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Karyotypes of Brown Frogs Distributed in Japan, Korea, Europe and North America

By

Midori NISHIOKA[1], Hitoshi OKUMOTO[1], Hiroaki UEDA[1] and Masashi RYUZAKI[2]

[1]*Laboratory for Amphibian Biology, Faculty of Science, Hiroshima University, Hiroshima 730, Japan*

and

[2]*Department of Biology, Kitasato University School of Medicine, Sagamihara, Kanagawa 228, Japan*

(With 12 Text-figures)

ABSTRACT

The karyotypes of five Japanese brown frog species, *Rana japonica, Rana ornativentris, Rana tsushimensis, Rana dybowskii* and *Rana chensinensis,* two Korean brown frog species, *Rana amurensis coreana* and *Rana dybowskii,* one European brown frog species, *Rana temporaria* and one North American brown frog species, *Rana sylvatica,* were compared with one another by examining the relative length (RL) and numerical value of the centromere position (NVC) of each chromosome pair. The differences between two species or local races in these items were checked by the method of HUBBS and HUBBS (1953) as well as by t-test (MATHER, 1965).

Rana japonica, Rana tsushimensis, Rana amurensis coreana, Rana temporaria and *Rana sylvatica* have 2n=26 chromosomes, while *Rana ornativentris, Rana dybowskii* and *Rana chensinensis* have 2n=24 chromosomes. The 26 or 24 chromosomes are divided into two groups in size; one contains five (Nos. 1~5) pairs of large chromosomes and the other contains eight (Nos. 6~13) or seven (Nos. 6~12) pairs of small chromosomes. Chromosome No. 9 of the species with 26 chromosomes and chromosome No. 10 of the species with 24 chromosomes have a secondary constriction in the long arm, except that No. 9 of *Rana sylvatica* has an additional secondary constriction in the short arm. The karyotypes of the five species with 26 chromosomes differ from one another with a statistical significance ($P \leqq 5\%$) in either RL or NVC of seven to all of the 13 chromosome pairs. The karyotypes of the three species with 24 chromosomes differ from one another with a statistical significance ($P \leqq 5\%$) in either RL or NVC of seven to all of the 12 chromosome pairs. The karyotypes of the species having 26 chromosomes were compared with those of the species having 24 chromosomes in RL and NVC by neglecting chromosomes Nos. 11, 12 and 13 of the former species and chromosomes Nos. 6 and 12 of the latter species, as these chromosomes are difficult to compare in size and shape. It was found that the karyotypes of the five species with 26 chromosomes differ with a statistical significance ($P \leqq 5\%$) from those of the three species with 24 chromosomes in either RL or NVC of five to all of the 10 chromosome pairs.

The differences in karyotype among the eight brown frog species are not always correlated with the genetic distances and the reproductive isolation among them.

INTRODUCTION

The Far East is characteristic in that many brown frog species are distributed in contrast to Europe and North America. In Japan alone, there are seven species, of which *Rana chensinensis* is distributed in Hokkaido and *Rana okinavana* in the Ryukyu Islands. *Rana japonica, Rana ornativentris* and *Rana tagoi* are abundant in Honshu, Shikoku and Kyushu, and *Rana tsushimensis* and *Rana dybowskii* are found in Tsushima alone. *Rana dybowskii* is also distributed in Korea together with *Rana amurensis coreana,* and *Rana japonica* is also distributed in China together with *Rana chensinensis.* On the other hand, *Rana temporaria* is widely distributed in Europe together with *Rana arvalis.* In North America, there is a brown frog species, *Rana sylvatica.*

The chromosomes of *Rana temporaria* have been observed by many investigators, such as WITSCHI (1922a, b, 1924, 1933), GALGANO (1933), WICKBOM (1945), and MORESCALCHI (1967). Of the Japanese brown frog species, the chromosome number of *Rana japonica* was reported by KAWAMURA (1939, 1940, 1943), KOBAYASHI (1962) and SETO (1965). The chromosomes of *Rana chensinensis* distributed in Sakhalin were described by KAWAMURA (1943). Thereafter, those of the same species from Hokkaido and those of *Rana ornativentris* were described by KOBAYASHI (1962), SETO (1965) and NISHIOKA, UEDA and RYUZAKI (1972). The chromosome numbers of *Rana tsushimensis, Rana amurensis coreana* and *Rana dybowskii* from Tsushima and Korea were reported by KAWAMURA and NISHIOKA (1973). The karyotype of *Rana sylvatica* was described by HENNEN (1964). These brown frog species have 2n=24 or 26 chromosomes, which are divided into two groups. One group includes five pairs of large chromosomes, while the other includes seven or eight pairs of small chromosomes. A comparative study on the karyotypes of these brown frog species has not yet been made, although the karyotypes of some of them have been described in detail.

A comparative study on the karyotypes of pond frogs distributed in Japan, Korea, Taiwan, Europe and North America was made by NISHIOKA, OKUMOTO and RYUZAKI (1987). In parallel with this, the present authors have made a similar study on eight brown frog species distributed in Japan, Korea, Europe and North America. The main results are reported in this paper.

MATERIALS AND METHODS

The following brown frogs belonging to eight species were used in order to examine their karyotypes.

1. *Rana japonica* GÜNTHER from the suburbs of Hiroshima. Three mature males and three mature females.
2. *Rana ornativentris* WERNER from mountainous areas of Hiroshima Prefecture. Two mature males and two mature females.
3. *Rana chensinensis* DAVID from Hokkaido. Two mature males and two

mature females.

4. *Rana tsushimensis* STEJNEGER from Tsushima Island. Three mature males and three mature females.
5. *Rana dybowskii* GÜNTHER from Tsushima Island. Two mature males and two mature females.
6. *Rana dybowskii* GÜNTHER from Korea. Two mature males and two mature females.
7. *Rana amurensis coreana* OKADA from Korea. A mature male and two mature females.
8. *Rana temporaria* L. from France. Three mature males and three mature females.
9. *Rana sylvatica* LE CONTE from North America. A mature male and a mature female.

Chromosome preparations were made by the air-drying method from bone marrow cells (OMURA, 1967) or leucocytes which were increased by the blood culture method (VOLPE and GEBHARDT, 1968; NISHIOKA, OKUMOTO and RYUZAKI, 1987). In some species, the chromosomes of tadpoles were observed in the tail-tips of tadpoles by the water-pretreatment squash method (NISHIOKA, 1972).

The total and arm lengths of each chromosome were measured by using enlarged microphotographs of well-spread metaphase chromosomes. The karyotypes of different species were compared with each other in relative length and centromere position of each chromosome. The mean of the lengths of two homologous chromosomes was regarded as the chromosome length of this pair. The sum total of the lengths of 12 or 13 pairs of chromosomes in each metaphase spread was regarded as the genome length. The relative length (RL) of each chromosome pair was shown by the percentage of the chromosome length to the genome length. The centromere position was shown by the percentage of the short-arm length to the chromosome length, that is, presented as a numerical value of the centromere position (NVC).

Enlarged microphotographs of 100~250 metaphase spreads were taken from one species or subspecies. Of these metaphase spreads, the best 50 were measured. The mean of measurements from these 50 metaphase spreads was regarded as the value of the species or subspecies. The length of a chromosome having a secondary constriction was measured by excluding the part of constriction.

The differences between two species or subspecies in relative chromosome length and centromere position were examined by the method of HUBBS and HUBBS (1953) as well as by t-test (MATHER, 1965). When the samples are 50 in number, $t \geqq 2.01$ indicates that the difference is statistically significant as it is $P \leqq 5\%$, while $t > 3.50$ indicates that the difference is highly significant, as it is $P < 0.1\%$.

OBSERVATION

I. Brown frogs having 26 chromosomes

Rana japonica, Rana tsushimensis, Rana amurensis coreana, Rana temporaria and *Rana sylvatica* were 26 in diploid chromosome number. The karyotype of each species consisted of five pairs of large chromosomes (Nos. 1~5) and eight pairs of small chromosomes (Nos. 6~13). Males and females of each species did not differ from each other in karyotype. The homologous chromosomes of each pair were identical in relative length and centromere position. Chromosomes were divided into four types, median, submedian, subterminal and terminal, on the basis that the numerical values of centromere positions were 50.0~37.5, 37.4~25.0, 24.9 ~12.5 and 12.4~0, respectively.

1. *Rana japonica*

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads obtained from three mature males and three mature females by the blood culture method are presented in Table 1. The 13 pairs of chromosomes (Nos. 1~13) were arranged in an order of relative length (Fig. 1).

Of the five pairs of large chromosomes, three were of median type, one was intermediate between median and submedian types and the remainder was of submedian type. The largest chromosome (No. 1) was 13.3~16.9, 14.73 on the average, in RL and 41.8~49.5, 45.73 on the average, in NVC, being of median type. Chromosome No. 2 was 11.4~14.2, 12.90 on the average, in RL. It was 37.8~42.2 in NVC, being of median type in 27 of the 50 metaphase spreads and 31.3~37.4, being of submedian type in the remaining 23. This chromosome was 37.48 on the average in NVC, being intermediate between median and submedian types in the 50 metaphase spreads. Chromosomes Nos. 3 and 4 were similar to each other in size; they were 10.9~12.9, 11.60 on the average, and 10.4~12.9, 11.40 on the average, in RL, respectively. However, the two chromosomes considerably differed from each other in centromere position. Chromosome No. 3 was 37.5~47.2 in NVC, being of median type in 37 of the 50 metaphase spreads and 33.1~37.0, being of submedian type in the other 13. This chromosome was 39.04 on the average in NVC, being of median type in the 50 metaphase spreads. On the other hand, chromosome No. 4 was 29.3~37.1 in NVC, being of submedian type in 49 of the 50 metaphase spreads and 39.5 in NVC, being of median type, in the remainder. This chromosome was 32.90 on the average in NVC, being of submedian type in the 50 metaphase spreads. Chromosome No. 5 was 8.7~10.8, 9.74 on the average, in RL and 39.0~47.4, 43.72 on the average, in NVC, being of median type.

Of the eight pairs of small chromosomes, three were of median type, three were of submedian type, one was intermediate between submedian and subterminal

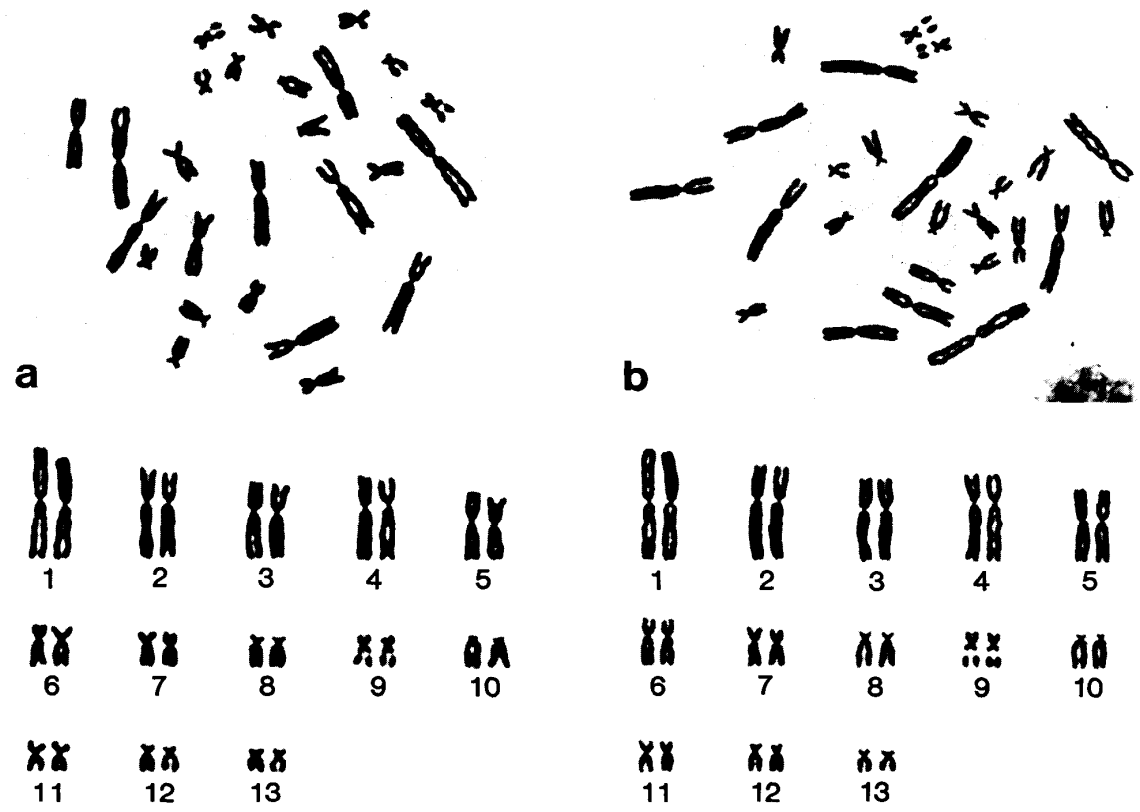


Fig. 1. Metaphase plates and the karyotypes of a female and a male *Rana japonica*.
a. Female b. Male ×1100

types and the remainder was of subterminal type. Chromosomes Nos. 6 and 7 significantly differed from each other in size; being 5.4~7.1, 6.35 on the average, and 5.0~6.2, 5.59 on the average, in RL, respectively. However, they were very similar to each other in shape. Chromosome No. 6 was 37.5~44.1 in NVC, being of median type in 37 of the 50 metaphase spreads and 31.6~37.4 in NVC, being of submedian type in the remaining 13. This chromosome was 39.24 on the average in NVC, being of median type. On the other hand, No. 7 was 37.5~44.4, being of median type in 39 of the 50 metaphase spreads and 33.3~37.4, being of submedian type in the other 11. On the average, this chromosome was 38.81 in NVC, being of median type. Chromosomes Nos. 8 and 9 were 4.4~5.7, 5.16 on the average, and 4.4~5.7, 5.08 on the average, in RL, respectively. Although no statistically significant difference in size could be demonstrated between these two chromosomes, they somewhat differed from each other in shape. Chromosome No. 8 was 16.2~24.7 in NVC, being of subterminal type in 29 of the 50 metaphase spreads and 25.0~35.9 in NVC, being of submedian type in the other 21. This chromosome was 25.02 on the average in NVC, being intermediate between submedian and subterminal types. Chromosome No. 9 was 25.3~37.4 in NVC, being of submedian type in 49 of the 50 metaphase spreads and 22.2, being of subterminal type in the remainder. This chromosome was 31.19 on the average in NVC, being of submedian type. Moreover, it had a secondary constriction in

TABLE 1

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana japonica*

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	13.3	16.9	14.73±0.11	1	41.8	49.5	45.73±0.22	m (50)
2	11.4	14.2	12.90±0.09	2	31.3	42.2	37.48±0.33	m (27) sm (23)
3	10.9	12.9	11.60±0.06	3	33.1	47.2	39.04±0.39	m (37) sm (13)
4	10.4	12.9	11.40±0.06	4	29.3	39.5	32.90±0.30	sm (49) m (1)
5	8.7	10.8	9.74±0.07	5	39.0	47.4	43.72±0.25	m (50)
6	5.4	7.1	6.35±0.05	6	31.6	44.1	39.24±0.38	m (37) sm (13)
7	5.0	6.2	5.59±0.04	7	33.3	44.4	38.81±0.33	m (39) sm (11)
8	4.4	5.7	5.16±0.05	8	16.2	35.9	25.02±0.42	st (29) sm (21)
9*	4.4	5.7	5.08±0.04	9*	22.2	37.4	31.19±0.45	sm (49) st (1)
10	4.3	5.3	4.84±0.03	10	11.8	17.7	14.01±0.18	st (47) t (3)
11	4.2	5.1	4.65±0.03	11	34.1	46.8	41.75±0.34	m (47) sm (3)
12	3.6	5.0	4.30±0.04	12	25.1	40.3	33.31±0.31	sm (44) m (6)
13	3.1	4.0	3.66±0.03	13	25.0	37.4	32.14±0.32	sm (50)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

the long arm. Chromosomes Nos. 10 and 11 were 4.3~5.3, 4.84 on the average, and 4.2~5.1, 4.65 on the average, in RL, respectively. Although these two chromosomes were very similar to each other in size, they remarkably differed from each other in shape. Chromosome No. 10 was 12.5~17.7 in NVC, being of subterminal type in 47 of the 50 metaphase spreads and 11.8~12.2, being of terminal type in the remaining three. On the average, this chromosome was 14.01 in NVC, being of subterminal type. Chromosome No. 11 was 38.0~46.8 in NVC, being of median type in 47 of the 50 metaphase spreads and 34.1~37.0, being of submedian type in the remaining three. This chromosome was 41.75 on the average in NVC, being of median type. Chromosomes Nos. 12 and 13 were 3.6~5.0, 4.30 on the average, and 3.1~4.0, 3.66 on the average, in RL, respectively. There was statistically a significant difference in size between these two chromosomes, although they were very similar to each other in shape. Chromosome No. 12 was 25.1~36.6 in NVC, being of submedian type, in 44 of the 50 metaphase spreads and 37.5~40.3, being of median type, in the other six. This chromosome was 33.31 on the average in NVC, being of submedian type. Chromosome No. 13 was 25.0~37.4, 32.14 on the average, in NVC, being of submedian type in all the 50 metaphase spreads.

2. *Rana tsushimensis*

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads obtained from three mature males and three mature females by the blood culture method are presented in Table 2. The karyotype of this species was very similar to that of *Rana japonica,* when the 13 pairs of chromosomes were arranged in an order corresponding to those of the latter species in size and shape (Fig. 2).

Of the five pairs of large chromosomes, four were of median type and the other was of submedian type. The largest chromosome (No. 1) was 14.0~17.0, 15.49 on the average, in RL and 42.7~49.1, 46.36 on the average, in NVC, being of median type. Chromosome No. 2 was 12.3~14.6, 13.23 on the average, in RL. While this chromosome was 37.5~42.9 in NVC, being of median type in 41 of the 50 metaphase spreads, it was 35.5~37.2, being of submedian type in the other nine. On the average, this chromosome was 39.53 in NVC, being of median type. Chromosomes Nos. 3 and 4 were 10.6~13.3, 11.87 on the average, and 10.4~12.5, 11.70 on the average, in RL, respectively. There was statistically no significant difference in size between these two chromosomes. Chromosome No. 3 was 37.8~44.7, 41.95 on the average, in NVC, being of median type. On the other

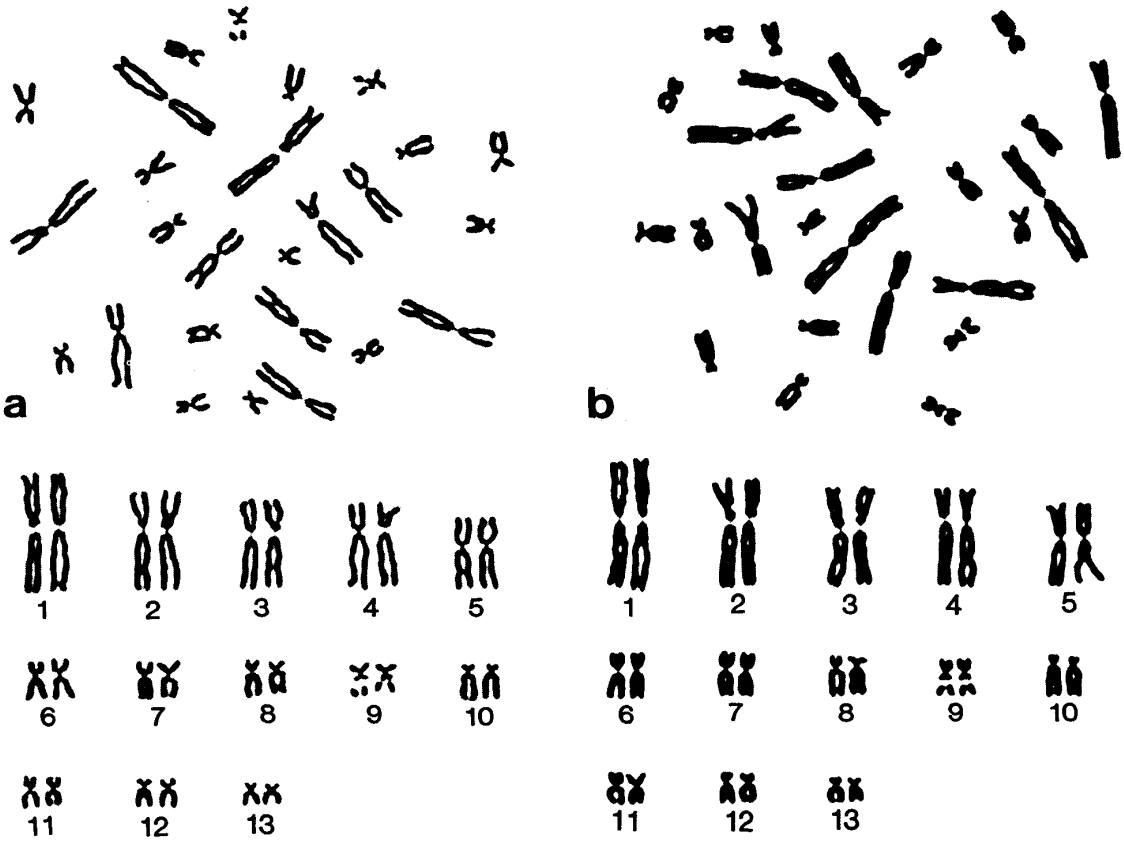


Fig. 2. Metaphase plates and the karyotypes of a female and a male *Rana tsushimensis*.
a. Female b. Male ×1100

TABLE 2

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana tsushimensis*

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	14.0	17.0	15.49±0.12	1	42.7	49.1	46.36±0.20	m (50)
2	12.3	14.6	13.23±0.08	2	35.5	42.9	39.53±0.28	m (41) sm (9)
3	10.6	13.3	11.87±0.07	3	37.8	44.7	41.95±0.26	m (50)
4	10.4	12.5	11.70±0.05	4	26.4	40.0	33.40±0.38	sm (45) m (5)
5	9.2	11.3	10.00±0.07	5	39.4	46.7	43.67±0.24	m (50)
6	5.2	7.1	6.04±0.05	6	30.6	44.4	38.86±0.39	m (35) sm (15)
7	5.0	6.0	5.41±0.04	7	31.3	44.4	38.69±0.45	m (34) sm (16)
8	4.2	5.6	4.81±0.05	8	20.3	34.5	26.53±0.41	sm (38) st (12)
9*	4.1	5.3	4.78±0.04	9*	26.3	42.9	32.81±0.43	sm (47) m (3)
10	4.3	5.3	4.73±0.04	10	12.8	28.9	17.87±0.42	st (49) sm (1)
11	3.8	5.7	4.53±0.05	11	30.6	44.4	40.15±0.40	m (44) sm (6)
12	3.4	5.1	4.01±0.04	12	26.3	40.0	33.21±0.47	sm (45) m (5)
13	2.9	4.0	3.41±0.03	13	25.2	40.0	30.74±0.50	sm (45) m (5)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

hand, chromosome No. 4 was 26.4~36.7 in NVC, being of submedian type in 45 of the 50 metaphase spreads and 37.5~40.0, being of median type in the remaining five. This chromosome was 33.40 on the average in NVC, being of submedian type. Chromosome No. 5 was 9.2~11.3, 10.00 on the average, in RL and 39.4~46.7, 43.67 on the average, in NVC, being of median type.

Of the eight pairs of small chromosomes, three were of median type, four others were of submedian type and the remainder was of subterminal type. Chromosomes Nos. 6 and 7 were 5.2~7.1, 6.04 on the average, and 5.0~6.0, 5.41 on the average, in RL, respectively. Although these two chromosomes statistically differed from each other in RL, they were similar to each other in centromere position. Chromosome No. 6 was 37.5~44.4 in NVC, being of median type in 35 of the 50 metaphase spreads and 30.6~37.3, being of submedian type in the other 15. On the other hand, chromosome No. 7 was also 37.5~44.4 in NVC, being of median type in 34 of the 50 metaphase spreads and 31.3~37.0 in NVC, being of submedian type in the other 16. Chromosomes Nos. 6 and 7 were 38.86 and 38.69 on the average in NVC, respectively, both being of median type. Chromosomes Nos. 8, 9 and 10 were 4.2~5.6, 4.81 on the average, 4.1~5.3, 4.78 on the average, and 4.3~5.3, 4.73 on the average, in RL, respectively. Although

these three chromosomes were very similar in size, they remarkably differed from one another in shape. Chromosome No. 8 was 25.0~34.5 in NVC, being of submedian type, in 38 of the 50 metaphase spreads and 20.3~24.6, being of subterminal type in the other 12. Chromosome No. 9 was characteristic in having a secondary constriction in the long arm. It was 26.3~37.4 in NVC, being of submedian type in 47 of the 50 metaphase spreads and 37.5~42.9, being of median type in the other three. Chromosome No. 10 was 12.8~23.1 in NVC, being of subterminal type, in 49 of the 50 metaphase spreads and 28.9, being of submedian type, in the remainder. On the average, chromosomes Nos. 8, 9 and 10 were 26.53, 32.81 and 17.87 in NVC, being of submedian, submedian and subterminal type, respectively. Chromosomes Nos. 11, 12 and 13 were 3.8~5.7, 4.53 on the average, 3.4~5.1, 4.01 on the average, and 2.9~4.0, 3.41 on the average, in RL, respectively. Statistically significant differences in size could be observed among these three chromosomes. Chromosome No. 11 was 37.5~44.4 in NVC, being of median type, in 44 of the 50 metaphase spreads and 30.6~37.3, being of submedian type in the other six. This chromosome was 40.15 on the average in NVC, being of median type. Chromosomes Nos. 12 and 13 were 26.3~36.8 and 25.2~36.3 in NVC, respectively, being of submedian type in 45 of the 50 metaphase spreads and 37.8~40.0 and 37.5~40.0, respectively, being of median type, in the other five. They were 33.21 and 30.74 on the average in NVC, respectively, both being of submedian type.

3. *Rana amurensis coreana*

Karyotype analysis was made in 25 metaphase spreads obtained from the tail-tips of 20 tadpoles by the squash method after water pretreatment in 1970 as well as in 25 metaphase spreads obtained from bone marrow cells of two mature females in 1976. When the 13 pairs of chromosomes were arranged in an order corresponding to those of *Rana japonica* in size and shape, the karyotype of *Rana amurensis coreana* obtained from bone marrow cells was very similar to those of *Rana japonica* and *Rana tsushimensis*. While the karyotype elucidated from 14 of the 25 metaphase spreads of tail-tip cells was the same as that elucidated from the metaphase spreads of bone marrow cells, the karyotype elucidated from the other 11 metaphase spreads of tail-tip cells differed from the latter in that the two partners of chromosome No. 9 were not homologous, although both of them had a secondary constriction in the long arm. While one of the partners was the same as that found in the metaphase spreads of bone marrow cells, the other partner was intermediate in RL between chromosomes Nos. 5 and 6 (Fig. 3). The larger chromosomes found in 11 metaphase spreads of tail-tip cells were 34.5~35.9, 35.48 on the average, in NVC, being of submedian type, while the smaller chromosomes were 48.4~50.0, 49.63 on the average, being of median type. The latter chromosomes did not differ in shape from those found in the other 14 metaphase spreads of tail-tip cells as well as all the 25 metaphase spreads of bone marrow cells. When the two non-homologous No. 9 chromosomes were compared with each other, there was no difference in length of the short arm, whereas in the long

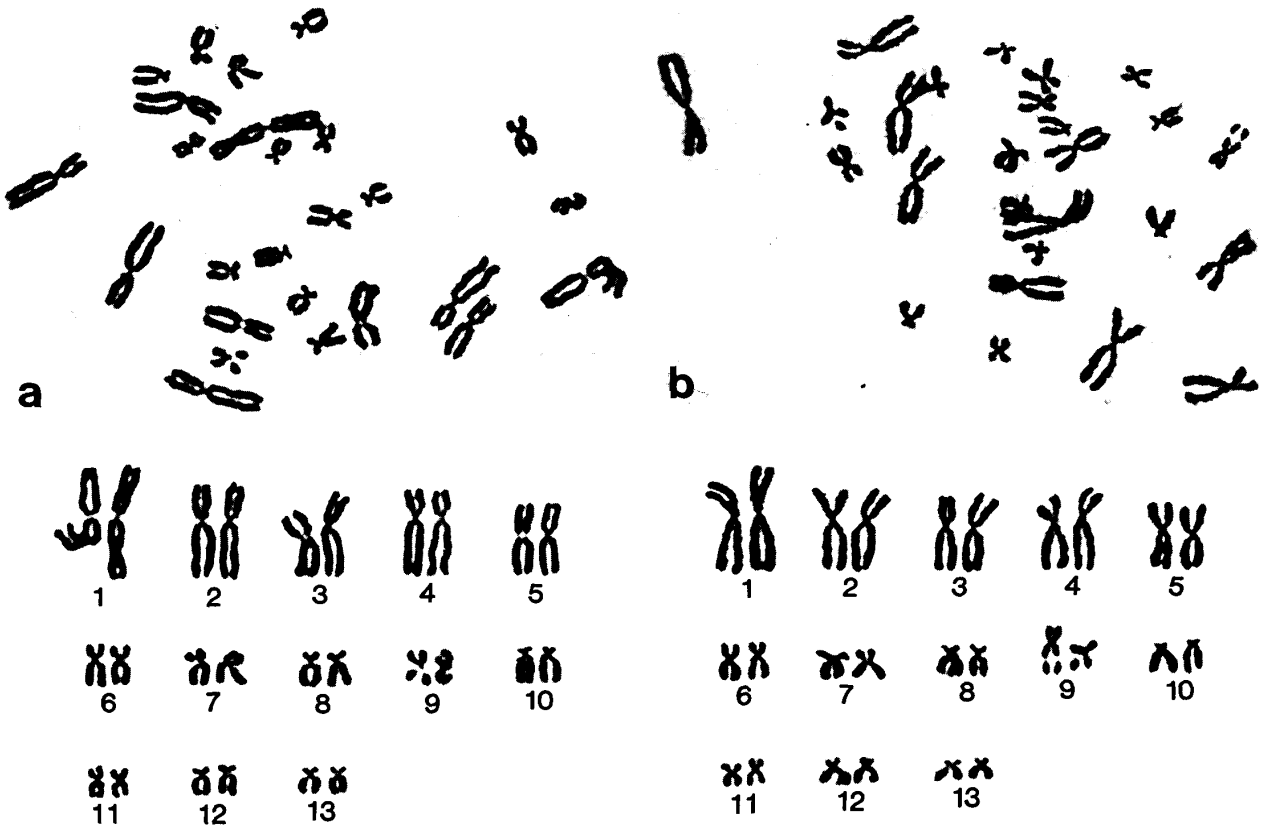


Fig. 3. Metaphase plates and the karyotypes of epidermal cells from *Rana amurensis coreana* tadpoles.
a. Two partners of chromosome No. 9 were homologous. ×1100
b. Two partners of chromosome No. 9 were not homologous.

arm of the longer partner, an additional segment was inserted between the centromere and the secondary constriction. The latter chromosome was 1.3~1.5 times longer than the shorter partner.

There were statistically no significant differences in size and shape of chromosomes except No. 9 between the karyotype from bone marrow cells and that from tail-tip cells. Thus, the relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes except the larger No. 9 in a total of 50 metaphase spreads are presented in Table 3.

Of the five pairs of large chromosomes, four were of median type and the remainder was of submedian type. The largest chromosome (No. 1) was 14.2 ~18.3, 15.60 on the average, in RL and 41.7~50.0, 46.55 on the average, in NVC, being of median type. Chromosome No. 2 was 12.4~15.1, 13.47 on the average, in RL and 37.5~46.8, 42.31 on the average, in NVC, being also of median type. Chromosomes Nos. 3 and 4 of *Rana amurensis coreana* corresponded to those of *Rana japonica* and *Rana tsushimensis* in NVC, although No. 3 was rather smaller in RL than No. 4. Chromosomes Nos. 3 and 4 were 10.4~12.7, 11.47 on the average, and 10.8~13.6, 11.96 on the average, in RL, respectively. There was statistically a slight difference in RL between these two chromosomes. Chromosome No. 3 was 38.1~46.7 in NVC, being of median type in 49 of the 50 metaphase spreads

TABLE 3

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana amurensis coreana*

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	14.2	18.3	15.60±0.14	1	41.7	50.0	46.55±0.28	m (50)
2	12.4	15.1	13.47±0.09	2	37.5	46.8	42.31±0.32	m (50)
3	10.4	12.7	11.47±0.08	3	36.7	46.7	41.63±0.34	m (49) sm (1)
4	10.8	13.6	11.96±0.09	4	27.9	40.5	34.18±0.47	sm (39) m (11)
5	8.4	11.0	9.94±0.09	5	42.3	50.0	44.87±0.27	m (50)
6	5.3	6.8	5.91±0.05	6	34.5	46.2	40.11±0.41	m (43) sm (7)
7	4.7	5.8	5.32±0.05	7	32.3	46.7	37.99±0.47	m (33) sm (17)
8	4.4	5.7	4.94±0.05	8	25.0	32.7	29.03±0.43	sm (50)
9*	4.4	5.5	4.97±0.04	9*	37.5	50.0	45.95±0.48	m (50)
10	3.9	5.3	4.62±0.05	10	12.5	21.4	16.43±0.44	st (50)
11	3.6	5.2	4.39±0.06	11	30.0	50.0	43.39±0.60	m (45) sm (5)
12	3.5	4.5	3.98±0.03	12	20.0	36.4	28.67±0.57	sm (48) st (2)
13	3.0	3.8	3.44±0.03	13	14.3	33.3	22.90±0.62	st (30) sm (20)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

and 36.7, being of submedian type in the remainder. On the other hand, chromosome No. 4 was 27.9~36.8 in NVC, being of submedian type in 39 of the 50 metaphase spreads and 37.5~40.5, being of median type in the other 11. On the average, these two chromosomes were 41.63 and 34.18, being of median and submedian type, respectively. Chromosome No. 5 was 8.4~11.0, 9.94 on the average, in RL and 42.3~50.0, 44.87 on the average, in NVC, being of median type.

Of the eight pairs of small chromosomes, four were of median type, two were of submedian type and the remaining two were of subterminal type. Chromosomes Nos. 6 and 7 were 5.3~6.8, 5.91 on the average, and 4.7~5.8, 5.32 on the average, in RL, respectively. Chromosome No. 6 was 37.5~46.2 in NVC, being of median type in 43 of the 50 metaphase spreads and 34.5~36.8, being of submedian type in the other seven, while chromosome No. 7 was 37.5~46.7 in NVC, being of median type in 33 of the 50 metaphase spreads and 32.3~36.4, being of submedian type in the other 17. On the average, these two chromosomes were 40.11 and 37.99 in NVC, respectively, both being of median type. They statistically differed from each other in both size and shape. Chromosomes Nos. 8 and 9 were 4.4~5.7, 4.94 on the average, and 4.4~5.5, 4.97 on the average, in RL, respectively. Although there was statistically no significant difference in size between these two

chromosomes, they distinctly differed from each other in shape. Chromosome No. 8 was 25.0~32.7, 29.03 on the average, in NVC, being of submedian type, while No. 9 was 37.5~50.0, 45.95 on the average, in NVC, being of median type. Moreover, No. 9 had a secondary constriction in the long arm. Chromosomes Nos. 10 and 11 were 3.9~5.3, 4.62 on the average, and 3.6~5.2, 4.39 on the average, in RL, respectively. There was statistically a slightly significant difference in size between these two chromosomes. They distinctly differed from each other in shape. Chromosome No. 10 was 12.5~21.4, 16.43 on the average, in NVC, being of subterminal type. Chromosome No. 11 was 37.5~50.0, being of median type in 45 of the 50 metaphase spreads and 30.0~36.2, being of submedian type in the other five. This chromosome was 43.39 on the average in NVC, being of median type. Chromosomes Nos. 12 and 13 were 3.5~4.5, 3.98 on the average, and 3.0~3.8, 3.44 on the average, in RL, respectively. Chromosome No. 12 was 25.0~36.4, being of submedian type, in 48 of the 50 metaphase spreads and 20.0 and 22.2, being of subterminal type, in the other two, while chromosome No. 13 was 14.3~23.8, being of subterminal type in 30 of the 50 metaphase spreads and 25.0~33.3, being of submedian type in the other 20. These two chromosomes were 28.67 and 22.90 on the average in NVC, being of submedian and subterminal type, respectively.

4. *Rana temporaria*

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads obtained from three mature males and three mature females by the blood culture method are presented in Table 4. The 13 pairs of chromosomes were arranged in an order corresponding to those of *Rana japonica* in size and shape (Fig. 4).

Of the five pairs of large chromosomes, three were of median type and the other two were of submedian type. The largest chromosome (No. 1) was 14.0~18.6, 16.50 on the average, in RL and 42.0~48.5, 45.16 on the average, in NVC, being of median type. Chromosome No. 2 was 12.0~14.5, 13.18 on the average, in RL. It was 33.0~37.4 in NVC, being of submedian type, in 42 of the 50 metaphase spreads and 37.5~40.5, being of median type, in the other eight. On the average, this chromosome was 35.84 in NVC, being of submedian type. Chromosomes Nos. 3 and 4 were 10.5~12.7, 11.59 on the average, and 10.1~12.8, 11.48 on the average, in RL, respectively; there was statistically no significant difference in size between these two chromosomes. Chromosome No. 3 was 37.5~45.1, being of median type, in 45 of the 50 metaphase spreads and 33.2~37.0, being of submedian type, in the other five. On the other hand, chromosome No. 4 was 28.0~36.4, being of submedian type, in 48 of the 50 metaphase spreads and 37.5 and 38.2, being of median type, in the remaining two. These two chromosomes were 39.82 and 32.40 on the average in NVC, being of median and submedian type, respectively. Chromosome No. 5 was 8.5~11.2, 9.95 on the average, and 38.5~47.5, 43.04 on the average, in NVC, being of median type in all the 50 metaphase spreads.

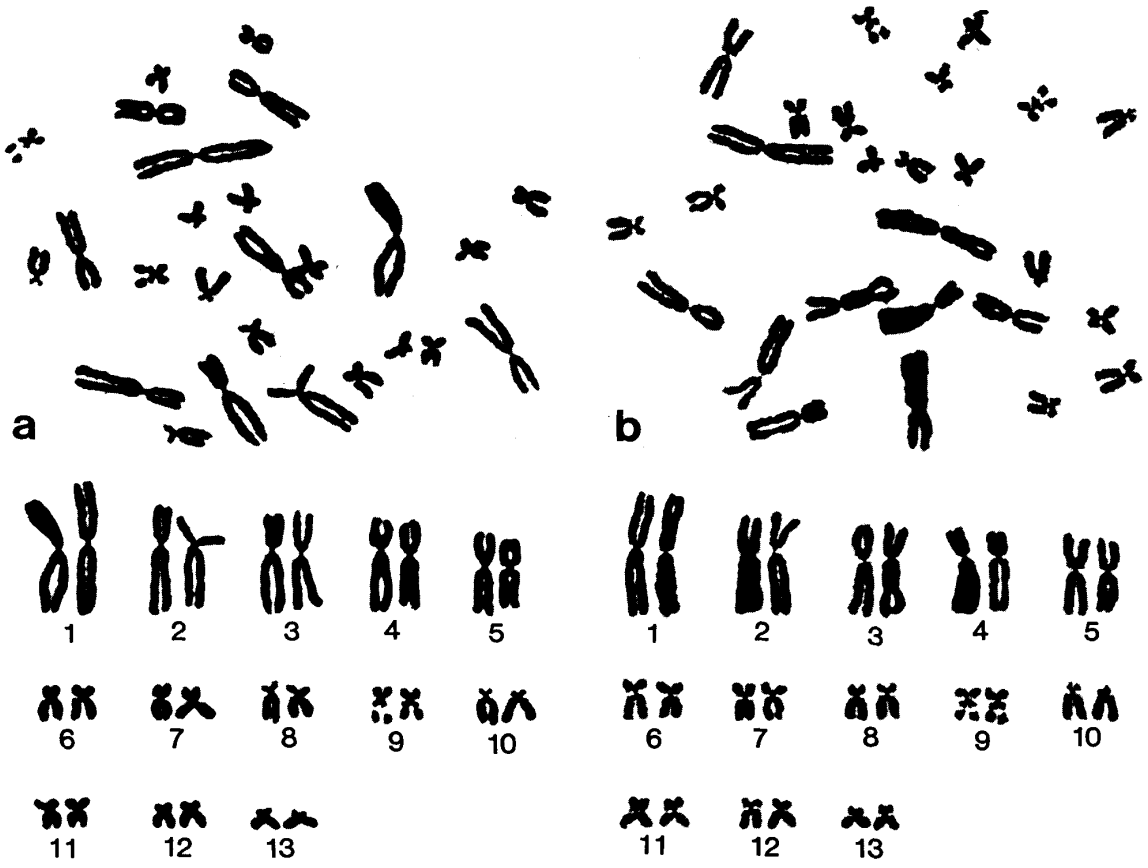


Fig. 4. Metaphase plates and the karyotypes of a female and a male *Rana temporaria*.
a. Female b. Male ×1100

Of the eight pairs of small chromosomes, two were of median type, one was intermediate between submedian and median types, four were of submedian type and the remainder was of subterminal type. Chromosome No. 6 was 4.8~6.7, 5.89 on the average, in RL. It was 30.6~37.3, being of submedian type, in 25 of the 50 metaphase spreads and 37.5~43.5, being of median type, in the other 25. On the average, this chromosome was 37.23 in NVC, being intermediate between submedian and median types. Chromosome No. 7 was 4.5~5.9, 5.33 on the average, in RL. It was 37.5~46.7 in NVC, being of median type, in 39 of the 50 metaphase spreads and 30.8~37.3, being of submedian type, in the other 11. This chromosome was 38.07 on the average in NVC, being of median type. Chromosomes Nos. 8 and 9 were 4.4~5.7, 4.95 on the average, and 4.0~5.5, 4.73 on the average, in RL, respectively. Although the difference in size between these two chromosomes was very slight, they distinctly differed from each other in shape, as No. 9 had a secondary constriction in the long arm. Chromosome No. 8 was 25.1~35.1 in NVC, being of submedian type, in 49 of the 50 metaphase spreads and 39.7, being of median type in the remainder, while No. 9 was 27.3~36.7, being of submedian type in 47 of the 50 metaphase spreads and 37.5~40.0, being of median type, in the other three. These two chromosomes were 30.27 and 32.17 on the average in NVC, respectively, both being of

TABLE 4

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana temporaria*

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	14.0	18.6	16.50±0.12	1	42.0	48.5	45.16±0.19	m (50)
2	12.0	14.5	13.18±0.09	2	33.0	40.5	35.84±0.22	sm (42) m (8)
3	10.5	12.7	11.59±0.06	3	33.2	45.1	39.82±0.30	m (45) sm (5)
4	10.1	12.8	11.48±0.10	4	28.0	38.2	32.40±0.30	sm (48) m (2)
5	8.5	11.2	9.95±0.07	5	38.5	47.5	43.04±0.32	m (50)
6	4.8	6.7	5.89±0.05	6	30.6	43.5	37.23±0.37	sm (25) m (25)
7	4.5	5.9	5.33±0.04	7	30.8	46.7	38.07±0.42	m (39) sm (11)
8	4.4	5.7	4.95±0.04	8	25.1	39.7	30.27±0.42	sm (49) m (1)
9*	4.0	5.5	4.73±0.05	9*	27.3	40.0	32.17±0.40	sm (47) m (3)
10	4.0	5.5	4.57±0.05	10	12.5	29.2	18.05±0.42	st (49) sm (1)
11	3.6	5.4	4.45±0.05	11	30.0	46.7	39.81±0.47	m (37) sm (13)
12	3.2	4.8	4.04±0.04	12	25.0	40.0	34.96±0.49	sm (33) m (17)
13	2.2	4.6	3.35±0.06	13	25.0	41.9	32.24±0.60	sm (39) m (11)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

submedian type. Chromosome No. 10 was 4.0~5.5, 4.57 on the average, in RL. It did not statistically differ in RL from No. 9 as well as No. 11 which was 3.6~5.4, 4.45 on the average. However, chromosome No. 10 was 12.5~22.4 in NVC, being of subterminal type, in 49 of the 50 metaphase spreads and 29.2, being of submedian type, in the remainder, while chromosome No. 11 was 38.5~46.7 in NVC, being of median type, in 37 of the 50 metaphase spreads and 30.0~36.7, being of submedian type, in the remaining 13. On the average, chromosomes Nos. 10 and 11 were 18.05 and 39.81 in NVC, being of subterminal and median type, respectively. Chromosomes Nos. 12 and 13 were 3.2~4.8, 4.04 on the average, and 2.2~4.6, 3.35 on the average, in RL, respectively. Chromosome No. 12 was 25.0~36.4 in NVC, being of submedian type, in 33 of the 50 metaphase spreads and 37.5~40.0, being of median type, in the other 17, while chromosome No. 13 was 25.0~36.6 in NVC, being of submedian type, in 39 of the 50 metaphase spreads and 37.5~41.9, being of median type, in the other 11. These two chromosomes were 34.96 and 32.24 on the average in NVC, respectively, both being of submedian type.

5. *Rana sylvatica*

The relative lengths (RL) and numerical values of centromere positions (NVC)

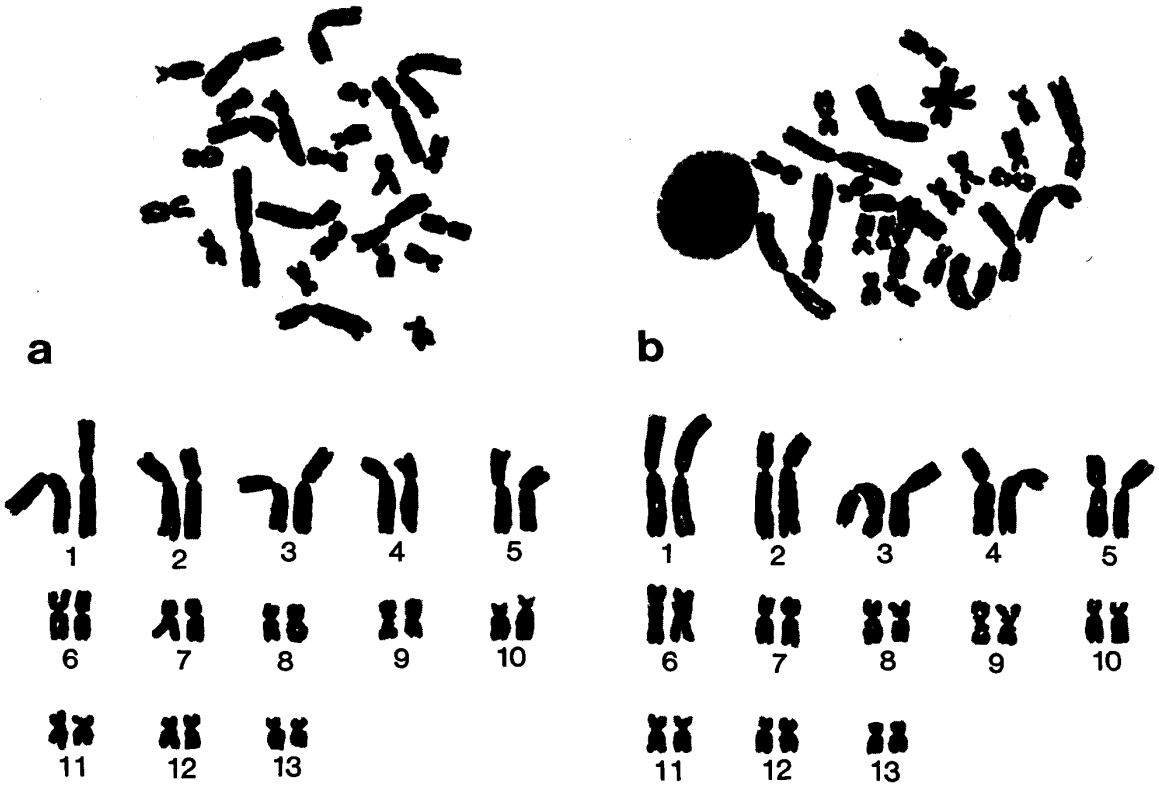


Fig. 5. Metaphase plates and the karyotypes of a female and a male *Rana sylvatica*.
a. Female b. Male ×1100

of the chromosomes in 50 metaphase spreads obtained from a mature male and a mature female by the blood culture method are presented in Table 5. The 13 pairs of chromosomes were arranged in an order corresponding to those of *Rana japonica* in size and shape (Fig. 5).

Of the five pairs of large chromosomes, three were of median type and the other two were of submedian type. The largest chromosome (No. 1) was 13.5~16.6, 15.04 on the average, in RL and 43.7~50.0, 47.23 on the average, in NVC, being of median type. Chromosome No. 2 was 11.5~13.9, 12.66 on the average, in RL. It was 32.3~37.4 in NVC, being of submedian type, in 38 of the 50 metaphase spreads, while 38.1~41.9, being of median type, in the other 12. It was 36.37 on the average in NVC, being of submedian type. Chromosomes Nos. 3 and 4 were 10.0~12.3, 11.19 on the average, and 10.2~13.0, 11.31 on the average, in RL, respectively. Although there was statistically no significant difference between these two chromosomes, they distinctly differed from each other in shape. Chromosome No. 3 was 38.5~47.4 in NVC, being of median type, in 49 of the 50 metaphase spreads and 35.0, being of submedian type, in the remainder. It was 42.36 on the average in NVC, being of median type. Chromosome No. 4 was 28.6~35.9, 32.28 on the average, in NVC, being of submedian type. Chromosome No. 5 was 8.6~10.7, 9.83 on the average, in RL and 38.6~47.4, 43.48 on the average, in NVC, being of median type.

TABLE 5

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana sylvatica*

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	13.5	16.6	15.04±0.12	1	43.7	50.0	47.23±0.30	m (50)
2	11.5	13.9	12.66±0.10	2	32.3	41.9	36.37±0.41	sm (38) m (12)
3	10.0	12.3	11.19±0.09	3	35.0	47.4	42.36±0.43	m (49) sm (1)
4	10.2	13.0	11.31±0.10	4	28.6	35.9	32.28±0.34	sm (50)
5	8.6	10.7	9.83±0.08	5	38.6	47.4	43.48±0.32	m (50)
6	5.3	7.4	6.45±0.05	6	37.0	50.0	44.84±0.47	m (49) sm (1)
7	4.9	6.6	5.60±0.06	7	33.3	46.9	40.22±0.46	m (45) sm (5)
8	4.3	5.7	5.03±0.05	8	31.3	50.0	40.67±0.42	m (42) sm (8)
9*	4.5	6.4	5.46±0.07	9*	38.5	50.0	46.66±0.48	m (50)
10	4.4	5.5	4.94±0.05	10	19.5	37.4	28.93±0.63	sm (45) st (5)
11	4.0	5.3	4.59±0.06	11	32.0	48.8	38.32±0.58	m (30) sm (20)
12	3.5	5.0	4.22±0.06	12	28.6	42.9	35.60±0.58	sm (33) m (17)
13	3.1	4.2	3.67±0.05	13	25.2	44.4	35.78±0.63	sm (32) m (18)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

Of the eight pairs of small chromosomes, five were of median type and the other three were of submedian type. Chromosome No. 6 was 5.3~7.4, 6.45 on the average, in RL. It was 38.8~50.0 in NVC, being of median type, in 49 of the 50 metaphase spreads and 37.0, being of submedian type, in the remainder. This chromosome was 44.84 on the average, in NVC, being of median type. Chromosome No. 7 was 4.9~6.6, 5.60 on the average, in RL. It was 37.5~46.9 in NVC, being of median type, in 45 of the 50 metaphase spreads and 33.3~37.4 in NVC, being of submedian type, in the other five. This chromosome was 40.22 on the average in NVC, being of median type. Chromosome No. 8 was 4.3~5.7, 5.03 on the average, in RL. It was 37.5~50.0 in NVC, being of median type, in 42 of the 50 metaphase spreads and 31.3~36.8, being of submedian type, in the other eight. This chromosome was 40.67 on the average in NVC, being of median type. Chromosome No.9 was 4.5~6.4, 5.46 on the average, in RL and 38.5~50.0, 46.66 on the average, in NVC, being of median type. This chromosome was larger than chromosome No. 8 and had a distinct secondary constriction in the long arm. Chromosome No. 10 was 4.4~5.5, 4.94 on the average, in RL and statistically similar to chromosome No. 8 in size. However, chromosome No. 10 remarkably differed in shape from Nos. 8 and 9. It was 25.0~37.4 in NVC, being of submedian type, in 45 of the 50 metaphase spreads and 19.5~23.5, being of

subterminal type, in the other five. This chromosome was 28.93 on the average in NVC, being of submedian type. Chromosome No. 11 was 4.0~5.3, 4.59 on the average, in RL. It was 37.5~48.8 in NVC, being of median type, in 30 of the 50 metaphase spreads and 32.0~36.6, being of submedian type, in the other 20. On the average, this chromosome was 38.32 in NVC, being of median type. Chromosomes Nos. 12 and 13 were 3.5~5.0, 4.22 on the average, and 3.1~4.2, 3.67 on the average, in RL, respectively. Although these two chromosomes significantly differed from each other in size, they were very similar to each other in shape. Chromosome No. 12 was 28.6~36.6 in NVC, being of submedian type in 33 of the 50 metaphase spreads and 37.5~42.9, being of median type in the other 17, while chromosome No. 13 was 25.2~36.4 in NVC, being of submedian type, in 32 of the 50 metaphase spreads and 37.5~44.4, being of median type, in the other 18. These two chromosomes were 35.60 and 35.78 on the average in NVC, respectively, both being of submedian type.

II. Brown frogs having 24 chromosomes

Rana ornativentris, Rana chensinensis and *Rana dybowskii* were 24 in diploid chromosome number. The karyotype of each species consisted of five pairs of large chromosomes (Nos. 1~5) and seven pairs of small chromosomes (Nos. 6~12), although there was no such distinct difference in size between chromosomes Nos. 5 and 6 as found in each species having 26 chromosomes. Chromosome No. 6 of the species having 24 chromosomes was intermediate in size between chromosomes Nos. 5 and 6 of the species having 26 chromosomes. Chromosomes Nos. 7~12 of the former species corresponded in size and shape to chromosomes Nos. 6~11 of the latter. Moreover, chromosome No. 6 of the former species almost corresponded to the sum of chromosomes Nos. 12 and 13 of the latter species in size.

1. *Rana ornativentris*

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads obtained from two mature males and two mature females by the blood culture method are presented in Table 6. The 12 pairs of chromosomes were arranged in an order corresponding to those of *Rana japonica* in size and shape (Fig. 6).

Of the five pairs of large chromosomes, four were of median type and the remainder was of submedian type. The largest chromosome (No. 1) was 13.4~17.6, 14.95 on the average, in RL and 44.3~49.5, 46.25 on the average, in NVC, being of median type. Chromosome No. 2 was 11.9~14.1, 12.86 on the average, in RL. It was 37.5~41.4 in NVC, being of median type, in 32 of the 50 metaphase spreads and 35.0~37.4, being of submedian type, in the other 18. This chromosome was 38.25 on the average in NVC, being of median type. Chromosome No. 3 was 10.8~12.2, 11.49 on the average, in RL and 37.5~44.4, 41.30 on the average, in NVC, being of median type. Chromosome No. 4 was

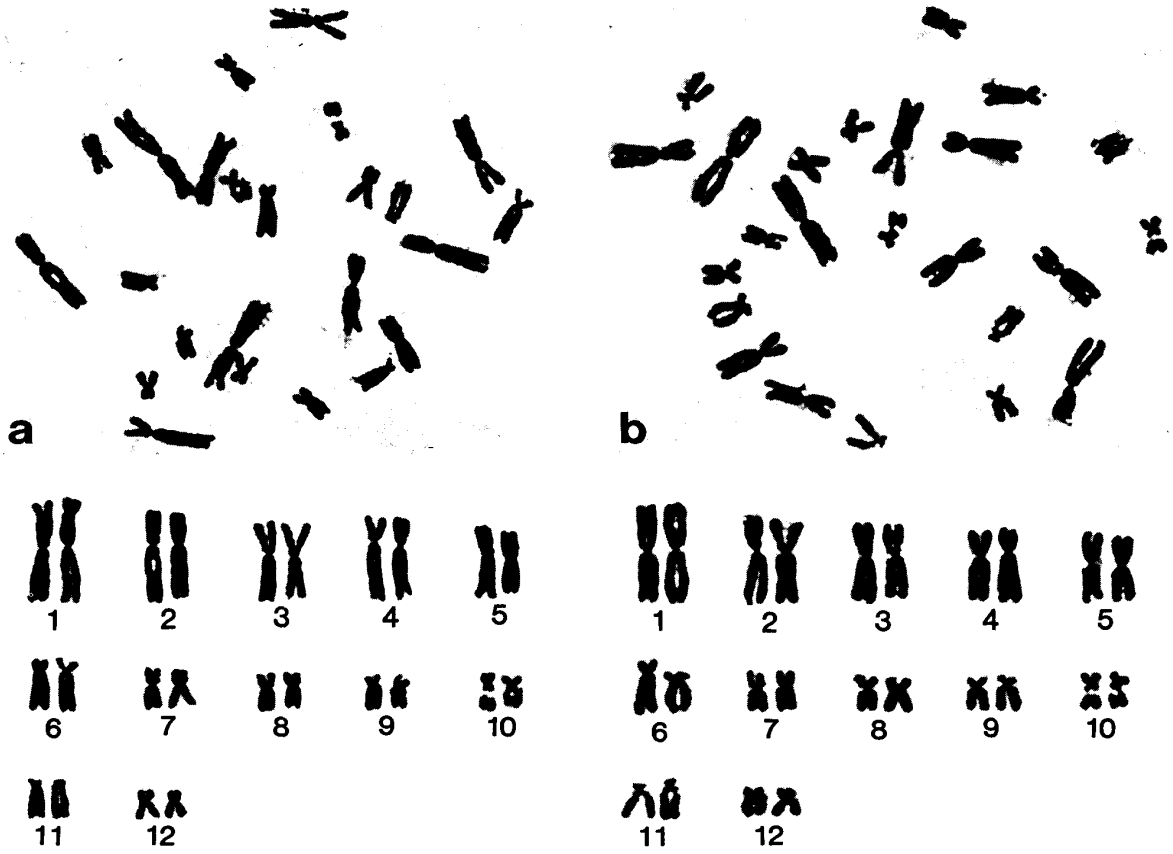


Fig. 6. Metaphase plates and the karyotypes of a female and a male *Rana ornativentris*.
a. Female b. Male ×1100

9.6~12.3, 10.94 on the average, in RL. It was 28.6~36.4 in NVC, being of submedian type, in 49 of the 50 metaphase spreads and 37.8, being of median type, in the remainder. This chromosome was 32.54 on the average in NVC, being of submedian type. Chromosome No. 5 was 9.1~11.2, 9.80 on the average, in RL and 38.0~47.0, 43.60 on the average, in NVC, being of median type.

Of the seven pairs of small chromosomes, two were of median type, three were of submedian type, one was of subterminal type and the remainder was intermediate between terminal and subterminal types. Chromosome No. 6 was 6.5~7.9, 7.27 on the average, in RL. It was 17.9~24.4 in NVC, being of subterminal type, in 41 of the 50 metaphase spreads and 25.0~27.3, being of submedian type in the other nine. On the average, it was 22.65 in NVC, being of subterminal type. Chromosome No. 7 was 5.5~7.2, 6.47 on the average, in RL. It was 37.5~50.0 in NVC, being of median type, in 48 of the 50 metaphase spreads and 35.5 and 35.7, being of submedian type, in the remaining two. On the average, it was 42.59 in NVC, being of median type. Chromosomes Nos. 8 and 11 were 5.1~6.2, 5.77 on the average, and 5.1~6.5, 5.83 on the average, in RL, respectively. There was statistically no significant difference in size between these two chromosomes, although they distinctly differed from each other in shape. Chromosome No. 8 was 37.5~46.7 in NVC, being of median type, in 48 of the 50 metaphase spreads and 34.4 and 36.4, being of submedian type, in the remaining

TABLE 6

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana ornativentris*

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	13.4	17.6	14.95±0.11	1	44.3	49.5	46.25±0.16	m (50)
2	11.9	14.1	12.86±0.06	2	35.0	41.4	38.25±0.21	m (32) sm (18)
3	10.8	12.2	11.49±0.05	3	37.5	44.4	41.30±0.23	m (50)
4	9.6	12.3	10.94±0.07	4	28.6	37.8	32.54±0.25	sm (49) m (1)
5	9.1	11.2	9.80±0.06	5	38.0	47.0	43.60±0.24	m (50)
6	6.5	7.9	7.27±0.04	6	17.9	27.3	22.65±0.28	st (41) sm (9)
7	5.5	7.2	6.47±0.05	7	35.5	50.0	42.59±0.40	m (48) sm (2)
8	5.1	6.2	5.77±0.04	8	34.4	46.7	42.03±0.37	m (48) sm (2)
9	4.4	6.0	5.05±0.05	9	17.4	33.3	26.48±0.37	sm (33) st (17)
10*	4.3	6.0	5.09±0.05	10*	22.2	33.3	27.97±0.36	sm (43) st (7)
11	5.1	6.5	5.83±0.05	11	7.7	15.4	12.23±0.26	t (25) st (25)
12	3.4	5.1	4.48±0.04	12	28.6	45.0	35.40±0.44	sm (38) m (12)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

two, while chromosome No. 11 was 7.7~12.2 in NVC, being of terminal type, in 25 of the 50 metaphase spreads and 12.5~15.4, being of subterminal type, in the other 25. On the average, chromosome No. 8 was 42.03 in NVC, being of median type, and chromosome No. 11 was 12.23 in NVC, being intermediate between terminal and subterminal types. Although chromosomes Nos. 9 and 10 were smaller than No. 11, they were arranged in this order on the basis of their shape. They were 4.4~6.0, 5.05 on the average, and 4.3~6.0, 5.09 on the average, in RL, respectively. Chromosome No. 9 was 25.0~33.3 in NVC, being of submedian type, in 33 of the 50 metaphase spreads and 17.4~24.6, being of subterminal type, in the other 17, while chromosome No. 10 was 25.0~33.3 in NVC, being of submedian type, in 43 of the 50 metaphase spreads and 22.2~24.7, being of subterminal type, in the remaining seven. These two chromosomes were 26.48 and 27.97 on the average in NVC, respectively, both being of submedian type. Chromosome No. 10 had a distinct secondary constriction in the long arm. Chromosome No. 12 was 3.4~5.1, 4.48 on the average, in RL. It was 28.6~37.3 in NVC, being of submedian type, in 38 of the 50 metaphase spreads and 37.5~45.0, being of median type, in the other 12. On the average, this chromosome was 35.40 in NVC, being of submedian type.

2. *Rana chensinensis*

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads obtained from two mature males and two mature females by the blood culture method are presented in Table 7. The 12 pairs of chromosomes were arranged in an order corresponding to those of *Rana ornativentris* in size and shape (Fig. 7).

Of the five pairs of large chromosomes, three were of median type and the other two were of submedian type. The largest chromosome (No. 1) was 13.8~17.9, 15.65 on the average, in RL and 43.0~49.2, 46.00 on the average, in NVC, being of median type. Chromosome No. 2 was 11.8~14.3, 13.14 on the average, in RL and 33.7~37.2 in NVC, being of submedian type, in 30 of the 50 metaphase spreads and 37.5~40.2, being of median type, in the other 20. On the average, chromosome No. 2 was 36.90 in NVC, being of submedian type. Chromosome No. 3 was 11.4~13.2, 12.11 on the average, in RL. It was 37.5~44.8 in NVC, being of median type, in 37 of the 50 metaphase spreads and 32.6~37.4, being of submedian type, in the other 13. This chromosome was 38.52 on the average in NVC, being of median type. Chromosome No. 4 was 10.5~12.5, 11.45 on the average, in RL and 28.2~37.0, 32.93 on the average, in NVC, being of submedian

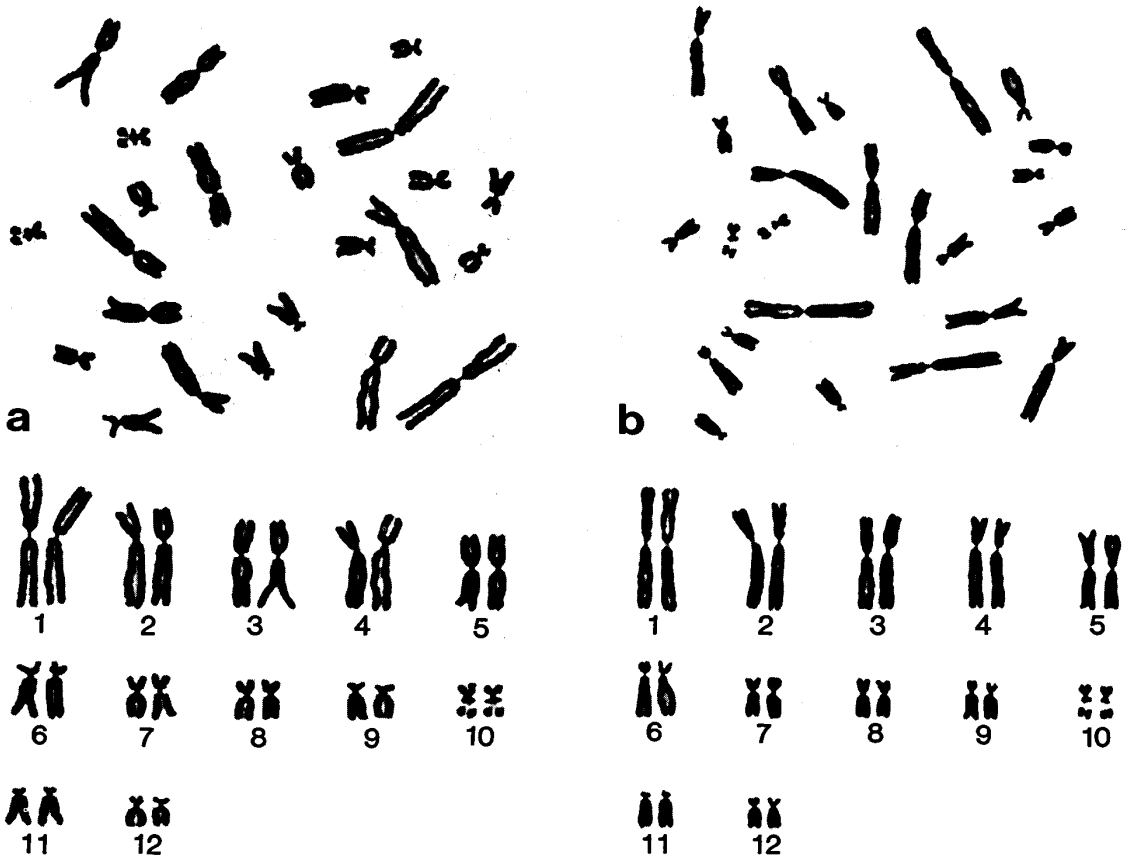


Fig. 7. Metaphase plates and the karyotypes of a female and a male *Rana chensinensis*.
a. Female b. Male ×1100

TABLE 7

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana chensinensis*

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	13.8	17.9	15.65±0.10	1	43.0	49.2	46.00±0.17	m (50)
2	11.8	14.3	13.14±0.07	2	33.7	40.2	36.90±0.24	sm (30) m (20)
3	11.4	13.2	12.11±0.06	3	32.6	44.8	38.52±0.33	m (37) sm (13)
4	10.5	12.5	11.45±0.06	4	28.2	37.0	32.93±0.28	sm (50)
5	9.1	11.7	9.96±0.08	5	39.2	46.7	43.35±0.30	m (50)
6	5.4	9.4	7.40±0.10	6	20.0	33.3	25.10±0.40	sm (25) st (25)
7	4.6	6.6	5.86±0.05	7	29.9	43.5	36.08±0.53	sm (25) m (25)
8	4.8	6.1	5.33±0.05	8	28.6	44.4	36.95±0.53	sm (25) m (25)
9	4.0	5.7	4.94±0.05	9	17.1	33.3	28.45±0.54	sm (43) st (7)
10*	4.1	5.7	4.94±0.05	10*	25.0	37.4	31.14±0.41	sm (50)
11	4.1	5.8	4.95±0.06	11	7.5	23.1	17.31±0.40	st (48) t (2)
12	3.1	4.9	4.24±0.05	12	21.1	37.0	29.05±0.58	sm (38) st (12)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

type. Chromosome No. 5 was 9.1~11.7, 9.96 on the average, in RL and 39.2~46.7, 43.35 on the average, in NVC, being of median type.

Of the seven pairs of small chromosomes, four were of submedian type, one was intermediate between submedian and median types, one was intermediate between submedian and subterminal types and the remainder was of subterminal type. Chromosome No. 6 was 5.4~9.4, 7.40 on the average, in RL. It was 25.0~33.3 in NVC, being of submedian type, in 25 of the 50 metaphase spreads and 20.0~24.6, being of subterminal type, in the other 25. This chromosome was 25.10 on the average in NVC, being intermediate between submedian and subterminal types. Chromosome No. 7 was 4.6~6.6, 5.86 on the average, in RL. It was 29.9~37.4 in NVC, being of submedian type, in 25 of the 50 metaphase spreads and 37.5~43.5, being of median type, in the other 25. On the average, this chromosome was 36.08 in NVC, being of submedian type. Chromosome No. 8 was 4.8~6.1, 5.33 on the average, in RL. It was 28.6~37.3 in NVC, being of submedian type, in 25 of the 50 metaphase spreads and 37.5~44.4, being of median type, in the other 25. This chromosome was 36.95 on the average in NVC, being intermediate between submedian and median types. Chromosomes Nos. 9, 10 and 11 were 4.0~5.7, 4.94 on the average, 4.1~5.7, 4.94 on the average and 4.1~5.8, 4.95 on the average, in RL, respectively. There were statistically no significant differences in

RL among these three chromosomes, whereas they differed from one another in shape. Chromosome No. 9 was 25.0~33.3 in NVC, being of submedian type, in 43 of the 50 metaphase spreads and 17.1~23.1, being of subterminal type in the remaining seven. This chromosome was 28.45 on the average in NVC, being of submedian type. Chromosome No. 10 was 25.0~37.4, 31.14 on the average, in NVC, being of submedian type. This chromosome had a distinct secondary constriction in the long arm. Chromosome No. 11 was 12.8~23.1 in NVC, being of subterminal type, in 48 of the 50 metaphase spreads and 7.5 and 10.0, being of terminal type, in the remaining two. This chromosome was 17.31 on the average in NVC, being of subterminal type. Chromosome No. 12 was 3.1~4.9, 4.24 on the average, in RL. It was 25.0~37.0 in NVC, being of submedian type, in 38 of the 50 metaphase spreads and 21.1~24.5, being of subterminal type, in the other 12. This chromosome was 29.05 on the average in NVC, being of submedian type.

3. *Rana dybowskii* from Tsushima

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads obtained from two mature males and two mature females by the blood culture method are presented in Table 8. The 12 pairs of chromosomes were arranged in an order corresponding to those of *Rana ornativentris* in size and shape (Fig. 8).

Of the five pairs of large chromosomes, four were of median type and the remainder was of submedian type. The largest chromosome (No. 1) was 14.0 ~18.3, 15.43 on the average, in RL and 40.5~48.1, 46.11 on the average, in NVC, being of median type. Chromosome No. 2 was 11.9~14.5, 13.09 on the average, in RL. It was 37.7~44.7 in NVC, being of median type, in 32 of the 50 metaphase spreads and 31.6~37.3, being of submedian type, in the other 18. This chromosome was 38.43 on the average in NVC, being of median type. Chromosomes Nos. 3 and 4 were 10.1~13.1, 11.65 on the average, and 10.3~12.8, 11.77 on the average, in RL, respectively. Although there was statistically no significant difference in size between these two chromosomes, they remarkably differed from each other in shape. Chromosome No. 3 was 38.0~46.3 in NVC, being of median type, in 46 of the 50 metaphase spreads and 34.8~36.4, being of submedian type, in the other four, while chromosome No. 4 was 26.0~37.1 in NVC, being of submedian type, in 48 of the 50 metaphase spreads and 37.5 and 38.3, being of median type, in the remaining two. These two chromosomes were 41.33 and 33.92 on the average in NVC, respectively, being of median and submedian type, respectively. Chromosome No. 5 was 9.0~12.3, 9.86 on the average, in RL and 40.7~48.0, 44.63 on the average, in NVC, being of median type.

Of the seven pairs of small chromosomes, one was of median type, one was intermediate between median and submedian types, two were of submedian type, two were intermediate between subterminal and submedian types, and the remaining one was of subterminal type. Chromosome No. 6 was 6.4~8.5, 7.56 on

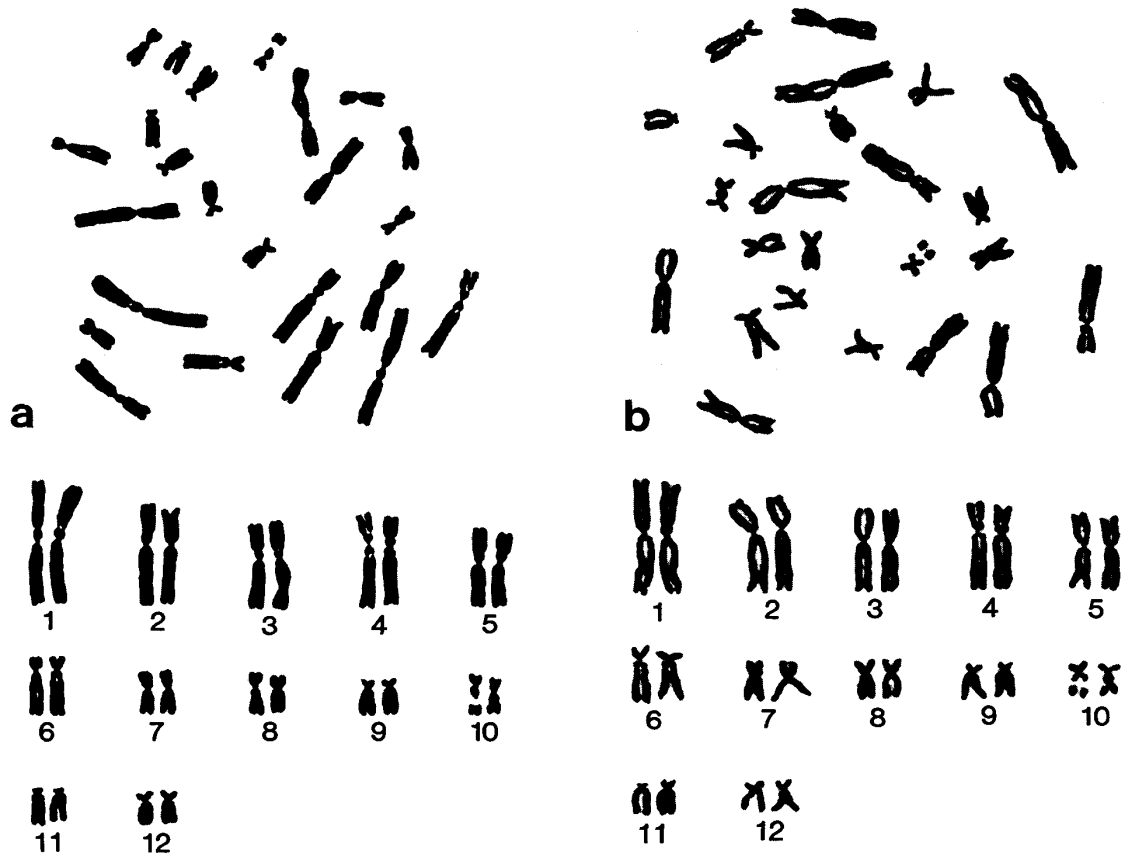


Fig. 8. Metaphase plates and the karyotypes of a female and a male *Rana dybowskii* from Tsushima.
a. Female b. Male ×1100

the average, in RL. It was 18.8~24.8 in NVC, being of subterminal type in 34 of the 50 metaphase spreads and 25.0~37.1, being of submedian type, in the other 16. This chromosome was 24.48 on the average in NVC, being intermediate between subterminal and submedian types. Chromosome No. 7 was 5.1~7.2, 6.38 on the average, in RL. It was 37.5~44.6 in NVC, being of median type, in 40 of the 50 metaphase spreads and 31.6~37.0, being of submedian type, in the other ten. This chromosome was 38.74 on the average in NVC, being of median type. Chromosome No. 8 was 4.7~6.1, 5.42 on the average, in RL. It was 37.5~44.6 in NVC, being of median type, in 32 of the 50 metaphase spreads and 26.8~37.0, being of submedian type, in the other 18. This chromosome was 37.52 on the average in NVC, being intermediate between median and submedian types. Chromosomes Nos. 9 and 10 were 4.1~5.6, 4.84 on the average, and 3.9~5.8, 4.75 on the average, in RL, respectively. There was statistically no significant difference in RL between these two chromosomes, although they remarkably differed from each other in shape. Chromosome No. 9 was 16.2~24.7 in NVC, being of subterminal type, in 26 of the 50 metaphase spreads and 25.0~29.4, being of submedian type in the other 24. This chromosome was 24.30 on the average in NVC, being intermediate between submedian and subterminal types. Chromosome No. 10 had a secondary constriction in the long arm. It was 25.0~36.8 in NVC, being of submedian type, in 42 of the 50 metaphase spreads and 21.7~24.4,

TABLE 8

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana dybowskii* from Tsushima

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	14.0	18.3	15.43±0.14	1	40.5	48.1	46.11±0.26	m (50)
2	11.9	14.5	13.09±0.10	2	31.6	44.7	38.43±0.40	m (32) sm (18)
3	10.1	13.1	11.65±0.09	3	34.8	46.3	41.33±0.39	m (46) sm (4)
4	10.3	12.8	11.77±0.08	4	26.0	38.3	33.92±0.33	sm (48) m (2)
5	9.0	12.3	9.86±0.09	5	40.7	48.0	44.63±0.28	m (50)
6	6.4	8.5	7.56±0.06	6	18.8	37.1	24.48±0.49	st (34) sm (16)
7	5.1	7.2	6.38±0.07	7	31.6	44.6	38.74±0.43	m (40) sm (10)
8	4.7	6.1	5.42±0.06	8	26.8	44.6	37.52±0.48	m (32) sm (18)
9	4.1	5.6	4.84±0.05	9	16.2	29.4	24.30±0.47	st (26) sm (24)
10*	3.9	5.8	4.75±0.06	10*	21.7	36.8	28.58±0.53	sm (42) st (8)
11	4.3	6.2	5.00±0.06	11	8.1	24.4	14.99±0.56	st (36) t (14)
12	3.7	5.1	4.27±0.04	12	21.2	36.5	28.98±0.55	sm (42) st (8)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

being of subterminal type, in the other eight. This chromosome was 28.58 on the average in NVC, being of submedian type. Chromosome No. 11 was 4.3~6.2, 5.00 on the average, in RL. It was 12.5~24.4 in NVC, being of subterminal type, in 36 of the 50 metaphase spreads and 8.1~12.3, being of terminal type, in the other 14. This chromosome was 14.99 on the average in NVC, being of subterminal type. Chromosome No.12 was 3.7~5.1, 4.27 on the average, in RL. It was 25.0~36.5 in NVC, being of submedian type, in 42 of the 50 metaphase spreads and 21.2~24.4, being of subterminal type, in the other eight. This chromosome was 28.98 on the average in NVC, being of submedian type.

4. *Rana dybowskii* from Korea

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads obtained from two mature males and two mature females by the blood culture method are presented in Table 9. The 12 pairs of chromosomes were arranged in an order corresponding to those of *Rana ornativentris* in size and shape (Fig. 9).

Of the five pairs of large chromosomes, four were of median type and the remainder was of submedian type. The largest chromosome (No. 1) was 13.6 ~17.0, 15.01 on the average, in RL and 43.1~49.5, 46.61 on the average, in NVC,

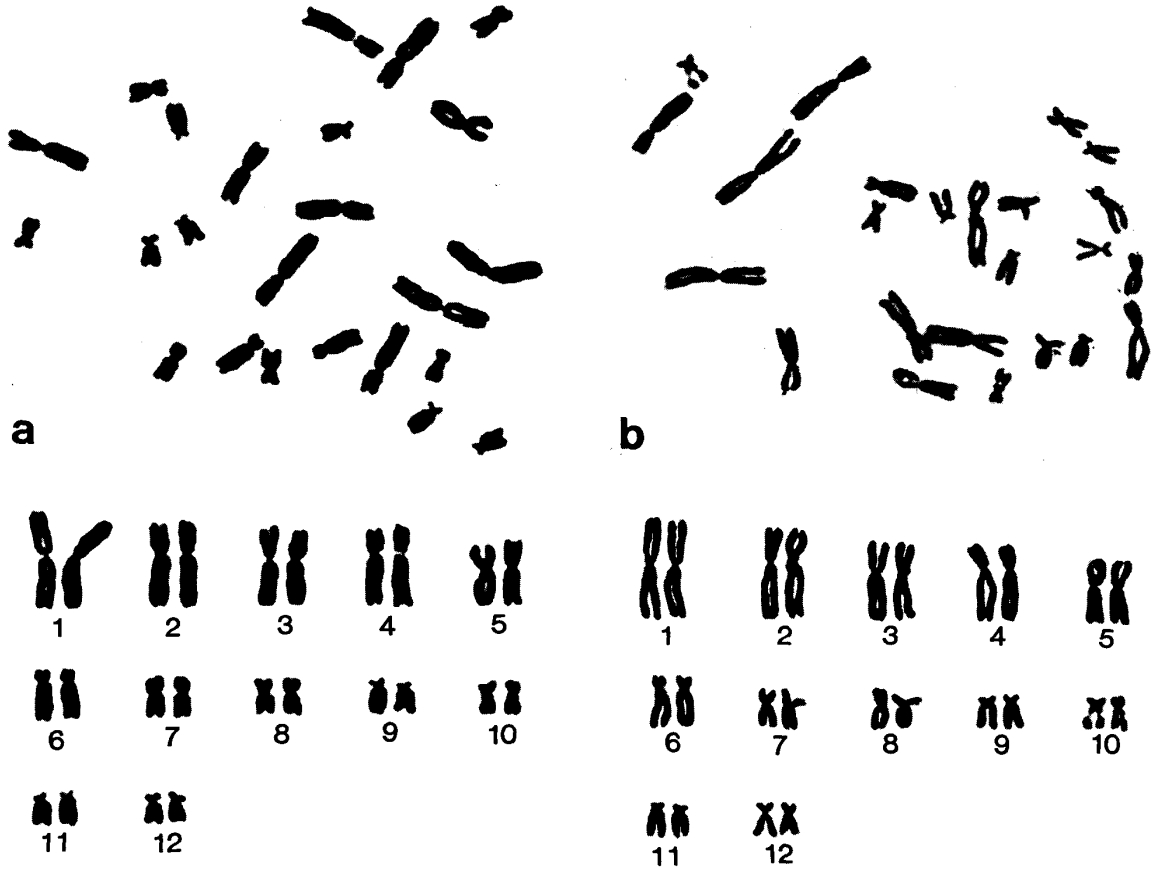


Fig. 9. Metaphase plates and the karyotypes of a female and a male *Rana dybowskii* from Korea.
a. Female b. Male ×1100

being of median type. Chromosome No. 2 was 12.4~13.9, 13.10 on the average, in RL. It was 37.5~42.3 in NVC, being of median type, in 37 of the 50 metaphase spreads and 33.3~37.4, being of submedian type in the other 13. This chromosome was 38.40 on the average in NVC, being of median type. Chromosomes Nos. 3 and 4 were 10.7~12.6, 11.58 on the average, and 10.5~12.9, 11.65 on the average, in RL, respectively. Although there was statistically no difference in size between these two chromosomes, they differed from each other in shape. Chromosome No. 3 was 37.6~43.2 in NVC, being of median type, in 45 of the 50 metaphase spreads and 35.9~37.3, being of submedian type, in the other five. While this chromosome was 39.89 on the average in NVC, being of median type, chromosome No. 4 was 28.1~35.5, 32.05 on the average, in NVC, being of submedian type. Chromosome No. 5 was 8.1~10.7, 9.78 on the average, in RL and 37.9~47.4, 43.29 on the average, in NVC, being of median type.

Of the seven pairs of small chromosomes, two were of median type, two others were of submedian type, one was intermediate between subterminal and submedian types and the remaining two were of subterminal type. Chromosome No. 6 was 6.8~8.4, 7.48 on the average, in RL. It was 18.5~24.8 in NVC, being of subterminal type in 30 of the 50 metaphase spreads, and 25.0~30.6, being of submedian type in the other 20. This chromosome was 24.59 on the average in

TABLE 9

Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana dybowskii* from Korea

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	13.6	17.0	15.01±0.10	1	43.1	49.5	46.61±0.17	m (50)
2	12.4	13.9	13.10±0.06	2	33.3	42.3	38.40±0.30	m (37) sm (13)
3	10.7	12.6	11.58±0.07	3	35.9	43.2	39.89±0.23	m (45) sm (5)
4	10.5	12.9	11.65±0.06	4	28.1	35.5	32.05±0.23	sm (50)
5	8.1	10.7	9.78±0.07	5	37.9	47.4	43.29±0.30	m (50)
6	6.8	8.4	7.48±0.05	6	18.5	30.6	24.59±0.43	st (30) sm (20)
7	5.5	7.1	6.08±0.05	7	33.3	44.4	39.17±0.32	m (44) sm (6)
8	4.4	6.5	5.58±0.04	8	33.3	46.2	38.08±0.49	m (32) sm (18)
9	4.1	5.6	4.89±0.05	9	9.6	24.4	17.61±0.48	st (47) t (3)
10*	4.3	5.8	4.99±0.06	10*	22.2	34.7	28.58±0.43	sm (44) st (6)
11	4.2	6.1	5.23±0.05	11	9.4	24.4	17.06±0.50	st (48) t (2)
12	4.2	5.3	4.69±0.04	12	22.2	34.5	29.10±0.57	sm (41) st (9)

$$RL = \frac{\text{Chromosome length}}{\text{Genome length}} \times 100 \qquad NVC = \frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$$

	NVC	Type
Chromosome type:	50.0~37.5	m
	37.4~25.0	sm
	24.9~12.5	st
	12.4~ 0	t

± Standard error of the mean
( ) No. of pairs
* Secondary constriction

NVC, being intermediate between subterminal and submedian types. Chromosomes Nos. 7 and 8 were 5.5~7.1, 6.08 on the average, and 4.4~6.5, 5.58 on the average, in RL, respectively. These two chromosomes differed from each other in size with a statistical significance. They also somewhat differed from each other in shape. While chromosome No. 7 was 37.5~44.4 in NVC, being of median type, in 44 of the 50 metaphase spreads and 33.3~37.3, being of submedian type, in the other six, chromosome No. 8 was 37.5~46.2 in NVC, being of median type, in 32 of the 50 metaphase spreads and 33.3~37.3, being of submedian type, in the other 18. These two chromosomes were 39.17 and 38.08 on the average in NVC, respectively, both being of median type. Chromosomes Nos. 9 and 10 were 4.1~5.6, 4.89 on the average, and 4.3~5.8, 4.99 on the average, in RL, respectively. Although there was statistically no significant difference in size between these two chromosomes, they remarkably differed from each other in shape. Chromosome No. 9 was 12.7~24.4 in NVC, being of subterminal type in 47 of the 50 metaphase spreads and 9.6~12.3, being of terminal type in the remaining three. This chromosome was 17.61 on the average in NVC, being of subterminal type. On the other hand, chromosome No. 10 had a secondary constriction in the long arm. It was 25.0~34.7 in NVC, being of submedian type, in 44 of the 50 metaphase spreads and 22.2~24.4, being of subterminal type, in the

remaining six. This chromosome was 28.58 on the average in NVC, being of submedian type. Chromosome No. 11 was 4.2~6.1, 5.23 on the average, in RL. It was 13.0~24.4 in NVC, being of subterminal type, in 48 of the 50 metaphase spreads and 9.4 and 10.0, being of terminal type, in the remaining two. This chromosome was 17.06 on the average in NVC, being of subterminal type. Chromosome No. 12 was 4.2~5.3, 4.69 on the average, in RL. It was 25.0~34.5 in NVC, being of submedian type, in 41 of the 50 metaphase spreads and 22.2~24.5, being of subterminal type, in the remaining nine. This chromosome was 29.10 on the average in NVC, being of submedian type.

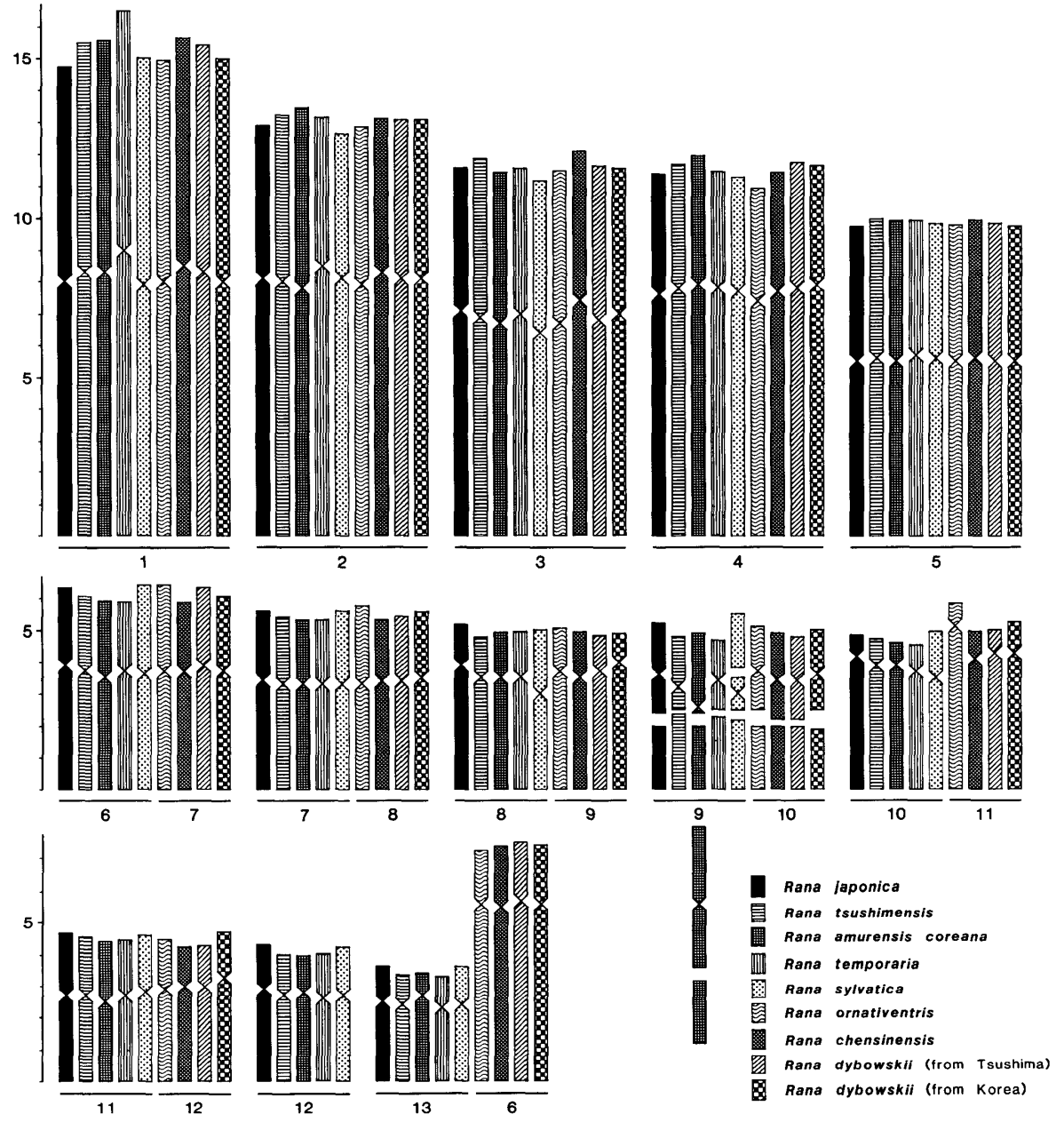


Fig. 10. Composite ideograms showing differences in relative chromosome length and centromere position among eight species and one local race of brown frogs.

Constrictions indicate centromere positions. Gaps indicate secondary constrictions.

III. Comparison of karyotypes among eight species and one local race

The karyotypes of eight species and one local race of brown frogs are graphically shown in Fig. 10. All these frogs had a secondary constriction in the long arm of chromosome No. 9 or No. 10. The differences in relative chromosome length and numerical value of centromere position among the eight species and the single local race are shown in accordance with HUBBS and HUBBS (1973) in Figs. 11 and 12. The result of examinations by the method of HUBBS and HUBBS on the presence of significant differences was in complete agreement with that by t-test.

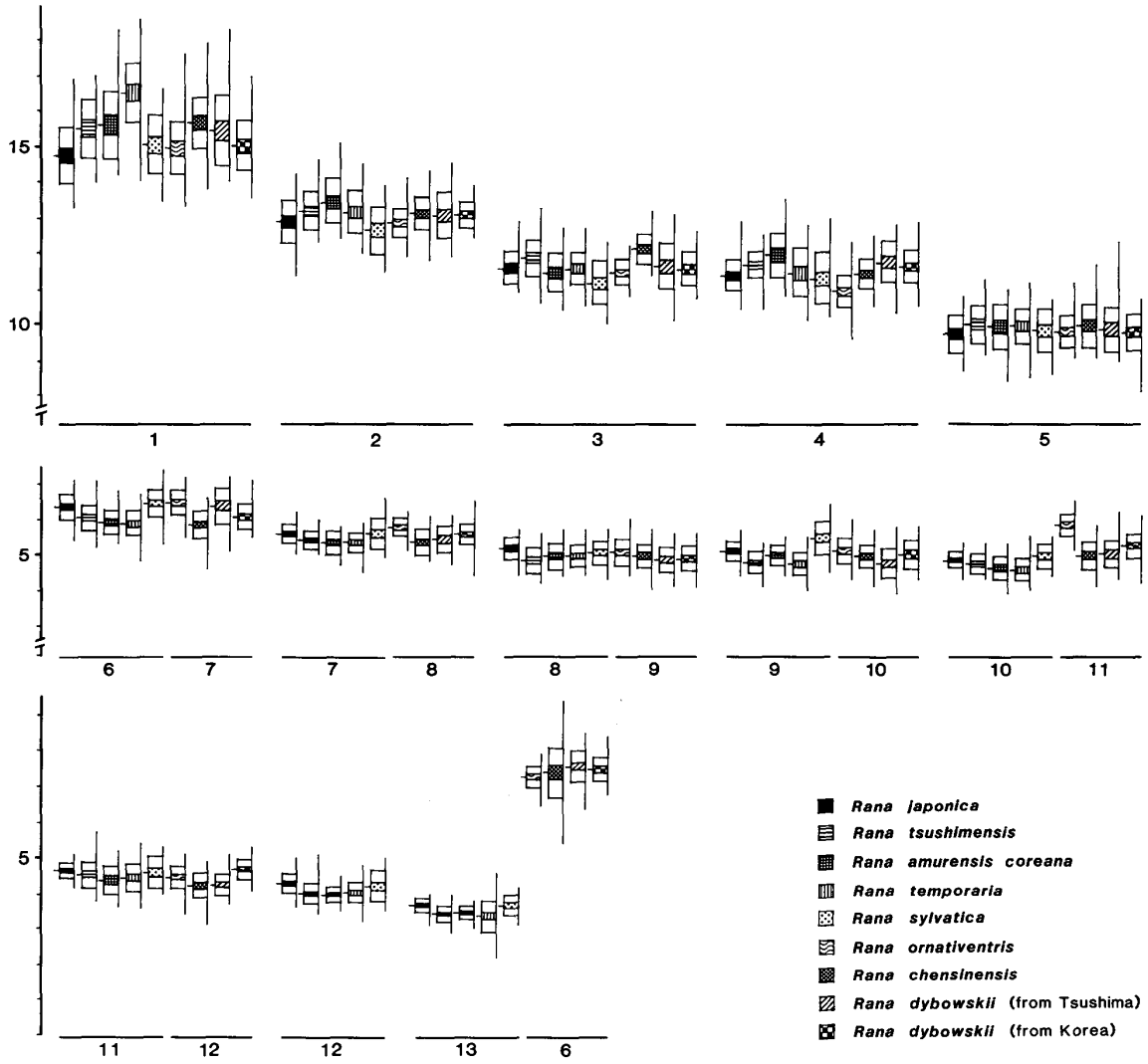


Fig. 11. Graphs showing differences in relative chromosome length among eight species and one local race of brown frogs.

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.

1. Brown frog species with 26 chromosomes

a. *Rana japonica* and others

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana japonica* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana tsushimensis* in nine chromosomes, Nos. 1, 3, 4, 6, 7, 8, 9, 12 and 13 ($t=2.07\sim4.17$, $P=5\%\sim0\%$), from those of *Rana amurensis coreana* in 10 chromosomes, Nos. 1, 2, 4, 6, 7, 8, 10, 11, 12 and 13 ($t=2.20\sim4.53$, $P=5\%\sim0\%$), from those of *Rana temporaria* in nine chromosomes, Nos. 1, 6, 7, 8, 9, 10, 11,12 and 13 ($t=2.32\sim7.69$, $P=5\%\sim0\%$) and from those of *Rana sylvatica* in two chromosomes, Nos. 3 and 9 ($t=2.68$, 3.33, $P=2\%$, 0.1%) (Fig. 11; Table 10).

The relative chromosome lengths of *Rana japonica* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana tsushimensis* in four chromosomes, No. 8 ($t=3.50$), No. 9 ($t=3.75$), No. 12 ($t=3.63$) and No. 13 ($t=4.17$), from those of *Rana amurensis coreana* in four chromosomes, No. 4 ($t=3.66$), No. 6 ($t=4.40$), No. 12 ($t=4.53$) and No. 13 ($t=3.67$) and from those of *Rana temporaria* in three chromosomes, No. 1 ($t=7.69$), No. 6 ($t=4.60$) and No. 9 ($t=3.87$), but did not differ with a high statistical significance from those of *Rana sylvatica* (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of the chromosomes of *Rana japonica* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana tsushimensis* in four chromosomes, Nos. 2, 3, 10 and 11 ($t=2.16\sim5.97$, $P=5\%\sim0\%$), from those of *Rana amurensis coreana* in eight chromosomes, Nos. 2, 3, 5, 8, 9, 10, 12 and 13 ($t=2.21\sim15.86$, $P=5\%\sim0\%$), from those of *Rana temporaria* in six chromosomes, Nos. 2, 6, 8, 10, 11 and 12 ($t=2.01\sim6.25$, $P=5\%\sim0\%$) and from those of *Rana sylvatica* in nine chromosomes, Nos. 1, 2, 6, 8, 9, 10, 11, 12 and 13 ($t=2.46\sim18.63$, $P=5\%\sim0\%$) (Fig. 12; Table 11).

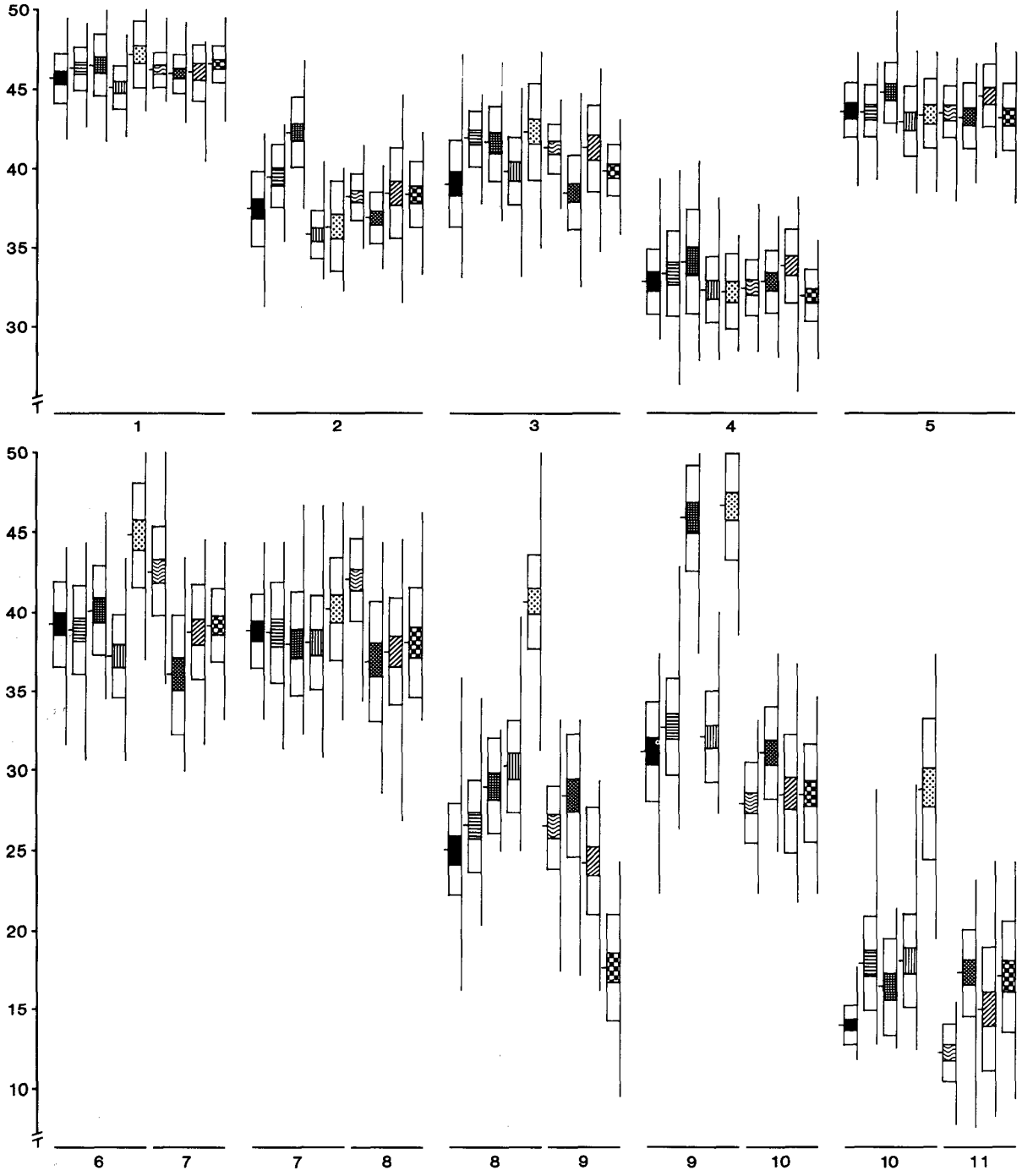
The centromere positions of the chromosomes of *Rana japonica* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana tsushimensis* in two chromosomes, No. 3 ($t=4.39$) and No. 10 ($t=5.97$), from those of *Rana amurensis coreana* in seven chromosomes, No. 2 ($t=7.43$), No. 3 ($t=3.54$), No. 8 ($t=4.72$), No. 9 ($t=15.86$), No. 10 ($t=3.60$), No. 12 ($t=5.06$) and No. 13 ($t=9.36$), from those of *Rana temporaria* in two chromosomes, No. 8 ($t=6.25$) and No. 10 ($t=6.25$) and from those of *Rana sylvatica* in seven chromosomes, No. 3 ($t=4.04$), No. 6 ($t=6.55$), No. 8 ($t=18.63$), No. 9 ($t=16.63$), No. 10 ($t=16.10$), No. 11 ($t=3.61$) and No. 13 ($t=3.64$) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana japonica* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana tsushimensis* in a total of 13 chromosomes including nine

in RL and four in NVC, from that of *Rana amurensis coreana* in a total of 18 chromosomes including 10 in RL and eight in NVC, from that of *Rana temporaria* in a total of 15 chromosomes including nine in RL and six in NVC, and from that of *Rana sylvatica* in a total of 11 chromosomes including two in RL and nine in NVC (Table 12).

The karyotype of *Rana japonica* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana tsushimensis, Rana amurensis coreana, Rana temporaria* and *Rana sylvatica* in RL or NVC of six, 11, five and seven chromosomes, respectively (Table 12).



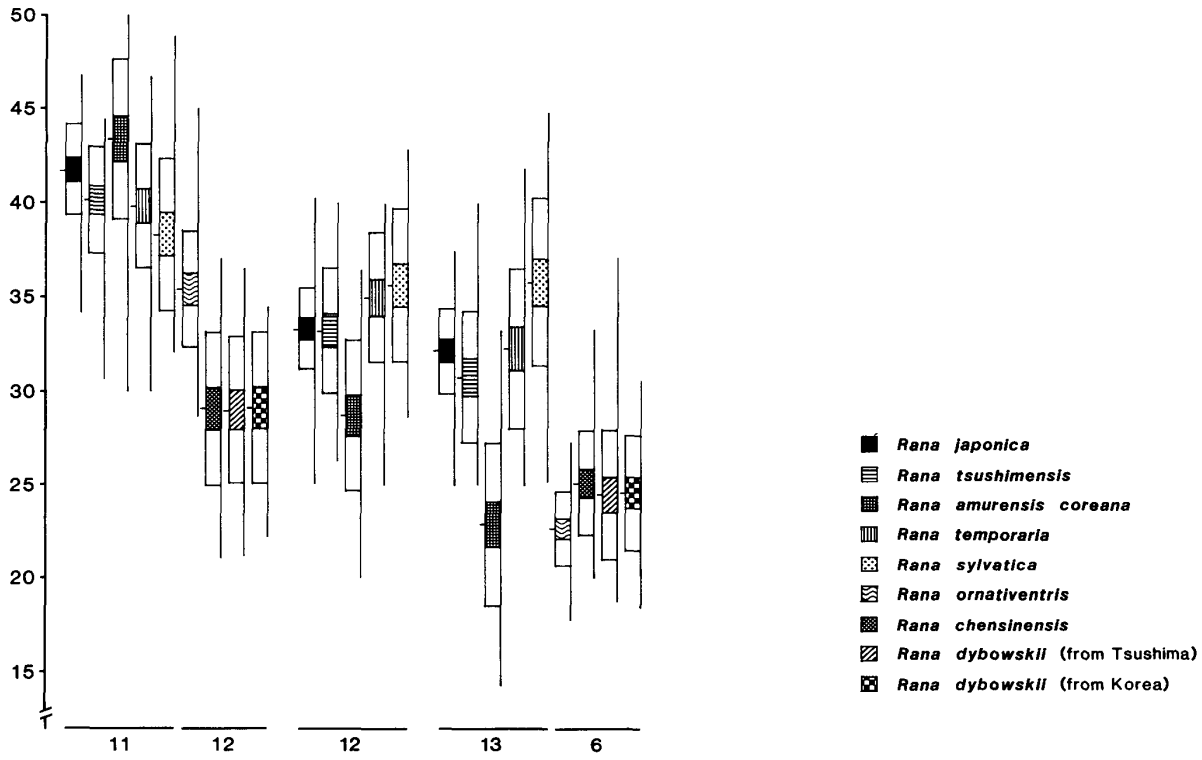


Fig. 12. Graphs showing differences in centromere position among eight species and one local race of brown frogs.

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.

b. *Rana tsushimensis* and others

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana tsushimensis* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana amurensis coreana* in two chromosomes, Nos. 3 and 9 ($t=2.66$, 2.38, $P<5\%$) and from those of *Rana temporaria* in two chromosomes, Nos.1 and 3 ($t=4.21$, 2.15, $P<5\%$) and from those of *Rana sylvatica* in nine chromosomes, Nos. 2, 3, 4, 6, 8, 9, 10, 12 and 13 ($t=2.06\sim5.96$, $P=5\%\sim0\%$). They also differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana temporaria* in chromosome No. 1 ($t=4.21$) and from those of *Rana sylvatica* in three chromosomes, No. 3 ($t=4.22$), No. 6 ($t=4.10$) and No. 9 ($t=5.96$), but did not differ with a high statistical significance from those of *Rana amurensis coreana* (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of the chromosomes of *Rana tsushimensis* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana amurensis coreana* in

TABLE 10

Numbers of chromosome pairs which significantly differ in relative length among eight species and one local race of brown frogs. Parentheses indicate a difference with a high significance

Ch. no.	Species	2n=26				2n=24			
		tsu.	*amur.*	*temp.*	*syl.*	*ornat.*	*chen.*	*dyb.* T	*dyb.* K
2n=26	*R. japonica*	9 (4)	10 (4)	9 (3)	2 (0)	3 (2)	5 (3)	4 (0)	4 (1)
	R. tsushimensis		2 (0)	2 (1)	9 (3)	9 (4)	2 (0)	2 (0)	5 (1)
	R. amurensis coreana			3 (0)	9 (3)	6 (5)	4 (1)	3 (1)	5 (1)
	R. temporaria				8 (4)	7 (5)	4 (2)	3 (3)	4 (2)
	R. sylvatica					4 (1)	6 (3)	4 (1)	6 (2)
2n=24	*R. ornativentris*						8 (5)	7 (2)	6 (3)
	R. chensinensis							3 (1)	6 (2)
	R. dybowskii from Tsushima								3 (1)

TABLE 11

Numbers of chromosome pairs which significantly differ in centromere position among eight species and one local race of brown frogs. Parentheses indicate a difference with a high significance

Ch. no.	Species	2n=26				2n=24			
		tsu.	*amur.*	*temp.*	*syl.*	*ornat.*	*chen.*	*dyb.* T	*dyb.* K
2n=26	*R. japonica*	4 (2)	8 (7)	6 (2)	9 (7)	5 (5)	4 (2)	2 (0)	4 (2)
	R. tsushimensis		7 (4)	5 (3)	7 (6)	5 (4)	3 (2)	3 (1)	4 (3)
	R. amurensis coreana			10 (5)	10 (7)	8 (4)	5 (4)	3 (3)	6 (3)
	R. temporaria				8 (5)	8 (6)	3 (0)	8 (3)	5 (4)
	R. sylvatica					7 (3)	7 (5)	7 (4)	7 (5)
2n=24	*R. ornativentris*						9 (7)	7 (3)	7 (5)
	R. chensinensis							7 (2)	5 (2)
	R. dybowskii from Tsushima								4 (1)

TABLE 12

Numbers of chromosome pairs which significantly differ in either relative length or centromere position among eight species and one local race of brown frogs. Parentheses indicate a difference with a high significance

Ch. no.	Species	2n=26				2n=24			
		tsu.	*amur.*	*temp.*	*syl.*	*ornat.*	*chen.*	*dyb.* T	*dyb.* K
2n=26	*R. japonica*	13 (6)	18 (11)	15 (5)	11 (7)	8 (7)	9 (5)	6 (0)	8 (3)
	R. tsushimensis		9 (4)	7 (4)	16 (9)	14 (8)	5 (2)	5 (1)	9 (4)
	R. amurensis coreana			13 (5)	19 (10)	14 (9)	9 (5)	6 (4)	11 (4)
	R. temporaria				16 (9)	15 (11)	7 (2)	11 (6)	9 (6)
	R. sylvatica					11 (4)	13 (8)	11 (5)	13 (7)
2n=24	*R. ornativentris*						17 (12)	14 (5)	13 (8)
	R. chensinensis							10 (3)	11 (4)
	R. dybowskii from Tsushima								7 (2)

seven chromosomes, Nos. 2, 5, 8, 9, 11, 12 and 13 (t=2.35~14.42, P=5%~0%), from those of *Rana temporaria* in five chromosomes, Nos. 1, 2, 3, 6, and 8 (t=2.14~7.33, P=5%~0%) and from those of *Rana sylvatica* in seven chromosomes, Nos. 2, 6, 8, 9, 10, 12 and 13 (t=2.26~17.03, P=5%~0%). They also differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana amurensis coreana* in four chromosomes, No. 2 (t=4.62), No. 9 (t=14.42), No. 12 (t=4.35) and No. 13 (t=6.96), from those of *Rana temporaria* in three chromosomes, No. 2 (t=7.33), No. 3 (t=3.79) and No. 8 (t=4.51) and from those of *Rana sylvatica* in six chromosomes, No. 2 (t=4.50), No. 6 (t=6.92), No. 8 (t=17.03), No. 9 (t=15.20), No. 10 (t=10.33) and No. 13 (t=4.43) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana tsushimensis* differed with a statistical significance (t≧2.01, P≦5%) from that of *Rana amurensis coreana* in a total of nine chromosomes including two in RL and seven in NVC, from that of *Rana temporaria* in a total of seven chromosomes including two in RL and five in NVC, and from that of *Rana sylvatica* in a total of 16 chromosomes including nine in RL and seven in NVC (Table 12).

The karyotype of *Rana tsushimensis* differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana amurensis coreana, Rana temporaria* and *Rana sylvatica* in RL or NVC of four, four and nine chromosomes, respectively (Table 12).

c. *Rana amurensis coreana* and others

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana amurensis coreana* differed with a statistical significance (t≧2.01, P≦5%) from those of *Rana temporaria* in three chromosomes, Nos. 1, 4 and 9 (t=2.52~3.45, P=2%~0.1%) and from those of *Rana sylvatica* in nine chromosomes, Nos. 1, 2, 4, 6, 7, 9, 10, 12 and 13 (t=2.15~5.40, P=5%~0%). They also differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana sylvatica* in three chromosomes, No. 2 (t=4.26), No. 6 (t=5.40) and No. 9 (t=4.30), but did not differ with a high statistical significance from those of *Rana temporaria* (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana amurensis coreana* differed with a statistical significance (t≧2.01, P≦5%) from those of *Rana temporaria* in 10 chromosomes, Nos. 1, 2, 3, 4, 5, 6, 9, 11, 12 and 13 (t=2.26~15.59, P=5%~0%) and from those of *Rana sylvatica* in 10 chromosomes, Nos. 2, 4, 5, 6, 7, 8, 10, 11, 12 and 13 (t=2.32~13.69, P=5%~0%). They also differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana temporaria* in five chromosomes, No. 2 (t=11.78), No. 6 (t=3.69), No. 9 (t=15.59), No. 12 (t=5.92) and No. 13 (t=7.65) and from those of *Rana sylvatica* in seven chromosomes, No. 2 (t=8.08),

No. 6 (t=5.36), No. 8 (t=13.69), No. 10 (t=11.50), No. 11 (t=4.30), No. 12 (t=6.03) and No. 13 (t=10.30) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana amurensis coreana* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana temporaria* in a total of 13 chromosomes including three in RL and 10 in NVC, and from that of *Rana sylvatica* in a total of 19 chromosomes including nine in RL and 10 in NVC. It also differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana temporaria* and *Rana sylvatica* in RL or NVC of five and 10 chromosomes, respectively (Table 12).

d. *Rana temporaria* and *Rana sylvatica*

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana temporaria* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana sylvatica* in eight chromosomes, Nos. 1, 2, 3, 6, 7, 9, 10 and 13 ($t = 2.61 \sim 6.08$, $P = 2\% \sim 0\%$). They also differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana sylvatica* in four chromosomes, No. 1 (t=6.08), No. 6 (t=5.60), No. 9 (t=6.00) and No. 10 (t=3.70) (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana temporaria* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana sylvatica* in eight chromosomes, Nos. 1, 3, 6, 7, 8, 9, 10 and 13 ($t = 2.44 \sim 16.40$, $P = 5\% \sim 0\%$). They also differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana sylvatica* in five chromosomes, No. 1 (t=4.12), No. 6 (t=9.00), No. 8 (t=12.38), No. 9 (t=16.40) and No. 10 (t=10.60) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana temporaria* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana sylvatica* in a total of 16 chromosomes including eight in RL and eight in NVC. It also differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from that of *Rana sylvatica* in a total of nine chromosomes including four in RL and five in NVC (Table 12).

2. Brown frog species with 24 chromosomes

a. *Rana ornativentris* and others

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana ornativentris* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana chensinensis* in eight chromosomes, Nos. 1, 2, 3, 4, 7, 8, 11 and 12 ($t = 2.15 \sim 7.97$, $P = 5\% \sim 0\%$), from those of *Rana dybowskii* from Tsushima in seven chromosomes Nos. 4, 6, 8, 9, 10, 11 and 12

(t=2.10~7.51, P=5%~0%) and from those of *Rana dybowskii* from Korea in six chromosomes, Nos. 4, 6, 7, 8, 11 and 12 (t=2.32~6.00, P=5%~0%) (Fig. 11; Table 10).

The relative chromosome lengths of *Rana ornativentris* differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana chensinensis* in five chromosomes, No. 3 (t=5.61), No. 4 (t=3.91), No. 7 (t=6.10), No. 8 (t=4.86) and No. 11 (t=7.97), from those of *Rana dybowskii* from Tsushima in two chromosomes, No. 4 (t=5.52) and No. 11 (t=7.51) and from those of *Rana dybowskii* from Korea in three chromosomes, No. 4 (t=5.45), No. 7 (t=3.90) and No. 11 (t=6.00) (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of the chromosomes of *Rana ornativentris* differed with a statistical significance (t≧2.01, P≦5%) from those of *Rana chensinensis* in nine chromosomes, Nos. 2, 3, 6, 7, 8, 9, 10, 11 and 12 (t=2.14~7.59, P=5%~0%), from those of *Rana dybowskii* from Tsushima in seven chromosomes, Nos. 4, 6, 7, 8, 9, 11 and 12 (t=2.35~6.45, P=5%~0%) and from those of *Rana dybowskii* from Korea in seven chromosomes, Nos. 3, 6, 7, 8, 9, 11 and 12 (t=2.67~10.35, P=2%~0%) (Fig. 12; Table 11).

The centromere positions of the chromosomes of *Rana ornativentris* differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana chensinensis* in seven chromosomes, No. 3 (t=4.92), No. 6 (t=3.57), No. 7 (t=6.97), No. 8 (t=5.59), No. 10 (t=4.12), No. 11 (t=7.59) and No. 12 (t=6.20), from those of *Rana dybowskii* from Tsushima in three chromosomes, No. 7 (t=4.64), No. 8 (t=5.26) and No. 12 (t=6.45) and from those of *Rana dybowskii* from Korea in five chromosomes, No. 7 (t=4.72), No. 8 (t=4.55), No. 9 (t=10.35), No. 11 (t=6.06) and No. 12 (t=6.19) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana ornativentris* differed with a statistical significance (t≧2.01, P≦5%) from that of *Rana chensinensis* in a total of 17 chromosomes including eight in RL and nine in NVC, from that of *Rana dybowskii* from Tsushima in a total of 14 chromosomes including seven in RL and seven in NVC and from that of *Rana dybowskii* from Korea in a total of 13 chromosomes including six in RL and seven in NVC (Table 12).

The karyotype of *Rana ornativentris* differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana chensinensis* and *Rana dybowskii* from Tsushima and *Rana dybowskii* from Korea in RL or NVC of 12, five and eight chromosomes, respectively (Table 12).

b. *Rana chensinensis* and others

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana chensinensis* differed with a statistical

significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana ornativentris* in eight chromosomes, as stated above. They also differed with the same statistical significance from those of *Rana dybowskii* from Tsushima and from Korea in three chromosomes, Nos. 3, 4 and 7 ($t=2.26\sim4.27$, $P=5\%\sim0\%$) and six chromosomes, Nos. 1, 3, 7, 8, 11 and 12 ($t=2.20\sim4.97$, $P=5\%\sim0\%$), respectively (Fig. 11; Table 10).

The relative chromosome lengths of *Rana chensinensis* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana dybowskii* from Tsushima in chromosome No. 7 ($t=4.27$) and from those of *Rana dybowskii* from Korea in two chromosomes, No. 3 ($t=4.06$) and No. 12 ($t=4.97$) (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana chensinensis* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana dybowskii* from Tsushima and from Korea in seven chromosomes, Nos. 2, 3, 5, 7, 9, 10 and 11 ($t=2.20\sim4.11$, $P=5\%\sim0\%$) and five chromosomes, Nos. 2, 3, 7, 9 and 10 ($t=2.24\sim10.63$, $P=5\%\sim0\%$), respectively (Fig. 12; Table 11).

They differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana dybowskii* from Tsushima and from Korea in two chromosomes, No. 3 ($t=3.87$) and No. 9 ($t=4.11$) and two chromosomes, No. 7 ($t=3.56$) and No. 9 ($t=10.63$), respectively (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana chensinensis* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana dybowskii* from Tsushima in a total of 10 chromosomes including three in RL and seven in NVC and from that of *Rana dybowskii* from Korea in a total of 11 chromosomes including six in RL and five in NVC (Table 12).

The karyotype of *Rana chensinensis* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana dybowskii* from Tsushima and from Korea in RL or NVC of three and four chromosomes, respectively (Table 12).

c. *Rana dybowskii* from Tsushima and Korea

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana dybowskii* from Tsushima differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of the same species from Korea in three chromosomes, Nos. 7, 11 and 12 ($t=2.08\sim5.25$, $P=5\%\sim0\%$). They also differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from the latter population in chromosome No. 12 ($t=5.25$) (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana dybowskii* from Tsushima differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of the same species from

Korea in four chromosomes, Nos. 3, 4, 5 and 9 ($t=2.25\sim7.04$, $P=5\%\sim0\%$). They also differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from the latter population in chromosome No. 9 ($t=7.04$) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana dybowskii* from Tsushima differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from that of the same species from Korea in a total of seven chromosomes including three in RL and four in NVC. It also differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from the latter population in a total of two chromosomes including one in RL and one in NVC (Table 12).

3. Brown frog species with 26 chromosomes and those with 24 chromosomes

The karyotypes of brown frog species with 26 chromosomes, including *Rana japonica, Rana tsushimensis, Rana amurensis coreana, Rana temporaria* and *Rana sylvatica,* were compared with those of brown frog species with 24 chromosomes, including *Rana ornativentris, Rana chensinensis* and *Rana dybowskii* from Tsushima and Korea, as shown in Fig. 10. In all these species, chromosomes Nos. 1, 3 and 5 of the five large ones are metacentric, while No. 4 is submetacentric. Chromosome No. 2 is metacentric or submetacentric. Of the eight pairs of small chromosomes in the species with 26 chromosomes, six pairs (Nos. 6~10 and No.12) are very similar in RL and NVC to six pairs (Nos. 7~12) of the seven pairs in the species with 24 chromosomes. Chromosome No. 6 of the latter species is distinctly larger than chromosome No. 6 of the former species and nearly the same in RL as the sum of two of the three smallest chromosomes of the former species. Chromosome No. 11 of these three chromosomes is metacentric in the five species with 26 chromosomes and remarkably differs in centromere position from the three smallest chromosomes in the four species with 24 chromosomes. Thus, chromosome No. 6 in the latter species seems principally to correspond to the sum of chromosomes Nos. 11 and 12 or 13 of the species with 26 chromosomes.

In order to simplify the comparison between the species with 26 chromosomes and those with 24 chromosomes, chromosomes Nos. 1~10 of the former species were compared with chromosomes Nos. 1~5 and 7~11 of the latter species in RL and NVC.

a. *Rana japonica* and the species with 24 chromosomes

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana japonica* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana ornativentris* in three chromosomes, Nos. 4, 7 and 10 ($t=2.25\sim12.01$, $P=5\%\sim0\%$), from those of *Rana chensinensis* in five chromosomes, Nos. 1, 3, 6, 7 and 8 ($t=2.20\sim4.90$, $P=5\%\sim0\%$), from those of *Rana dybowskii* from Tsushima in four chromosomes, Nos. 1, 4, 8 and 9 ($t=2.62\sim3.24$, $P=2\%\sim0.1\%$) and from those of *Rana dybowskii* from Korea in four chromosomes, Nos. 4, 6, 8 and 10 ($t=2.08\sim4.73$, $P=5\%\sim0\%$) (Fig. 11;

Table 10).

The relative chromosome lengths of *Rana japonica* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana ornativentris* in two chromosomes, No. 4 ($t=3.53$) and No. 10 ($t=12.01$), from those of *Rana chensinensis* in three chromosomes, No. 1 ($t=4.38$), No. 3 ($t=4.25$) and No. 6 ($t=4.90$) and from those of *Rana dybowskii* from Korea in chromosome No. 10 ($t=4.73$), but did not differ with a high statistical significance from those of *Rana dybowskii* from Tsushima (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana japonica* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana ornativentris* in five chromosomes, Nos. 3, 6, 7, 9 and 10 ($t=3.53\sim4.57$, $P=0.1\%\sim0\%$), from those of *Rana chensinensis* in four chromosomes, Nos. 6, 7, 8 and 10 ($t=2.11\sim5.32$, $P=5\%\sim0\%$), from those of *Rana dybowskii* from Tsushima in two chromosomes, Nos. 3 and 9 ($t=2.65\sim2.94$, $P=2\%\sim0\%$) and from those of *Rana dybowskii* from Korea in four chromosomes, Nos. 1, 8, 9 and 10 ($t=2.24\sim8.22$, $P=5\%\sim0\%$).

The centromere positions of *Rana japonica* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana ornativentris* in five chromosomes, No. 3 ($t=3.53$), No. 6 ($t=4.29$), No. 7 ($t=4.59$), No. 9 ($t=3.95$) and No. 10 ($t=3.98$), from those of *Rana chensinensis* in two chromosomes, No. 8 ($t=3.56$) and No. 10 ($t=5.32$), and from those of *Rana dybowskii* from Korea in two chromosomes, No. 8 ($t=8.23$) and No. 10 ($t=4.06$), but did not differ with a high statistical significance from those of *Rana dybowskii* from Tsushima (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana japonica* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from that of *Rana ornativentris* in a total of eight chromosomes including three in RL and five in NVC, from that of *Rana chensinensis* in a total of nine chromosomes including five in RL and four in NVC, from that of *Rana dybowskii* from Tsushima in a total of six chromosomes including four in RL and two in NVC and from that of *Rana dybowskii* from Korea in a total of eight chromosomes including four in RL and four in NVC (Table 12).

The karyotype of *Rana japonica* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana ornativentris, Rana chensinensis* and *Rana dybowskii* from Korea in RL or NVC of seven, five and three chromosomes, respectively, but did not differ with a high statistical significance from that of *Rana dybowskii* from Tsushima (Table 12).

b. *Rana tsushimensis* and the species with 24 chromosomes

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana tsushimensis* differed with a statistical

significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana ornativentris* in nine chromosomes, Nos. 1, 2, 3, 4, 6, 7, 8, 9 and 10 ($t=2.35 \sim 12.15$, $P=5\% \sim 0\%$), from those of *Rana chensinensis* in two chromosomes, Nos. 4 and 10 ($t=2.16$ and 2.26, $P=5\% \sim 2\%$), from those of *Rana dybowskii* from Tsushima in two chromosomes, Nos. 6 and 10 ($t=2.65$ and 2.79, $P=5\% \sim 0.1\%$), and from those of *Rana dybowskii* from Korea in five chromosomes, Nos. 1, 3, 7, 9 and 10 ($t=2.06 \sim 5.52$, $P=5\% \sim 0\%$) (Fig. 11; Table 10).

The relative chromosome lengths of *Rana tsushimensis* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris* in four chromosomes, No. 4 ($t=6.25$), No. 6 ($t=4.30$), No. 7 ($t=4.50$) and No. 10 ($t=12.15$), and from those of *Rana dybowskii* from Korea in chromosome No. 10 ($t=5.52$), but did not differ with a high statistical significance from those of *Rana chensinensis* and *Rana dybowskii* from Tsushima (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana tsushimensis* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana ornativentris* in five chromosomes, Nos. 2, 6, 7, 9 and 10 ($t=2.59 \sim 8.07$, $P=2\% \sim 0\%$), from those of *Rana chensinensis* in three chromosomes, Nos. 2, 3 and 6 ($t=2.99 \sim 5.77$, $P=1\% \sim 0\%$), from those of *Rana dybowskii* from Tsushima in three chromosomes, Nos. 8, 9 and 10 ($t=2.53 \sim 4.38$, $P=2\% \sim 0\%$) and from those of *Rana dybowskii* from Korea in four chromosomes, Nos. 3, 4, 8 and 9 ($t=2.15 \sim 9.99$, $P=5\% \sim 0\%$) (Fig. 12; Table 11).

The centromere positions of *Rana tsushimensis* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris* in four chromosomes, No. 6 ($t=4.72$), No. 7 ($t=4.05$), No. 9 ($t=6.10$) and No. 10 ($t=8.07$), from *Rana chensinensis* in two chromosomes, No. 2 ($t=5.04$) and No. 3 ($t=5.77$), from those of *Rana dybowskii* from Tsushima in one chromosome, No. 9 ($t=4.38$) and from those of *Rana dybowskii* from Korea in three chromosomes, No. 3 ($t=4.20$), No. 8 ($t=9.99$) and No. 9 ($t=4.92$) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana tsushimensis* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana ornativentris* in a total of 14 chromosomes including nine in RL and five in NVC, from that of *Rana chensinensis* in a total of five chromosomes including two in RL and three in NVC, from that of *Rana dybowskii* from Tsushima in a total of five chromosomes including two in RL and three in NVC and from that of *Rana dybowskii* from Korea in a total of nine chromosomes including five in RL and four in NVC.

The karyotype of *Rana tsushimensis* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris*, *Rana chensinensis*, *Rana dybowskii* from Tsushima and *Rana dybowskii* from Korea in RL or NVC of eight, two, one and four chromosomes, respectively (Table 12).

c. *Rana amurensis coreana* and the species with 24 chromosomes

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana amurensis coreana* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana ornativentris* in six chromosomes, Nos. 1, 2, 4, 6, 7 and 10 ($t=2.58 \sim 12.10$, $P=2\% \sim 0\%$), from those of *Rana chensinensis* in four chromosomes, Nos. 2, 3, 4 and 10 ($t=2.05 \sim 4.53$, $P=5\% \sim 0\%$), from those of *Rana dybowskii* from Tsushima in three chromosomes, Nos. 6, 9 and 10 ($t=2.16 \sim 3.86$, $P=5\% \sim 0\%$) and from those of *Rana dybowskii* from Korea in five chromosomes, Nos. 1, 2, 4, 7 and 10 ($t=2.03 \sim 6.10$, $P=5\% \sim 0\%$) (Fig. 11; Table 10).

The relative chromosome lengths of *Rana amurensis coreana* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris* in five chromosomes, No. 2 ($t=3.99$), No. 4 ($t=6.33$), No. 6 ($t=5.60$), No. 7 ($t=4.97$) and No. 10 ($t=12.10$), from those of *Rana chensinensis* in chromosome No. 3 ($t=4.53$), from those of *Rana dybowskii* from Tsushima in chromosome No. 6 ($t=3.86$) and from those of *Rana dybowskii* from Korea in chromosome No. 10 ($t=6.10$) (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana amurensis coreana* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana ornativentris* in eight chromosomes, Nos. 2, 4, 5, 6, 7, 8, 9 and 10 ($t=2.18 \sim 21.19$, $P=5\% \sim 0\%$), from those of *Rana chensinensis* in five chromosomes, Nos. 2, 3, 5, 6 and 9 ($t=2.66 \sim 16.59$, $p=2\% \sim 0\%$), from those of *Rana dybowskii* from Tsushima in three chromosomes, Nos. 2, 8 and 9 ($t=5.25 \sim 17.18$, $P=0.1\% \sim 0\%$) and from those of *Rana dybowskii* from Korea in six chromosomes, Nos. 2, 3, 4, 5, 8 and 9 ($t=2.77 \sim 19.06$, $P=1\% \sim 0\%$) (Fig. 12; Table 11).

The centromere positions of *Rana amurensis coreana* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris* in four chromosomes, No. 2 ($t=7.50$), No. 7 ($t=4.78$), No. 9 ($t=21.19$) and No. 10 ($t=5.81$), from those of *Rana chensinensis* in four chromosomes, No. 2 ($t=9.56$), No. 3 ($t=4.64$), No. 6 ($t=4.25$) and No. 9 ($t=16.59$), from those of *Rana dybowskii* from Tsushima in three chromosomes, No. 2 ($t=5.36$), No. 8 ($t=5.25$) and No. 9 ($t=17.18$) and from those of *Rana dybowskii* from Korea in three chromosomes, No. 2 ($t=6.30$), No. 8 ($t=12.53$) and No. 9 ($t=19.06$) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana amurensis coreana* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana ornativentris* in a total of 14 chromosomes including six in RL and eight in NVC, from that of *Rana chensinensis* in a total of nine chromosomes including four in RL and five in NVC, from that of *Rana dybowskii* from Tsushima in a total of six chromosomes including three in RL and

three in NVC and from that of *Rana dybowskii* from Korea in a total of 11 chromosomes including five in RL and six in NVC (Table 12).

The karyotype of *Rana amurensis coreana* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana ornativentris, Rana chensinensis,* and *Rana dybowskii* from Tsushima and *Rana dybowskii* from Korea in RL or NVC of nine, five, four and four chromosomes, respectively (Table 12).

d. *Rana temporaria* and the species with 24 chromosomes

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana temporaria* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana ornativentris* in seven chromosomes, Nos. 1, 2, 4, 6, 7, 9 and 10 ($t=2.09\sim12.60$, $P=5\%\sim0\%$), from those of *Rana chensinensis* in four chromosomes, Nos. 1, 3, 9 and 10 ($t=2.10\sim4.33$, $P=5\%\sim0\%$), from those of *Rana dybowskii* from Tsushima in three chromosomes, Nos. 1, 6 and 10 ($t=3.89\sim4.10$, $P=0.1\%\sim0\%$) and from those of *Rana dybowskii* from Korea in four chromosomes, Nos. 1, 7, 9 and 10 ($t=2.35\sim6.74$, $P=5\%\sim0\%$) (Fig. 11; Table 10).

The relative chromosome lengths of *Rana temporaria* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana ornativentris* in five chromosomes, No. 1 ($t=6.73$), No. 6 ($t=5.80$), No. 7 ($t=5.50$), No. 9 ($t=3.60$) and No. 10 ($t=12.60$), from those of *Rana chensinensis* in two chromosomes, No. 1 ($t=3.85$) and No. 3 ($t=4.33$), from those of *Rana dybowskii* from Tsushima in three chromosomes, No. 1 ($t=4.10$), No. 6 ($t=4.03$) and No. 10 ($t=3.89$) and from those of *Rana dybowskii* from Korea in two chromosomes, No. 1 ($t=6.74$) and No. 10 ($t=6.60$) (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana temporaria* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana ornativentris* in eight chromosomes, Nos. 1, 2, 3, 6, 7, 8, 9 and 10 ($t=2.77\sim8.33$, $P=1\%\sim0\%$), from those of *Rana chensinensis* in three chromosomes, Nos. 1, 2 and 3 ($t=2.06\sim2.33$, $P=5\%\sim2\%$), from those of *Rana dybowskii* from Tsushima in eight chromosomes, Nos. 1, 2, 3, 4, 5, 8, 9 and 10 ($t=2.09\sim6.70$, $P=5\%\sim0\%$) and from those of *Rana dybowskii* from Korea in five chromosomes, Nos. 1, 2, 6, 8 and 9 ($t=2.80\sim14.04$, $P=1\%\sim0\%$) (Fig. 12; Table 11).

The centromere positions of *Rana temporaria* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana ornativentris* in six chromosomes, No. 2 ($t=5.60$), No. 6 ($t=6.96$), No. 7 ($t=5.00$), No. 8 ($t=4.79$), No. 9 ($t=5.52$) and No. 10 ($t=8.33$), from those of *Rana dybowskii* from Tsushima in three chromosomes, No. 2 ($t=4.01$), No. 8 ($t=6.70$) and No. 9 ($t=3.82$) and from those of *Rana dybowskii* from Korea in four chromosomes, No. 1 ($t=4.02$), No. 2 ($t=4.87$), No. 8 ($t=14.04$) and No. 9 ($t=4.32$), but did not differ with a high statistical significance from those of *Rana chensinensis* (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana temporaria* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana ornativentris* in a total of 15 chromosomes including seven in RL and eight in NVC, from that of *Rana chensinensis* in a total of seven chromosomes including four in RL and three in NVC, from that of *Rana dybowskii* from Tsushima in a total of 11 chromosomes including three in RL and eight in NVC and from that of *Rana dybowskii* from Korea in a total of nine chromosomes including four in RL and five in NVC (Table 12).

The karyotype of *Rana temporaria* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris, Rana chensinensis,* and *Rana dybowskii* from Tsushima and *Rana dybowskii* from Korea in RL or NVC of 11, two, six and six chromosomes, respectively (Table 12).

e. *Rana sylvatica* and the species with 24 chromosomes

i) Difference in relative chromosome length

The relative chromosome lengths of *Rana sylvatica* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana ornativentris* in four chromosomes, Nos. 3, 4, 9 and 10 ($t = 2.06 \sim 8.90$, $P = 5\% \sim 0\%$), from those of *Rana chensinensis* in six chromosomes, Nos. 1, 2, 3, 6, 7 and 9 ($t = 2.44 \sim 6.01$, $P = 5\% \sim 0\%$), from those of *Rana dybowskii* from Tsushima in four chromosomes, Nos. 2, 3, 4 and 9 ($t = 2.15 \sim 5.45$, $P = 5\% \sim 0\%$), and from those of *Rana dybowskii* from Korea in six chromosomes, Nos. 2, 3, 4, 6, 9 and 10 ($t = 2.06 \sim 3.70$, $P = 5\% \sim 0\%$).

The relative chromosome lengths of *Rana sylvatica* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris* in chromosome No. 10 ($t = 8.90$), from those of *Rana chensinensis* in three chromosomes, No. 3 ($t = 6.01$), No. 6 ($t = 5.90$) and No. 9 ($t = 4.27$), from those of *Rana dybowskii* from Tsushima in chromosome No. 9 ($t = 5.45$), and from those of *Rana dybowskii* from Korea in two chromosomes, No. 6 ($t = 3.70$) and No. 9 ($t = 3.60$) (Fig. 11; Table 10).

ii) Difference in centromere position

The centromere positions of *Rana sylvatica* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana ornativentris* in seven chromosomes, Nos. 1, 2, 6, 7, 8, 9 and 10 ($t = 2.04 \sim 22.03$, $P = 5\% \sim 0\%$), from those of *Rana chensinensis* in seven chromosomes, Nos. 1, 3, 6, 7, 8, 9 and 10 ($t = 2.52 \sim 17.38$, $P = 2\% \sim 0\%$), from those of *Rana dybowskii* from Tsushima in seven chromosomes, Nos. 2, 4, 6, 7, 8, 9 and 10 ($t = 2.45 \sim 18.36$, $P = 5\% \sim 0\%$) and from those of *Rana dybowskii* from Korea in seven chromosomes, Nos. 2, 3, 6, 7, 8, 9 and 10 ($t = 2.25 \sim 25.57$, $P = 5\% \sim 0\%$) (Fig. 12; Table 11).

The centromere positions of *Rana sylvatica* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris* in three chromosomes, No. 8 ($t = 17.93$), No. 9 ($t = 22.03$) and No. 10 ($t = 17.33$), from those of *Rana chensinensis* in five chromosomes, No, 3 ($t = 5.01$), No. 6 ($t = 8.74$), No. 8

(t=12.63), No. 9 (t=17.38) and No. 10 (t=11.01), from those of *Rana dybowskii* from Tsushima in four chromosomes, No. 6 (t=6.77), No. 8 (t=18.36), No. 9 (t=17.88) and No. 10 (t=11.69) and from those of *Rana dybowskii* from Korea in five chromosomes, No. 3 (t=3.58), No. 6 (t=7.05), No. 8 (t=25.57), No. 9 (t=19.84) and No. 10 (t=10.44) (Fig. 12; Table 11).

iii) Difference in either relative chromosome length (RL) or centromere position (NVC)

The karyotype of *Rana sylvatica* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana ornativentris* in a total of 11 chromosomes including four in RL and seven in NVC, from that of *Rana chensinensis* in a total of 13 chromosomes including six in RL and seven in NVC, from that of *Rana dybowskii* from Tsushima in a total of 11 chromosomes including four in RL and seven in NVC and from that of *Rana dybowskii* from Korea in a total of 13 chromosomes including six in RL and seven in NVC (Table 12).

The karyotype of *Rana sylvatica* differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of *Rana ornativentris, Rana chensinensis,* and *Rana dybowskii* from Tsushima and *Rana dybowskii* from Korea in RL or NVC of four, eight, five and seven chromosomes, respectively (Table 12).

DISCUSSION

1. Comparison of karyotypes

In the Palearctic region, there are two groups of brown frogs, one of which is 26 in diploid chromosome number and the other is 24. The species having 26 chromosomes are usually sympatric with the species having 24 chromosomes. Of the six Far Eastern species whose karyotypes were examined in the present study, *Rana japonica, Rana tsushimensis* and *Rana amurensis coreana* are 26 in diploid number, while *Rana ornativentris, Rana dybowskii* and *Rana chensinensis* are 24 in diploid number. *Rana temporaria* from Europe and *Rana sylvatica* from North America which were used as materials in addition to these six species have 26 chromosomes.

The correct chromosome number of *Rana temporaria* was first reported by Witschi (1922a, b, 1924, 1933). He described that the 26 chromosomes were divided into two groups, five pairs of large chromosomes and eight pairs of small chromosomes. This diploid number of *Rana temporaria* was reconfirmed by Galgano (1933), Prokofiewa (1935), Wickbom (1945), Guillemin (1967), Morescalchi (1967), Ullerich (1967), and Nishioka, Ueda and Ryuzaki (1972). The chromosome number 2n=26 of *Rana japonica* was first reported by Kawamura (1939, 1940, 1943) and confirmed by Kobayashi (1962) and Seto (1965). The same chromosome number of *Rana tsushimensis* and *Rana amurensis coreana* was first reported by Kawamura and Nishioka (1973). The karyotype of *Rana sylvatica* distributed in North America was reported by Hennen (1964). This species is 26

in diploid chromosome number and has a pair of metacentric chromosomes with two prominent secondary constrictions in contrast with the other brown frog species, which have a pair of chromosomes with a clear secondary constriction on the long arm.

The chromosome number 2n=24 of *Rana chensinensis* which had been called *Rana temporaria* until KAWAMURA (1962) changed this name was first reported by KAWAMURA (1943) and confirmed by KOBAYASHI (1962) and SETO (1965). The same number of chromosomes of *Rana ornativentris* was counted by KOBAYASHI (1962), SETO (1965) and NISHIOKA, UEDA and RYUZAKI (1972). KAWAMURA and NISHIOKA (1973) have found that *Rana dybowskii* collected from Tsushima and the same species from Korea are 24 in diploid number.

In the present study, the karyotypes of the eight brown frog species described above were compared with one another by counting the number of chromosome pairs which significantly differ in relative length (Table 10) and centromere position (Table 11). In the eight species, the total numbers of chromosome pairs which differ significantly ($t \geqq 2.01$, $P \leqq 5\%$) or with a high significance ($t > 3.50$, $P < 0.1\%$) in either relative chromosome length (RL) or centromere position (NVC) are presented in Table 12. Of these numbers, the smaller one seems to indicate a closer similarity between the karyotypes of two species. When the species with 26 chromosomes are compared with one another by the total numbers of chromosome pairs which differ with a high statistical significance in either RL or NVC, the smallest number is four found between *Rana tsushimensis* and *Rana amurensis coreana* or *Rana temporaria,* and the second number is five found between *Rana japonica* or *Rana amurensis coreana* and *Rana temporaria.* The third number is six or seven found between *Rana japonica* and *Rana tsushimensis* or *Rana sylvatica.* The fourth numbers are 9~11 found between *Rana japonica* and *Rana amurensis coreana* and between *Rana tsushimensis, Rana amurensis coreana* or *Rana temporaria* and *Rana sylvatica.* On the other hand, when the species with 24 chromosomes are compared with one another by the total numbers of chromosome pairs which differ with a high statistical significance in either RL or NVC, the smallest number is two between *Rana dybowskii* from Tsushima and the same species from Korea. The second number is three or four found between *Rana chensinensis* and *Rana dybowskii* from Tsushima or Korea. The third number is five or eight found between *Rana ornativentris* and *Rana dybowskii* from Korea or Tsushima. The largest number is 12 found between *Rana ornativentris* and *Rana chensinensis.*

The karyotypes of the frogs having 26 chromosomes were compared with those of the frogs having 24 chromosomes in five pairs of large chromosomes and five pairs of small ones by excluding chromosome Nos. 6 and 12 of the latter species and chromosome Nos. 11, 12 and 13 of the former species, as chromosome No. 6 is nearly the same in relative length as the sum of chromosomes No. 11 and No. 12 or 13 of the species with 26 chromosomes.

When the total numbers of chromosome pairs which differ with a high statistical significance ($t > 3.50$, $P < 0.1\%$) in either RL or NVC are counted, the smallest number is zero or one found between *Rana japonica* or *Rana tsushimensis* and *Rana*

dybowskii from Tsushima. The second number is two found between *Rana tsushimensis* or *Rana temporaria* and *Rana chensinensis*. The third number is three or four found between *Rana japonica*, *Rana tsushimensis* or *Rana amurensis coreana* and *Rana dybowskii* from Korea, between *Rana amurensis coreana* and *Rana dybowskii* from Tsushima or between *Rana sylvatica* and *Rana ornativentris*. The fourth number is five or six found between *Rana japonica* or *Rana amurensis coreana* and *Rana chensinensis*, between *Rana temporaria* and *Rana dybowskii* from Tsushima or Korea or between *Rana sylvatica* and *Rana dybowskii* from Tsushima. The fifth number is seven or more found between *Rana japonica*, *Rana tsushimensis*, *Rana amurensis coreana* or *Rana temporaria* and *Rana ornativentris*, or between *Rana sylvatica* and *Rana chensinensis* or *Rana dybowskii* from Korea.

These findings seem to show that *Rana sylvatica* (2n=26) and *Rana ornativentris* (2n=24) most remarkably differ in karyotype from the other Palearctic species. Such a difference seems to have nothing to do with either their chromosome number or their DNA amount. It is known that *Rana sylvatica* is remarkably large and shows a great contrast to *Rana ornativentris* in diploid nuclear DNA amount (Bachmann and Nishioka, 1978; Oeldorf, Nishioka and Bachmann, 1978). The species with 26 chromosomes do not fundamentally differ in karyotype from those with 24 chromosomes. It is believed that the common ancestor of all the brown frog species would have 26 chromosomes and later was divided into two populations, one having 26 chromosomes and the other having 24 chromosomes. The latter number was probably obtained by union of chromosome No. 11 with chromosome No. 12 or 13. Each population of brown frogs evolved into several, different species and immigrated into suitable surrounding areas. This often resulted in sympatric distribution of two species which differ from each other in chromosome number. *Rana tsushimensis* (2n=26), *Rana chensinensis* (2n=24) and *Rana dybowskii* from Tsushima (2n=24) distinctly resemble each other in karyotype. *Rana japonica* (2n=26) is also very similar to *Rana dybowskii* from Tsushima. It is interesting to observe that the two populations of *Rana dybowskii* slightly differ from each other in karyotype, although they cannot be distinguished in morphology and reproduction.

2. Karyotypic difference, genetic distance and reproductive isolation

The karyotypic differences among the eight brown frog species were compared with genetic distances and the reproductively isolating mechanisms among them. The genetic distances among brown frogs distributed in the Palearctic region were calculated by Nishioka, Ueda and Sumida (1982) from the results of starch-gel electrophoretic analyses of nine enzymes extracted from the skeletal muscles and three blood proteins. It has been found that some of the karyotypic differences which are shown by differences in either RL or NVC are well correlated with the genetic distances, while the others are not correlated with the latter.

The genetic distances between *Rana ornativentris* and *Rana japonica*, *Rana tsushimensis* or *Rana temporaria* are 0.802, 1.678 and 1.239, respectively, while the numbers of chromosome pairs which differ with a high statistical significance

between these brown frog species are seven, eight and 11, respectively. The genetic distances between *Rana chensinensis* and *Rana japonica, Rana tsushimensis, Rana temporaria* or *Rana ornativentris* are 0.671, 1.623, 1.017 and 0.753, respectively, while the numbers of chromosome pairs which differ with a high statistical significance between these species are five, two, two and 12, respectively. The genetic distances between *Rana dybowskii* from Tsushima and *Rana japonica, Rana tsushimensis, Rana temporaria, Rana ornativentris* or *Rana chensinensis* are 0.811, 1.671, 1.321, 0.695 and 0.594, respectively, while the numbers of chromosome pairs which differ with a high statistical significance between these species are zero, one, six, five and three, respectively. The genetic distances between *Rana japonica* and *Rana tsushimensis,* between *Rana japonica* and *Rana temporaria* and between *Rana tsushimensis* and *Rana temporaria* are 1.135, 0.807 and 1.134, respectively, while the numbers of chromosome pairs which differ with a high statistical significance between these species are six, five and four, respectively.

These findings show that the karyotype of *Rana tsushimensis* does not always differ greatly from those of the other brown frog species, whereas the genetic distances between *Rana tsushimensis* and the other species are very large. The karyotypic differences of *Rana temporaria* from the other species are not always large, whereas the genetic distances between *Rana temporaria* and the other species are always large except that between *Rana temporaria* and *Rana japonica.* The number of chromosome pairs which differ with a high statistical significance between *Rana chensinensis* and *Rana dybowskii* from Tsushima is small and correlated with the smallest genetic difference, 0.594, between them. In contrast, such a number between *Rana ornativentris* and *Rana chensinensis* is 12, the largest of all, whereas the genetic distance is fairly small (0.753), as compared with the others. It seems evident that the difference in karyotype between two brown frog species is not correlated with the genetic distance between them.

The eight brown frog species seem to be isolated from one another by gametic isolation, hybrid inviability or hybrid sterility (KAWAMURA, 1943, 1950; KAWAMURA and KOBAYASHI, 1959, 1960; KAWAMURA and NISHIOKA, 1962, 1973, 1977; KOBAYASHI, 1962; KAWAMURA, NISHIOKA and UEDA, 1981). When viable hybrids are produced, they are always males which are completely sterile. *Rana tsushimensis* and *Rana amurensis coreana* cannot produce viable hybrids by crossing with the other species, although *Rana amurensis coreana* has not yet been crossed with *Rana temporaria* and *Rana sylvatica.* Among the species with 26 chromosomes, only female *Rana japonica* produces sterile male hybrids by mating with male *Rana temporaria.* The three species with 24 chromosomes can produce sterile male hybrids by crossing with one another. The hybrids between *Rana dybowskii* from Tsushima and the same species from Korea are viable and completely fertile.

While *Rana tsushimensis* shows extremely large genetic distances from *Rana chensinensis* and *Rana dybowskii* from Tsushima and produces inviable hybrids by crossing with these two species, the karyotypes of these three species are very similar to one another. The number of chromosome pairs which differ with a high statistical significance ($t>3.50$, $P<0.1\%$) in either RL or NVC between *Rana*

tsushimensis and *Rana chensinensis* or *Rana dybowskii* from Tsushima is two or one and similar to the number which is two between *Rana dybowskii* from Tsushima and the same species from Korea. Thus, it is evident that the differences in karyotype among the eight brown frog species are not always correlated with the genetic distances and the reproductive isolation among them.

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