Karyotype and chromosome banding in the reed frog *Hyperolius viridiflavus ommatostictus* (Amphibia, Anura, Hyperoliidae)[1]

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Summary. C-banding and mithramycin staining were used to characterize the karyotypes of 10 specimens of the African reed frog *Hyperolius viridiflavus ommatostictus* from Tanzania. The diploid chromosome number is 2n = 24. Although no heteromorphic sex chromosomes were present in the mitotic karyotypes, in many diakineses of male meiosis one or two bivalents exhibited an end-to-end arrangement. In the laboratory 7 out of 24 females changed sex spontaneously. This indicates that an XY/XX system of sex determination operates in *H. viridiflavus ommatostictus*.
Key words. Chromosome banding; karyotype; Hyperoliidae; Ranidae; Amphibia.

The 'superspecies' *Hyperolius viridiflavus* is found in most of sub-Saharan Africa. At least 50 species have been recognized[2]. The systematics of this group is still unresolved[2-4]. *Hyperolius viridiflavus ommatostictus* is found in East Africa between Mt. Kilimanjaro and Mt. Meru[3]. The family Hyperoliidae, with 14 genera, contains 206 recognized species and belongs to the highly evolved superfamily Ranidae. The group is considered to be derived independently from a leptodactylid stock[5].

The systematic classification and the relationships among the ranoid frogs (Ranidae, Rhacophoridae and Hyperoliidae) have not yet been determined with certainty. Cytogenetic studies of the ranoids have been carried out predominantly with conventionally-stained chromosomes, and have contributed to the understanding of the chromosomal evolution of many genera from this group[6-9]. This study describes the cytogenetic analysis of the hyperolid *Hyperolius viridiflavus ommatostictus*, using banding techniques. The criteria taken for comparison consider the number and morphology of the chromosomes, as was utilized for studies on the phylogenetic relationship of other ranoids[10,11].

Five male and five female laboratory-bred specimens were available for this investigation. The original stock was obtained in Tanzania. The chromosome preparations were made from bone marrow, testes, liver and gut epithelium by conventional air drying techniques[10].

The diploid chromosome number of the species is 2n = 24. After staining with orcein the chromosomes can be arranged into 12 pairs (fig. 1 A). The chromosome pairs 1 and 5–12 are metacentric to submetacentric and the pairs 2–4 clearly submetacentric. The analysis of the haploid genome of *Hyperolius viridiflavus ommatostictus* (table) shows some similarities to the hyperolids studied by Bogart and Tandy[9], such as a constant diploid chromosome number and a gradual size transition. However, these authors did not use banding patterns on chromosomes, and it is difficult to make any correlation on the basis of the morphology of the conventionally-stained chromosomes only.

The constitutive heterochromatin, stained by C-banding[10,12], is located in the centromeric region of each chromosome, in the intercalary region of pairs 1, 5, 8–12, and also in the terminal region of pairs 3, 6, 9 and 12 (figs 1 B and 2).

The fluorescence of the heterochromatic regions after double staining with the GC-specific fluorochromes mithramycin and distamycin[10,13] shows the nucleolus organizer regions localized pericentromerically in the long arm of pair 8 (fig. 1 C). Heteromorphic sex chromosomes have not been found in the specimens analyzed.

The analyses of 200 meiotic metaphases from two male individuals showed that only 11.5% of the diakineses had only bivalents with two terminal chiasmata, showing the typical ring-like configuration. Although no heteromorphic XY sex chromosomes were ever found, there were always about 34.6% of the diakineses with one bivalent and 53.4% with two bivalents exhibiting an end-

Relative lengths, arm ratio, centromere index and the type of metaphase chromosomes of *Hyperolius viridiflavus ommatostictus*.

Chromosome	% Length[a]	Arm ratio[b]	Index[c]	Type[d]
1	13.87	1.25	0.44	m
2	13.00	1.81	0.36	sm
3	11.67	2.47	0.28	sm
4	11.45	1.74	0.37	sm
5	9.47	1.53	0.39	m
6	7.71	1.03	0.49	m
7	7.04	1.13	0.40	m
8	7.04	1.46	0.41	m
9	6.61	1.31	0.43	m
10	6.17	1.15	0.46	m
11	5.28	1.01	0.50	m
12	4.84	1.02	0.49	m

[a] % Length is the percent that each chromosome represents with respect to the haploid genome length. The chromosomes of five high-quality aceto-orcein stained metaphases of one male and one female were used for the measurements. [b] Arm ratio is the centromeric ratio determined by dividing the length of the long arm by that of the short arm. [c] Index is the centromeric index determined by dividing the length of the short arm by the total length of the chromosome. [d] Type refers to the type of chromosome determined by centromeric ratio. Metacentric chromosomes (m) have a ratio of 1.00 to 1.69, submetacentric chromosomes (sm) have a ratio of 1.70 to 2.99, subtelocentric chromosomes (st) have a ratio of 3.00 to 6.99 and telocentric chromosomes (t) have a ratio of 7.00 or more.

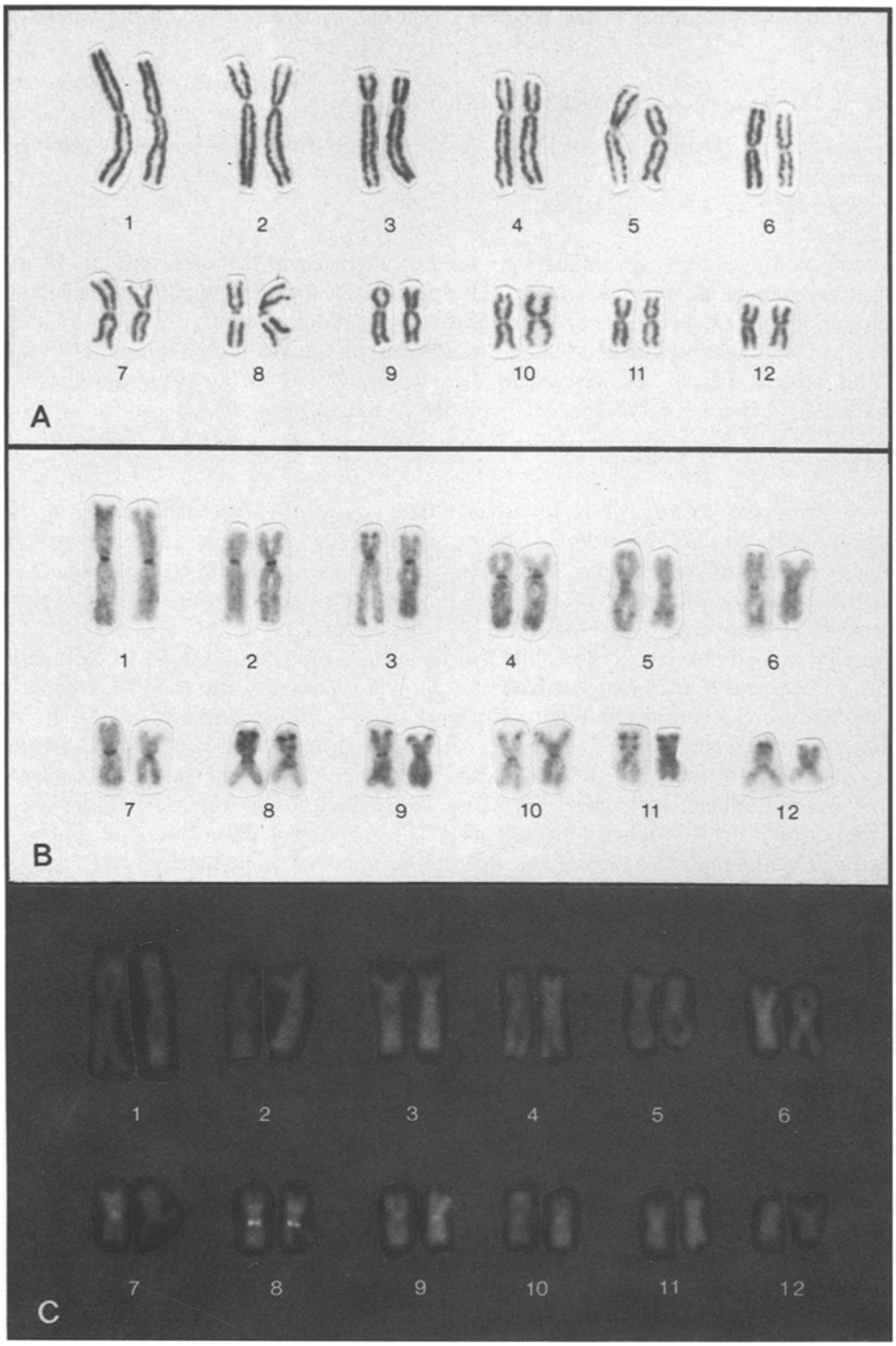

Figure 1. Karyotypes of *Hyperolius viridiflavus ommatostictus* showing (*A*) aceto-orcein stained, (*B*) C-banded and (*C*) mithramycin stained metaphase chromosomes. Note the bright mithramycin-fluorescence of the nucleolus organizer region in the long arm of chromosome pair 8.

to-end arrangement of the chromosomes. Only 0.5% of the metaphases were found to have more than 2 bivalents with an open configuration. It is possible that one of the diakinetic bivalents that exhibit the end-to-end association represents the XY pair[14].

Flow cytophotometric analysis of the DNA content of erythrocyte nuclei[14] yielded values of 8.33 ± 0.97 pg DNA per diploid nucleus, with an average of 59.71% of AT and 40.29% GC base pairs.

In the laboratory, seven out of 24 female individuals of *Hyperolius viridiflavus ommatostictus* changed sex after successfully laying multiple clutches[15]. This occurred without any hormone treatment. Secondary males produced fertile spermatozoa and fertilized four clutches.

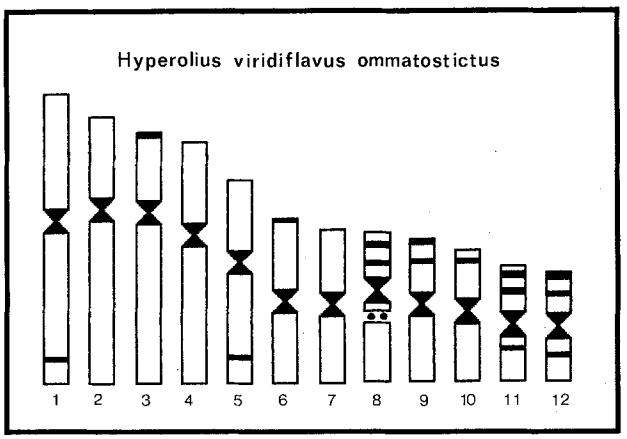

Figure 2. Diagrammatic representation of the maximum number of C-bands determined in the somatic metaphase chromosomes of male and female *Hyperolius viridiflavus ommatostictus*. Light areas: euchromatin; dark areas: constitutive heterochromatin; dark circles: nucleolus organizer regions.

Although none of the progeny from these breeding experiments could be raised and sexed we presume a male heterogametic sexual determination of the type XY based on the direction of sex change from female to male. This assumption is in accordance with successful breeding experiments with sex-reversed anurans using hormones[16]. In those experiments protandrous (male to female) sex reversal has been accomplished only in species showing a ZW system and protogynous (female to male) sex reversal in species with a XY system of determination.

Conflicting evidence comes from work by Richards on *Hyperolius viridiflavus viridiflavus*[17]. Breeding experiments showed a wide spectrum of sex ratios among different crosses. Richards interprets this high variance in sex ratio as perhaps indicating polyfactorial sex determination with environmental influence[17,18].

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Effect of low dose of 70 kVp X-rays on the intrauterine development of mice

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Summary. Pregnant Swiss albino mice were exposed to low doses of X-rays (~ 9 mGy) in the range used for diagnostic exposure, on day 3.5 of gestation (preimplantation period), day 6.5 (early organogenesis period) or day 11.5 (late organogenesis period). The fetuses were examined on the 18th day of gestation. Exposure at 3.5 days post coitus (d.p.c.) resulted in a significant increase in prenatal mortality, and an increased incidence of retarded fetuses was observed after exposure at 3.5 and 6.5 d.p.c. The major effect of exposure at 11.5 d.p.c. was a significant decrease in the fetal head size and brain weight.
Key words. Prenatal exposure; low dose X-rays; fetal anomalies.

Intrauterine development, particularly the period of organogenesis, is an especially radiosensitive phase in mammals. Even though the teratogenic effect of low doses of radiation has been demonstrated in children irradiated in utero by A-bomb radiation[1,2] and in laboratory animals[3–5], attempts to show a correlation between low doses in the range of diagnostic exposure and human fetal abnormalities have met with criticism[6]. Michel and Fritz-Niggli[7] showed that whole body exposure of pregnant mice to as little as 1 cGy of 140 kV X-rays and negative pions during organogenesis increased fetal abnormalities. However, data are lacking on the comparative response of the different stages of prenatal development to low doses at the levels that could result from