

# Chromosomal polymorphism of *Bufo bufo*: Karyotype and C-banding pattern of *B.b. verrucosissima*

V. J. Birstein & A. L. Mazin

Laboratory of Bioorganic Chemistry and Molecular Biology, Lomonosov State University, Moscow 117234, USSR

## Abstract

*Bufo bufo verrucosissima* has a karyotype consisting of 22 chromosomes (6 pairs of large and 5 pairs of small chromosomes which are meta- and submetacentric). By means of Ag-AS-staining nucleolar organizers were localized in the telomeric region of the long arms of the 6th pair of chromosomes. The karyotype differs from those of the other *B.bufo* subspecies by the form of the 4th pair, which is metacentric. A slight chromosomal polymorphism was shown also after C-banding of *B.b. verrucosissima* and *B.b.bufo* chromosomes.

## Introduction

*Bufo bufo verrucosissima* (Pall.) is a subspecies which inhabits the foot-hills and mountainous regions of the Caucasus (Terentjev & Chernov, 1949; Bannikov *et al.*, 1977). This subspecies is included in the group of four European forms of the *Bufo bufo* complex, i.e. *B.b. bufo*, *B.b. spinosus*, *B.b. gredosicola*, and *B.b. verrucosissima* (Mertens & Wermuth, 1960); it differs from the other three subspecies morphologically (Terentjev & Chernov, 1949). It is believed that *B.b. verrucosissima*, *B.b. spinosus*, *B.b. gredosicola* and *B.b. japonicus* form a group of ancient related preglacial subspecies and that *B.b. bufo* arose geologically more recently (Nikol'skii, 1918; Terentjev & Chernov, 1949). Although the first description of this form refers to the beginning of the past century (Pallas, 1811), *B.b. verrucosissima* remains one of the less known subspecies of the common toad (Tertyshnikov & Pisanetz, 1979) and its karyotype is still unknown. As shown earlier, the genome size of *B.b. verrucosissima* is somewhat greater than those of *B.b. bufo* and *B.b. asiaticus* (Mazin, 1980). In this paper, we describe the karyotype and the C-banding pattern of

*B.b. verrucosissima* chromosomes. The results are compared with the data for other forms of this species complex.

## Material and methods

*B.b. verrucosissima* (three males and one female) were collected in the Caucasian Wild Life Reserve (Guseripl), and *B.B. bufo* (one male and one female), in the Dmitrov district of the Moscow region. Chromosomal preparations of bone-marrow cells were obtained as previously described (Birstein, 1981). Chromosomes were stained with Giemsa. In the preparations obtained from each animal, 10 metaphase plates were analyzed. C-staining was performed according to the method of Schmid (1978). For Q-staining, a 2% aqueous solution of quinacrine (Gurr) or a Hoechst 33258 aqueous solution (0.5 µg/ml) was used; after 10 min of staining the slides were washed with phosphate-acetate buffer (pH 5.5). The Ag-AS-staining technique was used as described previously (Birstein, 1981).

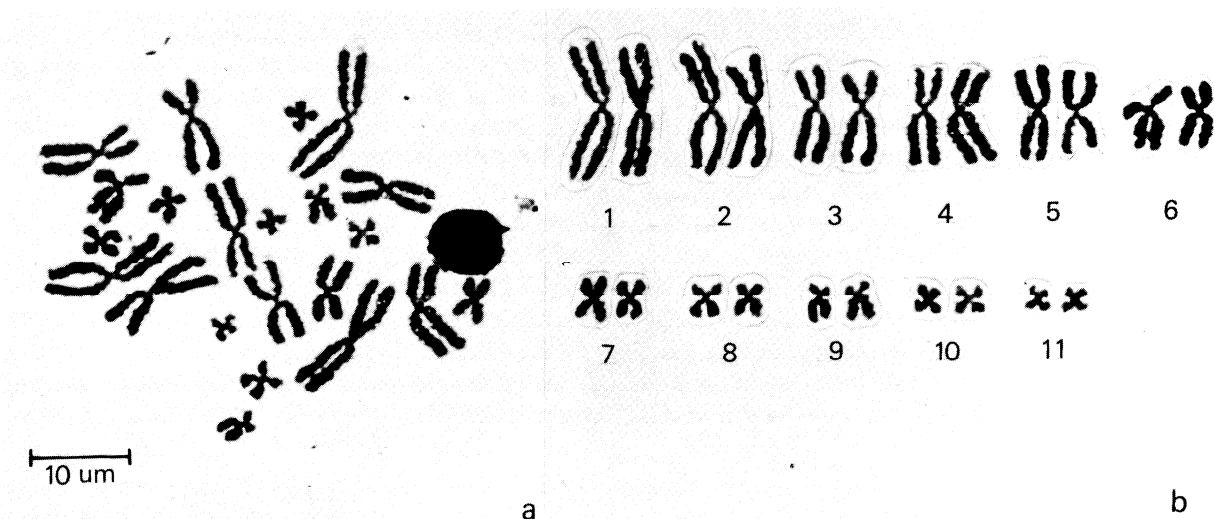


Fig. 1. *Bufo bufo verrucosissima*: (a) bone marrow metaphase (methanol-acetic acid, Giemsa); – (b) normal karyotype.

Table 1. Relative length and arm ratio (mean  $\pm$  one standard error) of *Bufo bufo verrucosissima* chromosomes.

No.	Relative length	Arm ratio	Chromosome
1	16.60 $\pm$ 0.30	1.16 $\pm$ 0.02	mc
2	14.55 $\pm$ 0.27	1.13 $\pm$ 0.01	mc
3	12.46 $\pm$ 0.13	1.55 $\pm$ 0.03	smc
4	11.92 $\pm$ 0.17	1.15 $\pm$ 0.02	mc
5	11.25 $\pm$ 0.07	1.21 $\pm$ 0.02	mc
6	9.36 $\pm$ 0.10	1.32 $\pm$ 0.04	mc
7	5.81 $\pm$ 0.07	1.15 $\pm$ 0.03	mc
8	5.26 $\pm$ 0.06	1.13 $\pm$ 0.02	mc
9	5.00 $\pm$ 0.10	1.63 $\pm$ 0.07	smc
10	4.09 $\pm$ 0.13	1.13 $\pm$ 0.03	mc
11	3.78 $\pm$ 0.10	1.09 $\pm$ 0.02	mc

\* mc, metacentric; smc, submetacentric

## Results and discussion

Figure 1 (a, b) shows the chromosome set of *B.b.verrucosissima*, and in Table 1 the results of the morphometry of the chromosomes are summarized (chromosomes of the best 10 plates were analysed). The *B.b.verrucosissima* karyotype consists of 11 pairs of chromosomes,  $NF = 44$ ; the same is true of the majority of other *Bufo* species (Bogart, 1972).

On the basis of the relative length, chromosomes can be easily divided in two groups: large chromosomes (pairs 1–6) and small ones (pairs 7–11). Large chromosomes of pairs Nos 1, 2, 4, 5 and 6 are nearly metacentric (centromeric index,  $CI = 1.13$ – $1.32$ ),

the homologues of pair No. 3 are large submetacentric chromosomes ( $CI = 1.55$ ); small chromosomes of pairs Nos 7, 8, 10 and 11 are practically metacentric and of pair No. 9 are submetacentric (Table 1). Chromosomes of pairs Nos 3 and 4, and Nos 8 and 9 are similar in size, but differ from each other in form. It is difficult to distinguish the homologues of pairs 7 and 8 and especially of pairs 10 and 11. No heteromorphic pairs of chromosomes were found in the karyotype.

On the whole, the *B.b.verrucosissima* karyotype is similar to those of the other *B.bufo* subspecies (Ullerich, 1966; Morescalchi, 1973; Obara *et al.*, 1975; Matsui, 1980). At the same time, a few characteristic features of this karyotype can be pointed out. In particular, the relative length of the chromosomes of pair No. 1 is a little greater than that of the chromosomes of pair No. 2 (16.60% and 14.55% respectively). In the case of the *B.b.bufo* karyotype the chromosomes of the first and second pairs could hardly be distinguished (Ullerich, 1966; Morescalchi, 1973). The relative length of these chromosomes in other *B.bufo* subspecies is also rather similar (Matsui, 1980). With respect to this feature, *B.b.verrucosissima* resembles most closely *B.b.japonicus*. The sum of the relative lengths of all *B.b.verrucosissima* small chromosomes is a little greater than that in other subspecies.

The comparison of the arm ratios of *B.b.verrucosissima* chromosomes and of those of the other *B.bufo* subspecies (Matsui, 1980) allows



Fig. 2. C-banding pattern of *B.b.verrucosissima* karyotype.

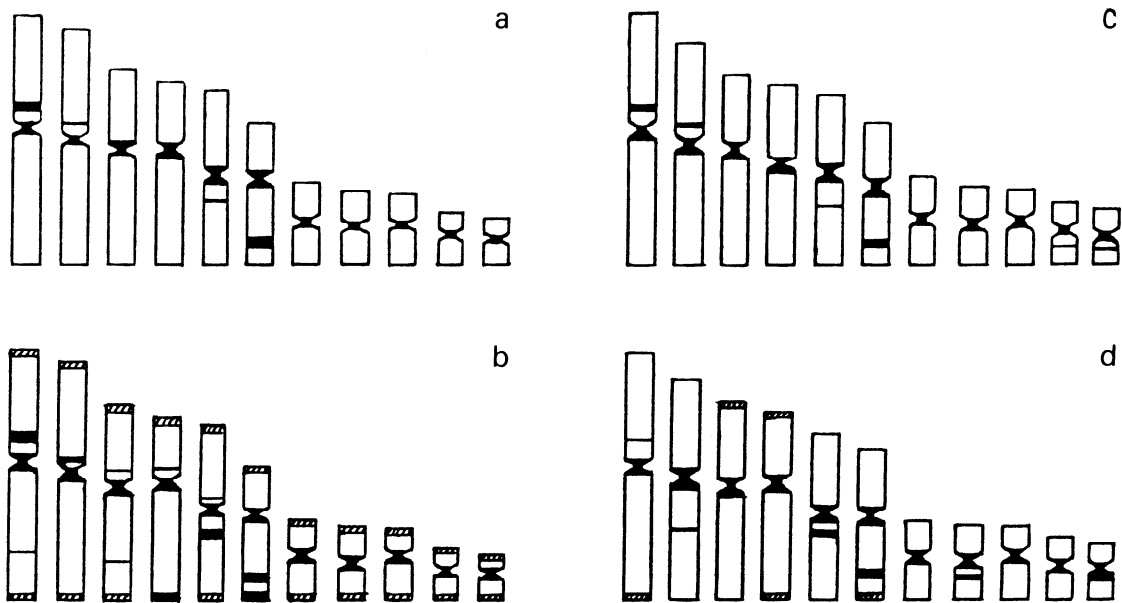


Fig. 3. A diagrammatic representation of haploid C-banded karyotypes of *B.b.bufo* (a, our results; b, according to Schmid, 1978), of *B.b.verrucosissima* (c, our results) and of *B.b.japonicus* (d, according to Obara et al., 1975).

the conclusion to be made that the form of some chromosomes remains unaltered, e.g. CI for the homologues of pair No. 1 of all subspecies is 1.16–1.26; of pair No. 2, 1.13–1.25; of pair No. 5, 1.07–1.21 and of pair No. 8, 1.13–1.26. The arm ratio of the chromosomes of other pairs may vary considerably. Thus, the homologues of the 4th pair of *B.b.verrucosissima* are metacentric (CI = 1.15), and in other subspecies they are submetacentric (CI = 1.74–1.82) (Matsui, 1980). On the contrary, CI of

the 3rd chromosome pair of *B.b.verrucosissima* (1.55) is a little higher than that of the other subspecies (1.35–1.48). Therefore, by the relative length and form of chromosomes, the *B.v.verrucosissima* karyotype differs clearly from the other investigated *B.bufo* karyotypes.

The C-banding pattern of *B.b.verrucosissima* chromosomes seems to be rather complicated (Fig. 2 and Fig. 3,a). The centromeres and the pericentric regions of all chromosomes were positively C-

banded. In addition interstitial C-bands were seen on the short arms of the chromosomes of the 1st and 2nd pairs. Also, a clear C-band was obtained in the distal part of the long arm of the No. 6 pair (the region of a secondary constriction?). Faint C-bands were seen in the proximal part of the long arm of pair No. 5 and clear thin C-bands, in the proximal region of the long arms of pairs Nos 10 and 11.

On the whole, the C-banding pattern of *B.b. verrucosissima* differs somewhat from that of the *B.bufo bufo* karyotype (Fig. 3a, b). Under our conditions, the centromeres of all *B.b. bufo* chromosomes and pericentric regions of the short arms of pairs Nos 3 and 5 and of the long arm of the 4th pair and of both arms of pair No. 6 were stained. A distinct C-band was seen in the distal part of the 1st pair of chromosomes and less strongly stained C-bands were observed in the distal parts of the 2nd pair of chromosomes; a faint C-band was seen in the proximal region of the long arm of pair No. 5.

It should be emphasized that the C-banding pattern of *B.b. bufo* chromosomes obtained by us differed somewhat from that obtained by other authors (Schmid, 1978; Grafodatsky *et al.*, 1978). In Schmid's experiments (1978) besides C-banded centromeres and interstitial bands on pairs No. 1, 2, 5 and 6, terminal C-bands and a few additional interstitial bands on the 1st, 3d-5th pairs were seen (Fig. 3,b). On the other hand, Grafodatsky *et al.* (1978) failed to obtain any interstitial C-bands on *B.b. bufo* chromosomes; in their experiments only centromeres were C-banded. All these data point to the instability of C-bands on Anuran chromosomes, a fact emphasized by other authors (Grafodatsky *et al.*, 1978; Schmid, 1978; Birstein, 1981). On the whole, our results resemble those of Schmid (1978) in that there are no C-bands on the small chromosomes of *B.b. bufo* and that the shape of pericentric C-banded blocks on *B.b. bufo* and *B.b. verrucosissima* chromosomes is a little different (Fig. 3, a-c).

On the small chromosomes, C-bands have been obtained on one homologue of the 8th pair of *B.b. japonicus* chromosomes (Obara *et al.*, 1975). It seems possible that there is also a C-band on the 11th pair of chromosomes of this subspecies because of a very wide pericentric C-stained block. The distribution of other C-bands on *B.b. japonicus* chromosomes partly coincides with that of *B.b. bu-*

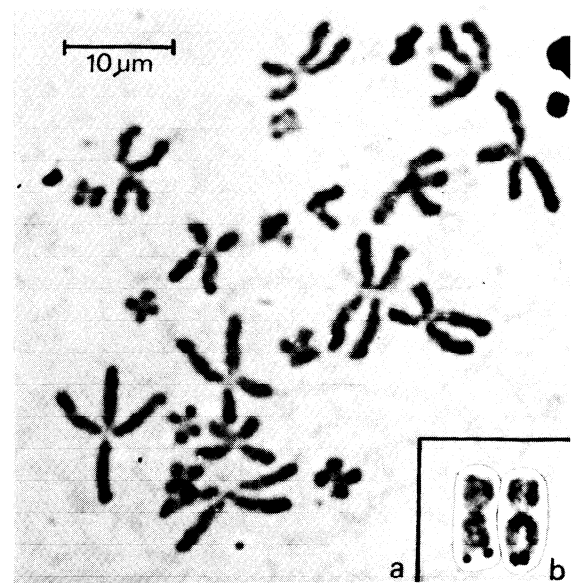


Fig. 4. Ag-AS stained metaphase chromosomes of *Bufo bufo*: (a) *B.b. verrucosissima*, bone marrow, arrow indicates NOR; - (b) *B.b. bufo*, 6th pair of chromosomes, black spots are stained NORs.

*fo* chromosomes (Fig. 3,d).

After Q-staining of *B.b. verrucosissima* and *B.b. bufo* chromosomes with quinacrine and Hoechst 33258, no difference between these subspecies was observed. In both cases all parts of the chromosomes fluoresced unequally: small intensively fluorescing regions were interspersed with small regions which fluoresced weakly.

According to the results of Ag-AS-staining, nucleolar-organizer regions (NORs) occupy an almost telomeric or practically telomeric position on the long arm of the homologues of the 6th pair of *B.p. verrucosissima* and *B.b. bufo* (Fig. 4). It is interesting that in one of the *B.b. verrucosissima* males only one homologue of the 6th pair was stained (Fig. 4a). By the same Ag-AS-method Schmid (1978) localized NORs in the telomeric regions of the 6th pair of *B.b. bufo* chromosomes.

The described difference in karyotype and C-banding pattern between *B.b. verrucosissima* and *B.b. bufo*, as well as the difference from the *B.b. japonicus* karyotype and C-banding (Obara *et al.*, 1975; Matsui, 1980), show intraspecific chromosomal polymorphism of *B. bufo*. This conclusion is in accordance with the data on genome size difference between *B. bufo* subspecies (Mazin, 1980): the

nuclear DNA content in erythrocytes of *B.b.bufo* is  $12.4 \pm 0.7$  pg, of *B.b.verrucosissima*,  $13.6 \pm 0.3$  pg and of *B.b.asiaticus* (subspecies of *B.bufo* or a separate species, which lives to the East of the Baikal Lake – Borkin & Roschin, 1981) is  $11.2 \pm 0.4$  pg. All these data favour the assumption that *B.bufo* subspecies are extremely isolated from each other and that many forms of the *B.bufo* complex should be treated as separate species (Matsui, 1980; Borkin & Roschin, 1981).

Earlier Matsui (1981) pointed to the karyological divergence between Eastern Asian *B.bufo* forms and European *B.bufo*. To our regret, the karyotypes of two ancient European *B.bufo* subspecies, i.e. *B.b.gredosicola* and *B.b.spinossus* are not described, that is why it is impossible to compare the karyotypes of all European *B.bufo* toads. Our results show that the karyotype of *B.b.verrucosissima* is probably more related to the *B.b.japonicus* karyotype, than to the karyotype of *B.b.bufo*. This supports the view that *B.b.verrucosissima* and *B.b.japonicus* are ancient related forms, and *B.b.bufo* is a geologically young subspecies which diverged from ancient European forms and spread in its contemporary area in the postglacial epoch (Nikol'skii, 1918; Terentjev & Chernov, 1949). But any conclusions about the real relatedness of the karyotypes of the ancient *B.bufo* forms will only be valid after investigation of *B.b.gredosicola* and *B.b.spinossus* karyotypes.

The above-mentioned difference in the C-banding pattern between *B.bufo* subspecies (forms) is rather interesting. Variations in the distribution of C-bands are characteristic of chromosomes of mammalian subspecies (Dev *et al.*, 1975; Mandahl, 1979). In Amphibia one case of inter-population difference in the C-banding pattern was described only for an urodelan *Triturus italicus* (Ragghianti *et al.*, 1980); on the whole, the karyotypes of the *Triturus* species are very conservative and the karyotypes of many species may be distinguished only after C-banding (Mancino *et al.*, 1977). The same karyotypic conservatism is characteristic of many systematic groups of Amphibia (Morescalchi, 1973; 1979). A great variability of C-banding pattern of mammalian chromosomes in comparison with amphibian ones lends support to the idea that the rate of chromosomal evolution in Mammalia is higher than in Amphibia (Wilson *et al.*, 1974).

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