KARYOTYPE AND GENOME SIZE OF PELODYTES CAUCASICUS (AMPHIBIA, PELOBATIDAE)

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The amount of nuclear DNA in *Pelodytes caucasicus* is 4.0 ± 0.4 pg. *P. caucasicus* has a karyotype of 24 chromosomes with 7 pairs of large and 5 of small chromosomes. The secondary constriction is localized in the middle of the shorter arms of the 2nd pair of the submetacentric chromosomes.

Introduction

Pelodytes caucasicus Boulenger 1896 is one of the least known endemic species of Caucasian anurans. Its only relative, *P. punctatus*, is widely spread in Western Europe. The karyotype of *P. punctatus* and of some other Pelobatids have been described by Morescalchi et al. (1977); of some species the nuclear DNA content has been determined (Olmo, 1973). The chromosome set and the amount of nuclear DNA of *P. caucasicus* have been determined by us for the first time.

Material and methods

The animals (one female and four males) were collected in the period of spawning at the end of June 1977 in the Caucasian Wild Life Reserve (Guseripl). Chromosome preparations were made from bone marrow and spleen cells of colchicinized animals (Haertel et al., 1974). In several preparations obtained from each animal, 10-15 metaphase plates were analyzed. The Ag-AS method was used to reveal the nucleolar orga-

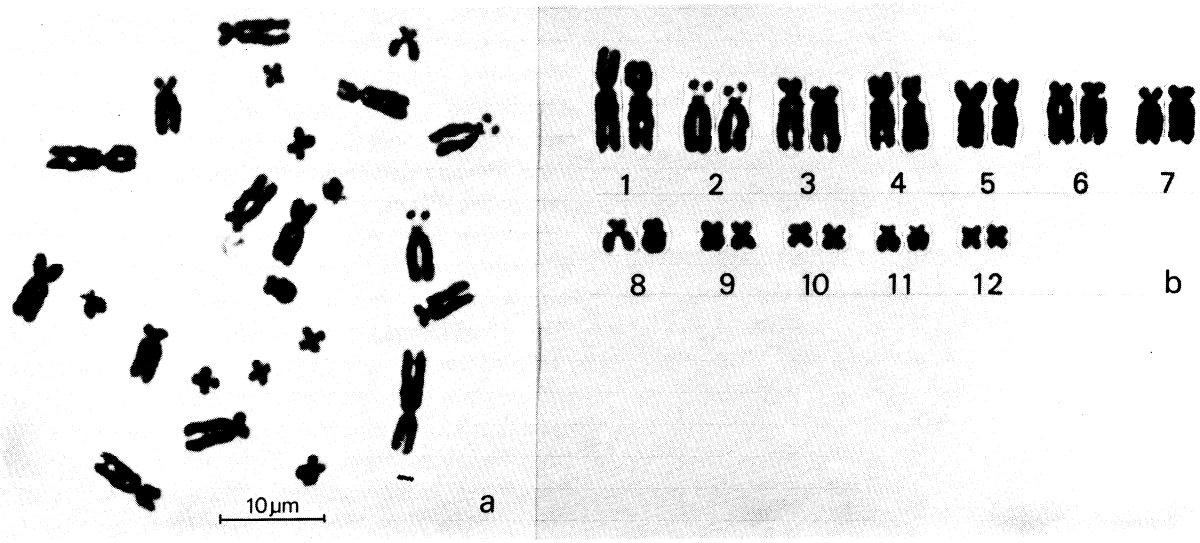

Fig. 1. *Pelodytes caucasicus*, (a) bone marrow metaphase (methanol-acetic acid, Giemsa); – (b) normal karyotype: 7 large and 5 small chromosomes. x 2.310.

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nizer region (NOR) (Ward, 1977; Schmid, 1978). DNA was measured photometrically on erythrocytes from Feulgen-stained blood smears. The blood smears were fixed with 4% buffered formalin or methanol-acetic acid (3:1) and hydrolyzed in 5 N HCl at 37°C during 16 minutes. The dye content of the Feulgen-stained nuclei was determined at 546 nm using a scanning and integrating microdensitometer (SIM-1). At least 25 nuclei were measured on each slide. In each series of experiments blood smears of *Pelobates fuscus* (7.8 pg) (Olmo, 1973; Morescalchi et al., 1977) and *Xenopus laevis* (6.3 pg) (Dawid, 1965; Thiébaud & Fischberg, 1977) were used for reference.

Results and discussion

Figure 1 (a, b) shows the chromosome set of *P. caucasicus*: 2n = 24, NF = 48. All chromosomes are biarmed (meta-, submeta- and subtelocentric). Two groups of chromosomes distinctly different in size can be discerned. The first includes seven pairs of larger chromosomes, out of which three pairs (1, 4 and 5) are submetacentric and four pairs (2, 3, 6 and 7) subtelocentric. The second group consists of five pairs of smaller chromosomes, more or less equal in size. Two pairs (10 and 12) in this group are metacentric, two pairs (8 and 9) submetacentric and pair eleven subtelocentric. No distinct differences were revealed between the karyotype of the males and the female.

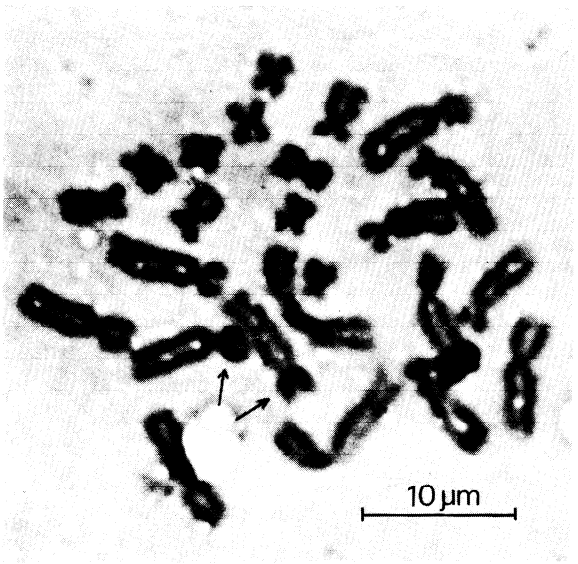

Fig. 2. *Pelodytes caucasicus*, Ag-AS stained metaphase bone marrow; arrows indicate nucleolar organizer regions.

The karyotype of *P. caucasicus* is largely typical of Pelobatidae. All the species studied, excluding *Leptobrachium pelodytoides* and *P. punctatus* (2n = 24), possess a set of 26 biarmed chromosomes (Morescalchi et al., 1977). Five or six pairs of chromosomes usually form a group of larger chromosomes and seven or eight pairs a group of smaller chromosomes (6+7 and 5+8 karyotypes). In *Pelodytes* the ratio of large and small chromosomes is in the same range (7+5), but it is different than in other species of this family. *P. caucasicus* and *P. punctatus* are somewhat different karyotypically: in the former the group of larger chromosomes consists of four pairs of submetacentric and three pairs of subtelocentric homologs, whereas in the latter it consists of three and four pairs, respectively. In addition, the localization of secondary constrictions in these species is different.

As is seen in Figures 1 and 2, the second pair of larger subtelocentric chromosomes has a secondary constriction in the middle of the shorter arm which can be detected in 90% of metaphase plates. In the sites of the secondary constriction characteristic black dots can be seen after silver staining which testify to the presence of nucleolar organizers (Schmid, 1978). In other species of this family, secondary constrictions can be found in one of the large and in one of the small chromosomes (Morescalchi et al., 1977). For example, in *P. punctatus* one of the secondary constrictions is localized in the long arm of the 7th pair of chromosomes and the other in the middle of the long arm of the 9th pair of small chromosomes. Such well expressed karyological differences between *Pelodytes* indicate that they should be regarded as individual species (Elzen, 1975).

Judging from our data, the nuclear DNA content in erythrocytes of *P. caucasicus* is 4.0 ± 0.4 pg. This is almost twice as low as in *P. fuscus* (7.8 ± 0.9 pg). Thus *P. caucasicus* has a smaller genome than many other Anura and in this character is close to Hylidae and Leptodactylidae. Within its own family *P. caucasicus* has an intermediate position with respect to the DNA amount between *Scaphiopus* and *Pelobates* (Olmo, 1973; Morescalchi et al., 1977). In conjunction with the karyological data, this may indicate that *Pelodytes* should be regarded as belonging to a specific evolutionary branch of Pelobatoidea.

References

Dawid, I. (1965). Deoxyribonucleic acid in amphibian eggs. *J. molec. Biol.* 12: 581-599.

Elzen, P. (1975). Contribution à la connaissance de Pelodytes punctatus (Daudin, 1802) (Amphibia, Pelobatidae) étudié en Camargue. *Bull. Soc. zool. Fr.* 100: 691-692.

Haertel, T.D., A. Owerarzak & R.M. Storm (1974). A comparative study of the chromosomes from five species of the genus Rana (Amphibia, Salientia). *Copeia* 1: 109-114.

Morescalchi, A., E. Olmo & V. Stingo (1977). Trends of karyological evolution in Pelobatoid frogs. *Experientia* 33: 1577-1578.

Olmo, E. (1973). Quantitative variations in the nuclear DNA and phylogenesis of the Amphibia. *Caryologia* 26: 43-68.

Schmid, M. (1978). Chromosome banding in Amphibia. I. Constitutive heterochromatin and nucleolus organizer regions in Bufo and Hyla. *Chromosoma* 66: 361-388.

Thiébaud, C.H. & M. Fischberg (1977). DNA content in the genus Xenopus. *Chromosoma* 59: 253-257.

Ward, O.G. (1977). Dimorphic nucleolar organizer regions in the frog Rana blairi. *Can. J. Genet. Cytol.* 19: 51-57.