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The Karyotypes of Four *Rhacophorus* Species Distributed in Japan

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(With 7 Text-figures)

INTRODUCTION

Of the anurans distributed in Japan, six species belong to the family Rhacophoridae. Although these species are placed at present into a single genus *Rhacophorus* by Japanese taxonomists, they can be divided into two groups according to color and pattern of the body as well as spawning habit. Three species of the first group are green in dorsal color and have no dark cross-bars on the hind legs. Eggs are laid as a foamy mass. Three species of the second group are dull brown in dorsal color and have dark cross-bars on the hind legs. They lay eggs enveloped in transparent jelly-like substance. While two species of the first group, *Rh. schlegelii* and *Rh. arboreus*, and one species of the second, *Rh. buergeri*, are distributed in the main islands of Japan, the remaining three species are distributed in the southwestern archipelagos. One of the latter, *Rh. japonicus*, belongs to the second group. *Rhacophorus arboreus* resembles *Rh. schlegelii* so closely in morphological characters that it had been classified as a variety or subspecies until KAWAMURA (1962) distinguished it as a valid species. These two species are sympatric and are very similar to each other in the environmental condition of habitats. *Rhacophorus japonicus* differs to some extent from *Rh. buergeri* in morphological characters as well as in the environmental condition of habitats, although it belongs to the same group as the latter. It has been interesting to the author to determine how morphological and ecological differences among these four species are reflected to their karyotypes. The results of observations on this question are presented in this paper.

MATERIALS AND METHODS

The karyotypes of the four species, *Rhacophorus schlegelii* (GÜNTHER), *Rh. arboreus* (OKADA and KAWANO), *Rh. buergeri* (SCHLEGEL) and *Rh. japonicus* (HALLOWELL), were examined by making use of their tadpoles. The tadpoles of *Rh. schlegelii* and *Rh. arboreus* were those which developed from eggs obtained in the field. While egg masses of *Rh. schlegelii* were collected from the suburbs of Hiroshima City, those of *Rh. arboreus* were from the northern part of Hiroshima

Prefecture. The tadpoles of *Rh. buergeri* and *Rh. japonicus* were obtained by artificial fertilization in our laboratory. Parents of *Rh. buergeri* were collected from the northern part of Hiroshima Prefecture, while those of *Rh. japonicus* were from Amami Oshima, Kagoshima Prefecture.

Squash preparations were made from the tail-tips of tadpoles basically according to the method of MAKINO and NISHIMURA (1952). The procedure is as follows: tadpoles are reared in a 50 mg/l colchicine (Merck) solution for 15~18 hours at room temperature. Tail-tips are cut off, immersed in distilled water for 60~120 minutes, and stained with 1% orcein (Chroma) dissolved in 45% acetic acid for 30~60 minutes on a slide glass. They are squashed under a cover glass after heated for 20~30 seconds and then mounted with PVLB*.

Karyotype analysis was carried out by making use of enlarged photographs of metaphase spreads. The length of each chromosome was measured and then the average length of homologous chromosomes was calculated. The genome length was obtained by totaling the average lengths of all the kinds of chromosomes. A hundred-times quotient of the average length of each chromosome divided by the genome length was called the relative chromosome length (RL). The position of the centromere of each chromosome was expressed by a numerical value (NVC) that is a hundred-times quotient of the short-arm length divided by the chromosome length.

The karyotype of *Rh. schlegelii* was analyzed on 52 metaphase spreads of 29 tadpoles. Forty-seven of these spreads were utilized to calculate the relative chromosome length and the centromere position of each chromosome. The karyotype of *Rh. arboreus* was analyzed on 62 metaphase spreads of 35 tadpoles. Fifty of these spreads were used for measurements of each chromosome. The karyotype analyses as well as the measurements of chromosomes in *Rh. buergeri* and *Rh. japonicus* were made on 51 metaphase spreads of 15 tadpoles and 51 metaphase spreads of 25 tadpoles, respectively. The karyotypes of the four species were compared with one another in terms of relative chromosome length and centromere position by the method of HUBBS and HUBBS (1953).

OBSERVATION

I. Karyotype of each species

The chromosomes of the four species, *Rh. schlegelii*, *Rh. arboreus*, *Rh. japonicus* and *Rh. buergeri*, are all 26 in diploid number. Their karyotypes are shown in Figs. 1~4, where the chromosomes are arranged in order of length. Each of 13 pairs consists of two homologous chromosomes of the same size and shape. They are divided into two groups according to their size. Group 1 consists of five large chromosomes, Nos. 1 to 5, while group 2 of eight small chromosomes, Nos. 6 to 13. On the other hand, the 13 pairs of chromosomes are divided into three types, median, submedian and subterminal according to the numerical values of

* PVLB: paraffin, vaseline, lanolin and Canadian balsam=2: 1: 1: 1

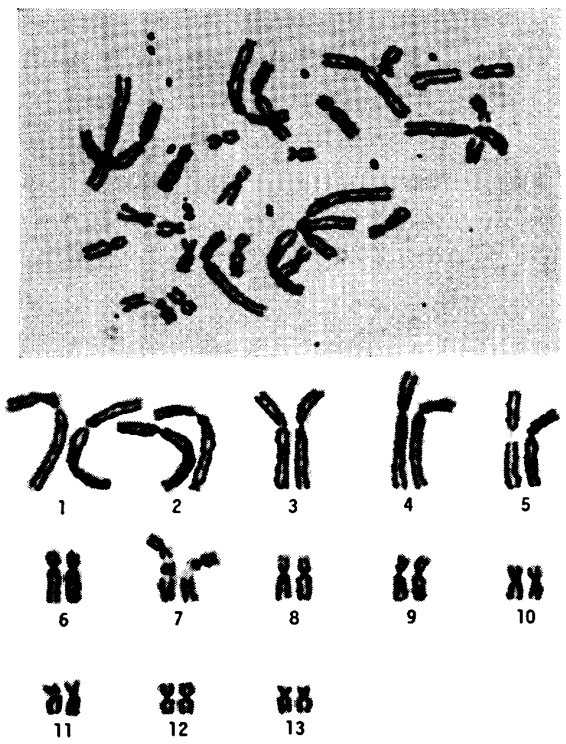

Fig. 1. Metaphase plate and the karyotype of an epidermal cell from a *Rhacophorus schlegelii* tadpole. × 1300.

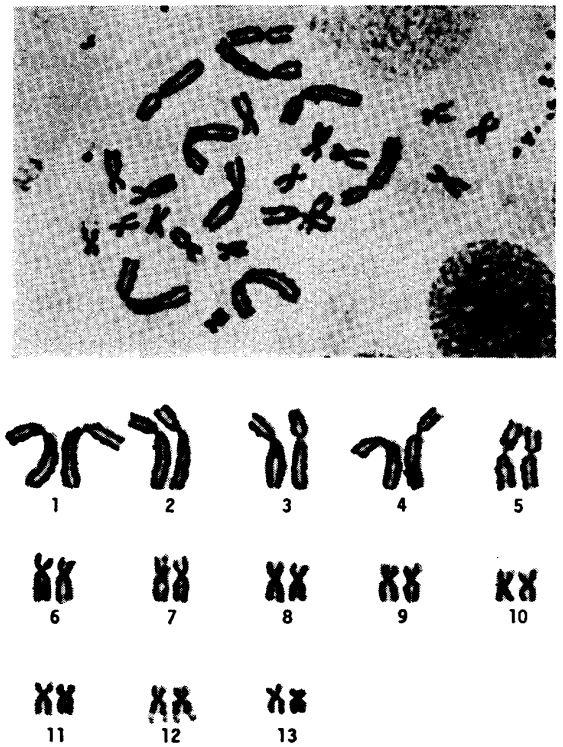

Fig. 2. Metaphase plate and the karyotype of an epidermal cell from a *Rhacophorus arboreus* tadpole. × 1300.

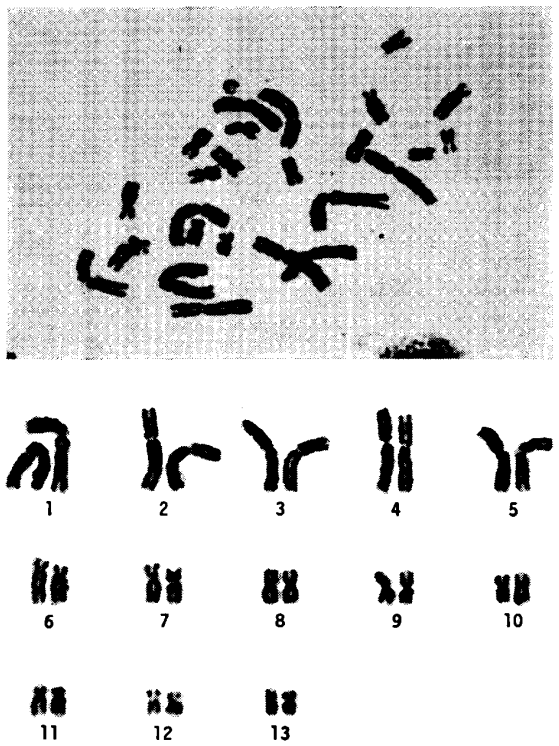

Fig. 3. Metaphase plate and the karyotype of an epidermal cell from a *Rhacophorus japonicus* tadpole. × 1300.

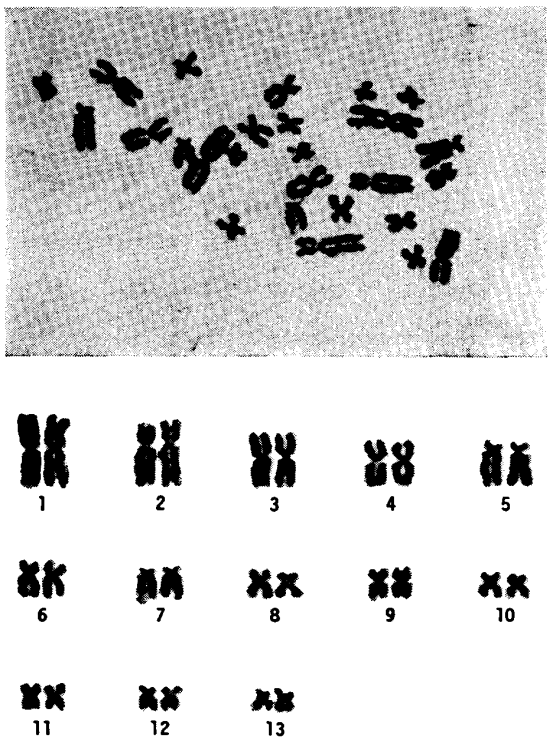

Fig. 4. Metaphase plate and the karyotype of an epidermal cell from a *Rhacophorus buergeri* tadpole. × 1300.

their centromere positions, 50.0~37.5, 37.5~25.0, 25.0~12.5, respectively. *Rhacophorus schlegelii* and *Rh. arboreus* have chromosomes of the median, submedian and median or submedian type, while *Rh. japonicus* and *Rh. buergeri* have those of the subterminal and subterminal or submedian type besides the three kinds of chromosomes found in *Rh. schlegelii* and *Rh. arboreus*. Each species has either a secondary constriction or a satellite in a chromosome pair of group 2. Such a secondary constriction or satellite gives a specific feature to the karyotype of each species. No heteromorphic pair of chromosomes considered to be that of sex chromosomes is found in the four species. In Tables 1 and 2, RL and NVC of the chromosomes of the four species are presented, respectively.

TABLE 1

Relative lengths of metaphase chromosomes of four *Rhacophorus* species

Rh. schlegelii				*Rh. arboreus*			
Chromosome no.	Minimum	Maximum	Mean	Chromosome no.	Minimum	Maximum	Mean
1	13.1	16.4	15.0±0.11	1	13.6	16.5	15.2±0.08
2	11.8	14.3	13.2±0.08	2	12.1	14.4	13.3±0.07
3	10.2	12.8	11.8±0.07	3	11.2	13.5	12.1±0.07
4	10.5	13.2	11.5±0.07	4	10.9	12.9	11.8±0.07
5	8.7	10.6	9.7±0.06	5	9.1	11.0	9.8±0.05
6	5.6	6.9	6.1±0.05	6	5.8	7.1	6.3±0.04
7	5.6	7.1	6.1±0.05	7	5.1	6.1	5.5±0.03
8	4.7	5.7	5.3±0.04	8	4.8	5.7	5.3±0.03
9	4.6	5.8	5.1±0.03	9	4.6	5.6	5.0±0.03
10	3.7	5.2	4.3±0.04	10	3.7	4.6	4.3±0.03
11	3.6	5.2	4.3±0.04	11	3.4	4.7	4.2±0.03
12	3.6	4.4	4.0±0.03	12	3.2	4.2	3.6±0.03
13	3.1	4.3	3.6±0.04	13	2.8	4.2	3.6±0.04
Rh. japonicus				*Rh. buergeri*			
Chromosome no.	Minimum	Maximum	Mean	Chromosome no.	Minimum	Maximum	Mean
1	13.5	16.2	14.8±0.10	1	14.3	17.3	15.5±0.10
2	11.9	15.0	13.2±0.08	2	10.9	14.8	12.2±0.10
3	10.4	13.4	11.7±0.09	3	9.8	12.2	11.1±0.07
4	10.7	12.9	11.7±0.07	4	9.0	10.6	9.8±0.05
5	9.6	11.3	10.3±0.06	5	8.6	10.7	9.6±0.07
6	5.6	6.6	6.0±0.04	6	6.9	7.7	7.3±0.03
7	5.0	6.3	5.7±0.04	7	4.8	6.3	5.7±0.05
8	4.7	5.8	5.3±0.04	8	5.3	6.2	5.7±0.03
9	4.3	5.9	5.0±0.05	9	4.9	5.9	5.4±0.03
10	3.6	5.2	4.5±0.05	10	4.4	5.7	5.0±0.04
11	3.7	4.8	4.2±0.04	11	4.1	5.4	4.6±0.05
12	2.6	4.7	3.7±0.07	12	3.6	5.2	4.3±0.05
13	3.0	4.4	3.7±0.04	13	3.2	4.4	3.8±0.04

Relative chromosome length: $\frac{\text{Each chromosome length}}{\text{Genome length}} \times 100$

±Standard error of the mean

TABLE 2

Centromere positions represented by numerical values and types of metaphase chromosomes of four *Rhacophorus* species

Rh. schlegelii					*Rh. arboreus*				
Chromosome no.	Minimum	Maximum	Mean	Type	Chromosome no.	Minimum	Maximum	Mean	Type
1	34.9	41.5	38.1±0.20	m	1	35.6	42.2	39.2±0.19	m
2	29.7	37.5	33.7±0.22	sm	2	29.5	37.7	33.8±0.24	sm
3	36.7	42.3	39.3±0.20	m	3	34.6	41.5	37.6±0.22	m or sm
4	32.5	38.8	35.8±0.21	sm	4	27.7	38.2	34.0±0.27	sm
5	41.5	46.5	44.3±0.15	m	5	40.3	45.7	43.6±0.19	m
6	29.5	38.5	34.2±0.31	sm	6	28.9	37.1	34.5±0.23	sm
7	31.7	39.7	36.0±0.28	sm	7	32.7	41.8	36.8±0.31	sm
8	32.1	41.8	37.6±0.33	m or sm	8	36.8	44.0	39.4±0.22	m
9	36.0	43.7	40.5±0.26	m	9	36.8	45.4	40.6±0.27	m
10	41.7	48.5	45.2±0.23	m	10	38.5	47.8	45.2±0.20	m
11	35.3	45.7	40.0±0.33	m	11	36.5	44.1	39.5±0.22	m
12	43.9	48.9	46.9±0.18	m	12	41.1	47.9	44.9±0.24	m
13	29.3	40.3	36.3±0.31	sm	13	33.3	40.0	36.9±0.22	sm
Rh. japonicus					*Rh. buergeri*				
Chromosome no.	Minimum	Maximum	Mean	Type	Chromosome no.	Minimum	Maximum	Mean	Type
1	40.5	48.2	44.2±0.26	m	1	42.2	46.9	44.6±0.18	m
2	33.2	45.0	38.1±0.29	m	2	25.8	33.8	30.2±0.28	sm
3	38.6	46.2	42.4±0.23	m	3	42.3	47.7	45.1±0.19	m
4	33.9	43.2	38.8±0.26	m	4	42.0	48.5	45.0±0.23	m
5	39.6	47.8	44.8±0.22	m	5	16.0	25.0	20.4±0.29	st
6	17.1	30.1	24.8±0.37	st or sm	6	35.1	43.5	39.3±0.29	m
7	35.6	44.9	41.2±0.25	m	7	19.4	33.3	27.0±0.41	sm
8	40.0	49.3	45.1±0.29	m	8	36.5	46.1	43.3±0.28	m
9	40.2	50.0	47.6±0.30	m	9	38.0	50.0	45.0±0.35	m
10	38.9	50.0	47.8±0.23	m	10	42.5	49.4	46.9±0.25	m
11	21.3	41.7	35.1±0.49	sm	11	41.0	49.3	45.6±0.31	m
12	33.3	49.1	43.7±0.56	m	12	30.5	44.3	37.9±0.43	m or sm
13	34.8	50.0	45.3±0.43	m	13	35.2	48.3	43.0±0.43	m

Numerical value of centromere position (NVC): $\frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$

±Standard error of the mean

Chromosome type:

NVC	Type
50.0～37.5	m
37.5～25.0	sm
25.0～12.5	st
12.5～ 0.0	t

1. *Rhacophorus schlegelii*

Of the chromosomes of group 1, three pairs are of the median type and two of the submedian type. The largest chromosome No. 1 is 15.0 in RL, and of the median type with NVC of 38.1, while No. 2 is 13.2 in RL and of the submedian type with NVC of 33.7. Chromosomes Nos. 3 and 4 are similar to each other in relative length, that is, 11.8 and 11.5, respectively. They differ from each other in the position of centromere; No. 3 is of the median type with NVC of 39.3, while No. 4 is of the submedian type with NVC of 35.8. Chromosome No. 5 is the smallest in group 1. It is 9.7 in RL and of the median type with NVC of 44.3.

In group 2, the chromosomes gradually become smaller with the advance of their numbers from 6 to 13. Of these eight pairs, four are of the median type,

three of the submedian type and one of the median or submedian type. Chromosomes Nos. 6 and 7 are the largest in group 2; both of them are 6.1 in RL and are of the submedian type, being 34.2 and 36.0 in NVC. Chromosome No. 7 is peculiar in having a distinct secondary constriction in the long arm. This is the most remarkable feature of the karyotype of *Rh. schlegelii*. Chromosomes Nos. 8 and 9 are 5.3 and 5.1 in RL, and 37.6 and 40.5 in NVC, respectively. They resemble each other so closely that they are often indistinguishable from each other. Chromosomes Nos. 10, 11 and 12 are 4.3, 4.3, 4.0 in RL and 45.2, 40.0 and 46.9 in NVC, respectively. No. 11 is distinguishable from the others, as its centromere is more eccentrically located. The smallest chromosome No. 13 is 3.6 in RL and of the submedian type with NVC of 36.3.

2. *Rhacophorus arboreus*

Of the five pairs of chromosomes belonging to group 1, two are of the median type, two of the submedian type and one of the median or submedian type, differing from those of *Rh. schlegelii*. The largest chromosome No. 1 is 15.2 in RL and of the median type with NVC of 39.2, while No. 2 is 13.3 in RL and of the submedian type with NVC of 33.8. Chromosomes Nos. 3 and 4 are 12.1 and 11.8 in RL, respectively. Although these two are similar to each other in relative chromosome length, they differ in the position of centromere. While No. 3 is of the median or submedian type with NVC of 37.6, No. 4 is of the submedian type with NVC of 34.0. Chromosome No. 5 is 9.8 in RL and of the median type with NVC of 43.6.

Seven of the 8 pairs of chromosomes belonging to group 2 are the same as those of *Rh. schlegelii* in chromosome type; Nos. 6, 7 and 13 are of the submedian type, while Nos. 9, 10, 11 and 12, are of the median type. Chromosome No. 6 is the largest in group 2; it is 6.3 in RL and 34.5 in NVC. Chromosomes Nos. 7, 8 and 9 are 5.5, 5.3 and 5.0 in RL, and 36.8, 39.4 and 40.6 in NVC, respectively. It is sometimes difficult to distinguish No. 7 from No. 8, and also No. 8 from No. 9. Chromosomes Nos. 10 and 11 are 4.3 and 4.2 in RL, respectively. The centromere of No. 10 which is 45.2 in NVC is more centrally located than that of No. 11 which is 39.5 in NVC. Chromosomes Nos. 12 and 13 are the smallest, being both 3.6 in RL. No. 12 is of the median type with NVC of 44.9, while No. 13 is of the submedian type with NVC of 36.9. The No. 12 chromosome has a satellite at the end of the long arm. This is the most remarkable feature of the karyotype of *Rh. arboreus*.

3. *Rhacophorus japonicus*

The five pairs of chromosomes belonging to group 1 are all of the median type, differing from those of *Rh. schlegelii* and *Rh. arboreus*. The largest No. 1 chromosome is 14.8 in RL and 44.2 in NVC. Chromosome No. 2 is 13.2 in RL and of the median type near the submedian type being 38.1 in NVC. Chromosomes Nos. 3 and 4 are similar in size, being both 11.7 in RL. They are 42.4 and 38.8 in NVC, that is, of the median type, although No. 4 is near the submedian type.

The smallest No. 5 chromosome of group 1 is 10.3 in RL and 44.8 in NVC.

Differing from the chromosomes of group 2 in *Rh. schlegelii* and *Rh. arboreus*, those of *Rh. japonicus* consist of 6 pairs of the median type, one pair of the submedian type and one pair of the subterminal or submedian type. Chromosome No. 6 is 6.0 in RL and 24.8 in NVC, that is, the largest in group 2 is of the subterminal or submedian type. Chromosomes Nos. 7, 8, 9 and 10 are 5.7, 5.3, 5.0 and 4.5 in RL and 41.2, 45.1, 47.6 and 47.8 in NVC, respectively. They are all of the median type and so similar to one another in size and shape that they are often indistinguishable, except No. 7 whose centromere is more eccentrically located than those of the other chromosomes. Chromosome No. 11 is 4.2 in RL and of the submedian type with NVC of 35.1. Chromosomes Nos. 12 and 13 are equal in size, being 3.7 in RL, and are 43.7 and 45.3 in NVC, respectively. No. 12 differs from No. 13 in possessing a slight secondary constriction in the short arm.

4. *Rhacophorus buergeri*

The chromosomes of group 1 in *Rh. buergeri* consist of 3 pairs of the median type, one pair of the submedian type and one pair of the subterminal type, differing from those of the other three species. Chromosome No. 1 is 15.5 in RL and of the median type with NVC of 44.6, while No. 2 is 12.2 in RL and of the submedian type with NVC of 30.2. No. 3 is 11.1 in RL and of the median type with NVC of 45.1. Chromosome No. 4 is also of the median type with NVC of 45.0, but it is 9.8 in RL. Chromosome No. 5 is nearly equal in size to No. 4; it is 9.6 in RL. However, No. 5 is of the subterminal type with NVC of 20.4. In this respect, the No. 5 chromosome is not only peculiar among the five pairs of chromosomes of group 1 in *Rh. buergeri*, but also such a chromosome is not found among those in the other three *Rhacophorus* species.

The chromosomes of group 2 in *Rh. buergeri* consist of 6 pairs of the median type, one pair of the submedian type and one pair of the median or submedian type, differing from those of the other three species. Between Nos. 6 and 7, there is an evident difference in size. The largest No. 6 chromosome is 7.3 in RL and of the median type with NVC of 39.3. Chromosomes Nos. 7 and 8 are both 5.7 in RL. While No. 7 is 27.0 in NVC, being of the submedian type near the subterminal, No. 8 is of the median type with NVC of 43.3. Chromosome No. 7 has a satellite at the end of the long arm. Chromosomes Nos. 9, 10 and 11 are 5.4, 5.0 and 4.6 in RL and 45.0, 46.9 and 45.6 in NVC, respectively; they are similar in size and all of the median type. Chromosome No. 12 is 4.3 in RL and of the median or submedian type with NVC of 37.9, while No. 13 is 3.8 in RL and of the median type with NVC of 43.0.

II. Comparison of the karyotypes

When the karyotypes of the four *Rhacophorus* species are compared with one another, it can be found at a glance that there is a secondary constriction in the long arm of chromosome No. 7 of *Rh. schlegelii* and in the short arm of No. 12 of

Rh. japonicus, while there is none in the chromosomes of the other two species. On the other hand, there is a satellite at the long arm of No. 12 of *Rh. arboreus* and at the long arm of No. 7 of *Rh. buergeri*, while there is none in the chromosomes of the other two species (Fig. 5).

When the four species are compared with one another in terms of the relative length of chromosomes, a distinct difference is found between *Rh. buergeri* and the other three species, as shown in Fig. 6. There are statistically significant dif-

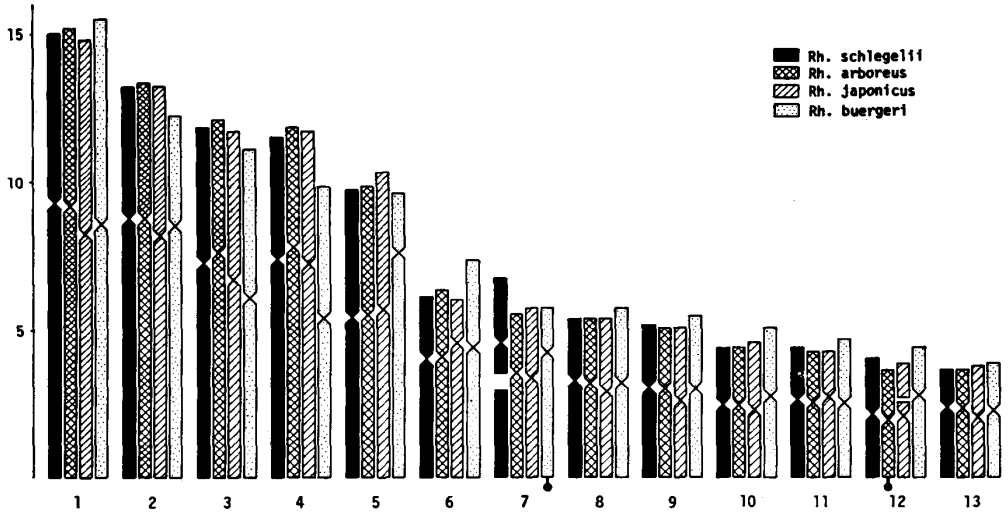

Fig. 5. Composite ideogram showing differences in relative chromosome length and centromere position among four *Rhacophorus* species. Constrictions indicate centromere position. Gaps indicate secondary constrictions. Small black circles indicate satellites.

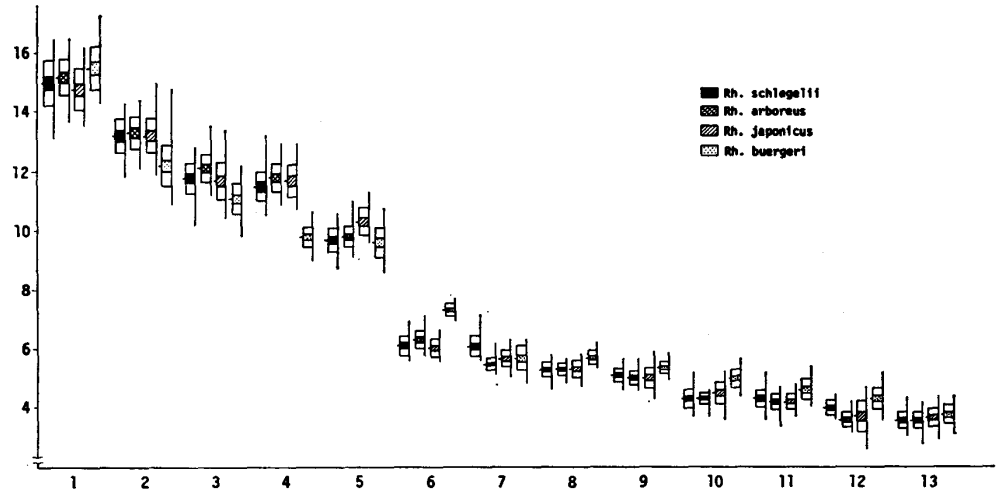

Fig. 6. Graph showing differences in relative chromosome length among four *Rhacophorus* species. In each foursome, a vertical line shows the range of relative chromosome lengths; a short horizontal line, the mean of the latter; an open rectangle on both sides of the horizontal line, the standard deviation; a small solid rectangle or the like on both sides of the horizontal line, two times the standard error of the mean. In general, if two solid rectangles or the likes do not overlap each other, the difference in relative length between the two chromosomes is statistically significant.

ferences between *Rh. buergeri* and the three other species in 9 chromosomes, Nos. 2, 3, 4, 6, 8, 9, 10, 11 and 12. On the contrary, when comparisons are made among the three other species, *Rh. schlegelii*, *Rh. arboreus* and *Rh. japonicus*, small differences are found among them. *Rhacophorus schlegelii* differs from the others in two chromosomes Nos. 7 and 12, *Rh. arboreus* differs from the others in three chromosomes Nos. 3, 6 and 7, and *Rh. japonicus* differs from the others in two chromosomes Nos. 5 and 10.

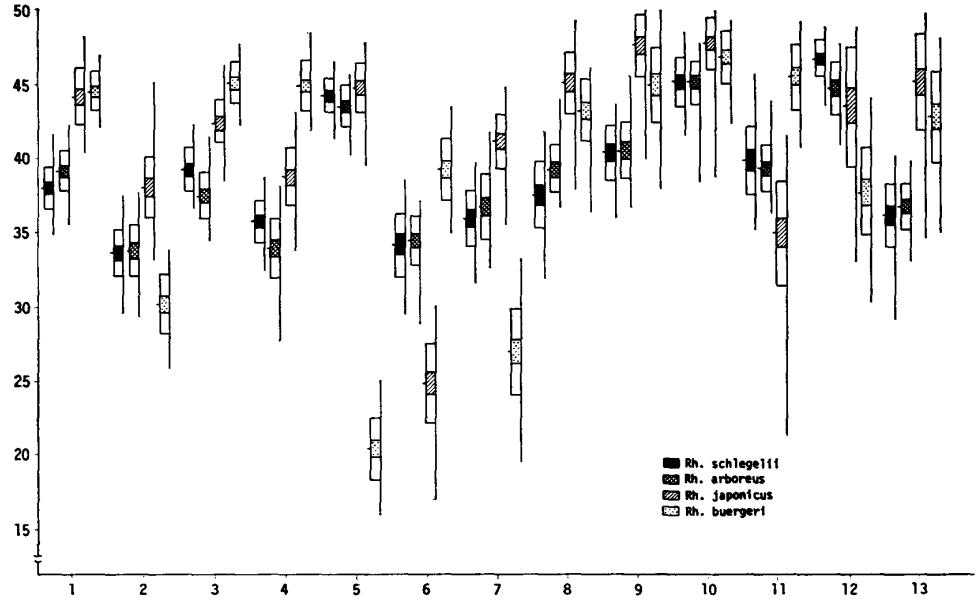

Fig. 7. Graph showing differences in centromere position among four *Rhacophorus* species. In each foursome, a vertical line shows the range of numerical values of centromere position; a short horizontal line, the mean of the numerical values; an open rectangle on both sides of the horizontal line, the standard deviation; a small solid rectangle or the like on both sides of the horizontal line, two times the standard error of the mean. In general, if two solid rectangles or the likes do not overlap each other, the difference in centromere position between the two chromosomes is statistically significant.

In the position of centromere, *Rh. schlegelii* and *Rh. arboreus* are very similar to each other, as shown in Fig. 7, although there are small differences in six chromosomes Nos. 1, 3, 4, 5, 8 and 12. Of these six chromosomes, No. 3 is of the median type in *Rh. schlegelii*, while it is of the median or submedian type in *Rh. arboreus*; the No. 3 chromosomes of the two species are 39.3 ± 0.20 and 37.6 ± 0.22 in NVC. Chromosome No. 8 is of the median or submedian type in *Rh. schlegelii*, while it is of the median type in *Rh. arboreus*; the No. 8 chromosomes of the two species are 37.6 ± 0.33 and 39.4 ± 0.22 in NVC. On the other hand, there are large differences in the position of centromere between the green-colored species, *Rh. schlegelii* and *Rh. arboreus*, and the brown-colored species, *Rh. japonicus* and *Rh. buergeri*. Statistically significant differences are found in all the chromosomes excluding Nos. 5 and 12. The differences are remarkably large in ten of the 11 chromosomes. Between *Rh. japonicus* and *Rh. buergeri* there are also statistically significant differences in the centromere position of all the chromo-

somes excluding Nos. 1 and 10. These differences are especially large in 6 of the 11 chromosomes.

DISCUSSION

The arboreal green-frog, *Rhacophorus arboreus* was first described by OKADA and KAWANO (1924) as a variety of *Rh. schlegelii* and later classified as a subspecies of the latter by OKADA (1931). The present status as a valid species was given by KAWAMURA (1962). Although these two species closely resemble each other in morphological characters, *Rh. arboreus* is considerably larger than *Rh. schlegelii*. These two species are sympatric and seasonally and ecologically isolated from each other. The breeding season of *Rhacophorus schlegelii* is usually about two weeks earlier than that of *Rh. arboreus*. While the former species lays a foamy egg mass in a hole formed underground, the latter does on tree leaves over water. According to NAKANE (1953, '54), the two species are completely isolated from each other by gametic isolation and hybrid inviability.

The two brown-colored species, *Rh. japonicus* and *Rh. buergeri* were classified as *Polypedates japonicus* (HALLOWELL) and *P. buergeri* (SCHLEGEL) by STEJNEGER (1907). This classification by STEJNEGER was followed by OKADA (1931). Later, NAKAMURA and UENO (1963) and KAWAMURA (1965) moved their genus name from *Polypedates* to *Rhacophorus*. However, LIEM (1970) placed these two species as *Buergeria japonica* and *B. buergeri*, by separating them from the genus *Polypedatus* or *Rhacophorus*.

There are a few reports concerning the chromosomes of three of the above four species. MAKINO (1932) has reported that the spermatogonia of *Rh. schlegelii* have 26 chromosomes consisting of 10 large and 16 small ones. SATO (1934) has also observed 26 chromosomes in spermatogonia and 13 bivalents in spermatocytes of *Rh. buergeri*. The author made a preliminary report on the karyotypes of *Rh. schlegelii* and *Rh. arboreus* (1967). Recently, YAMAMOTO and MASUDA (1973) have observed the karyotypes of *Rh. arboreus* and *Rh. buergeri*. According to YAMAMOTO and MASUDA, *Rh. buergeri* differs from *Rh. arboreus* in the centromere position of chromosomes Nos. 1 to 5 and No. 7. The difference in relative chromosome length between No. 5 and No. 6 of *buergeri* is very small as compared with that of *arboreus*. In view of these facts, YAMAMOTO and MASUDA have registered their reservation toward placing these two species in the same genus.

According to the author's observation, *Rh. arboreus* is very similar to *schlegelii* in the centromere position as well as in the relative length of all the chromosomes except a few. One of the brown-colored species *Rh. buergeri* differs more or less distinctly from these two green-colored species in the centromere position of all the chromosomes. The other brown-colored species *Rh. japonicus* differs also from *Rh. arboreus* and *Rh. schlegelii* in the centromere position of all the chromosomes except Nos. 5 and 12. On the other hand, *Rh. japonicus* differs more or less distinctly from *Rh. buergeri* in the centromere position of all the chromosomes except Nos. 1 and 10. *Rhacophorus japonicus* is so similar to *Rh. schlegelii* and

Rh. arboreus in relative length of all the chromosomes that differences are scarcely observed among the three species. Contrarily, *Rh. buergeri* is remarkably different in this respect from the two species, *Rh. schlegelii* and *Rh. arboreus*. Concerning a secondary constriction and a satellite, special difference is scarcely found between the green-colored species and the brown-colored ones, although the four species differ from one another in these respects.

From the results of observation on the karyotypes of the four species, it seems unquestionable that the two green-colored species, *Rh. schlegelii* and *Rh. arboreus* are very closely allied to each other. However, it does not seem reasonable to move the two brown-colored species, *Rh. japonicus* and *Rh. buergeri* from the genus *Rhacophorus* into either *Polypedates* or *Buergeria*, as there are large differences in karyotype between the two species.

Differing from the state of affairs in *Rhacophorus*, there are many reports on the chromosomes of *Rana* species which are nearly related to *Rhacophorus* species. While *Rana* species are usually 26 in diploid number and have 10 large and 16 small chromosomes, three brown-frog species, *Rana arvalis* (WITSCHI, 1933), *Rana chensinensis* (KAWAMURA, 1943) and *Rana ornativentris* (KOBAYASHI, 1946), are 24 and *Rana namiyei* (KURAMOTO, 1972) is 22. The karyotypes of the species having 26 chromosomes are very similar to one another in the shape of 5 pairs of large chromosomes, except those of three species, *R. dalmatina* (GUILLEMIN, 1967), *R. subaspere* and *R. holsti* (KURAMOTO, 1972), in which a pair of large chromosomes have a secondary constriction, differing from the others. In 14 *Rana* species, when the centromere position is shown as in the present paper, chromosomes Nos. 1 and 5 are of the median type, and No. 2 is of the submedian or median type near the submedian type. Nos. 3 and 4 are usually similar to each other in size; one of them is always of the median type, while the other is of the submedian or median type near the submedian type. The small chromosomes in these species are of the median, submedian and subterminal types. They differ in the combination of types from species to species. In *Rhacophorus schlegelii* and *Rh. arboreus*, chromosomes Nos. 1 and 5 are of the median type, and Nos. 2 and 4 are of the submedian type. Chromosome No. 3 of *Rh. schlegelii* is of the median type, while that of *Rh. arboreus* is of the median or submedian type. Besides, Nos. 3 and 4 of these two species are similar to each other in size. Therefore, it can be said that *Rh. schlegelii* and *Rh. arboreus* are very similar to most *Rana* species in the composition of large chromosomes. On the other hand, *Rh. japonicus* differs from the latter in that all the five pairs are of the median type. However, the centromeres of chromosomes Nos. 2 and 4 are somewhat eccentrically situated, while those of Nos. 1, 3 and 5 are very close to the center. Besides, Nos. 3 and 4 are similar to each other in size. From these points, it can be said that *Rh. japonicus* somewhat resemble *Rana* species. Differing remarkably from most *Rana* species as well as the three other *Rhacophorus* species, *Rh. buergeri* has chromosomes Nos. 3 and 4 which are distinctly different from each other in size; No. 4 rather resembles No. 5. Moreover, No. 5 is of the subterminal type, while Nos. 1, 3 and 4 are of the median type.

In the southwestern archipelagos, there are two *Rhacophorus* species, besides *Rh. japonicus*. They are a brown-colored frog, *Rh. eiffingeri* (BOETTGER) and a green-colored one, *Rh. viridis* (HALLOWELL) including three subspecies. *Rhacophorus viridis* is very similar to *Rh. schlegelii* and *Rh. arboreus* in shape and structure of the egg mass, while *Rh. eiffingeri* has a peculiar spawning habit. It is interesting to investigate the karyotypes of these species and subspecies in order to clarify the phylogeny of *Rhacophorus* distributed in Japan and the adjacent area. This problem is being studied by the author.

SUMMARY

1. The karyotypes of four frog species, *Rhacophorus arboreus, Rh. buergeri, Rh. japonicus* and *Rh. schlegelii* were studied by the squash method applied to the tail-tips of tadpoles.

2. These four species are 26 in diploid chromosome number and have five pairs of large chromosomes (Nos. 1~5) and eight pairs of small chromosomes (Nos. 6~13). The karyotypes of the four species are more or less different from one another in relative length and centromere position of chromosomes.

3. In *Rh. schlegelii*, chromosomes Nos. 1, 3 and 5 are of the median type and Nos. 2 and 4 are of the submedian type. Chromosome No. 7 has a secondary constriction in the long arm. The karyotype of *Rh. arboreus* is very similar to that of *Rh. schlegelii*. Although there is no chromosome with a secondary constriction, No. 12 has a satellite at the end of the long arm. The karyotype of *Rh. japonicus* differs remarkably from those of the other three species; all the five large chromosomes are of the median type. Chromosome No. 12 has a secondary constriction in the short arm. The karyotype of *Rh. buergeri* also differs sharply from those of the other three species; Nos. 1, 3 and 4 are of the median type, No. 2 is of the submedian type and No. 5 of the subterminal type. Chromosome No. 7 has a satellite at the end of the long arm.

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