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KARYOTYPES OF TWO TAIWAN RHACOPHORID FROGS, *RHACOPHORUS TAIPEIANUS* AND *R. MOLTRECHTI*

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Mitsuru Kuramoto (1985) Karyotypes of two Taiwan rhacophorid frogs, *Rhaco phorus taipeianus* and *R. moltrechti*. *Bull. Inst. Zool., Academia Sinica* **24**(1): 63-68. Two endemic Taiwan rhacophorid frogs, *Rhacophorus taipeianus* and *R. moltrechti*, have $2n=26$ chromosomes with five large and eight small pairs. Their karyotypes differ significantly in six of 13 chromosome pairs. Although *R. moltrechti* has acoustic characteristics very similar to those of *R. owstoni* occurring in the Ryukyu Islands, the karyological divergence between the two species is as large as that between *R. moltrechti* and *R. taipeianus*.

The karyotype is one of the species specific characteristics reflecting the phylogenetic relationships of organisms. For example, the karyotype of a frog *Rana kuhlii* of Taiwan is nearly identical to that of *R. namiyei* of the Ryukyu Islands, indicating that the two have derived from a common ancestral stock (Kuramoto, 1984a).

The frog genus *Rhacophorus* includes about 20 species ranging widely in southeastern Asia (Liem, 1970). Karyotypes of four Japanese species have been given by Kuramoto (1977) and Okumoto (1977), but those of Taiwan species have not been reported. In this paper I describe the karyotypes of two endemic Taiwan species, *Rhacophorus taipeianus* and *R. moltrechti*, and compare them with each other and with the karyotype of *R. owstoni*, a relative occurring in southwestern Ryukyu Islands (Kuramoto, 1977; Kuramoto and Utsunomiya, 1981).

MATERIALS AND METHODS

Specimens of *R. taipeianus* were obtained from Mucha (4 males) and Nankang (3 males), Taipei and those of *R. moltrechti* from Shitou (4 males), Nantou-Sien. Chromosome spreads were prepared from bone marrow cells according to Omura's (1967) method. Length of long arm and short arm of each chromosome was measured with a digitizer (MYPAD-A3) on enlarged photomicrographs of metaphase chromosome spreads, and relative length (length of a chromosome pair with total length of a haploid set being 100) and arm ratio (long arm length/short arm length) were calculated chromosome pairs were numbered in the order of decreasing mean relative length and chromosome shape was described based on Levan *et al.* (1964).

RESULTS

R. taipeianus and *R. moltrechti* have $2n=26$ chromosomes with five large and eight small pairs (Fig. 1). Pair nos. 3, 7, 8 and 11 of *R. taipeianus* and nos. 6, 8 and 13 of *R. moltrechti* are submedian, and the other pairs are median (Table 1). In three of seven spreads analyzed, *R. taipeianus* had a secondary constriction on the short arm of pair no. 11, whereas no clear secondary constrictions were observed in *R. moltrechti*. No heteromorphic pairs could be detected in both karyotypes.

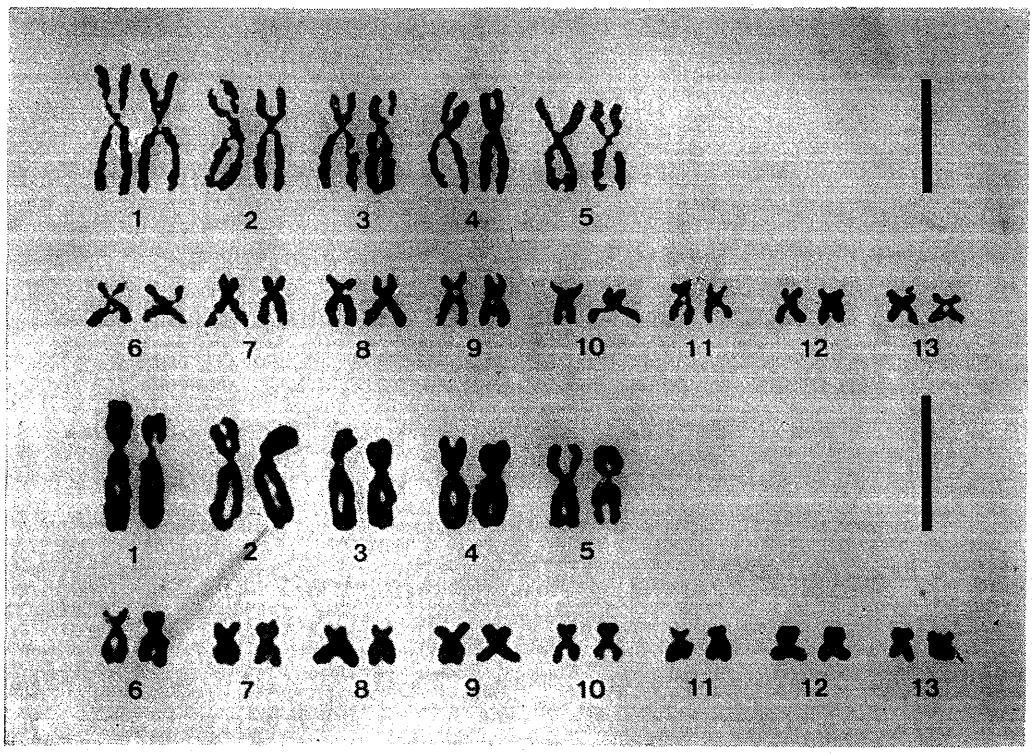

Fig. 1. Karyotypes of two Taiwan rhacophorid frogs, *R. taipeianus* (upper) and *R. moltrechti* (lower). Scales equal 10 μm.

TABLE 1

Relative length and arm ratio ($\bar{x}\pm$SE) of chromosomes of two Taiwan rhacophorid frogs

Pair no.	*R. taipeianus* (N=7)		*R. moltrechti* (N=10)	
	Relative length	Arm ratio*	Relative length	Arm ratio*
1	13.07±0.25	1.43±0.04 m	14.58±0.19	1.53±0.03 m
2	11.79±0.24	1.59±0.04 m	12.53±0.26	1.66±0.05 m
3	11.07±0.07	1.78±0.03 sm	11.28±0.16	1.60±0.03 m
4	10.46±0.20	1.51±0.03 m	10.45±0.12	1.58±0.04 m
5	9.55±0.20	1.27±0.02 m	9.61±0.12	1.36±0.03 m
6	6.86±0.18	1.21±0.02 m	6.28±0.09	1.84±0.08 sm
7	6.28±0.09	1.87±0.02 sm	6.00±0.09	1.36±0.04 m
8	5.83±0.17	1.77±0.04 sm	5.60±0.07	2.07±0.09 sm
9	5.46±0.22	1.60±0.04 m	5.42±0.08	1.42±0.04 m
10	5.31±0.12	1.35±0.05 m	5.05±0.11	1.52±0.03 m
11	5.23±0.17	1.75±0.03 sm	4.71±0.08	1.69±0.05 m
12	4.85±0.18	1.33±0.03 m	4.37±0.10	1.57±0.04 m
13	4.25±0.12	1.39±0.04 m	4.12±0.08	1.84±0.08 sm

* m and sm indicate median and submedian respectively.

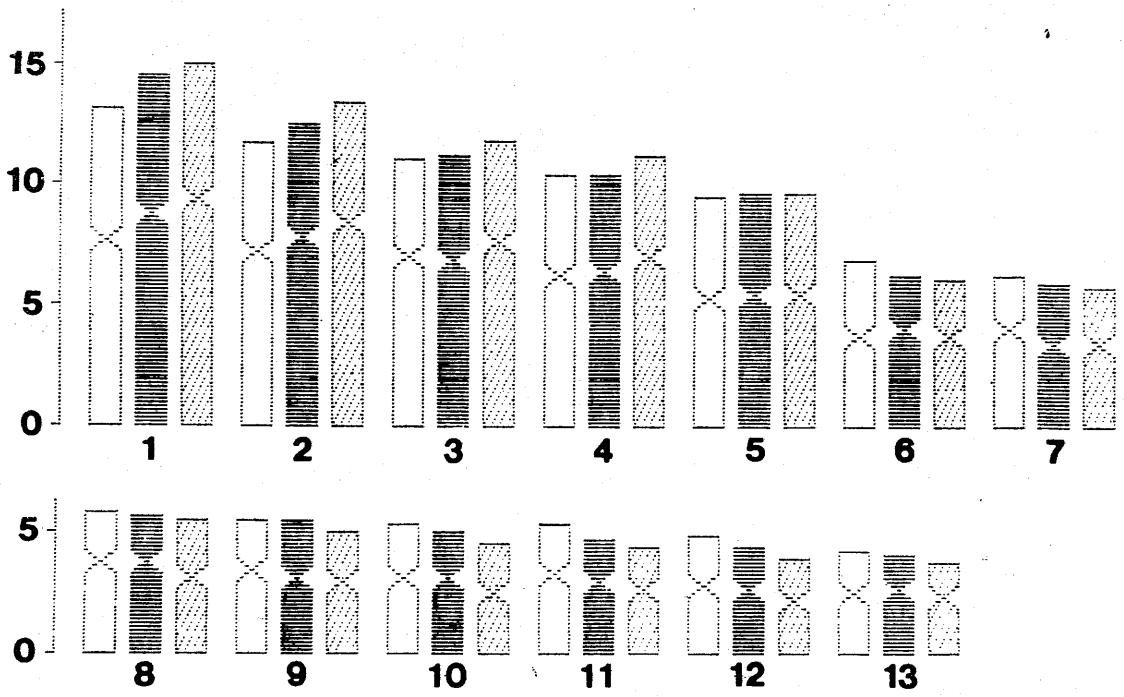

Fig. 2. A computor-depicted idiogram comparing karyotypes of *R. taipeianus* (hollow), *R. moltrachti* (solid) and *R. owstoni* (hatched).

There are no distinct differences between karyotypes of the two species; they have no specific chromosomes characterizing each species. A statistical test, however, revealed that six of 13 pairs differed significantly in either relative length or arm ratio, or in both, between the two species (t-test, $p<0.05$). Pair nos. 2, 3, 5, 7, 8, 9 and 10 of *R. taipeianus* correspond respectively to nos. 3, 4, 5, 6, 8, 10 and 9 of *R. moltrechti* in both relative length and arm ratio. This suggests that the chromosomes of the two species have differentiated through minor structural changes.

Fig. 2 compares karyotypes of the two rhacophorid frogs of Taiwan and *R. owstoni* of the Ryukyu Islands. The karyological data of *R. owstoni* are taken from Kuramoto (1977). The karyotype of *R. owstoni* differs from those of Taiwan species; the numbers of chromosome pairs that do not differ in both relative length and arm ratio were eight between *R. owstoni* and *R. taipeianus* and seven between *R. owstoni* and *R. moltrechti* ($p<0.05$). Mean total length of large chromosome pairs was larger in *R. owstoni* ($\bar{x}=61.3$) than that of *R. moltrechti* ($\bar{x}=58.5$) and the latter was larger than that of *R. taipeianus* ($\bar{x}=55.9$); the differences were highly significant ($p<0.01$).

DISCUSSION

Including *R. maculatus* of India (Singh, 1974) and *R. pardalis* of Philippines (Kuramoto, unpubl.), karyotypes of eight *Rhacophorus* species are now available for comparison. All of them have 26 chromosomes consisting of five large and eight small chromosomes. In this respect the genus *Rhacophorus* is similar to a common frog genus *Rana* (Kuramoto, 1972; Lin and Huang, 1979), suggesting a close relationship of two families, Rhacophoridae and Ranidae.

Because the rhacophorid frogs of Taiwan and Japan are similar in size, shape, dorsal color, habitat and breeding ecology, the taxonomic status of each member has been confusing (Inger, 1947; Chen, 1956). Acoustic studies (Kuramoto, 1975, 1984b; Kuramoto and

Utsunomiya, 1981) revealed that *R. taipeianus* described by Liang and Wang (1978) is distinct species and that *R. owstoni* of the Ryukyu Islands is related to *R. moltrechti*, not to *R. taipeianus*. Karyological comparisons, however, do not show clearly that *R. moltrechti* resembles *R. owstoni*. The karyotypes of the two species differ from each other as the karyotype of *R. taipeianus* differs from that of *R. moltrechti* or *R. owstoni*.

Kuramoto (1977), comparing karyotypes of *R. owstoni*, *R. viridis* and *R. schlegelii*, showed that the numbers of chromosome pairs that do not differ in both relative length and arm ratio are 7-10. The corresponding numbers for three species here compared are within this range. Apparently *R. owstoni* has diverged karyologically from *R. moltrechti*, although the two have essentially identical cell structures. Since all Japanese species have large chromosome pairs that are much longer in total length than in Taiwan species (Kuramoto, 1977), it seems that some structural changes occurred after the ancestral form invaded the Ryukyu Islands.

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兩種樹蛙 *Rhacophorus taipeianus* 和 *R. moltrechti* 之染色體型

倉 本 滿

兩種本地特有種之樹蛙，*Rhacophorus taipeianus* 和 *R. moltrechti*，各具有 2n=26 個染色體，五對大的和八對小的染色體。這兩種樹蛙的染色體型很明顯地有六對染色體不相同，雖然在琉球，*R. molrechti* 和 *R. moltrechti* 之叫聲非常相似，但是這兩種樹蛙之染色體型比 *R. taipeianus* 和 *R. moltrechti* 之染色體型分化得遠。