and 2. that they are exceptional external parasiticides with a potential as animal systemic insecticides applied by dermal, oral or parenteral routes of administration.

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Amounts of nuclear DNA in anurans of the USSR

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Summary. The amounts of nuclear DNA in blood erythrocytes of 18 species of Anura from the USSR have been determined to be in the range of 4.0-20.6 pg (10^{-12} g). Brown frogs of genus *Rana* have lower mean genome sizes than green frogs. Palaearctic Anura, as a whole, have a greater content of nuclear DNA than the species of the same families from regions further south.

Only 22 species of about 2000 living anurans inhabit the USSR (3 species of *Bombina,* 2 of *Hyla,* 4 of *Bufo,* 2 of *Pelobates,* 1 of *Pelodytes,* and 10 of *Rana*). The fauna of this vast region of the world, occupying a considerable part of the Palaearctic area, was formed during the postglacial period. Some of the frogs, e.g., *Rana arvalis, R. temporaria*[1] and *Bufo bufo*[2], were able to move far to the North and cross the Polar Circle.

The present work is concerned with determination of the content of nuclear DNA in 18 species of Anura that constitute more than 80% of the USSR fauna. The data obtained fill the gap in the knowledge of Anura from this region and give additional information about palaearctic species, as a whole[3,4].

Material and methods. The animals were collected by us in various regions of the USSR in the period from 1974 to 1978. *Bufo raddei* was kindly given to us by Dr T.O. Alexandrovskaya (the Zoological Museum of Moscow State University), *B. orientalis, H. japonica, R. dalmatina, R. chensinensis* by Dr L.Ya. Borkin (the Zoological Institute, USSR Academy of Sciences, Leningrad). Blood smears were fixed with a mixture of methanol and acetic acid (3:1) or with 4% buffered formalin. In each series of experiments, smears from *Xenopus laevis* were used as a standard. The size of the *X. laevis* genome was assumed to be 6.3 pg[5]. Hydrolysis of the smears was carried out in 5 N HCl at 37±0.5 °C for 15-35 min, the time interval corresponding to the middle part of the hydrolysis curves of the majority of species. The dye content of the Feulgen stained nuclei was measured at 546 nm by a scanning and integrating microdensitometer, type SIM-I. Not less than 100 nuclei of intact erythrocytes of each species were measured on 4-5 slides hydrolyzed for various periods of time; the results obtained were averaged.

Results and discussion. As seen from the table, the content of nuclear DNA in the species studied varies from 4.0 to 20.6 pg and the range of chromosomal sets in them from 22 to 26. The genome of *Bombina bombina* was found to be the largest, i.e. 20.6±1.5 pg in size. *B. variegata* that forms hybrids with *B. bombina* in the sympatric zone[6] has a smaller genome. The Far East species *B. orientalis,* that is allopatric to it, contains a similar amount of DNA. Discoglossidae seem to have the highest content of nuclear DNA among diploid *Anura*[3,4].

The Caucasian endemic species *Pelodytes caucasicus* has the smallest genome among all the species studied by us, i.e. 4.0±0.4 pg, which is almost half the size of that of *Pelobates fuscus* (7.8±0.9 pg) belonging to the same family. *P. caucasicus,* together with *P. punctatus,* whose karyotypes are of a similar structure[7,8], form one of the isolated evolutionary branches of Pelobatidae. The latter occupies an intermediate position between *Scaphiopus* (1.6-3.6 pg)[3] and the true *Pelobates* (5.6-9.0 pg)[3,7]. The Caucasian relict subspecies, *Bufo bufo verucosissima,* (13.6±0.3 pg) differs by its genome value from the Far East form *B.b. asiaticus* (11.2±0.4 pg). Another Far East species, *B. raddei,* is close to *B. viridis* which is characterized by the lowest content of DNA among the species of toads studied (table). Despite significant discrepancies in the literature data (e.g., for *Bufo bufo* from 9.2 to 15.5 and for *B. viridis* from 6.7 to 13.3 pg[3,9]), there is an impression that *Bufo* species from the tropics, like those of *Rana*[4,10], have smaller DNA values than those from the temperate zone.

The 2 allopatric species of tree frogs, *Hyla arborea* and *H. japonica,* which, probably, were a single species in the preglacial epoch, hardly differ in the genome size. The DNA content in them is higher than the average amount of DNA (5.7 pg) in the 30 studied species (most of which are tropical) of this family[3].

Among brown (grass) frogs, the Far East *R. chensinensis* and the Carpathian *R. dalmatina* have the smallest genomes and the Caucasian *R. macrocnemis* has the largest one. Sympatric *R. ridibunda* and *R. lessonae,* whose hybrid is known as *R. esculenta,* differ significantly in the size of their genomes (table). It is noteworthy that, in this case, as well as in the case of *Bombina, Bufo* and a number of other species, significant differences in genome size between parental species cannot prevent natural interspecific hybrids from being formed.

As seen from the table, brown frogs, on the whole, contain a smaller amount of DNA (9.7 pg), compared with green frogs (13.6 pg). Summarizing the data of Bachmann and Nishioka[4] and ours, we can give the mean values for 11 species of brown frogs and for 8 pond ranids, which are

Amounts of nuclear DNA in some anurans of the USSR, with *Xenopus laevis* as a standard of reference

Species	Number of chromosomes	Diploid DNA content in pg/nucleus	
		Mean ± SD	From literature
Discoglossidae			
Bombina bombina	24	20.6 ± 1.5	16.5-18.8[3]
B. orientalis		16.8 ± 0.6	20.5[4]
B. variegata	24	17.5 ± 0.5	15.0-17.1[3]
Pelobatidae			
Pelodytes caucasicus	24	4.0 ± 0.4	
Pelobates fuscus	26	7.8 ± 0.9	8.2- 8.9[3,7]
Bufonidae			
Bufo bufo asiatica	22	11.2 ± 0.4	
B.b. bufo	22	12.4 ± 0.7	9.2-15.5[3]
B. b. verucosissima		13.6 ± 0.3	
B. raddei		9.8 ± 0.9	
B. viridis	22	8.8 ± 1.2	6.7-13.3[3,9]
Hylidae			
Hyla arborea	24	8.8 ± 0.6	6.8- 7.8[3]
H. japonica	24	8.0 ± 0.7	
Ranidae			
Rana arvalis	24	10.3 ± 0.3	8.1-10.8[3]
R. dalmatina	26	8.9 ± 0.6	
R. macrocnemis	26	11.5 ± 0.5	
R. chensinensis	24	8.0 ± 0.8	11.6[4]
R. temporaria	26	9.8 ± 0.8	6.4-10.5[3,4]
R. lessonae	26	12.6 ± 1.1	
R. nigromaculata	26	12.1 ± 0.2	12.0[4]
R. ridibunda	26	16.2 ± 1.8	
Pipidae			
Xenopus laevis	36	6.3	6.3[5]

9.9 and 13.5 pg, respectively. This favours the idea of the genetic isolation of these 2 groups[11].

The analysis of the data obtained shows that the content of nuclear DNA in diploid species varies not only within order, e.g. Salientia (4.0-20.6 pg), but also within family, e.g., Pelobatidae (4.0-7.8 pg), and even within genus, e.g., *Bufo* (8.8-13.6 pg) and *Rana* (8.0-16.2 pg). The high degree of variability in the nuclear DNA is a characteristic feature of Amphibia which distinguishes these from most other Tetrapoda.

The results obtained by us, and those reported in the literature[3,4,10], allow the assumption to be made that Palaearctic Anura have a larger genome compared with that of related species from regions further south. A similar situation was observed by Stebbins[12] for vascular plants and, recently, by Bachmann and Nishioka[4] for ranids. We shall illustrate this assumption by our data. Thus, 2 subarctic species of brown frogs, *R. arvalis* and *R. temporaria*, as well as Caucasian highland species *R. macrocnemis*, have larger genomes compared with those of *R. dalmatina* and *R. chensinensis* which occur further south. The size of the genome of *B.b. bufo*, which penetrated into the North of Karelia beyond the Polar Circle[2], is much larger than that of *B. raddei* and *B. viridis* whose areas are further south. *R. ridibunda* has a larger genome compared with that of Middle Asian *R. nigromaculata*. A similar conclusion can be derived from comparing palaearctic and tropical species of Hylidae, Bufonidae, Ranidae, etc.

There is a close correlation between the size of genome of anurans and the minimal duration of their larval period[13]. The paradox consists in the fact that subarctic species, which have to spawn during a brief and cold northern summer, are characterized by both a larger genome and a faster larval development compared with the same species from zones further south[14]. The same is probably true of the highland amphibians[15]. As shown by our experiments[16], the duration of the first mitotic cycle in synchronous cleavage divisions of oocytes is the same for *R. temporaria*, *R. arvalis* and *R. ridibunda* at, respectively, 17.5, 18.5 and 21.5 °C, i.e. the temperatures that correspond to the midpoint of the optimal temperature range of development of these species. Hence, for their development to be more rapid, Palaearctic species seem to use other ways of adaptation than decrease in the amount of their nuclear DNA.

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