

Karyological Studies of an Anuran Species, *Bufo kangii* Yoon

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물두꺼비의 핵형에 관한 研究

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적 요

空氣乾燥法을 이용하여 *B. kangii* Yoon의 骨髓細胞에서 分裂像을 얻었다.

本種의 핵형에 관한 특징은 다음과 같다.

- (1) 本種의 2倍體數는 22로서 큰 染色體 6쌍과 작은 染色體 5쌍으로 구분할 수 있다.
- (2) 모든 相同染色體는 metacentric 이었다.
- (3) 染色體에 第2收縮環이 없다.
- (4) 특이한 性染色體를 찾아볼 수 없었다.

INTRODUCTION

Karyological studies on vertebrates have been utilized not only to identify a species in relation to others, but also have provided a useful information in searching of their evolutionary processes. Though studies on anuran species have been extensively studied by many authors (Mana and Bhunya 1966, Bogart 1967, Beckert and Doyle 1967, 1970, Griffin *et al.* 1970), Beckert and Doyle (1967) were first able to provide a reliable elucidation of karyotypes on Bufonidae by means of air-drying technique.

The present paper is concerned with the karyological characteristics of *Bufo kangii* Yoon, which is one of two species in the family Bufonidae described in Korea.

MATERIALS AND METHODS

Adult male and female organisms of *B. kangii* Yoon collected from Mt. Sulak

were used for this study. Chromosome preparations were made from bone marrow cells with a slight modification described by Ojima *et al.* (1964) and Beckert and Doyle (1967).

The animals were received 0.01 ml/g body weight of 1% colchicine into peritoneal cavities, and killed an hour later. The femurs were cut off, and the bone marrow cells were removed from the shafts by amphibian Ringer's solution. The cell suspension was centrifuged at 1,500 rpm for 10 minutes (5°~7°C), and treated with the hypotonic solution (0.075 M KCl) for 30 minutes at 20°~25°C, and then fixed in acetic alcohol (1:3). A drop of the fixed cells was placed on a wet slide kept in 50% alcohol, dried quickly over a spirit flame, and stained with Giemsa. Well spread 30 metaphases were selected, photographed, and analyzed for idiogram, using the criteria established by Levan *et al.* (1964) and Ullerich (1966).

RESULTS AND DISCUSSION

The distribution of chromosome number of *B. kangii* Yoon is shown in table 1. Chromosome number of this species was ranged from 18 to 22, but the majority of the cells represented twenty-two chromosomes (81%). Thus the diploid number for this species was determined to be twenty-two.

Table 1. Distribution of chromosome numbers in *B. kangii* Yoon

Case	Sex	Total metaphase scored	Distribution of chromosome number				
			18	19	20	21	22
1	M	30	2	4	2	1	21
2	M	62	1	1	2	2	56
3	M	24	1	1	2	1	19
4	F	13	1	0	2	2	8
5	F	35	0	0	2	2	31
6	F	35	1	1	2	4	27
Total		199	6	7	12	12	162
%		100	3	3.5	4	4	81

Table 2 shows the measurements of relative length and arm ratio in each homologous chromosomes obtained from thirty metaphases. As shown in the table 2, the karyotype of *B. kangii* Yoon appears to have several consistent characteristics— (1) all chromosome pairs had similar arm ratios and were metacentrics; (2) karyotypic sexual dimorphism that may reflect sex chromosome was not observed; (3) the characteristic achromatic region of all homologues was not found.

Male and female karyotypes are shown in Figs.1 and 2. Ullerich(1966), Beckert and Doyle(1967, 1970), working with various species of *Bufo*. ordered the pairs of chromosomes in a series of decending size. This system was also followed in these figures.

In comparing the karyotypes of *B. bufo*, *B. viridis*, *B. calamita* and *B. marinus*, identified by Ullerich(1966) and Beckert and Doyle(1967) with this species, the following conclusions were made— (1) the diploid number of all five species was found to be twenty-two; (2) in *B. kangii* Yoon there was a steady decline in relative length of chromosomes, but the size was largely divided into two groups. i.e. six large (12-6 μ) and five small (3.5-2 μ) homologues. This characteristic was consistent with those of *B. bufo*, *B. viridis*, and *B. calamita* and differed from that of *B. marinus* which was reported as having five large six small pairs; (3) the secondary constriction reported for *B. bufo* and *B. viridis* on the sixth chromosome pair was not observed; (4) the achromatic region reported for *B. marinus* was not found in this species.

Table 2. Mean karyotype measurements from thirty random spreads

Chromosome number	Relative length (%)	Ratio	$\frac{\text{Length of long arm}}{\text{Length of short arm}}$
1	17.2 \pm 1.6		1.10 \pm 0.06 m
2	15.6 \pm 0.5		1.25 \pm 0.08 m
3	13.5 \pm 0.3		1.50 \pm 0.20 m
4	12.1 \pm 0.3		1.47 \pm 0.50 m
5	10.7 \pm 0.3		1.16 \pm 0.09 m
6	9.2 \pm 0.4		1.34 \pm 0.14 m
7	5.2 \pm 0.6		1.21 \pm 0.05 m
8	4.6 \pm 0.3		1.13 \pm 0.06 m
9	4.4 \pm 0.1		1.36 \pm 0.15 m
10	4.0 \pm 0.1		1.33 \pm 0.18 m
11	3.7 \pm 0.3		1.07 \pm 0.08 m

m: metacentric chromosome

SUMMARY

Metaphases were obtained from the bone marrow cells of *B. kangii* Yoon, by means of direct air-drying technique. The karyological characteristics of this species were as follows;

- 1) The diploid chromosome number was 22 (2n=22) which might be divided into six large and five small homologous chromosomes.
- 2) All homologous chromosomes of this species were metacentrics.

- 3) The secondary constriction was not found in all members of chromosomes.
- 4) There was no evidence for the existence of a specific sex chromosome pair in this species.

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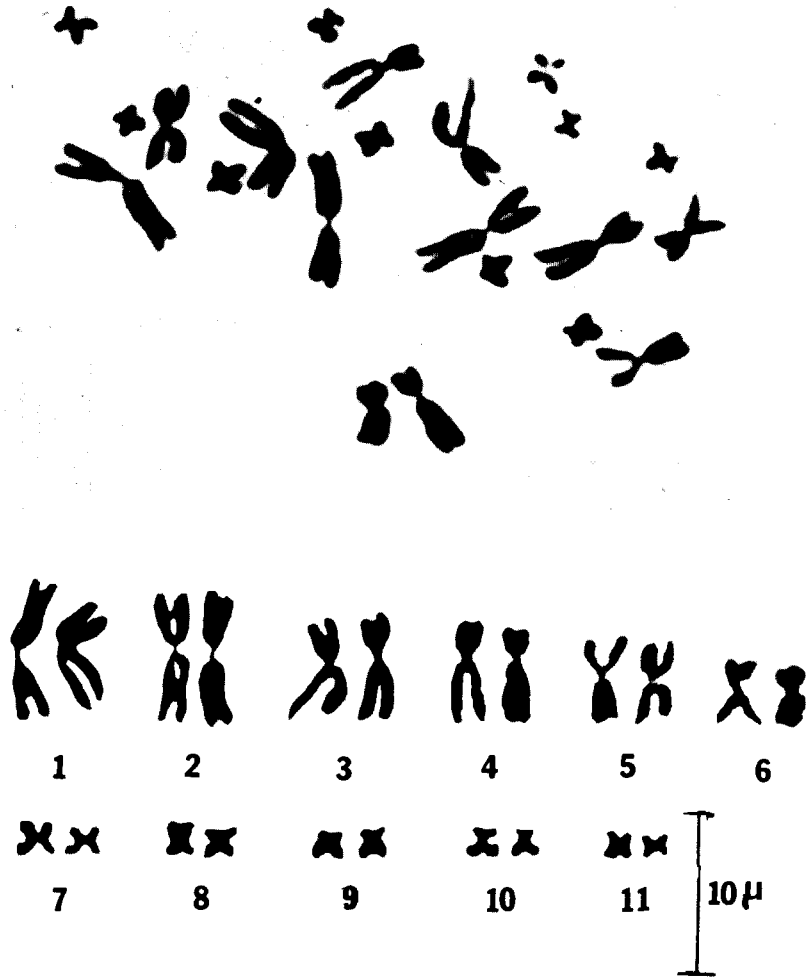


Fig.1. The metaphase chromosome and idiogram of *Bufo kangii* (♂)

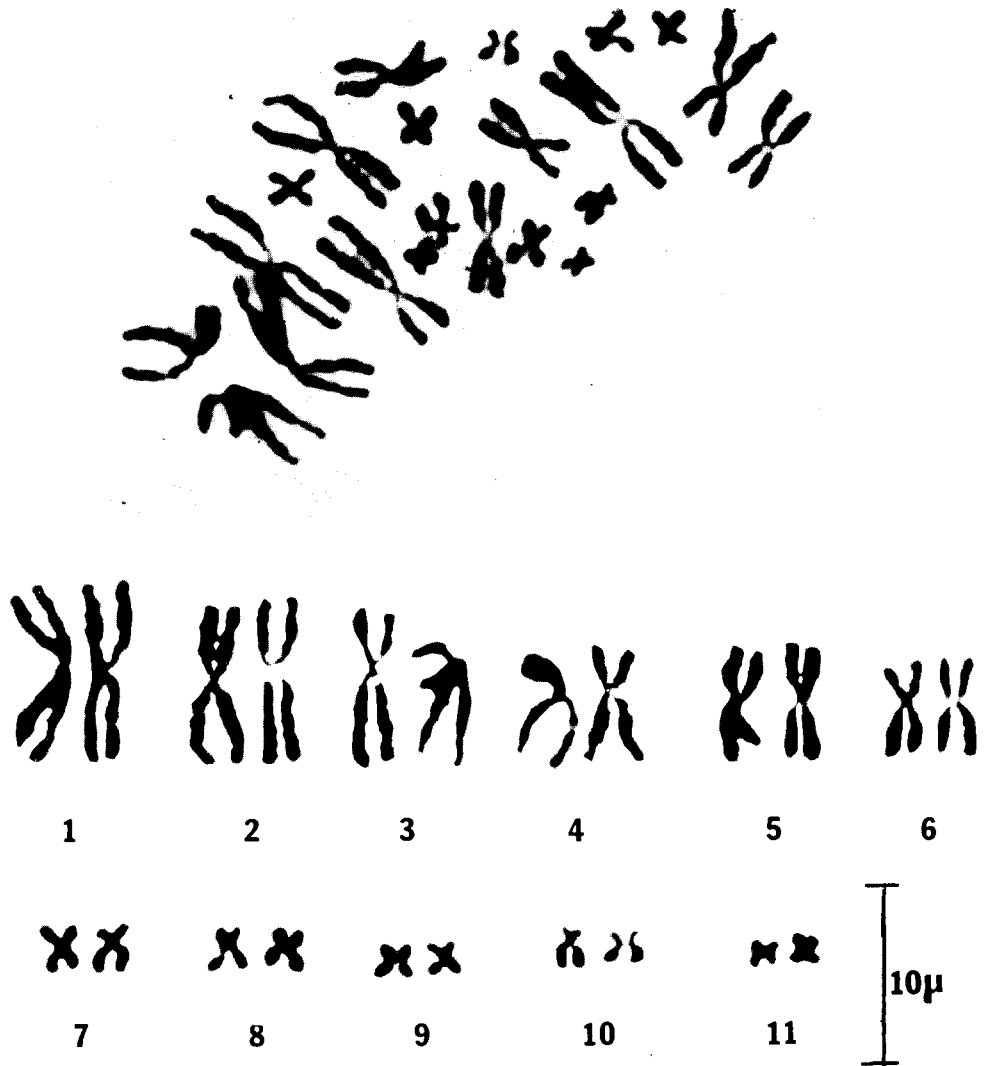


Fig.2. The metaphase chromosome and idiogram of *Bufo kangii*(♀)