Somatic Chromosomes of *Bufo arenarum*

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In the genus *Bufo* several investigators (Makino 1932, Minouchi 1931, Stholer 1928) have described the existence of 22 chromosomes and in *Bufo arenarum* Saez and Col., by studying the testicular meiosis, showed the presence of twelve large and ten decresent size chromosomes (Saez *et al.* 1934, Saez *et al.* 1936).

The present work deals with the study of somatic chromosomes of *Bufo arenarum* using the recent technical advances in cytogenetics.

Materials and methods

Twenty *Bufo arenarum* embryos from stages 21 to 23 (Rugh 1952), obtained by artificial fertilization, were kept for twenty hours at room temperature in a 0.1% colchicine (Merck) solution in tap water. Afterwards, the embryos were sectioned in small pieces less than 1 mm and suspended in a 0.50% trypsin (BBL) in Holfreter without Ca for 20 minutes at 37°C. A fine cellular suspension was obtained by gentle pipetting during this step. After trypsinization the cells were centrifugated, the supernatant drawn off, the sediment washed in 5 cc of Holfreter solution, centrifugated and suspended in 2 cc of distilled water. After 30 minutes at room temperature the cells were centrifugated, the supernatant drawn off, the sediment fixed in 3/1 ethanol-acetic acid for 20 minutes. At last the cells were centrifugated again, the precipitated disrupted in 0.2 cc of ethanol-acetic acid, dropped on wet slides and dried on a Bunsen flame. In none of the cases were the cells squashed, so as to avoid if possible, excessive dispersion and loss of chromosomes. The preparations were stained 30 minutes at 60°C with natural orcein (Gurr) in 50% acetic acid. Coverglasses were mounted with Canada balsam. All the centrifugations were made at 700 r/m for 5 minutes.

Results

Chromosome counts of each embryo are listed in Table 1. A total of 196 suitable metaphases were counted from the twenty tadpoles. Of these metaphases 170 (87%) had a diploid number of 22; 21 (10%) had 21 and 5 (3%) cells showed 20 chromosomes.

The identification and karyotype of chromosomes were made on 70 good spread metaphases (Figs. 1 and 2). The chromosomes of *Bufo arenarum*

Table 1. *Chromosomes of Bufo arenarum*

Embryos	Number of chromosomes, $2n$			Total number of cells counted
	20	21	22	
1	—	1	4	5
2	1	2	7	10
3	—	2	7	9
4	—	—	5	5
5	—	1	6	7
6	—	—	7	7
7	1	—	8	9
8	—	—	11	11
9	—	1	14	15
10	—	2	11	13
11	1	3	14	18
12	—	1	11	12
13	1	3	15	19
14	—	2	12	14
15	1	—	11	12
16	—	—	4	4
17	—	1	9	10
18	—	—	5	5
19	—	—	4	4
20	—	1	5	6
Total	5	21	170	196

Fig. 1. Metaphase of *Bufo arenarum*. Black granules are melanin. Aceto-orcein 1200 $\times$.

can be arranged in three groups. The group I is composed by 5 pairs of long chromosomes. Pairs 1 and 5 metacentrics and 2, 3 and 4 submetacentrics. The group II is formed by four pairs of medium size metacentric chromosomes. In this group, the seventh pair shows a constant secondary constriction in one of its arms which makes it a different one (Fig. 3). Group III is composed of two pairs of short metacentric chromosomes.

No evident chromosome differences were found that would

suggest the existence of heterogametic sex.

Discussion

Although only 87% of the metaphases were found containing 22 chromosomes, we think that the sample is sufficiently large to come to the conclusion that 22 is the modal number in *Bufo arenarum*, as were postulated by Saez and col. (1934, 1936).

Otherwise the chromosome identification in the cells with 20 and 21 elements (3 and 10% respectively), showed a variable chromosome loss and so were interpreted as

technical artifacts. Court Brown and col. (1960) in a large series of human cells have demonstrated that there was a distribution about the modal number and that the distribution was negatively skewed. They found that 12% had a count below the modal number percentage that closely resembles to the results in this investigation.

Fig. 3. Part of a metaphase showing two members of the seventh pair with secondary constrictions. Aceto-orcein 2400 $\times$.

overlooked by the cited authors because they studied the strongly condensed testicular meiotic chromosomes. Di Berardino (1962) has found secondary constrictions in several pairs of somatic chromosomes of *Rana pipiens*.

The absence of sexual dimorphism in the chromosomes of *Bufo arenarum* agrees with the finding of Saez and col. (1936) in the same species and Di

Fig. 2. Karyotype of *Bufo arenarum*. 1000 $\times$.

Berardino (1960) in *Rana pipiens*. Yosida (1957) in *Hyla arborea* found sex chromosome dimorphism in the male and recently Weiler and col. (1962) were able to identify the sex chromosomes in *Xenopus laevis* describing the male as homogametic and the female as heterogametic according with what Chang and col. (1956) have postulated. New experiences will be necessary to identify the homo- and heterogametic sex in *Bufo arenarum*.

Summary

Chromosomes were analysed in 20 *Bufo arenarum* embryos. The modal number found was 22 which could be arranged in 3 groups. Group I has 5 pairs of long chromosomes, Group II 4 pairs of medium-sized chromosomes, Group III 2 pairs of short chromosomes. Pairs 2, 3 and 4 were sub-metacentrics, the remainder metacentrics. The pair 7 showed a constant secondary constriction in one of its arms. No evidence of sexual dimorphism was found.

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