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Karyotype and Genome Size in the *Bufo viridis* Group

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Usually it has been accepted that three species of green toad group inhabit the USSR: *Bufo calamita* (west of the European part), *B. raddei* (Transbaikalia and Far East) and *B. viridis viridis* (the European part and Middle Asia eastwards to the Altai). However, Hemmer et al. (1978) described a new subspecies, *B. v. turanensis* and also recorded *B. latastii* for the first time for the USSR. Simultaneously the taxonomy of green toads of the USSR was revised by Pisanetz and Szczerbak (1979) who described a new subspecies, *B. v. asiomontanus*. Recognizing the validity of *B. v. turanensis* they pointed out the incorrect identification of several green toad populations from Soviet Middle Asia as *B. latastii*. Discovery of polyploidy was one of most important steps in the study of green toads. These first tetraploid toads were found in Kirghizia (Masik et al., 1976; Bachmann et al., 1978) and then in Turkmenia. The latter were described as a new species, B. danatensis Pisanetz, 1978.

MATERIALS AND METHODS

A total of 93 toads from 29 localities were karyotyped. Chromosomes were counted in dividing bone marrow or spleen cells and stained using Giemsa or Romanowski techniques. DNA content (79 toads) was determined by flow cytofluorimetry. Details of our technique will be published elsewhere. Mice thymocytes and blood cells of *Rana temporaria* were used for the standardization of the measurements. The coefficient of variation of DNA histograms was about 1.5 per cent. DNA differences of less than 5-8 per cent were considered significant only after additional examination of the blood cell mixtures from the toads compared. Totally 42 pair mixtures from 20 toads samples were analysed.

RESULTS

KARYOTYPES: B. calamita - 13 toads from: 1 - Ainazi, Latvia, 2 - Svityaz Lake, Volyn region, Ukraine. B. raddei - 9 toads from: 1 - Verkhny. Torey, Buryatia, southern Siberia, USSR, 2 - Shamar and 3 - small oase Gua-Bulag, Gobi Desert, from Mongolia, 4 - Khanka Lake, Soviet Far East.

Diploid *B. viridis* - 35 toads from 12 localities. Previous data on distribution of green toads were published without regard to their ploidy. Therefore we list 24 populations (identification by the chromosome and/or DNA content analyses). 1 - Riga, Latvia, 2 - Pushchino, Moscow region, 3 - Golaya Pristan, Kherson region, 4 - Melitopol and 5 - Makovka, Zaporozhye region, 6 - Planerskoye and Karadagh, the Crimea, 7 - Sochi, 8 - Chervlyonnye Buruny, Kalmykia, 9 - Kaspijsk, Daghestan, 10 - Akhaldaba and 11 - Kumisi Lake from Georgia, 12 - Kuchak, 13 - Zolokary, 14 - Tsovagyukh, Sevan Lake (Nos 12-14 are in Armenia), 15 - Sarykamysh Lake, 16 - Shakh-Senem, 17 - Danata, Kyuren-Dagh Range, 18 – oase Ai-Derenear Kara-Kala, 20 - Ashkhabad (Nos 15-20 are in Turkmenia), 23 - Chirik and 24-90 km to southwest of Dushanbe, Kafirnigan River valley, from Tadjikistan, 28 - Samarkand, Uzbekistan, 32 - Chu River valley, 650 m and Tyulyok (Toktosunov, 1984; our data) and 33 - Frunze, from Kirghizia (Bachmann et al., 1978; our data).

The diploid *B. calamita*, *B. raddei* and *B. viridis* have similar karyotypes: 2n = 22, NF = 44, 6 pairs of larger and 5 pairs of smaller chromosomes. In *B. calamita* a secondary constriction is located on the long arms of the chromosomes of the 7th pairs. However, we failed to see the secondary constrictions on the chromosomes of any other diploid and tetraploid green toads. Heteromorphic chromosomes were not found. The morphometric analysis of the karyotypes will be published elsewhere.

Tetraploid toads are preliminarily united by us under the name *B. danatensis*. 36 toads from 12 localities were karyotyped. 4n = 44, NF s 88, 6 tetrads of larger and 5 tetrads of smaller chromosomes. We list 21 localities (the data from Mongolia were obtained by Borkin, Terbish & Zaune in 1982). 17 - Danata, Kyuren-Dagh Range (the type locality for B. danatensis), 18 - oase Ai-Dere, 19 - Kurukhsudon Reserve, Kopeth-Dagh Range, 21 - Badkhyz Reserve, Akar-Cheshme Point (Nos 17-21 are in Turkmenia), 22-30 km from the mouth of Tupalang River, Gissar Range, 800 m, Uzbekistan, 25 - Ziddi, Gissar Range, 3000 m, 26 - Romit Canyon, Chuligaram, 27 -mouth of Komarou River, 2000 m (Nos 25-27 are in Tadjikistan), 29 - Tashkent, 30 -Kuraminsky Range, 3000 m, Uzbekistan, 31 - Toktogul valley, 900 m, 33 - Frunze, 34 - Arpa velley, 3590 m, 35 - Kara-Kudzhur valley, 3000 m, 36 - Issyk-Kul Lake, 1670 m, 37 - Kemin valley, 2500 m /Nos 31-37 are in Kirghizia/, 38 - Kapchagai, Hi River, 39 - desert near Burylbaital, southern Balkhash Lake region, 40 - Aksiir Farm, Zaissan (Nos 38-40 are in Kazakhstan), 41 - oase Khug-Bulag, Bulgan district, Khovd region, Mongolia. (Nos 33 from Bachmann et al., 1978; Nos 31, 34-37 from Toktosunov, 1984; No 39 from Egemberdieva, 1983; the rest - our data).

DNA CONTENT: Our measurements are partly represented in the Tab. 1. Assuming DNA content of a mouse equal to 6.8 pg/nucleus we can convert our data. Thus, the absolute DNA content in B. calamita is equal to 10.81 pg, in B. viridis to 11.42 - 14.01, in B. danatensis to 23.60 - 25.36 pg/nucleus. These estimates are similar to data of Bachmann et al. /1978/. We found intraspecific variation in DNA content. Three groups were discovered among diploid B. viridis: I. 1.68 - 1.72 relative units /r.u./ofDNA or 11.42 - 11.70 pg /the samples Nos. 2, 9, 10, 12 - 14, 18, 32a/; II. 1.76 - 1.81 r.u. or 11.97 - 12.31 pg /Nos. 1, 4 - 6, 8, 16a, 32b, 33/; III. 2.06 r.u. or 14.1 pg /Nos. 16b/. In the tetraploid B. danatensis, two groups were found: Turkmenian /Nos. 18, 19/and Tashkent /Nos. 29/ toads. DNA content in Kapchagai toads was measured earlier, before the experiments with mixtures. Therefore, estimates of their position (third group?) requires new experiments. The intraspecific variation in DNA content was already mentioned for animals (Mazin and Borkin, 1979; Sherwood and Patton, 1982).

Table 1. DNA content in various populations of the green toads. Mice thymocytes were used as a standart. *, ** two series of simultaneous measurements of blood cell pair mixtures

Species and locality	N	Relative DNA amount (x)
Bufo calamita (2n)		
1. Ainazhi, Latvia	9	1,59**
Bufo viridis (2n)		
1. Riga, Latvia	9	1,76**
2. Pushchino, Moscow region	3	1,69*
4. Melitopol, Zaporozhye region	3	1,81*
5. Makovka, Zaporozhye region	2	1,77**
6. Planerskoye, Crimea	2	1,78**
8. Chervlyonnye Buruny, Kalmyk	1	1,80
9. Kaspijsk, Daghestan	3	1,72**
10. Akhaldaba, Georgia	1	1,68*
12. Kuchak, Armenia	2	1,72*
13. Zolokary, Armenia	3	1,68*
14. Tsovagukh, Armenia	4	1,70**
16a. Shakh-Senem, Turkmenia	2	1,81**
16b. Shakh-Senem, Turkmenia	2	2,06**
18. Ai-Dere, Turkmenia	1	1,71**
32a. Tyulyok, Kirghizia	1	1,68*
32b. Tyulyok, Kirghizia	1	1,76*
33. Frunze, Kirghizia	2	1,78**
Bufo danatensis (4n)		
18. Ai-Dere, Turkmenia	3	3,63**
19.Kurukhsudon, Turkmenia	2	3,60**
29. Tashkent, Uzbekistan	9	3,73**
38. Kapchagai, Kazakhstan	2	3,47

TAXONOMY: To solve a number of taxonomic problems it would be necessary first of all to clarify the degree of ploidy of numerous nominal (sub) species beyond Soviet Asia. The toads identified by Hemmer et al. (1978) as *B. latastii* (4 localities in the USSR) seem to belong to tetraploid *B. danatensis*. This is proved for example by karyotyping of toads from the population (No. 24) mentioned by Hemmer et al. (1978) as *B. latastii*. We also failed to assign the toads "*B. latastii*" sensu Hemmer et al. (op. cit.) to true *B. latastii* Boulenger after the examination of the syntypes of the last species.

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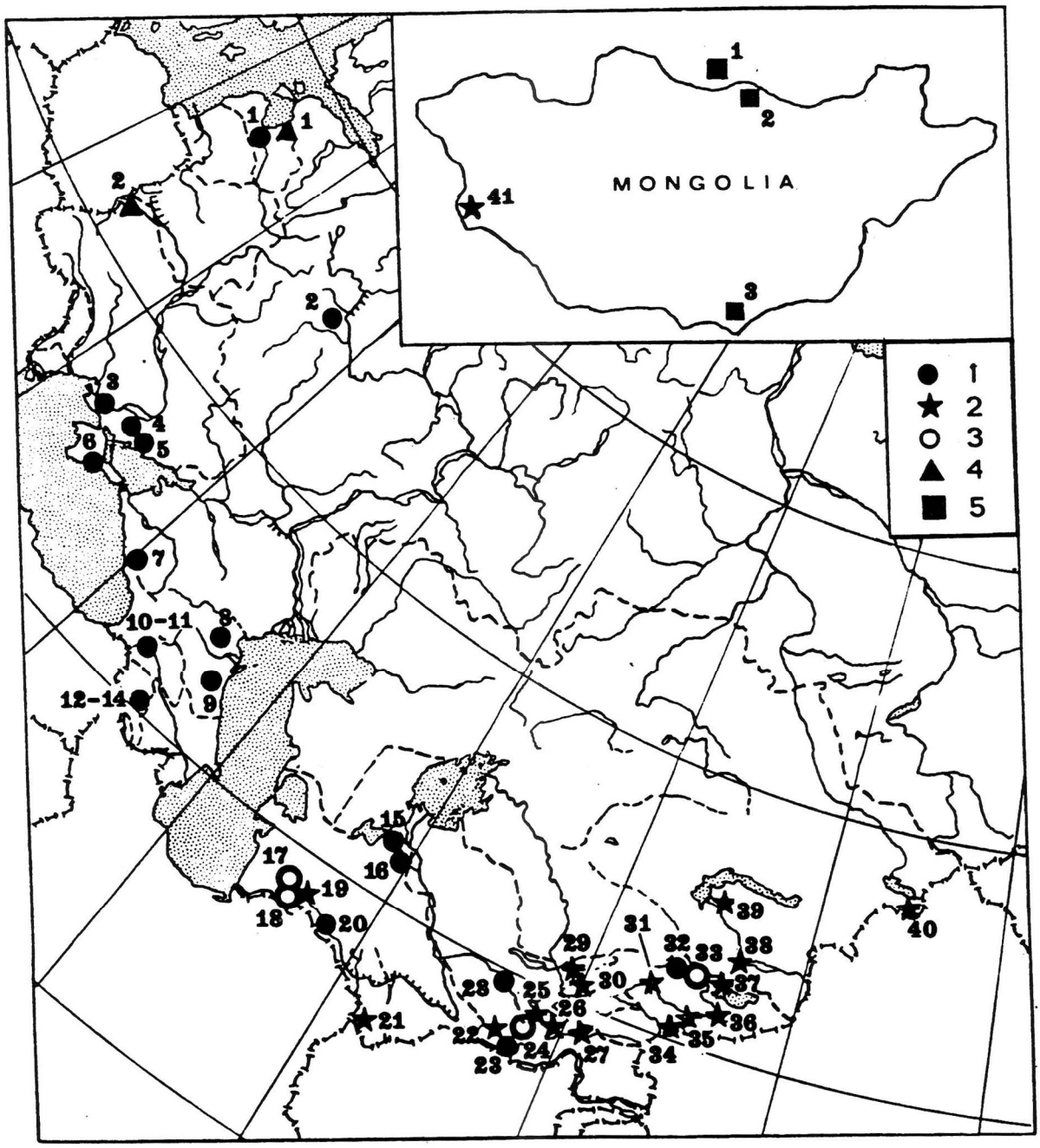

Distribution of the green toads examined by the chromosome or DNA content analyses. 1 - Bufo viridis /2n/, 2 - B. danatensis /4n/, 3 - sympatric localities of the both species, 4 - B. calamita and 5 - B. raddei. Locality numbers correspond to numbers in text and table.

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