences between averaged values reached 13.6%.

The majority of frogs under the study (247 specimens, or 52%) were assigned to *R. esculenta*. These hybrids produced four peaks associated with various ploidy and parental species genome composition (Fig. 4). Diploid frogs (LR), males and females, were intermediate between *R. lessonae* and *R. ridibunda*, and their genome size values were equal to 14.72 – 15.15 pg. Four specimens had somewhat higher value (15.27 – 15.44 pg) at the diploid level (Table 2). Thus, the diploid class provided almost 64% out of *R. esculenta*, examined by us in 2002 – 2004 (Figs. 5 and 6).

Among hybrids, the share of polyploid individuals was equal to 36% ($n = 89$). According to genome size (Fig. 4, Table 2), they were composed of 88 triploids (21.46 – 23.25 pg) and one tetraploid specimen (29.58 pg).

Triploid hybrids formed two groups (Figs. 4, 7, and 8; Table 2) having a little different animal numbers. The

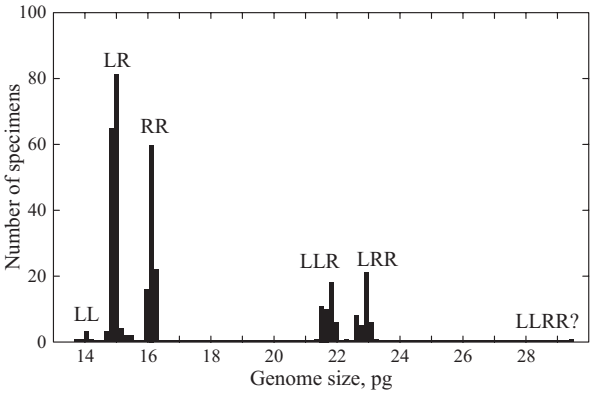

Fig. 4. The distribution of genome size in the *Rana esculenta* complex of eastern Ukraine according to species, ploidy level, and species genome composition. LL, *R. lessonae*; RR, *R. ridibunda*; LR, LLR, LRR, and LLRR?, four groups of hybrid *R. esculenta*.

group ($n = 46$, or 52% of all polyploids) with lesser genome size was assigned to the triploid class with the LLR genome composition, i.e., two *lessonae* genomes and one *ridibunda* genome, because its averaged value (21.77 pg) was in agreement with theoretically expected estimate (L + LR → 7.035 + 14.96 = 22.00 pg). Another group ($n = 41$, or 46% of all polyploids) with larger genome size (22.93 pg) seemed to have the LRR genome structure, i.e., two *ridibunda* genomes and one *lessonae* genome, respectively (R + LR → 8.06 + + 14.96 = 23.02 pg). Genome size values in both triploid groups ranged without overlap. However, one triploid had an intermediate position between the both groups (LRx; Table 2).

Both kinds of triploids were represented by males and females.

A sole tetraploid specimen (an juvenile) showed the genome size value (29.58 pg) between theoretically expected values for hybrids with symmetrical ge-

TABLE 2. Genome Size Variation (in picograms) in Parental and Hybrid Species of the *Rana esculenta* Complex in Eastern Ukraine ($N = 473$)

Species	Type	*N*	Mean ± SD	Range	CV, %
lessonae	LL	5	14.07 ± 0.09	13.97 – 14.20	0.6
lessonae	LL?	1	13.68		
esculenta	LR	154	14.96 ± 0.08	14.72 – 15.15	0.5
esculenta	LR?	4	15.38 ± 0.08	15.27 – 15.44	0.5
esculenta	LLR	46	21.77 ± 0.15	21.46 – 22.03	0.7
esculenta	LRx	1	22.36		
esculenta	LRR	41	22.93 ± 0.16	22.64 – 23.25	0.7
esculenta	LLRR?	1	29.58		
ridibunda	RR	220	16.12 ± 0.09	15.74 – 16.35	0.6

Fig. 5. A diploid male of *Rana esculenta* from Liptsy, Kharkov Province. Photo by Spartak N. Litvinchuk.

Fig. 6. A diploid male of *Rana esculenta* from Biostantsiya, Kharkov Province. Photo by Spartak N. Litvinchuk.

Fig. 7. A triploid female of *R. esculenta* with the LLR genome composition from Kreidyanka, Kharkov Province. Photo by Dmitry A. Shabanov and Alexey V. Korshunov..

Fig. 8. A triploid male of *R. esculenta* with the LRR genome composition from Kreidyanka, Kharkov Province. Photo by Dmitry A. Shabanov and Alexey V. Korshunov.

nome structure, LLRR (LL + RR → 14.07 + 16.12 = = 30.19 pg) and for frogs of the LLLR genome type (LLL + R → 21.11 + 8.06 = 29.17 pg).

The genome size variation in all, both diploid and triploid, groups were quite similar: values of the coefficient of variation (CV) ranged from 0.5 to 0.7% (Table 2).

We failed to observe any correlation between sex or age (adults or juveniles), on the one hand, and the genome size variation, on the other hand.

Green Frog Composition in Localities

The majority of localities, examined in 2002 – 2004, under the study was inhabited by *R. ridibunda* or, in lesser extent, by *R. esculenta*, whereas *R. lessonae* was recorded in three localities only, 8% (Fig. 2, Tables 1 and 3). Formerly, in 1996, the latter species was found in Russkii Orchik as well (Fig. 2). Twenty two localities were shared by two or three species of green frogs. We also found one locality (Mikhailovskoe) where both parental species were recorded together without hybrids. However, we could suggest that such system existed in other locality as well (Gorodnoe). Our samples from this locality, examined by DNA flow cytometry, were represented by *R. ridibunda* only. Nevertheless, we identified another species, *R. lessonae*, in the collection of the Museum of Nature, Kharkov University. In contrast, *R. esculenta* coexisted with *R. ridibunda* in 13 localities, whereas the occurrence of all members of the *R. esculenta* complex in the same localities was registered in two cases (Table 1). Moreover, all three members of the complex were recorded in Russkii Orchik (Fig. 2). Some localities (larger samples with ten or more specimens) were represented by one species only: either by *R. ridibunda* (3 cases) or by *R. esculenta* (3 cases).

Triploids were found in 14 localities (37%) of Kharkov, Donetsk, and Lugansk Provinces (Fig. 9). Their occurrence was associated with *R. ridibunda* in 11 cases, whereas both parental species coexisted with triploids in two localities only (Biostantsiya and Velikaya Gomolsha — Fig. 10; Tables 1 and 4). Samples from two localities (Bezlyudovka and Izbitskoe) contained diploid and triploid hybrids only, without any parental species.

Unlike diploid *R. esculenta*, triploids were recorded along the middle part of Severskiy Donets River only. The most distant localities with triploids were separated by the distance of above 450 km (from Izbitskoe village to Stanichno-Luganskoe village — Fig. 9).

A tetraploid frog was found in the locality Gomolshanskii, Kharkov Province (Fig. 9, Table 1 and 4) where both kinds of triploids co-existed with parental species.

Triploid Genom Structure and Population Systems

Triploids of the LLR composition only were revealed in three types of population systems (Table 4), namely: in the E type (for instance, Izbitskoe), the R-E type (for instance, Svyatogorsk, Novokondrashovka, and Stanichno-Luganskoe), as well as in the L-E-R type (for instance, Velikaya Gomolsha).

Triploids with the LRR genome structure only were found in the L-E-R type of population systems (Biostantsiya and Sukhaya Gomolsha — Table 4).

Both kinds of triploids together were revealed in two types of population systems (Table 4). These were mixed populations of the R-E type (for instance, Kreidyanka — Fig. 11, and Ol'khovatka) and the L-E-R type (for instance, Gaidary).

DISCUSSION

Historical Background

Based on his field observations in years 1848 and 1849, Alexander Czernay (1852), Extraordinary Professor of Kharkov University, classified all green frogs, distributed in Kharkov Government, to the same species *R. viridis* Roesel, with three so called varieties.

TABLE 3. Frequency of Green Frog Species in Localities of Eastern Ukraine (population systems are based on field, museum and genome size data)

No.	Locality	Population systems	Samples examined by DNA flow cytometry			
			N (total)	Species, %		
				lessonae	*esculenta*	*ridibunda*
1	Kozievka	RL	1			100
2	Gorodnoe	RL	16			100
3	Gubarevka	R	11			100
4	Mikhailovskoe	RL	5	80		20
5	Sharovka	RE	3		100	
6	Liptsy	RE	9		67	33
7	Murom	RE	10		10	90
8	Russkaya Lozovaya	RE	7		100	
9	Lesnoe	R	1			100
10	Tsirkuny	R	2			100
11	Pyatikhatka	R	2			100
12	Ol'khovka	R	1			100
13	Bezlyudovka	E	11		100	
14	Izbitskoe	E	15		100	
15	Verkhnyaya Pisarevka	R	6			100
16	Pershotravnevoe	R	10			100
17	Martovoe	R	7			100
18	Artemovka	R	8			100
19	Pechenegi	RE	17		6	94
20	Pyatnitskoe	R	3			100
21	Kitsevka	E	1		100	
22	Merefa	E	7		100	
23	Levkovka	R	4			100
24	Zhadanovka	RE	18		17	83
25	Biostantsiya	REL	27	4	54	42
26	Gaidary	RE	106		46	54
27	Gomolshanskii	RE	27		89	11
28	Velikaya Gomolsha	REL	6	17	67	17
29	Sukhaya Gomolsha	RE	6		75	25
30	Kreidyanka	RE	19		90	10
31	Chervonaya Gusarovka	RE	16		44	56
32	Ol'khovatka	RE	22		100	
33	Veseloe	R	1			100
34	Chervony Shakhter	RE	16		81	19
35	Petropol'e	R	1			100
36	Svyatogorsk	RE	14		64	36
37	Novokondrashovka	RE	4		75	25
38	Stanichno-Luganskoe	RE	10		20	80

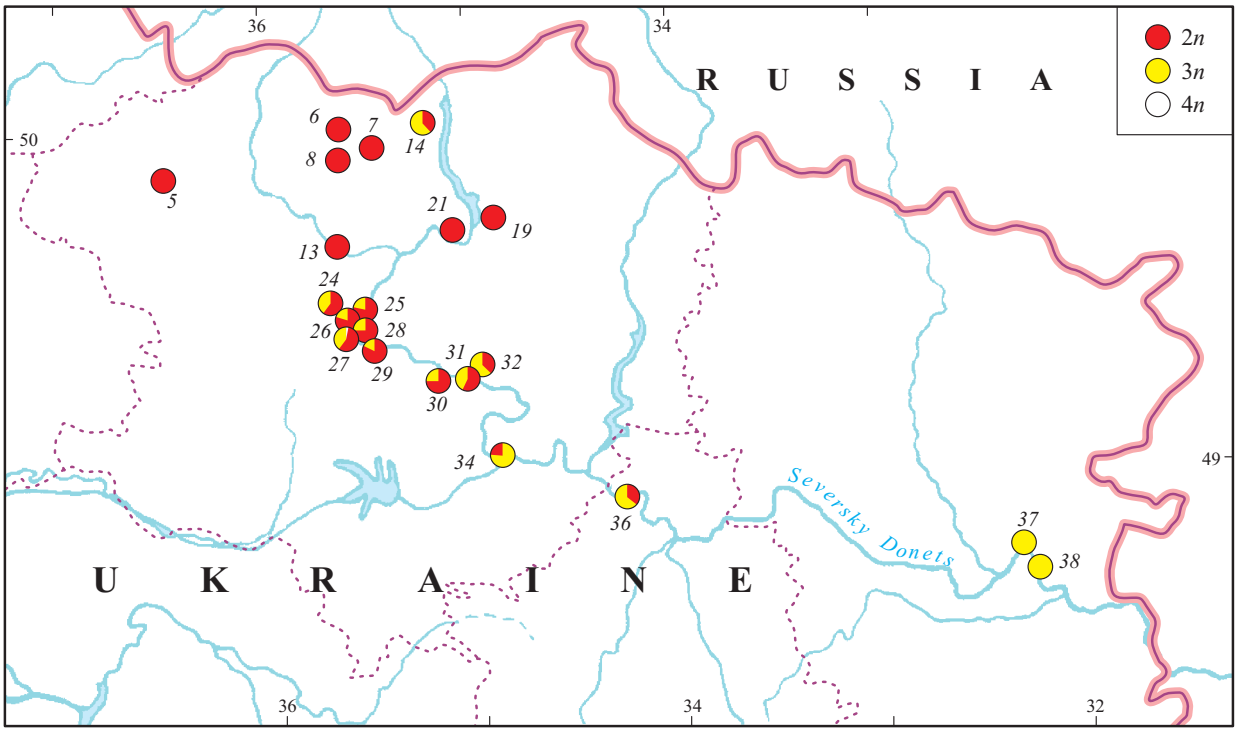

Fig. 9. The distribution of diploid and polyploid hybrids in eastern Ukraine (based on genome size data).

TABLE 4. Incidence of Diploid and Triploid Hybrids in Localities of Eastern Ukraine

No.	Locality	Population systems	*N* (hybrids)	Hybrid type, %				
				2*n*	LLR	LRR	LRx	LLRR?
5	Sharovka	RE	3	100				
6	Liptsy	RE	6	100				
7	Murom	RE	1	100				
8	Russkaya Lozovaya	RE	7	100				
13	Bezlyudovka	E	11	100				
14	Izbitskoe	E	15	40	60			
19	Pechenegi	RE	1	100				
21	Kitsevka	E	1	100				
22	Merefa	E	7	100				
24	Zhadanovka	RE	3	67	33			
25	Biostantsiya	REL	15	80		13	7	
26	Gaidary	RE	68	74	4	22		
27	Gomolshanskii	RE	24	42	17	38		4
28	Velikaya Gomolsha	REL	4	75	25			
29	Sukhaya Gomolsha	RE	8	83		17		
30	Kreidyanka	RE	21	74	11	16		
31	Chervonaya Gusarovka	RE	7	57	14	29		
32	Ol'khovatka	RE	22	41	41	18		
34	Chervony Shakhter	RE	13	23	38	38		
36	Svyatogorsk	RE	9	33	67			
37	Novokondrashovka	RE	3		100			
38	Stanichno-Luganskoe	RE	2		100			

Fig. 10. A floodplain of Seversky Donets River near Biostantsiya. The locality was inhabited by *Rana lessonae* (occasional), *R. ridibunda*, and *R. esculenta*. Hybrids were represented by both diploids and triploids (LLR and LRR). Photo by Dmitry A. Shabanov.

Fig. 11. A floodplain of Seversky Donets River near the village Kreidyanka, Kharkov Province. The locality was inhabited by *Rana ridibunda* and *R. esculenta*. Hybrids were represented by both diploids and triploids (LLR and LRR). Photo by Dmitry A. Shabanov.

The eminent Russian herpetologist Alexander Nikolsky, Professor of Kharkov University (since 1903) also applied the single-species concept to European green frogs (with three subspecies). He suggested that European part of Russian Empire, including Kharkov Government was inhabited by *R. esculenta ridibunda* only (Nikolsky, 1918).

Pavel Terentjev (1927) introduced two-species scheme, with *R. ridibunda* and *R. esculenta*, each species was splitted in two subspecies. Based on St. Petersburg (Leningrad) and Moscow collections, he listed the former species for Zmiyov, Kharkov Province ("Zmiersk, gub. Kharkow"). According to P. V. Terentjev, European part of the Soviet Union was inhabited by *R. ridibunda ridibunda* and *R. esculenta lessonae*. This scheme was widely accepted by Soviet zoologists after the publication of the classic guide book on the herpetofauna of the USSR co-authored by P. V. Terentjev and S. A. Chernov (1949).

Valery Vedmederja (1984) from the Museum of Nature, Kharkov State University, was able to distinguish three species of green frogs occurred in Kharkov Province. He applied morphological and ecological (habitat) approaches to identify these species. *Rana lessonae* preferred to inhabit various forest stagnant water bodies in Krasnokutsk District (Vladimirskoe Forestry, a "pure" population), as well as in Zachepilovka, Kharkov, and Volchansk Districts in the northwest, southwest and northeast of Kharkov Province. In the latter two districts, the species was recorded with *R. esculenta* together, often in the same habitat. The lake frog, *R. ridibunda* was distributed in many districts of the province, except the northern Volchansk, Krasnokutsk, and Bogodukhov Districts. As a rule, the species liked running water bodies. The hybrid *R. esculenta* inhabited both types of water bodies, coexisting with *R. ridibunda* or *R. lessonae*. All three species occupied "Orchik" (= Russkii Orchik) site in Zachepilovka District. However, Iskov Pond near Gaidary village was settled by *R. esculenta* only. Vedmederya (1984) also mentioned that this species was represented by two morphological forms with *lessonae*-like and *ridibunda*-like frogs. The both forms can occur in the same water bodies, often with *R. ridibunda* together.

More detailed historical sketch of green frog studies, in the 20th century in particular, has been published by Korshunov et al. (2004).

In 1983 – 1994, we studied green frogs from the vast territory of the Central Chernozem ("black soil" in Russian) Region of Russia, including Kursk, Belgorod, and Voronezh Provinces which bordered with eastern Ukraine (Lada et al., 1995). To extent that research geographically, in 1995 and 1996, we (G. A. Lada) collected two samples from the large Iskov Pond and from Koryakov Yar situated in Zmiyov District (Lada, 1998, 1999). The points "Orchik" in Zachepilovka District, and "Ol'khovaya balka" in Kharkov District were visited as well. These field trips to Kharkov Province were stimulated by V. I. Vedmederja's published data. In 2002 our current cooperative project started.

Polyploidy

1. Geographic distribution of triploidy

Some authors mentioned the strange geographic distribution of triploidy in wild populations of *Rana esculenta*. Such an enigmatic situation is without serious explanation. Indeed, triploids were often revealed in many localities of northern France, the Netherlands, northern Germany, Denmark, southern Sweden, Poland, western Slovakia, and western Hungary (Table 5). However, triploids are virtually absent in surveyed areas of Austria, Italy, ex-Yugoslavia, and the former Soviet Union (Graf and Polls Pelaz, 1989a). Triploids are abundant especially in the northwestern part of the range of *R. esculenta*, while they are less frequent in other European regions. It seems that triploids are totally absent from eastern Europe, the Italian and Balkan peninsulas (Mikulíček and Kotlík, 2001). Obviously, triploids are common in the western Baltic area.

Curiously, five triploids, or 1.5%, out of 336 *R. lessonae* frogs were reported from the Netherlands (Blommers-Schlösser, 1990). Later, Okulova et al. (1997) found two triploid animals, or 4.3%, of this species in Ivanovo Province, central European Russia ($n = 47$). Both cases were based on erythrocyte size only. However, verification is needed by karyotyping or DNA cytometry. Triploidy was reported for North African green frogs (*R. saharica*) as well (Hemmer et al., 1980). Moreover, this phenomenon was found in *R. grafi*, another hybrid form in western Europe (France) arosen from the hybridization between *R. perezi* and *R. ridibunda* (Schmeller et al., 2001).

Three triploid *R. esculenta* identified by the albumin electrophoresis were reported from Lower Austria; the sample size is unknown (Schneider and Tunner, 1982). Occasional frogs with triploid erythrocyte size were found in Bohemia and Moravia, Czech Republik (Souček et al., 1993; Zavadil, 1994; Zavadil et al., 1999). Two obviously occasional triploid or tetraploid specimens confirmed by genome size and chromosome count, respectively, were also recorded from the eastern Baltic area, Kaliningrad Province of Russia and Latvia, respectively (Borkin et al., 1979; our unpublished data). Using erythrocyte size, Okulova et al. (1997) suggested occasional triploidy in *R. esculenta* (single specimen) from Ivanovo Province, central European Russia, al-

TABLE 5. Geographical Distribution of Triploidy in Natural Populations of European Hybrid Green Frogs *Rana esculenta* and *R. grafi*, and Methods of Its Identification (occasional occurrences of triploids are not included)

Country, References	Chr	PE	M	CS	GS
1. *Rana esculenta*					
France					
Regnier and Neveu (1986)				+	
Polls Pelaz and Graf (1988)	+	+		+	
Graf and Polls Pelaz (1989a, 1989b)	+				+
Polls Pelaz and Graf (1989)		+			
L. J. Borkin, A. Dubois, A. Ohler, and A. E. Vinogradov (unpublished data)					+
The Netherlands					
Blommers-Schlösser (1990)		+		+	
Germany					
Günther (1970)	+				
Günther (1975a)				+	
Günther and Hähnel (1976)		+			
Hemmer (1977)		+			
Günther et al. (1979)		+		+	
Eikhorst (1981)				+	
Eikhorst (1988)		+		+	
Berger and Günther (1988, 1991 – 1992)				+	
Günther and Plötner (1989 – 1990)				+	
Vinogradov et al. (1990)					+
Plötner and Klinkhardt (1992)	+			+	
Plötner et al. (1994)				+	
Berger and Berger (1994)				+	
Rybacki (1995d)				+	
Sweden					
Ebendal and Uzzell (1982)		+	+	+	
L. J. Borkin, P. Sjogern, and A. E. Vinogradov (unpublished data)					+
Denmark					
Wickbom (1945)	+				
Knudsen and Scheel (1975)	+				
Fog (1995)		+	+	+	
Rybacki (1995a)				+	
L. J. Borkin, K. Fog and A. E. Vinogradov (unpublished data)					+
Poland					
Günther et al. (1979)		+		+	
Berger (1988)				+	
Rybacki (1995b, 1995c, 1995d, 1995e)				+	
Ogielska et al. (1995, 2001)				+	+
Schröer (1996)				+	+
Rybacki and Berger (2001)				+	
Slovakia					
Mikulíček and Kotlík (2001)	+	+		+	
Hungary					
Tunner and Heppich-Tunner (1992)	+	+			
Ukraine					
This paper					+
2. *Rana grafi*					
France					
Schmeller et al. (2001)				+	+

Note. Chr, chromosome count; PE, protein electrophoresis; M, external morphometric characters; CS, cell (erythrocyte) size; GS, genome size.

though our DNA flow cytometric analysis did not registered any triploidy among Ivanovo frogs. Apart from these data, no polyploid green frogs have yet been reported from enormous European territory of the former Soviet Union (Mazin and Borkin, 1979; Borkin et al., 1987; Vinogradov et al., 1988, 1990, 1991; Caune and Borkin, 1993; Lada et al., 1995; Borkin et al., 2003a, 2003b). Several studies, which were focused on green frog populations of Ukraine, showed no triploidy (Mazin and Borkin, 1979; Mezhzherin and Morozov-Leonov, 1992, 1993, 1996; Morozov-Leonov and Mezhzherin, 1995; Lada, 1999; our unpublished data).

In his PhD dissertation abstract, Morozov-Leonov (1998) provided a brief mention that six out of 25 hybrid frogs from Ukrainian Transcarpathians displayed the gene-dose effect in albumin, which was suggested to be an evidence for triploidy ("allotriploidy"). Unfortunately, no other data or comments about the case were given. In recent paper (Morozov-Leonov et al., 2003) devoted to hybrids of the same region, the karyotype analysis indicated diploid level of all hybrids examined. We failed to reveal any triploidy among *R. esculenta* from Ukrainian Transcarpathians (our unpublished data).

Rybacki and Berger (2001) provided an important analysis of the distribution of triploids in Poland. The authors mentioned that the picture seen in the western and eastern parts of the country is similar to the distribution patterns found in the corresponding neighbouring areas. In Poland the highest triploid proportion was observed in the northwestern districts Szczecin and Gdańsk (23% and 21% among all water frogs examined, respectively). This region is close to northeastern Germany where triploids are especially numerous (up to 90%). In southern Poland, as in the Czech Republic and Slovakia, triploids are usually rare. In this respect the only exception was the Wrocław District (whith 11% of triploids). In the eastern districts of Poland, only a few triploids were found, and in the territory of the former Soviet Union triploid *R. esculenta* seems to be almost absent. However, Schröer (1996) found that 45% out of 50 individuals examined from eastern Poland, Białystok District, bordered with Byelorussia, proved to be triploids. We found no triploids in Byelorussia (Borkin et al., 1986; our unpublished data).

Thus, our finding of mass occurrence of triploids in Ukraine is quite surprising. This should be regarded as the first reliable record of triploid *R. esculenta* in eastern Europe in general, and in eastern Ukraine in particular. It is worth noting that these triploids are isolated from the nearest 3*n* localities approximately by the distances of 1000 (eastern Poland) and 1500 km (western Hungary).

Apart from numerous triploids, a tetraploid hybrid was revealed by us in 2003 in Verkhnii Gomolshanskii pond, Zmiyov District, Kharkov Province (Figs. 2 and 4; Tables 2 and 3). This throws new light on previous tetraploid record from the environs of Riga, Latvia, where a *R. esculenta* male with four chromosome sets was found among 32 animals (Borkin et al., 1979). Both localities are separated by a distance of about 1120 km. No case of natural tetraploidy in *R. esculenta* is known in other parts of Europe.

2. Methods of polyploidy identification

Various authors applied different techniques to check ploidy level in green frog samples (Table 5). To evaluate the reliability and importance of the published data, we give the brief comparison of methods.

Chromosome number. The chromosome counting, of course, provides direct evidence for any kind of polyploidy (e.g., Günther, 1970). However, this method would be quite labour-intensive to examine numerous samples containing tens or hundreds of animals.

Erythrocyte size. Since the mid 1970es, the cell size has been proposed as a useful tool for ploidy determination in green frogs. That cell size varies with ploidy level in amphibians has long been known. The difference is particularly marked in red blood cells (erythrocytes), and the size of these cells has been used previously to distinguish diploid and triploid ambystomes (Uzzell, 1963) and green frogs (Günther, 1975a; Uzzell and Berger, 1975). The positive correlation between erythrocyte size and ploidy level in green frogs has been confirmed by several authors because cell size data were in correspondence with the chromosome number, protein electrophoresis or nuclear DNA cytometry data (Uzzell et al., 1975; Günther, 1977; Ogielska-Nowak, 1978; Polls Pelaz and Graf, 1988; Plötner and Klinkhardt, 1992). Moreover, the cell size technique allowed to make air dried blood smears for tadpoles and frogs of various age, including postmetamorphosed froglets, by removing tips of tails or digits, respectively. Importantly, such an approach allows working with animals in the field and laboratory, without killing them. Therefore, it was no wonder that this very easy and cheap tool has been widely accepted as a good indicator of ploidy level, and the majority of triploidy records was evidenced by erythrocye size only (Table 5).

However, in young animals the difference in blood cells size between diploids and triploids was not so distinct (Ogielska-Nowak, 1978). Sometimes, different conditions during the cell drying process might result in cell parameters (Plötner and Klinkhardt, 1992). Later, Schröer (1996) argued that the comparison of erythrocyte size was inadequate to identify different ploidy level. Ogielska et al. (2001) found that the correlation

between erythrocyte area and DNA content was quite high ($r = 0.82$), however, large cells may have low DNA content, and vice versa. The authors concluded that the erythrocyte size is not always a good indicator of ploidy. Finally, results presented by Schmeller et al. (2001) supported significant influence of several factors (like body length, geographic location, organic matter of substratum, and relative amount of oxygen) on the erythrocyte size in hybrids. Even within single populations up to 16.7% of individuals may be wrongly affiliated to ploidy level. These authors agreed with Schröer's criticism that the cell planimetry is less suitable for ploidy determination. Taking into considerations such recent conclusions, we should use the literature data based on cell size only with some precaution. It is worth noting that cell size does not enable to distinguish triploids with different genome composition, LLR and LRR, respectively.

Morphometric indices. Some experienced authors used the external morphological characters expressed, for instance, by DP/CI and T/CI indices to distinguish diploid and triploid hybrids as well as both kinds of triploids because genome-dosage effects are seen in morphological features (e.g., Berger et al., 1978; Berger and Truszkowski, 1980; Fog, 1995; Rybacki and Berger, 2001). Indeed, for instance, Berger and Günther (1988) demonstrated that individuals with the same genome composition had similar indices in every study population, and those with different genome composition showed clearly marked differences. However, formerly, Günther (1975a, p. 157) stated that "among the triploid edible frogs there was a greater morphological overlap towards *lessonae* and *ridibunda* than among the diploids, although most of the 3*n* individuals could be included within the range of variability of the 2*n* animals." Later, Plötner and Klinkhardt (1992) postulated that a clear classification of triploids into LLR and LRR genotypes is not possible, and only slight differences exist between triploid and diploid *R. esculenta*. In fact, in several studies, morphological identification did not correspond with electrophoretic data, and geographic variation in external traits has been suggested (e.g., Hemmer, 1977; Regnier and Neveu, 1986; Günther et al., 1991). Rybacki (1995a, 1995b) provided the ranges of DP/CI and T/CI indices, with obvious overlap between diploid and triploid *R. esculenta*, as well as triploids with *R. ridibunda* (for LR and LLR also see Table 5 in Berger and Truszkowski, 1980).

According to Schröer (1997), the results of this method varied strongly in different populations, and increased overlap between *R. lessonae*, diploid and triploid *R. esculenta* was observed (Schröer, 1996). In contrast to progeny of triploids from Poland, marked overlap in morphometric characters (DP/CI against T/CI) between diploid hybrids and their parental species was evidenced for that from eastern Germany (Günther et al., 1979). Pagano and Joly (1999), based on specimens identified by allozyme analysis, found that morphological indices in *R. esculenta* and *R. ridibunda* widely overlapped, and most of the hybrids could not be distinguished from *R. ridibunda* using these indices. They asserted that morphometric identification might be far from being secure. In our studies performed by means of DNA flow cytometry, we also revealed that the ranges of indices in diploid *R. esculenta* and their parental species may overlap, and the identification of some individuals by morphometric characters may be confused (Lada et al., 1995; Borissovsky et al., 2000; Nekrasova and Morozov-Leonov, 2001).

It is widely accepted that, according to the gene-dosage effect, the LLR *esculenta* should be more similar to *R. lessonae*, while for LRR frogs, in contrary, a higher morphometric similarity to *R. ridibunda* can be expected. However, for triploids from some East German populations, this rule was contested (Plötner et al., 1994).

Protein electrophoresis. Some authors checked ploidy level in hybrid green frogs used the electrophoretical data. In accordance with the morphological traits, gene-dosage effects visible in more heavily stained albumin (or other proteins) bands indicate that such specimens were triploid. The technique also allows to recognize the genome composition in triploids, LLR or LRR. Curiously, a specimen of *R. esculenta* with diploid erythrocyte size displayed the triploid effect in the lactate dehydrogenase locus LDH-B (Günther and Hähnel, 1976), while several frogs with larger cells were diploid (Gubányi and Korsós, 1992). However, obvious gene-dosage effects in some triploids may be not revealed (Ebendal and Uzzell, 1982; Plötner and Klinkhardt, 1992; Mikulíček and Kotlík, 2001; see also Schröer, 1996). This gene dosage effect can be demonstrated by cellogel or polyacrylamide gel electrophoresis, but is not always clear with strach gel electrophoresis (Hemmer et al., 1980). This technique is quite labor-intensive to treat many large samples, and sometimes, the data interpretation may be not simple. Only few authors applied the method to recognize triploidy (e.g., Blommers-Schlösser, 1990; Tunner and Heppich-Tunner, 1992).

Genome size. The first papers based on the nuclear DNA content (genome size) in green frogs were published in late 1970s (Tunner and Dobrowsky, 1976; Ogielska-Nowak, 1978; Mazin and Borkin, 1979). Several cytophotometric techniques exist, like microdensitometry with staining nuclei by Feulgen method (Ogielska-Nowak, 1978; Hemmer et al., 1980; Konrad et al., 1980) or microfluorimetry with use of fluorescent Schiff

reagent (Mazin and Borkin, 1979; Ogielska et al., 2001), DNA flow cytometry (our studies since 1985). Currently, this is the most reliable method which can provide relatively quickly the next data set about each specimen: 1) species identification, 2) ploidy level, 3) genome composition in hybrids, 4) type of gametes (spermatozoa) in terms of ploidy and genome composition. The application of this method can allow to solve various problems of population genetics, gametogenesis, etc., associated with the *R. esculenta* complex. However, the laboratory equipment (the cytophotometer with computers) is quite expensive, and the work needs a good experience. The future progress of the approach will be associated with the development of cheaper portable cytophotometer and taking blood cells without animal killing.

Bioacoustic approach. Some differences in mating call were found between diploid and triploid *R. esculenta* from Austria; triploidy was evidenced from plasma albumin staining intensity (Schneider and Tunner, 1982).

Some conclusion can be drawn from these brief comments. External morphology, erythrocyte size, and protein electrophoresis, certainly, can indicate the existence of triploidy among green frog populations. However, real data, based on indices or cell size in particular, may be biased. In fact, karyotype and genome size analyses are more appropriate for exact ploidy determination (Schmeller et al., 2001). The application of DNA flow cytometry, being the most precise technique, may provide new opportunities for the study on green frogs, especially in connection with other methods. Frankly speaking, any choice of approach should be adequate to the problem, which should be solved.

We agreed with Ogielska et al. (2001) that the problem of ploidy is more complicated than it was assumed, and we cannot rule out the existence of aneuploids or mosaics among the hybrids (Berger and Ogielska, 1994). Another serious problem is the instability or irregularities in clonal inheritance mechanisms, which, probably, occur more frequently in populations with triploids (Günther, 1975b; Uzzell et al., 1977; Vinogradov et al., 1990).

3. Mass occurrence of triploidy in eastern Ukraine

Based mainly on erythrocyte size measurements, Günther (1975a) calculated that in the northeastern part of Germany, the share of triploids varied between 15% and 83% among all hybrids ($2n + 3n$ together) in populations. As a whole, the percentage of triploids was equal to about 39% out of *R. esculenta* (which was examined in the region). Numerous triploids (62 – 100%, recalculated by us) were identified by Berger and Günther (1988) among hybrids collected near Serrahn, Mecklenburg, with sample size between 6 and 88 individuals (100% hybrid occurrence was registered in the smallest sample). Using various techniques, including chromosome counting, Plötner and Klinkhardt (1992) found that triploidy reached about 61% among 46 frogs taken from artificial forest pools in northern Germany as well. Hemmer (1977) and Eikhorst (1981) reported that triploids were very common in some localities in northwestern Germany (between 21.5% and 83%).

In a pure *R. esculenta* population existed in the south of the Netherlands, triploids were about 64% (Blommers-Schlösser, 1990). In France triploid hybrids of the LLR composition reached 19% in a population with *R. lessonae* (Polls Pelaz and Graf, 1989).

In central Europe, the frequency of triploid *R. esculenta* obviously decreases from northern Germany to the south and to the east (Rybacki and Berger, 2001:Fig. 2). For instance, in the westernmost part of Slovalia, 3*n* frogs composed 4 – 12% out of hybrids (recalculated by us from Table 1; Mikulíček and Kotlík, 2001). High incidence of triploids in northeast Poland (58 – 84%) reported by Schröer (1996) was disputed by Rybacki and Berger (2001). However, in western Hungary, 50 triploids, or 96%, were detected electrophoretically and karyologically among 52 hybrids (Tunner and Heppich-Tunner, 1992).

In eastern Ukraine, among all hybrids, the share of triploid individuals was equal to 36% (Table 2), ranging from 20% to 76% in localities (Table 4). In two small samples (Novokondrashovka and Stanichno-Luganskoe, with 3 and 2 hybrids, respectively), they composed 100%. Therefore, the triploid portion in east-Ukrainian populations was similar to that in northern Germany or Hungary. Thus, the trend of decreasing occurrence of triploids did not correct for eastern Ukraine. Nevertheless, such a mass occurrence in the region is quite surprising because we failed to reveal the triplody occurrence in adjacent territories of Russia despite of our extensive screening frog populations in the Central Chernozem Territory and in Volga River Basin (Lada et al., 1995; Borissovsky et al., 2000, 2001; Borkin et al., 2003a, 2003b).

4. Triploidy and population systems

In his remarkable paper about the triploidy, Günther (1975a) reported on the occurrence of triploid hybrids in several types of population systems. These were so-called "pure" E type consisting of *R. esculenta* only, i.e., without any parental species, the L-E type (*e-l* and *l-e* of Günther), where hybrids co-existed with *R. lessonae*, as well as the R-E type (*e-r*) consisting of hybrids and *R. ridibunda*.

It should be noted that Günther (1975a) proposed a detailed classification of population systems, which re-

flected the genome structure, sex ratio, and relative frequencies of species in mixed populations. In contrast to this paper, we use more simplified designation of population systems based on the species existence only. In these systems, the portion of triploids was equal to 20 – 83% (*E*), 15 – 43% (L-E), and 41% (R-E).

Two other localities with high share of triploids in northern Germany were occupied by the pure E type of population systems (Eikhorst, 1981; Plötner and Klinkhardt, 1992). However, in Hungary high incidence of triploidy was associated with the R-E type (Tunner and Heppich-Tunner, 1992).

In France (Polls Pelaz and Graf, 1989), the RRL triploidy was identified in the L-E type.

In Poland (Rybacki and Berger, 2001, Table 2), triploids are known from various kinds of the E, L-E, and R-E types, as well as, rarely, in the L-E-R type of population systems.

In eastern Ukraine, triploids were revealed in three types of population systems. These were the E type (60%), the R-E type (27 – 76%, and 100% in two samples of small size), as well as the L-E-R type (17 – 55%). Thus, unlike in northern Germany, triploids under the study did not reach the highest frequency in pure hybrid populations. Moreover, triploids were recorded from the mixed population system of the L-E-R type, where all members of the *R. esculenta* complex exist together. Interestingly, this complicated system is very rare in western and central Europe. However, the system is widely distributed in the Central Chernozem Territory and Volga River Basin in Russia (Lada et al., 1995; Borissovsky et al., 2001; Borkin et al., 2003a). Nevertheless, unlike in Ukraine, we failed to find any triploids in these regions.

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