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Intraspecific and Interspecific Genome Size Variation in Hynobiid Salamanders of Russia and Kazakhstan: Determination by Flow Cytometry

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Abstract. - The amount of DNA per diploid nucleus in *Salamandrella keyserlingii* and *Onychodactylus fischeri* from Russia, as well as in *Ranodon sibiricus* from Kazakhstan was determined by flow cytometry. *Onychodactylus fischer*i had the highest genome size, *Ranodon sibiricus* the lowest, and *Salamandrella keyserlingii* was intermediate. Obvious geographic variation in genome size was revealed for *Salamandrella keyserlingii* and *Ranodon sibiricus*. The comparison of nuclear DNA content in eleven hynobiid species supported the genus *Onychodactylus* as a separate lineage.

Key words. - nuclear DNA content, genome size, flow cytometry, Caudata, Hynobiidae, *Onychodactylus fischeri*, *Salamandrella keyserlingii*, *Ranodon sibiricus*, Russia, Kazakhstan.

Introduction

Diploid genome size, measured in picograms (pg; 1 pg = 10^{-12} g) of DNA per nucleus, varies widely in vertebrates. Living amphibians represent the largest range of genome size variability among terrestrial vertebrates, with a minimum of 1.7 pg in male frogs of *Eleutherodactylus shrevei* (Schmid et al., 2002) and maximum of 241 pg in *Necturus lewisi* (Olmo, 1973). Some extinct amphibians may have had maximum DNA contents greater (approximately 300 pg) than the maximum known in living forms (Thoson and Muraszko, 1978).

Various methods are used for the measuring of genome size in amphibians (for example, biochemical analysis, Feulgen densitometry, static cell fluorometry, ultraviolet microscopy, etc.). However, modern studies are based on a comparatively new and very precise method of flow DNA cytometry. As a rule, standard errors of this method (peak mean ratios) are less than 0.5% (Rosanov and Vinogradov, 1998). Among approximately 415 amphibian species examined, nuclear DNA contents were studied by flow cytometry in about 143 species only (Gregory, 2001a).

Among some specific applications, DNA flow cytometry has been used to examine ploidy levels (Borkin et al., 1986, 1996, 2001a; Vinogradov et al., 1990; Sharbel et al., 1997; Litvinchuk et al., 1998, 2001), to identify morphologically similar species (Sharbel et al., 1995; MacCulloch et al., 1996; Litvinchuk et al., 1997, 1999; Murphy et al., 1997; Borkin et al., 2001b, 2003; Khalturin et al., 2003), and to search for hybrid individuals (Borkin et al., 1987, 2002; Litvinchuk et al., 2003). Many authors have noted a relationship between genome size and some biological parameters, such as cellular and nuclear sizes, replication time, cell-cycle length, cell division rate, metabolic rate, longevity, morphological complexity in the brain, etc. (Van't Hoff and Sparrow, 1963; Goin et al., 1968; Olmo and Morescalchi, 1975; Bachmann and Nishioka, 1978; Sessions and Larson, 1987; Shakhbazov and Gapchenko, 1990; Licht and Lowcock, 1991; Nevo and Beiles, 1991; Roth et al., 1994; Vinogradov, 1999; Gregory, 2001b, 2001c; Griffith et al., 2003). However, an adaptive value of genome size is most clearly shown in its relationship with the timing of embryonic development in amphibians (Bachmann, 1972; Oeldorf et al., 1978; Horner and Macgregor, 1983; Pagel and Jonstone, 1992; Jockusch, 1997; Chipman et al., 2001; Gregory, 2002, 2003).

This research is focused on salamanders of the family Hynobiidae, which consists of approximately 42 species. Among modern amphibians, hynobiids are recognized to be one of the primitive urodelans (e.g., Duellman and Trueb, 1986; Larson and Dimmick, 1993). Hynobiids inhabit various mountain regions of Palaearctic Asia, where they have quite restricted ranges. The territory of Russia and Kazakhstan are only inhabited by three hynobiid species. Other family members, such as the Siberian Salamander (*Salamandrella keyserlingii*), is widely distributed from Northeastern European Russia to the Kamchatka Peninsula. The Ussuri Clawed Salamander (*Onychodactylus fischeri*) can be found in the southern part of Russian Far East and in the Korean Peninsula. The Semirechensk salamander (*Ranodon sibiricus*) inhabits the Dzhungarsky Alatau

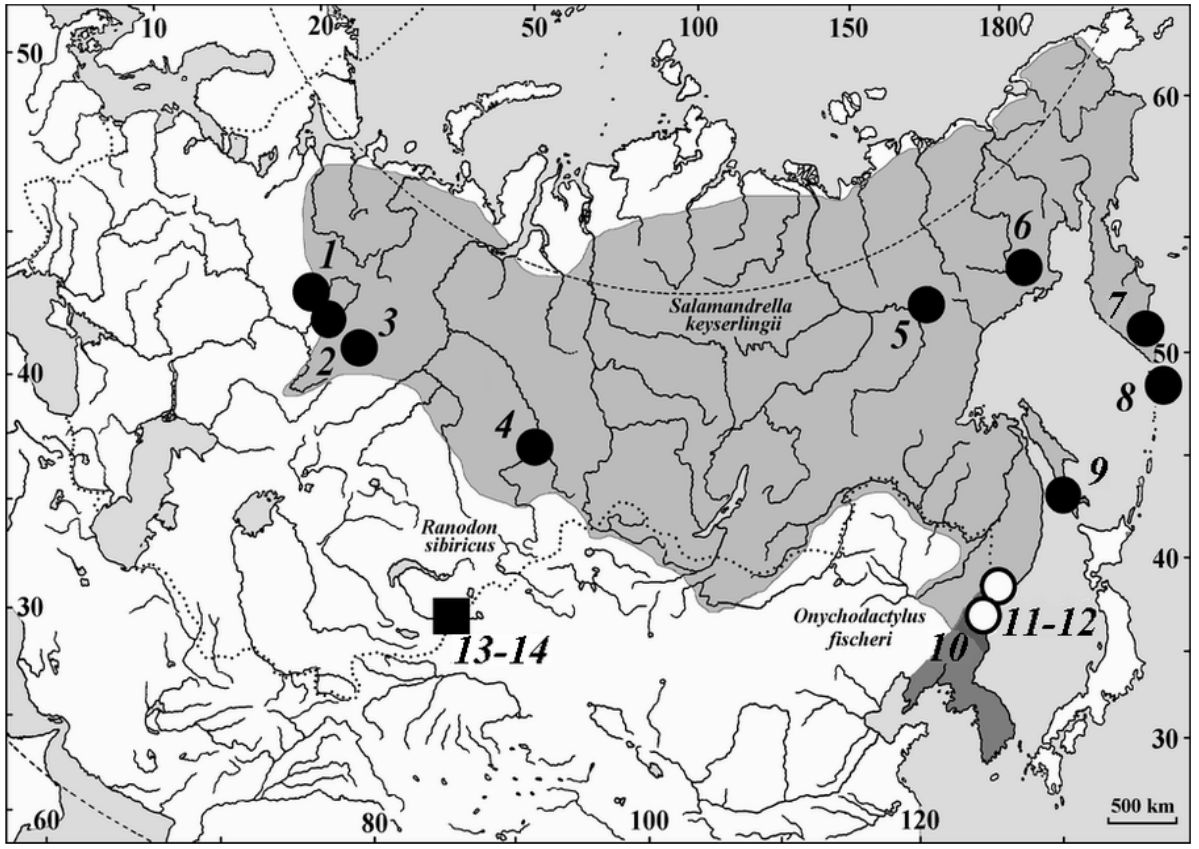

Figure 1. Distribution of *Salamandrella keyserlingii* (grayish area), *Ranodon sibiricus* (dark square), and *Onychodactylus fischeri* (dark-grayish area), with localities studied (locality number as in Table 1). Dark circles designate the "Euro-Siberian" type of *Salamandrella keyserlingii*, white ones - "Primorsky" type.

Mountains in southeastern Kazakhstan and adjacent China. The last two species are included in the Russian and Kazakhstan Red Data Books, respectively. *Ranodon sibiricus* is also included in the IUCN Red List of Threatened Animals under the category "vulnerable" (Borkin, 1998; Kuzmin, 1999). The goal of present paper is to evaluate the intra- and interspecific genome size variation in three hynobiid species of Russia and Kazakhstan, with flow cytometry.

Materials and methods

Eighty-eight metamorphosed specimens from 13 populations of three species distributed in Russia and Kazakhstan were used in this study of genome size variation. Blood was taken from the clipped tail tip. The samples of *Salamandrella keyserlingii* and *Onychodactylus fischeri* studied are kept at the collections of the Department of Herpetology, Zoological Institute, Russian Academy of Sciences. All specimens of *Ranodon sibiricus* were obtained from the Almaty Zoo (Kazakhstan), and after preparation of blood samples, the live salamanders were sent to the Moscow Zoo.

Peripheral blood cells of specimens of the Ribbed Newt, *Pleurodeles waltl*, received from Prof. J. C. Lacroix (Université Paris VI, Paris, France) were used as a reference standard. Details of the technique have been published previously (Vinogradov et al., 1990; Rosanov and Vinogradov, 1998; Borkin et al., 2001). The relative genome size differences (RD) were calculated with use of formula: RD=(m1-m2)/(m1+m2) × 200%, where m1 and m2 are the sample means.

Results

The genome size variation in three hynobiid species varied between 54.5 and 109.0 pg (Table 1). The lowest nuclear DNA content was recorded in *Ranodon sibiricus* (in average 55.4 pg), whereas the highest was in *Onychodactylus fischeri* (107.7 pg). *Salamandrella keyserlingii* was intermediate in size (66.4 pg).

Differences between populations were studied in two species, represented by more than one sample. In *S. keyserlingii*, ten samples were allocated to two main, distinct groups with different genome sizes (Table 1 and Fig. 1). The geographic distribution of these two groups

Table 1. Locality of origin ("Lat" is latitude, and "Lon" is longitude), sample size, genome size (in picograms; SD is standard deviation) with the coefficient of variation (CV; in percents) for 13 populations of three hynobiid species.

Province/Region	Locality	Lat	Lon	n	Mean	SD	Range	CV
***Salamandrella keyserlingii* (Russia)**								
The "Euro-Siberian" group								
1 Nizhny Novgorod	Pizhma	57°52'	47°05'	5	66.7	0.2	66.5 - 66.9	0.3
2 Udmurtia	Chur	57°06'	52°59'	10	67.5	0.4	66.7 - 68.1	0.6
3 Ekaterinburg	Ekaterinburg	56°51'	60°43'	15	66.6	0.5	65.5 - 67.7	0.8
4 Tomsk	Tomsk	56°31'	84°58'	11	66.6	0.6	66.0 - 68.0	0.9
5 Yakutia	Khandyga	62°39'	135°33'	2	67.8		67.5 - 68.0	
6 Kamchatka	Petropavlovsk	53°02'	158°38'	1	66.8			
7 Kurile Islands	Paramushir Island	50°41'	156°07'	2	67.1		66.8 - 67.4	
8 Sakhalin Island	Aniva	46°43'	142°31'	2	66.5		66.3 - 66.6	
TOTAL (for the group)				48	66.9	0.6	65.5 - 68.1	0.9
The "Primorsky" type								
9 Primorsky	Kedrovaya Pad' Reserve	43°07'	131°32'	11	64.8	0.3	64.4 - 65.2	0.4
10 Primorsky	Ussuriysk Reserve	43°38'	132°09'	2	64.8		64.7 - 64.8	
	TOTAL (for the group)			13	64.8	0.2	64.4 - 65.2	0.4
	TOTAL (for the species)			61	66.4	1.0	64.4 - 68.1	1.5
Onychodactylus fischeri (Russia)								
11 Primorsky	Ussuriysk Reserve	43°38'	132°09'	7	107.7	0.7	106.7 - 109.0	0.7
***Ranodon sibiricus* (Kazakhstan)**								
12 Taldy-Kurgan	Oy-Saz River	44°51'	79°02'	10	54.8	0.2	54.5 - 55.1	0.3
13 Taldy-Kurgan	Borokhudzir River	44°30'	79°28'	10	56.0	0.6	55.1 - 56.7	1.0
	TOTAL			20	55.4	0.7	54.5 - 56.7	1.3

Table 2. The genome size (GS; in picograms) of hynobiids, referred by some authors.

Species	Locality	GS	Reference
Hynobius (Hynobius) dunni	unknown	33.76[2]	Olmo, 1973
H. (H.) nebulosus	unknown	38.40[2]	Olmo, 1973
H. (H.) tsuensis	unknown	32.96[2]	Olmo, 1973
H. (Satobius) retardatus	unknown	38.31[2]	Olmo, 1973
H. (Pseudosalamandra) naevis	unknown	40.90[2]	Olmo, 1973
Onychodactylus fischeri	Ussuriysk Reserve	45.5[2]	Mazin, 1978
	-//-	95.08[1]	Vinogradov, 1998
	-//-	107.7[1]	Present paper
O. japonicus	unknown	102-106[2]	Olmo, 1983
Paradactylodon gorganensis	Shirabad cave	34.77[1]	Stöck, 1999
P. mustersi	unknown	43.3[2]	Morescalchi et al., 1979
Ranodon sibiricus	Tekeli	50.7[2]	Morescalchi et al., 1979
	-//-	45.591[1]	Vinogradov, 1998
	2 localities	55.4[1]	Present paper
Salamandrella keyserlingii	Yakutia	42.5[2]	Mazin, 1978
	Ekaterinburg	42.3[2]	Morescalchi et al., 1979
	unknown	38[2]	Grafodatsky and Grigoriev, 1982
	Ekaterinburg	33.2[3]	Vladychenskaya et al., 1988
	-//-	55.481[1]	Vinogradov, 1998
	10 localities	66.41[1]	Present paper

[1]Flow cytometry; [2]Feulgen's densitometry; [3]Kinetics reassociation.

Table 3. Genome size (2C), range of egg diameters (mm), and time of embryonic ("Embr.") and larval ("Larv.") development (days) in some hynobiids.

Taxon	2C	Reference	Egg	Embr.	Larvae	Reference
Hynobius dunni	33.8	Olmo, 1973	2.0	-	-	Thorn, 1969
H. nebulosus	38.4	Olmo, 1973	2.6-5.0	25-30[3]	138-274[1]	Thorn, 1969
H. tsuensis	33.0	Olmo, 1973	4.0	-	90-150[1]	Thorn, 1969
H. retardatus	38.3	Olmo, 1973	2.3-3.1	21-28[2]	80-720[2]	Sasaki, 1924; Mikamo, 1956
H. naevis	40.9	Olmo, 1973	5.0-5.3	8-41[1]	120[1]	Thorn, 1969
Onychodactylus fischeri	107.7	Present paper	5.0-8.0	360[2]	780-1500[2]	Smirina et al., 1994; Griffin and Solkin, 1995; Kuzmin, 1995
O. japonicus	104.0	Olmo, 1983	4.5-5.4	120-150[3]	~1000	Iwasawa and Kera, 1980; Hayase and Yamane, 1982
Paradactylodon gorganensis	34.8	Stöck, 1999	-	-	>360[2]	Stöck, 1999
P. mustersi	43.3	Morescalchi et al., 1979	-	-	>360[2]	Reilly, 1983
Ranodon sibiricus	55.4	Present paper	3.0-5.0	21-42[2]	230-720[2]	Lebedkina, 1964; Brushko, Narbaeva, 1988; our data
Salamandrella keyserlingii	66.4	Present paper	2.1-3.5	14[4]	78-110[1]	Ishchenko et al., 1995a,b; Berman, 1996

[1]Laboratory conditions (temperature unknown); [2]nature conditions; [3]t = 10°; [4]t = 16°.

Table 4. Genome size (2C), diploid number of chromosomes (2n) and number of uniarmed macrochromosomes (UM) in some hynobiids.

Taxon	2C	Reference	2n	UM	Reference
Hynobius dunni	33.8	Olmo, 1973	56	0	Morescalchi et al., 1979; Seto et al., 1986
H. nebulosus	38.4	Olmo, 1973	56	0-2	Seto et al., 1986; Kuro-o et al., 1987
H. tsuensis	33.0	Olmo, 1973	56	0	Seto et al., 1986
H. retardatus	38.3	Olmo, 1973	40	0	Azumi and Sasaki, 1971; Kuro-o et al., 1987
H. naevis	40.9	Olmo, 1973	58	0	Kohno et al., 1987
Onychodactylus fischeri	107.7	Present paper	78	0	Iizuka and Yazawa, 1994
O. japonicus	104.0	Olmo, 1983	78	0	Morescalchi et al., 1979; Kohno et al., 1991
Paradactylodon gorganensis	34.8	Stöck, 1999	62	0	Stöck, 1999
P. mustersi	43.3	Morescalchi et al., 1979	62	10	Morescalchi et al., 1979
Ranodon sibiricus	55.4	Present paper	66	18	Morescalchi et al., 1979
Salamandrella keyserlingii	66.4	Present paper	62	26	Kohno et al., 1991

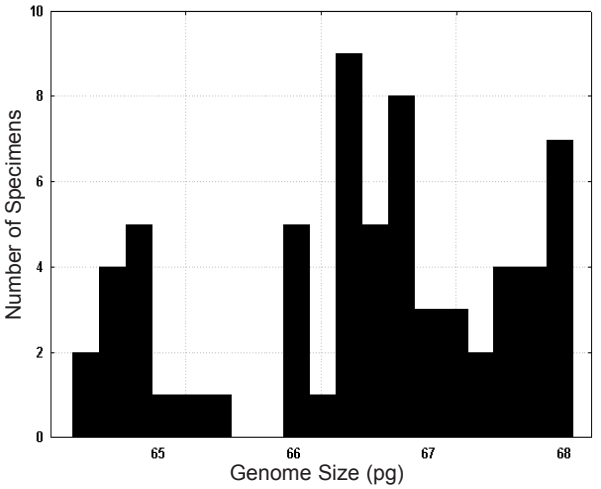

Figure 2. Genome size distribution in *Salamandrella keyserlingii.*

of samples proved to not be chaotic and the "Euro-Siberian" (8 samples) and "Primorsky" (2 samples) groups were recognized. The data ranges of the groups did not overlap, and the gap between both groups was equal to 0.3 pg (Fig. 2). The "Primorsky" samples were characterized by smaller genome size in comparison with the "Euro-Siberian" ones: 64.4-65.2 pg vs. 65.5-68.1 pg; the means were 64.8 pg vs. 66.9 pg. The differences (RD) between means were equal to 3.2%, and were observed in five separate comparisons. Therefore, these groups were shaped both by genome size and geographically.

The samples of *R. sibiricus* were taken from two semi-isolated populations from upper parts of Borokhudzir and Oy-Saz rivers. Both samples demonstrated different genome sizes (Table 1). The ranges of genome size values in these two samples slightly overlapped. The Oy-Saz sample was characterized by smaller genome size in comparison with the Borokhudzir sample: 54.5-55.1 pg vs. 55.1-56.7 pg; the means are 54.8 pg vs. 56.0 pg. The differences (RD) between means were equal to 2.2%.

The coefficient of variation (CV) ranged between 0.3% and 0.9% in *S. keyserlingii*, between 0.3% and 1.0% in *R. sibiricus*, and was equal to 0.7% in the sample of *O. fischeri* (Table 1). The overall within-species genome size variation in *S. keyserlingii* and *R. sibiricus* were quite similar (1.5% and 1.3%, respectively). Among the "Euro-Siberian" samples of *S. keyserlingii*, the CVs ranged between 0.3% (Nizhny Novgorod Province) and 0.9% (Tomsk Province), whereas it was equal to 0.4% in the "Primorsky" samples of *S. keyserlingii*.

Discussion

1) Genome size values in hynobiids: literature data

Presently, the nuclear DNA content has been determined for eleven hynobiid species. However, among them, only four species were examined through flow cytometry (Table 2). The first data collected about genome size of nine hynobiid species was obtained by Italian researchers with an application of Feulgen densitometry (Olmo, 1973, 1983; Olmo and Morescalchi, 1975; Morescalchi et al., 1979). According to these data, genome size in hynobiids ranged from 32.96 pg in *Hynobius tsuensis* to 102-106 pg in *Onychodactylus japonicus* (Table 2). According to Mazin (1978), the nuclear DNA content in two Russian species, *Onychodactylus fischeri* and *Salamandrella* ("*Hynobius*") *keyserlingii*, measured by Feulgen densitometry, were similar to each other (45.5 and 42.5 pg, respectively). Grafodatsky and Grigoriev (1982) estimated genome size of *Salamandrella keyserlingii* (38 pg), perhaps, by means of Feulgen densitometry. Vladychenskaya et al. (1988) studied kinetics of DNA reassociation; they found that the nuclear DNA content of *Salamandrella keyserlingii* was equal to 33.2 pg. Using DNA flow cytometry, Vinogradov (1998) estimated genome size values as 95.08 pg for *Onychodactylus fischeri*, 45.59 pg for *Ranodon sibiricus*, and 55.48 pg for *Salamandrella keyserlingii*. Finally, Stöck (1999) determined that genome size of *Batrachuperus gorganensis* is 34.77 pg, using DNA flow cytometry as well.

The literature values of genome size for *Onychodactylus fischeri*, *Ranodon sibiricus*, and *Salamandrella keyserlingii* expressed in absolute units vary sometimes more than two-fold (ranges are 45.5-95.1 pg, 45.6-50.7 pg, and 33.2-55.5 pg, respectively). Therefore, the comparison of data provided by various authors should be made cautiously. The contradictions may be explained by an application of different techniques (Feulgen densitometry, kinetics reassociation, and flow cytometry), dyes, and laboratory conditions (cell preparation methods, devices for measurements, types of reference cell standards, etc.). It has been shown that genome size measured with fluorochromes of different nucleotide specificity may differ markedly (e.g., Johnston et al., 1987; Birshtein et al., 1993; Vinogradov and Borkin, 1993). For instance, the determination of genome size by means of flow cytometry for cell samples of *Salamandrella keyserlingii* stained with olivomycin and Hoechst (GC- and AT-specific fluorochromes, respectively) provided 6.68 and 4.77 arbitrary units (*Rana temporaria* was taken as an internal reference; Vinogradov, 1998). To exclude the influence

of AT/GC-structure, it is necessary to use ethidium bromide or propidium iodide (Vinogradov and Borkin, 1993), which were used in our research.

To convert genome size from the relative units to picograms, it is necessary to have data about genome size of reference cells. Such data should be obtained without using stains. Unfortunately, the data available today, mentioned by various authors, do not correspond to each other. For instance, Vinogradov (1998) reported a genome size of reference standard *Mus musculus* to be 6.5 pg. Our estimations of genome size of some mammals (*Homo sapiens*, *Mus musculus*, *Rattus norvegicus*) were the closest to that mentioned by Bianchi et al. (1983). In our work, we used the genome size of males of *Mus musculus* (C57B1) as a basic reference standard with value of 6.8 pg. Other authors preferred other reference standards, which have different base-pair-specificity of some stains widely used in flow cytometry. Vinogradov and Borkin (1993) listed the AT- and GC-pair specific DNA contents (CAT and CGC) for many species of amphibians. For instance, CAT/CGC was equal to 1.42 in *Xenopus laevis* (Mazin's reference standard), and 1.00 in *Rana lessonae* (= *Rana* "*esculenta*"; Olmo's and Morescalchi's standard).

The estimations of Italian authors (Olmo, 1973, 1983; Morescalchi et al., 1979) and our genome size values for *Ranodon sibiricus* (50.7 and 54.5-56.7 pg, respectively) and members of genus *Onychodactylus* (102.0-106.0 and 106.7-109.0 pg, respectively) are quite similar. However, data for *Salamandrella keyserlingii* (42.3 and 64.4-68.1 pg, respectively) are in obvious discordance. The genome size of *Onychodactylus fischeri*, *Ranodon sibiricus*, and *Salamandrella keyserlingii* mentioned by Vinogradov (1998), was lower than our values, who used other stains and lower genome size estimation of basic reference standard.

Unfortunately, some authors did not supply any information about sample sizes and localities. However, some differences in genome size might be influenced by intra-population and geographic variation as well.

2) Within-species variation

Some authors discussed the levels of intraspecific variation in genome size. We recognized two kinds of such a variation; namely, the "within-population" variation and "between-population" (or geographical) variation.

A) *Within Population Variation*

Among amphibians, the greatest intrapopulational variation (CV = 7.5%, the data of Licht and Lowcock, 1991 were recalculated by us) was recorded for the Western Red-back Salamander (*Plethodon vehiculum*). However, the variation in other amphibian species was considerably lower (Licht and Lowcock, 1991; MacCulloch et al., 1996; Murphy et al., 1997; Lizana et al., 2000). The variation within populations of three hynobiid species (CVs were 0.3-1.0%, mean was 0.67 ± 0.12%) was quite similar to that in salamandrids (range is 0.1-1.7%, mean is 0.64 ± 0.03%, 99 populations studied), pelobatids, and other anurans studied in our laboratory at the same conditions (Litvinchuk et al., 1997, 1999, 2001a,b, 2003; Rosanov and Vinogradov, 1998; Borkin et al., 2001b, 2003; our data).

Sexual dimorphism in genome size has been registered in some amphibian species (Schmid et al., 2002; our data). Unfortunately, in the hynobiids examined by us, sexual differences are not expressed in external characters beyond the breeding time. Our study was based mostly on juvenile and non-breeding adult animals, and, therefore, we failed to reliably identify the sex without anatomical dissections.

B) *Geographical Variation*

The significant geographical variation in genome size was revealed for several amphibian species (Licht and Lowcock, 1991; Murphy et al., 1997; Litvinchuk et al., 1999, 2001b). In a few cases, differences (RD) exceeded 8%. However, in the majority of species studied, such differences were about 1% (Licht and Lowckock, 1991; Borkin et al., 1997, 2000, 2001, 2003; Litvinchuk et al., 1997, 1999, 2001b, 2003; our data). In *Salamandrella keyserlingii*, the maximum genome size difference (RD = 4.5%) was found between samples from the Khandyga (Yakutia Republic) and Kedrovaya Pad' Reserve (Primorsky Territory). The average differences (RD) between the "Euro-Siberian" and the "Primorsky" sample groups of the species were equal to 3.2%.

3) Interspecies differences: developmental and karyological correlations

Eleven species of the family Hynobiidae may be divided into two groups by their genome size. Two species of the genus *Onychodactylus* form a group with the largest genomes (104-108 pg). They also have the longest embryonic and larval development periods (Table 3), as well as the greatest number of chromosomes (Table 4).

Another group includes the remaining nine species (33-67 pg). Among them, *Salamandrella keyserlingii* has the largest genome size, and the seven species from the genera *Hynobius* and *Batrachuperus* have smaller sizes. *Ranodon sibiricus* has an intermediate genome size. Such a distribution of genome sizes in the second group does not seem to be associated with ovum diameter, and, perhaps, with time of embryonic and larval development (Table 3).

The comparison of genome size values in the second group with karyological data evidenced no signifi-

cant relations between the nuclear DNA content and diploid chromosome numbers (Table 4). Nevertheless, we found the positive correlation (r = 0.9998) between genome size and uniarmed macrochromosome numbers.

4) Intergeneric relationships

Based on recent studies (Fei and Ye, 2000a; Fu et al., 2001), approximately eight or ten genera of the hynobiids could be recognized. The most speciose genus, *Hynobius,* consists of about 24 species, which may be arranged into three subgenera (Matsui et al., 1992; Mizuno et al., 1995; Borkin, 1999), namely *Pseudosalamandra* (7 species from Japan and Taiwan), *Satobius* (1 species from Hokkaido Island), and *Hynobius* (11 species from Japan, and, perhaps, 5 species from Korea and China). The family also includes the genera *Liua* (1 species), *Onychodactylus* (2 species), *Pachyhynobius* (1 species), *Protohynobius* (1 species), *Pseudohynobius* (2 species), *Ranodon* (1 species), *Salamandrella* (1 species, which penetrates to eastern Europe), and *Batrachuperus* (sensu lato) (about 9 species). The taxonomic position of *Ranodon*, *Liua*, and *Pseudohynobius* was discussed (Fei and Ye, 2000b; Kuzmin and Thiesmeier, 2001). Based on mitochondrial DNA study, T. Papenfuss (Pers. comm. in Fu et al., 2001) showed that the genus *Batrachuperus* is paraphyletic, and consists of two groups with distinct geographic distributions. The Eastern (Chinese) group belongs to *Batrachuperus* (sensu stricto) and consists of about six species (Fei and Ye, 2000b; Fu et al., 2001; Song et al., 2001). The western (Iran and Afganistan) group includes two or three species (Stöck, 1999), and may be allocated to *Paradactylodon*.

Various authors suggested several configurations of evolutionary relationships among hynobiids. For instance, based on reproductive biology characters, Thorn (1969) proposed to recognize two families: Ranodontidae, with the genus *Ranodon* only, and Hynobiidae, with remaining genera. Using a set of morphological and biological data, Zhao and Hu (1984) separated two "natural" groups among Chinese species: the *Hynobius* group with predominantly terrestrial species (*Hynobius* and *Salamandrella*), and the *Ranodon* group with predominantly aquatic inhabitants (*Ranodon*, *Onychodactylus*, *Batrachuperus*, and *Liua*). Later, Zhao and Zhang (1985) assigned the genus *Pachyhynobius* to a third group. Combining morphological characters and mitochondrial DNA data, Larson and Dimmick (1993) found the closest relationships between the genera *Salamandrella* and *Hynobius*, thus confirming the traditional acceptance of the similarity of these taxa. However, the genus *Onychodactylus* was more closely related to that lineage, whereas *Batrachuperus* (the eastern group) proved to be more distant. Recently, Fei and Ye (2000a) have erected a new subfamily for the newly discovered *Protohynobius puxiongensis*, whereas all other hynobiids were allocated to another subfamily.

Genome size data are not in agreement with all these suggestions, which did not recognize separate position of the genus *Onychodactylus*. Apart from the largest genome size, distincton of the genus was also supported by morphological and karyological data (Morescalchi et al., 1979; Olmo, 1983; Kohno et al., 1991; Iizuka, Yazawa, 1994; Kuro-o et al., 2000; Litvinchuk and Borkin, 2003).

5) Taxonomic considerations

Based on the intraspecific analysis, Bassarukin and Borkin (1984), and Borkin (1994) outlined the peculiarities of *Salamandrella keyserlingii* from the southern part of the Russian Far East. Moreover, these authors suggested that local populations would be considered as a geographic race of the species (the species type territory is the Kultuk Village, Baikal Lake, Irkutsk Province, Russia; restricted by Borkin, 1994). Indeed, the "Euro-Siberian" and "Primorsky" groups of populations are different in allozymes (Litvinchuk et al., 2001a), in some morphological characters (Ostashko, 1981; Bassarukin and Borkin, 1984; Borkin, 1994; Litvinchuk and Borkin, 2003), in breeding sites (Kuzmin, 1990), and in the shape of egg sacs and time of larval development (Korotkov, 1977; Bassarukin and Borkin, 1984; Sapozhnikov, 1990; Vorobyeva et al., 1999). Therefore, geographic differences in genome size revealed by us are in concert with differences in other characters. Based on a concordance of various characters, including genome size, we would recognize a distinct status of the "Primorsky" samples, at least, of a subspecific rank. Formerly, Nikolsky (1906) and Dybowski (1928) have coined the names *Salamandrella keyserlingii* var. tridactyla and *Salamandrella keyserlingii* var. kalinowskiana, respectively, for animals from the Russian Far East. Obviously, the first name has the priority. Therefore, populations from Russian Far East (Primorsky Territory) should be named *Salamandrella keyserlingii tridactyla* Nikolsky, 1906.

The range of *Ranodon sibiricus* is quite limited. Nevertheless, it consists of several semi-isolated areas (Brushko et al., 1988; Kuzmin et al., 1998; our data). The populations from the upper parts of Borokhudzir and Oy-Saz Rivers are separated from each other by a mountain range. Therefore, it is not surprising that the genome size difference (RD) between them is relatively large (2.2%). However, geographical variation in the species is poorly studied, and so the taxonomic value of that difference is still unclear.

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