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A Comparative Study on the Karyotypes of Pond Frogs Distributed in Japan, Korea, Taiwan, Europe and North America

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(With 9 Text-figures)

ABSTRACT

The karyotypes of *Rana nigromaculata, Rana brevipoda, Rana plancyi chosenica, Rana plancyi fukienensis, Rana lessonae* and *Rana pipiens* were compared with one another by examining the relative length (RL) and the numerical value of the centromere position (NVC) of each chromosome pair. The differences between two species or subspecies in these items were checked by the method of HUBBS and HUBBS (1953) as well as by t-test (MATHER, 1965). All these five species and one subspecies are 26 in diploid chromosome number and have no heteromorphic pairs of chromosomes. These 26 chromosomes are divided into two groups in size, of which one contains five pairs (Nos. 1~5) of large chromosomes and the other contains eight pairs (Nos. 6~13) of small chromosomes. When they are divided into four types, median, submedian, subterminal and terminal, all the species and subspecies are the same in type of seven chromosome pairs, while they are not always the same in type of the other six chromosome pairs. All the species and subspecies have a pair of small chromosomes having a secondary constriction.

The karyotypes of the five species and one subspecies differ from one another with a statistical significance ($P \leqq 5\%$) in RL or NVC of two to all of the 13 chromosome pairs. They also differ from one another with a high statistical significance ($P < 0.1\%$) in RL or NVC of one to 10 of the 13 chromosome pairs. The total numbers of chromosome pairs which differ with a high statistical significance ($P < 0.1\%$) in either RL or NVC are six between *brevipoda* and *nigromaculata,* five between *brevipoda* and *chosenica,* nine between *brevipoda* and *fukienensis,* eight between *brevipoda* and *lessonae,* and 16 between *brevipoda* and *pipiens*; three between *nigromaculata* and *chosenica,* 12 between *nigromaculata* and *fukienensis,* eight between *nigromaculata* and *lessonae,* and 18 between *nigromaculata* and *pipiens*; eight between *chosenica* and *fukienensis,* eight between *chosenica* and *lessonae,* and 14 between *chosenica* and *pipiens*; 13 between *fukienensis* and *lessonae,* 15 between *fukienensis* and *pipiens*; and 18 between *lessonae* and *pipiens.*

Similarity in karyotypes does not always reflect weakness in reproductive isolation nor shortness in genetic distance based on electrophoretic patterns of various kinds of proteins, although there is an agreement to some extent between these affairs.

INTRODUCTION

The pond frogs distributed in the Far East can be included in the *Rana esculenta* group found in Europe, as they are very similar to the latter in morphology, ecology and reproductive physiology. The Far Eastern and European pond frogs are now divided into seven species and several subspecies and usually produce natural or artificial interspecific hybrids between two species which are various in combination.

All these species and subspecies of pond frogs are 26 in diploid chromosome number. This number was first reported by IRIKI (1928, 1932) in *Rana nigromaculata*. As *Rana brevipoda*, another Japanese pond frog species, had been included in *Rana nigromaculata* at that time and, moreover, these two species are sympatric with each other in Kyoto district, where IRIKI made his studies, it is obscure which species was used as material by IRIKI. The fact that *Rana nigromaculata* has 2n=26 chromosomes was confirmed by KAWAMURA (1937, 1939) in the Hiroshima population which includes no *Rana brevipoda*. The chromosomes of *Rana brevipoda* were first described by NISHIOKA (1972). *Rana plancyi chosenica* had been called *Rana nigromaculata chosenica* until SHANON (1956) placed this as a subspecies of *Rana plancyi*. *Rana plancyi fukienensis* was at first described as a new species, *Rana fukienensis*, by POPE (1929) and later placed to the subspecies rank by BORING (1938–1939). TING (1939) has reported that both *Rana nigromaculata* and *Rana plancyi* collected from North China have 2n=26 chromosomes consisting of five pairs of long rod-shaped or V-shaped chromosomes and eight pairs of short rod-shaped or V-shaped chromosomes. The 2n=26 chromosomes of *Rana plancyi fukienensis* were recently observed by LIN and HUANG (1979). The same number of chromosomes was also observed in *Rana plancyi chosenica* by NISHIOKA (1983) and NISHIOKA and OKUMOTO (1983).

In Europe, DALCQ (1930), GALGANO (1933a, b) and WICKBOM (1945) have reported that the chromosomes of *Rana esculenta* which is probably *Rana lessonae* are 26 in diploid number. The leopard frog, *Rana pipiens*, which is an American frog species fairly resembling the above Palearctic pond frogs in appearance, has 2n=26 chromosomes (PARMENTER, 1920, 1925, 1933).

The five species and one subspecies, *Rana nigromaculata, Rana brevipoda, Rana plancyi chosenica, Rana plancyi fukienensis, Rana lessonae* and *Rana pipiens*, are very similar to one another in having 26 chromosomes consisting of five pairs of large chromosomes and eight pairs of small chromosomes. In the present study, the karyotypes of these species and subspecies are compared with one another in relative chromosome length and centromere position in order to elucidate the differences in chromosome morphology. These differences were collated with the degrees of reproductive isolation and the genetic distances calculated on the basis of electrophoretic patterns of various enzymes and blood proteins among the five species and one subspecies.

MATERIALS AND METHODS

A total of 28 mature frogs were used as materials. They included three male and three female *Rana nigromaculata* HALLOWELL from Hiroshima, Japan, three male and three female *Rana brevipoda* ITO from Konko, Okayama Prefecture, Japan, two male and two female *Rana plancyi chosenica* OKADA from Seoul, Korea, two male and two female *Rana plancyi fukienensis* POPE from Chiagi, Taiwan, three male and three female *Rana lessonae* CAMERANO from Luxembourg and a male and a female *Rana pipiens* SCHREBER from Vermont, USA.

Chromosome preparations were made from blood cultivated principally by VOLPE and GEBHARDT's method (1968). The method adopted by the present authors was as follows. After the skin surrounding the shoulder-joint was cleansed with 70% alcohol, the subclavian artery is punctured with a sterile needle through the skin. Blood is discharged into a sterile heparinized capillary tube, 1.4~1.6 mm in diameter, to a length of about 5 cm, and then poured into a cylindrical glass vial, 1.2 cm in diameter and 7 cm in height, containing 4.5 ml of a sterile culture medium. This vial is corked and left to stand for about four days at 25°C. The culture medium consists of 75 ml of EAGLE's minimum essential medium (MEM, Nissui Pharmacy Co.) containing a high concentration of kanamycin, 6 ml of whole egg ultrafiltrate, 5 ml of phytohemagglutinin M, 24 ml of redistilled (double-distilled) water, 30 ml of 2% lactoalbumin hydrolysate and 14 ml of fetal calf serum. Twelve hours before the cultivated cells are harvested, the culture is added with colchicine solution so that the final concentration of colchicine becomes 10^{-5}~10^{-7} M.

The medium in each culturing vial is decanted into a 15 ml centrifuge tube and centrifuged at 800 rpm for 5 minutes. The supernatant fluid is discarded and replaced by 5 ml of HANKS' balanced salt solution. The suspension is centrifuged at 800 rpm for 5 minutes. All but one ml of the supernatant fluid is discarded and replaced by 3 ml of distilled water. After agitated well, the cell suspension is allowed to stand at room temperature for 13~15 minutes, to which is gently added 4 ml of fixative consisting of a mixture of absolute methanol and glacial acetic acid at a rate of 3:1. The suspension is centrifuged at 800 rpm for 8 minutes. The supernatant fluid is discarded and slowly replaced by 5 ml of fixative. The cell suspension is gently agitated and centrifuged at 800 rpm for 5 minutes. This procedure is repeated two or three times. The supernatant fluid is drawn off as far as possible and replaced by 0.5~1.5 ml of fixative. After the cell suspension is gently agitated, one or two drops of suspension are placed on a clean microscopic slide and quickly dried by igniting the alcohol contained in the suspension. As soon as the flame dies out, the remaining fluid is shaken off from the slide.

The staining and mounting were made as follows. The slide attached with fixed cells is subjected to hydrolysis in 1 N HCl at 56°C for 10 minutes and then rinsed with distilled water. The chromosomes are stained for 6 minutes with a mixture of 6.0 ml of GIEMSA, 1.5 ml of 0.15 M NH_4OH and 92.5 ml of distilled

water. Dehydration and clearing are made by the routine methods (VOLPE and GEBHARDT, 1968).

The karyotypes of different species and subspecies were compared with each other by examining the chromosome lengths and centromere positions. The total and arm lengths of each chromosome were measured by using enlarged photomicrographs of well-spread metaphase chromosomes. 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 the 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 150~200 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. Karyotypes of five species and one subspecies

Chromosome preparations of *Rana nigromaculata, Rana brevipoda, Rana plancyi chosenica, Rana lessonae* and *Rana pipiens* were made in June, July and August, 1972, while those of *Rana plancyi fukienensis* were made in July, 1975. All these species and subspecies were 26 in diploid chromosome number (Figs. 1~6). The 13 pairs of homologous chromosomes were numbered No. 1 to No. 13. The homologous chromosomes of each pair were equal to each other in relative length and centromere position. They were divided into two groups in size. Group 1 consisted of five pairs of large chromosomes, Nos. 1~5, while group 2 consisted of eight pairs of small chromosomes, Nos. 6~13. When chromosomes were divided into four types, median, submedian, subterminal and terminal, on the basis of the numerical values of centromere positions which were 50.0~37.5, 37.4~25.0, 24.9~12.5 and 12.4~0, respectively, each of the 13 pairs of chromosomes in the five species and one subspecies was of median type, submedian type, subterminal type, median or submedian type, or submedian or subterminal type. While seven pairs of chromosomes of each species or subspecies were the same in type as those of the others, the remaining six pairs of chromosomes of each species or subspecies

were not always the same as those of the others. All the species and subspecies had a pair of small chromosomes having a secondary constriction. Males and females of each species did not differ from each other in karyotype.

1. *Rana brevipoda* ITO

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads from three male and three female *Rana brevipoda* are presented in Table 1. The chromosomes are arranged as those reported by NISHIOKA (1972) in tadpoles (Fig. 1).

Of group 1, chromosomes Nos. 1, 4 and 5 were of median type and Nos. 2 and 3 were of submedian type. The largest chromosome (No. 1) was 14.1~17.3, 15.97 on the average, in RL and 43.6~49.9, 46.41 on the average, in NVC. In all the 50 metaphase spreads examined, chromosome No. 1 was always of median type. Chromosome No. 2 was 11.9~14.6, 13.44 on the average, in RL and 31.3~37.4 in NVC, being of submedian type, in 36 of the 50 metaphase spreads and 37.5~39.1 in NVC, being of median type in the remaining 14. Chromosome No. 3 was 10.6~13.6, 11.88 on the average, in RL and 28.7~35.2, 31.43 on the average, in NVC. There were distinct differences in RL among chromosomes Nos. 1, 2 and 3. Chromosome No. 4 was 10.7~12.5, 11.63 on the average, in RL and 37.7~45.0, 41.52 on the average, in NVC. Although the difference in RL between

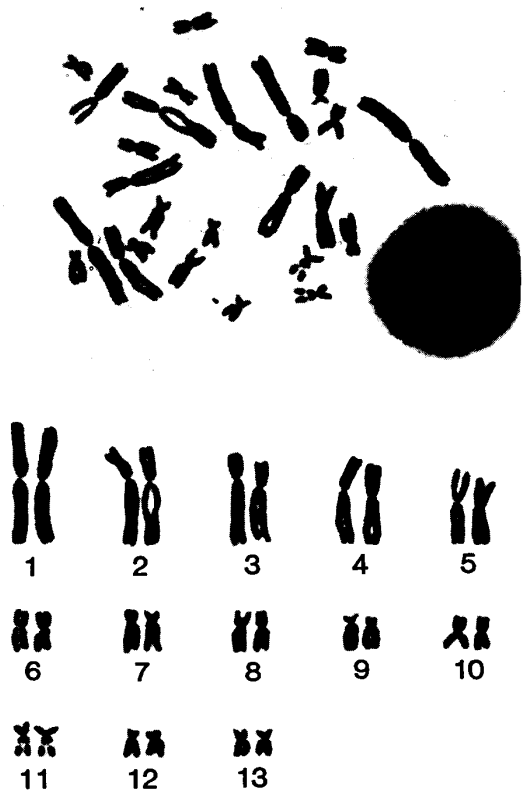

Fig. 1. Metaphase plate and the karyotype of a *Rana brevipoda*. ×1000

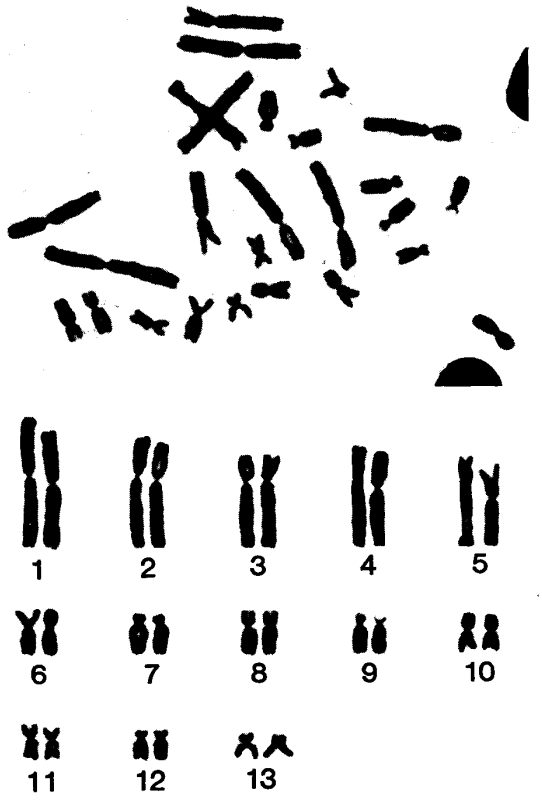

Fig. 2. Metaphase plate and the karyotype of a *Rana nigromaculata*. ×1000

TABLE 1

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

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	14.1	17.3	15.97±0.09	1	43.6	49.9	46.41±0.17	m (50)
2	11.9	14.6	13.44±0.08	2	31.3	39.1	36.53±0.21	sm (36) m (14)
3	10.6	13.6	11.88±0.08	3	28.7	35.2	31.43±0.21	sm (50)
4	10.7	12.5	11.63±0.06	4	37.7	45.0	41.52±0.22	m (50)
5	9.0	10.5	9.82±0.06	5	38.5	44.1	41.35±0.18	m (50)
6	5.0	6.2	5.70±0.04	6	38.1	46.0	42.95±0.23	m (50)
7	5.0	6.0	5.45±0.03	7	25.3	32.6	29.23±0.25	sm (50)
8	4.7	5.9	5.22±0.04	8	37.5	46.8	42.19±0.31	m (50)
9	3.9	5.4	4.62±0.04	9	24.5	33.8	28.35±0.32	sm (47) st (3)
10	3.9	5.2	4.45±0.03	10	39.8	50.0	45.01±0.28	m (50)
11*	3.4	4.7	4.12±0.04	11*	41.7	50.0	44.99±0.27	m (50)
12	3.1	4.3	3.80±0.04	12	30.8	40.4	35.26±0.33	sm (42) m (8)
13	3.4	4.5	3.90±0.04	13	35.6	49.3	41.22±0.37	m (48) sm (2)

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

	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

chromosomes Nos. 3 and 4 was not statistically significant, chromosome No. 4 distinctly differed from chromosome No. 3 in centromere position. Chromosome No. 5 also remarkably differed from chromosome No. 4 in RL, as No. 5 was 9.0~10.5, 9.82 on the average, in RL (Table 1).

Of the eight pairs of small chromosomes belonging to group 2, chromosomes Nos. 6, 8, 10, 11 and 13 were of median type and Nos. 7, 9 and 12 were of submedian type. Chromosomes Nos. 7 and 9 were 29.23 and 28.35 on the average in NVC, respectively. Although they were of submedian type from these mean values, chromosome No. 9 was 24.5 or 24.7 in NVC, being of subterminal type in three of the 50 metaphase spreads. Chromosome No. 11 was characteristic in having a secondary constriction. The difference in RL between chromosomes Nos. 12 and 13 was not statistically significant. While chromosome No. 12 was smaller than No. 13 in 16 of the 50 metaphase spreads, it was equal to or larger than the latter in the remaining spreads. These two chromosomes slightly differed in centromere position. Chromosome No. 12 was 30.8~37.4 in NVC, being of submedian type in 42 of the 50 metaphase spreads and 37.5~40.4 in NVC, being of median type in the remaining eight spreads. Chromosome No. 13 was 37.5~49.3 in NVC, being of median type in 48 of the 50 metaphase spreads

and 35.6 or 37.4 in NVC, being of submedian type in the remaining two spreads (Table 1).

2. *Rana nigromaculata* HALLOWELL

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads from three male and three female *Rana nigromaculata* are presented in Table 2, where they are arranged as those reported by NISHIOKA (1972). The karyotype of *Rana nigromaculata* is shown in Fig. 2. The 13 pairs of chromosomes were very similar to those of *Rana brevipoda* in RL and NVC.

Of five large chromosomes belonging to group 1, Nos. 1, 2, 4 and 5 were of median and No. 3 was of submedian type. The largest chromosome (No. 1) was 14.3~17.5, 15.83 on the average, in RL and 42.4~48.4, 46.26 on the average, in NVC, being of median type. Chromosome No. 2 was 11.8~14.8, 13.21 on the average, in RL and 36.2~47.6, 38.61 on the average, in NVC, the mean being of median type near submedian. However, it was 36.2~37.4 in NVC, being of submedian type, in nine of the 50 metaphase spreads and 37.5~47.6, being of median type in the remaining 41. The difference in RL between two chromosomes Nos. 3 and 4 was not statistically significant. In 21 of the 50 metaphase

TABLE 2

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

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	14.3	17.5	15.83±0.11	1	42.4	48.4	46.26±0.19	m (50)
2	11.8	14.8	13.21±0.09	2	36.2	47.6	38.61±0.24	m (41) sm (9)
3	10.5	12.5	11.53±0.07	3	28.8	34.4	31.28±0.18	sm (50)
4	10.6	12.8	11.72±0.08	4	34.7	44.9	41.66±0.28	m (48) sm (2)
5	8.6	10.6	9.66±0.06	5	40.6	45.6	42.78±0.17	m (50)
6	5.4	6.5	5.89±0.04	6	34.6	45.8	42.16±0.36	m (47) sm (3)
7	4.8	6.2	5.31±0.04	7	22.0	31.6	26.49±0.29	sm (43) st (7)
8	4.9	6.1	5.32±0.04	8	35.2	44.6	40.38±0.30	m (49) sm (1)
9	4.2	5.5	4.79±0.04	9	22.0	32.8	26.31±0.34	sm (35) st (15)
10	4.1	5.7	4.80±0.04	10	40.4	48.5	44.27±0.27	m (50)
11*	3.8	5.3	4.19±0.05	11*	38.8	49.2	44.33±0.32	m (50)
12	3.6	5.0	4.11±0.04	12	28.1	38.8	31.98±0.34	sm (49) m (1)
13	3.1	4.6	3.65±0.04	13	34.5	44.4	39.24±0.31	m (42) sm (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

spreads, chromosome No. 3 was larger than No. 4, while it was slightly smaller than the latter in the remaining 29. Chromosome No. 4 was 34.7~44.9, 41.66 on the average, in NVC, the mean being of median type, although it was 34.7 or 37.0 in NVC, being of submedian type in two of the 50 metaphase spreads. Chromosome No. 5 was 8.6~10.6, 9.66 on the average, in RL and 40.6~45.6, 42.78 on the average, in NVC (Table 2).

Of the eight pairs of small chromosomes belonging to group 2, chromosomes Nos. 6, 8, 10, 11 and 13 were of median type and Nos. 7, 9 and 12 were of submedian type, as found in *Rana brevipoda*. Chromosome No. 6 was 37.5~45.8 in NVC, being of median type, in 47 of the 50 metaphase spreads and 34.6~37.0, being of submedian type in the remaining three spreads. Although chromosomes Nos. 7 and 8 could not be distinguished from each other in relative length, they distinctly differed in centromere position. Chromosome No. 7 was 25.0~31.6 in NVC, being of submedian in 43 of the 50 metaphase spreads and 22.0~24.7 in NVC, being of subterminal in the remaining seven spreads. Chromosome No. 8 was 35.2~44.6, 40.38 on the average, in NVC, being of median type except one spread. In this exceptional spread, it was 35.2 in NVC, being of submedian type. While there was no difference in size between chromosomes Nos. 9 and 10, they clearly differed from each other in centromere position. Chromosome No. 9 was 25.0~32.8 in 35 of the 50 metaphase spreads, being of submedian type and 22.0~24.5, being of subterminal type in the remaining 15. Chromosome No. 11 was characteristic in having a secondary constriction. The difference in RL between chromosomes Nos. 12 and 13 was statistically significant. Chromosome No. 12 was 28.1~38.8, 31.98 on the average, in NVC, being of submedian type except one spread, in which it was 38.8 in NVC, being of median type. Chromosome No. 13 was 37.5~44.4, being of median type in 42 of the 50 metaphase spreads and 34.5~37.4, being of submedian type in the other eight (Table 2).

3. *Rana plancyi chosenica* Okada

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads from two male and two female *Rana plancyi chosenica* are presented in Table 3. The chromosomes are arranged in an order corresponding to those of *Rana brevipoda* and *Rana nigromaculata* in size and shape (Fig. 3).

Of five pairs of large chromosomes belonging to group 1, Nos. 1, 2, 4 and 5 were of median type and No. 3 was of submedian type as found in *Rana nigromaculata*. The largest chromosome (No. 1) was 15.0~18.1, 16.24 on the average, in RL and 43.7~49.0, 46.89 on the average, in NVC, being of median type. Chromosome No. 2 was 11.7~13.7, 12.73 on the average, in RL. It was 37.5~41.9 in NVC, being of median type in 43 of the 50 metaphase spreads and 35.4~37.4, being of submedian type in the remaining seven. Chromosomes Nos. 3 and 4 were very similar to each other in RL, while they remarkably differed from each other in NVC. Chromosome No. 5 was remarkably smaller than No. 4 in RL, while both

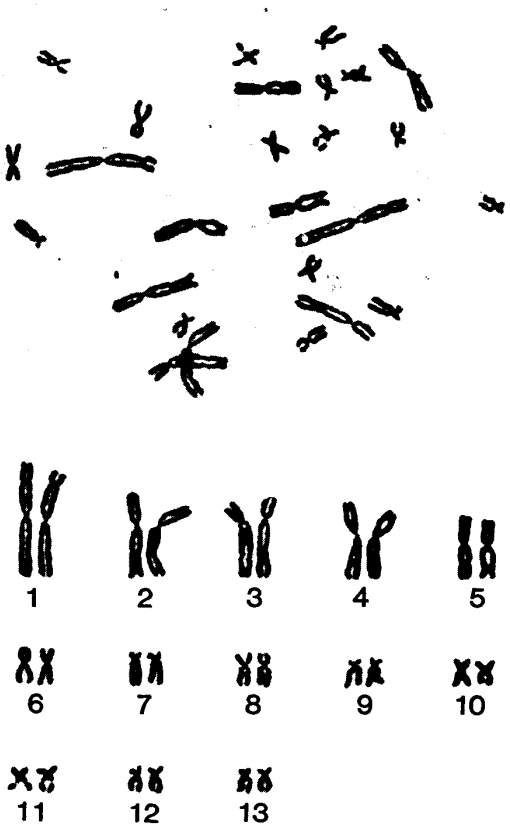

Fig. 3. Metaphase plate and the karyotype of a *Rana plancyi chosenica*. ×1000

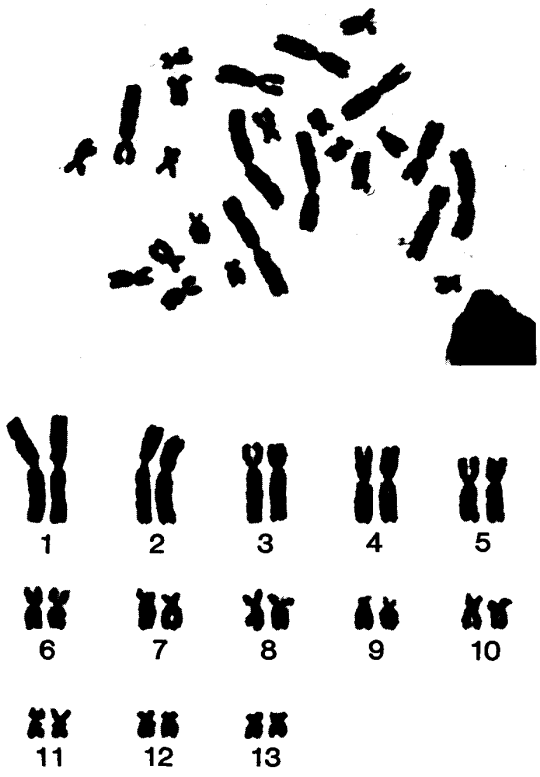

Fig. 4. Metaphase plate and the karyotype of a *Rana plancyi fukienensis*. ×1000

chromosomes were of median type (Table 3).

Of eight pairs of small chromosomes belonging to group 2, Nos. 6, 8, 10, 11 and 13 were of median type and Nos. 7, 9 and 12 were of submedian type as found in *Rana brevipoda* and *Rana nigromaculata*. The differences in RL among chromosomes Nos. 6, 7 and 8 were statistically significant, although they were slight. There was no significant difference in NVC between chromosomes Nos. 6 and 8. While chromosomes Nos. 9 and 10 were very similar to each other in RL, they remarkably differed in centromere position. Chromosome No. 9 was 24.6~32.3, 28.20 on the average, in NVC, being of submedian type except one spread which was of subterminal type. Chromosome No. 11 was characteristic in having a secondary constriction. The difference in RL between chromosomes Nos. 12 and 13 was not statistically significant. However, they slightly differed from each other in centromere position. Chromosome No. 12 was 31.0~37.4 in NVC in 46 of the 50 metaphase spreads, being of submedian type and 37.5~41.8, being of median type in the other four. Chromosome No. 13 was 37.5~45.1 in NVC, being of median type in 44 of the 50 metaphase spreads and 34.4~37.4, being of submedian type in the other six (Table 3).

4. *Rana plancyi fukienensis* Pope

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

TABLE 3

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

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	15.0	18.1	16.24±0.10	1	43.7	49.0	46.89±0.17	m (50)
2	11.7	13.7	12.73±0.07	2	35.4	41.9	38.86±0.18	m (43) sm (7)
3	10.7	12.6	11.63±0.07	3	28.5	34.1	31.66±0.18	sm (50)
4	10.4	12.5	11.32±0.06	4	40.2	45.3	42.56±0.17	m (50)
5	8.6	10.4	9.65±0.05	5	40.7	48.0	43.55±0.23	m (50)
6	5.4	6.6	5.92±0.04	6	39.2	47.0	43.82±0.23	m (50)
7	4.9	6.1	5.48±0.03	7	25.3	33.3	29.83±0.24	sm (50)
8	4.8	5.8	5.30±0.03	8	37.6	46.9	41.86±0.28	m (50)
9	4.2	5.4	4.71±0.03	9	24.6	32.3	28.20±0.25	sm (49) st (1)
10	4.2	5.2	4.74±0.03	10	41.4	48.4	44.87±0.24	m (50)
11*	3.7	4.8	4.25±0.03	11*	41.7	48.4	44.91±0.24	m (50)
12	3.5	4.6	4.09±0.03	12	31.0	41.8	34.80±0.32	sm (46) m (4)
13	3.5	4.5	3.95±0.03	13	34.4	45.1	39.91±0.34	m (44) sm (6)

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

	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 chromosomes in 50 metaphase spreads from two male and two female *Rana plancyi fukienensis* are presented in Table 4. The chromosomes are arranged in an order corresponding to those of *Rana brevipoda* and *Rana nigromaculata* in size and shape (Fig. 4).

Of five pairs of large chromosomes belonging to group 1, Nos. 1, 2, 4 and 5 were of median type and No. 3 was of submedian type, as found in *Rana nigromaculata* and *Rana plancyi chosenica*. The largest chromosome (No. 1) was 14.7~16.5, 15.79 on the average, in RL and 44.8~49.4, 48.24 on the average, in NVC, being of median type. Chromosome No. 2 was 12.2~14.2, 13.33 on the average, in RL and 39.6~43.3, 41.61 on the average, in NVC, being of median type. The difference in RL between chromosomes Nos. 3 and 4 was not statistically significant, but these two chromosomes differed from each other in NVC. While chromosome No. 3 was of submedian type, chromosome No. 4 was of median type. Chromosome No. 5 was 8.9~10.7, 9.51 on the average, in RL and was of median type (Table 4).

Of eight pairs of small chromosomes belonging to group 2, Nos. 6, 8, 10, 11 and 13 were of median type and Nos. 7, 9 and 12 were of submedian type, as found in *Rana brevipoda, Rana nigromaculata* and *Rana plancyi chosenica*. The difference in RL between chromosomes Nos. 6 and 7 was not statistically significant, but they

TABLE 4

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

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	14.7	16.5	15.79±0.07	1	44.8	49.4	48.24±0.16	m (50)
2	12.2	14.2	13.33±0.08	2	39.6	43.3	41.61±0.17	m (50)
3	10.3	11.8	11.21±0.06	3	31.0	35.2	33.08±0.18	sm (50)
4	10.0	11.7	10.92±0.06	4	41.0	45.8	43.09±0.18	m (50)
5	8.9	10.7	9.51±0.05	5	39.4	45.6	42.20±0.26	m (50)
6	5.4	6.2	5.82±0.05	6	41.3	47.4	43.44±0.22	m (50)
7	5.2	6.0	5.64±0.05	7	25.6	32.4	29.35±0.24	sm (50)
8	5.0	5.8	5.41±0.04	8	39.6	45.7	42.42±0.28	m (50)
9	4.3	5.4	4.82±0.04	9	28.0	34.6	31.22±0.29	sm (50)
10	4.7	5.7	5.23±0.04	10	41.5	48.3	44.76±0.17	m (50)
11*	3.7	4.5	4.12±0.03	11*	43.0	48.6	46.54±0.19	m (50)
12	3.6	4.6	4.16±0.04	12	30.9	39.7	35.18±0.37	sm (46) m (4)
13	3.6	4.3	3.88±0.03	13	40.3	47.5	43.24±0.33	m (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

distinctly differed from each other in centromere position. Chromosomes Nos. 9 and 10 were 4.3~5.4, 4.82 on the average, and 4.7~5.7, 5.23 on the average, in RL, respectively. Chromosome No. 10 was larger than chromosome No. 9; the difference in RL between these two chromosomes was statistically significant. While chromosome No. 9 was 28.0~34.6, 31.22 on the average, in NVC, being of submedian type, chromosome No. 10 was 41.5~48.3, 44.76 on the average, in NVC, being of median type. Chromosome No. 11 was characteristic in having a secondary constriction. Although the difference in RL between chromosomes Nos. 12 and 13 was slight, it was statistically significant. Chromosome No. 12 was 30.9~37.4 in NVC, being of submedian type in 46 of the 50 metaphase spreads and 37.5~39.7, being of median type in the remaining four. Chromosome No. 13 was 40.3~47.5, 43.24 on the average, in NVC, being of median type (Table 4).

5. *Rana lessonae* CAMERANO

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads from three male and three female *Rana lessonae* are presented in Table 5. The chromosomes are arranged in an

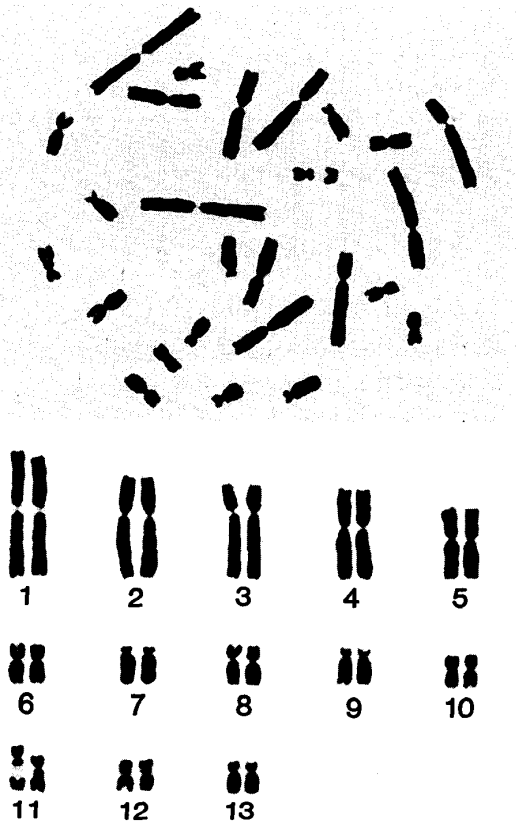

Fig. 5. Metaphase plate and the karyotype of a *Rana lessonae*. ×1000

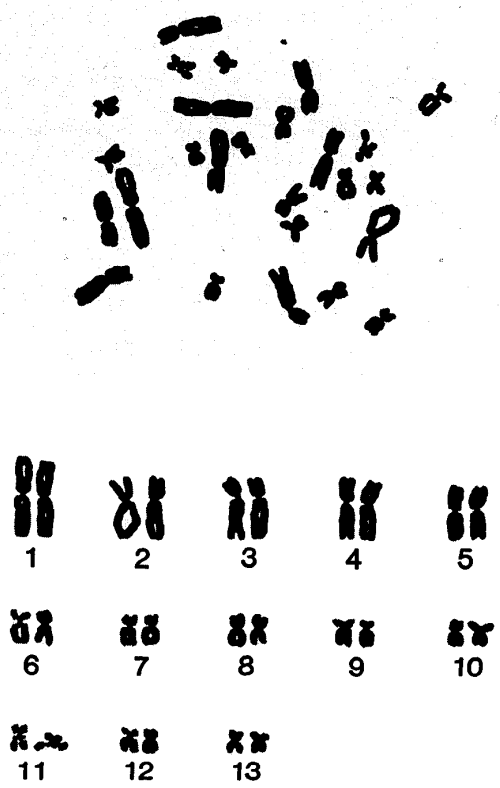

Fig. 6. Metaphase plate and the karyotype of a *Rana pipiens*. ×1000

order corresponding to those of *Rana brevipoda* and *Rana nigromaculata* in size and shape (Fig. 5).

Of five pairs of large chromosomes belonging to group 1, Nos. 1, 2, 4 and 5 were of median type and No. 3 was of submedian type, as found in *Rana nigromaculata* and two others. The largest chromosome (No. 1) was 14.0~18.0, 15.51 on the average, in RL and of median type. Chromosome No. 2 was 37.5~42.1 in NVC, being of median type in 35 of the 50 metaphase spreads and 34.6~37.3, being of submedian type in the other 15. The difference in RL between chromosomes Nos. 3 and 4 was not statistically significant. Chromosome No. 3 was of submedian type, while chromosome No. 4 was of median type. Chromosome No. 5 was 8.3~10.7, 9.50 on the average, in RL and of median type (Table 5).

Of eight pairs of small chromosomes of group 2, Nos. 6, 8 and 10 were of median type, No. 11 was intermediate between submedian and median types, Nos. 9, 12 and 13 were of submedian type and No. 7 was of subterminal type. Chromosomes Nos. 7 and 8 were very similar to each other in RL, but they remarkably differed from each other in centromere position. Chromosome No. 7 was 19.2~24.7 in NVC, being of subterminal type in 34 of the 50 metaphase spreads and 25.0~32.9, being of submedian type in the other 16. This chromosome was 23.94 on the average in NVC, being of subterminal type. On the other hand, chromosome No. 8 was 36.8~48.0, 42.21 on the average, in NVC, being of median

TABLE 5
Relative lengths, centromere positions represented by numerical values and types of metaphase chromosomes in *Rana lessonae*

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	14.0	18.0	15.51±0.13	1	43.2	48.3	45.38±0.19	m (50)
2	12.1	15.2	13.38±0.10	2	34.6	42.1	38.63±0.24	m (35) sm (15)
3	10.7	14.0	11.77±0.08	3	26.7	37.3	31.58±0.26	sm (50)
4	10.0	13.0	11.63±0.09	4	38.9	46.9	43.11±0.22	m (50)
5	8.3	10.7	9.50±0.08	5	41.7	49.2	44.42±0.18	m (50)
6	5.2	6.8	5.90±0.05	6	39.7	50.0	44.96±0.27	m (50)
7	4.6	5.9	5.30±0.04	7	19.2	32.9	23.94±0.35	st (34) sm (16)
8	4.6	5.9	5.29±0.04	8	36.8	48.0	42.21±0.28	m (49) sm (1)
9	4.3	5.4	4.81±0.04	9	22.9	33.0	27.98±0.31	sm (47) st (3)
10	3.8	5.6	4.41±0.04	10	37.5	50.0	45.63±0.31	m (50)
11*	4.0	6.1	4.83±0.05	11*	31.8	43.3	37.32±0.32	sm (30) m (20)
12	3.5	4.5	4.04±0.04	12	30.4	43.4	34.81±0.34	sm (47) m (3)
13	2.8	4.0	3.63±0.04	13	30.4	40.7	35.16±0.33	sm (46) m (4)

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

	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 except one spread. Chromosome No. 9 was very similar to No. 11 in RL, although they distinctly differed in shape. Chromosome No. 9 was 25.0~33.0 in NVC, being of submedian type in 47 of the 50 metaphase spreads and 22.9~24.5, being of subterminal type in the remaining three. Chromosome No. 11 was 4.0~6.1, 4.83 on the average, in RL and slightly larger than No. 10 which was 3.8~5.6, 4.41 on the average, in RL and of median type. Chromosome No. 11 was characteristic in having a secondary constriction in the long arm. It was 31.8~37.4 in NVC, being of submedian type in 30 of the 50 metaphase spreads and 37.5~43.3, being of median type in the other 20. This chromosome was 37.32 on the average in NVC, being intermediate between submedian and median types. The difference in RL between chromosomes Nos. 12 and 13 was statistically significant. Although these two chromosomes were of submedian type from the mean values in NVC, they were of median type in three and four of the 50 metaphase spreads, respectively (Table 5).

6. *Rana pipiens* SCHREBER

The relative lengths (RL) and numerical values of centromere positions (NVC) of the chromosomes in 50 metaphase spreads from a male and a female *Rana pipiens*

are presented in Table 6. The chromosomes are arranged in an order corresponding to those of *Rana brevipoda* and *Rana nigromaculata* in size and shape (Fig. 6).

Of five pairs of large chromosomes of group 1, Nos. 1, 4 and 5 were of median type and Nos. 2 and 3 were of submedian type. The largest chromosome (No. 1) was 13.7~15.6, 14.67 on the average, in RL and 45.1~49.6, 47.12 on the average, in NVC, being of median type. Chromosome No. 2 was 34.1~37.4 in NVC, being of submedian type in 42 of the 50 metaphase spreads and 37.5~39.8, being of median type in the other eight. While chromosomes Nos. 3 and 4 were very similar in RL, they distinctly differed from each other in NVC. Chromosome No. 5 was remarkably smaller than No. 4 and of median type (Table 6).

Of eight pairs of small chromosomes of group 2, Nos. 6, 8, 9, 10, 11 and 13 were of median type, No. 12 was intermediate between median and submedian types, and No. 7 was of submedian type. Chromosome No. 7 was 32.6~37.4 in NVC, being of submedian type in 39 of the 50 metaphase spreads and 37.6~39.6 in NVC, being of median type, in the other 11. This chromosome was 35.83 on the average in NVC, being of submedian type. Although chromosomes Nos. 8 and 9 were very similar in size, they considerably differed from each other in centromere position. While chromosome No. 8 was 40.4~49.3, 46.37 on the average, in

TABLE 6

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

Relative length (RL)				Numerical value of centromere position (NVC)				
Chromosome no.	Minimum	Maximum	Mean±	Chromosome no.	Minimum	Maximum	Mean±	Type
1	13.7	15.6	14.67±0.07	1	45.1	49.6	47.12±0.15	m (50)
2	11.5	13.8	12.49±0.06	2	34.1	39.8	36.29±0.19	sm (42) m (8)
3	10.8	12.7	11.51±0.05	3	30.4	35.5	32.99±0.15	sm (50)
4	10.6	12.1	11.15±0.05	4	39.9	45.4	42.64±0.17	m (50)
5	8.8	10.6	9.72±0.05	5	44.1	49.1	46.97±0.17	m (50)
6	5.9	6.8	6.34±0.03	6	40.0	49.2	44.52±0.23	m (50)
7	5.4	6.2	5.84±0.03	7	32.6	39.6	35.83±0.27	sm (39) m (11)
8	4.8	6.0	5.30±0.04	8	40.4	49.3	46.37±0.24	m (50)
9	4.7	5.7	5.30±0.03	9	33.0	44.9	39.29±0.33	m (39) sm (11)
10	4.1	5.2	4.72±0.04	10	42.3	48.7	45.28±0.23	m (50)
11*	3.3	5.1	4.36±0.05	11*	37.7	46.3	41.27±0.27	m (50)
12	4.0	5.4	4.49±0.03	12	32.8	43.8	37.85±0.31	m (29) sm (21)
13	3.7	4.6	4.11±0.03	13	40.2	49.3	45.06±0.29	m (50)

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

Chromosome type:

NVC	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 of median type in all the 50 metaphase spreads, chromosome No. 9 was 33.0~37.4 in NVC, being of submedian type in 11 of the 50 metaphase spreads, and 37.5~44.9, being of median type in the other 39. This chromosome was 39.29 on the average in NVC, being of median type. Chromosomes Nos. 11 and 12 were 3.3~5.1, 4.36 on the average and 4.0~5.4, 4.49 on the average, in RL, respectively. Chromosome No. 11 was of median type and had a secondary constriction in the long arm. Chromosome No. 12 was 37.5~43.8 in NVC, being of median type, in 29 of the 50 metaphase spreads and 32.8~37.4, being of submedian type in the other 21. This chromosome was 37.85 on the average in NVC, being intermediate between median and submedian types. Chromosome No. 13 was of median type (Table 6).

II. Comparison of karyotypes among five species and one subspecies

The karyotypes of five species and one subspecies are graphically shown in Fig.

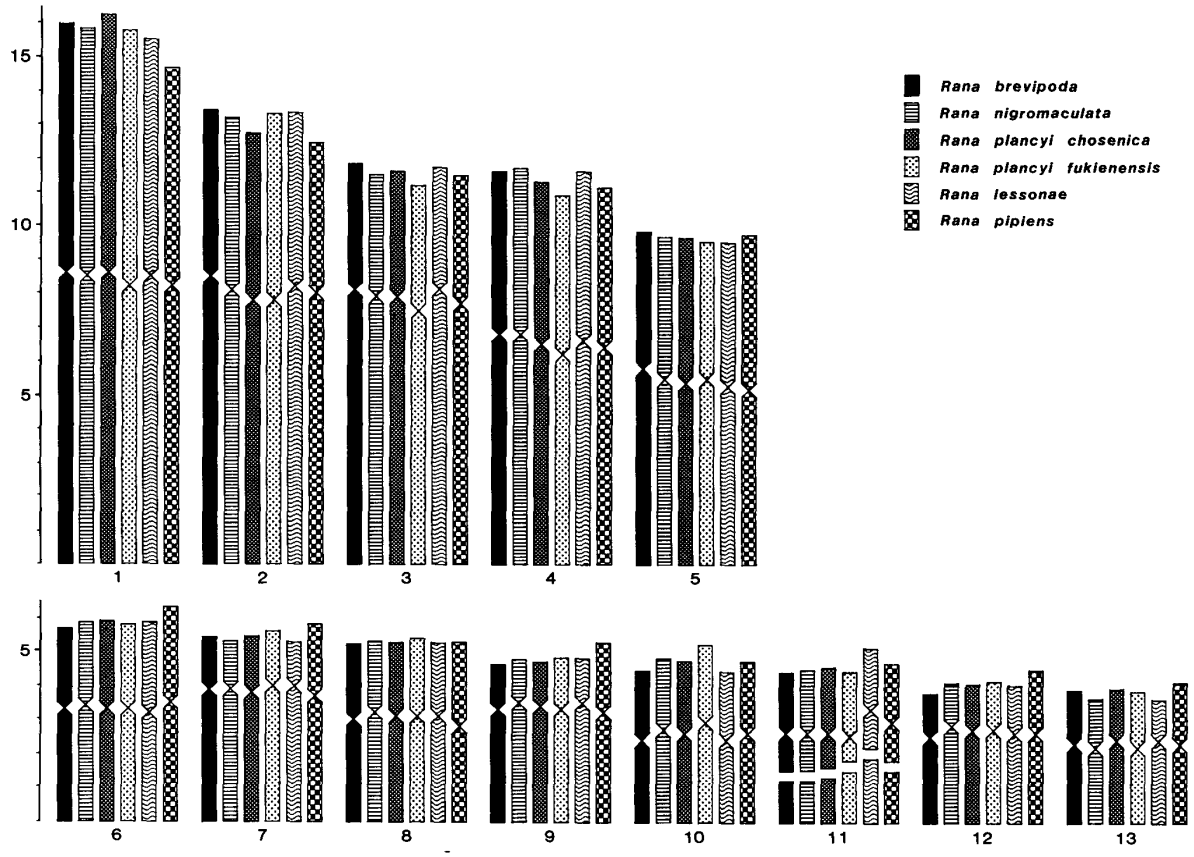

Fig. 7. Composite ideograms showing differences in relative chromosome length and centromere position among five species and one subspecies of pond frogs.

Constrictions indicate centromere positions. Gaps indicate secondary constrictions.

7. All these species and subspecies had a secondary constriction in the long arm of chromosome No. 11. The differences in relative chromosome length and numerical value of centromere position among the five species and the single

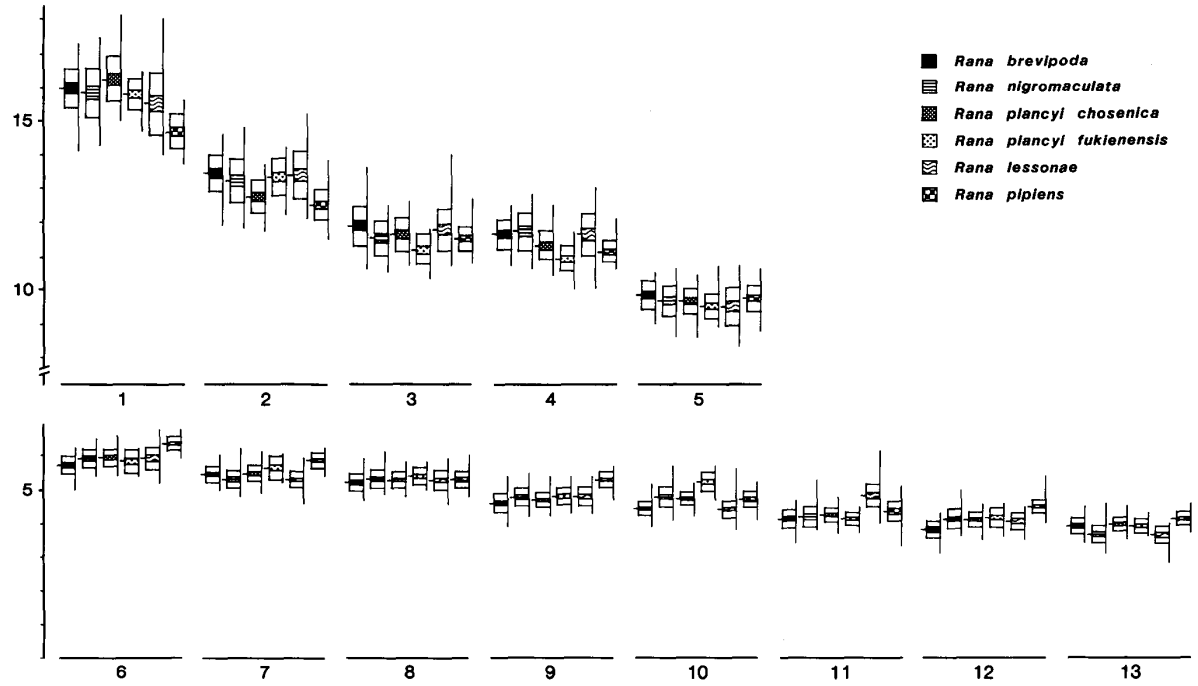

Fig. 8. Graphs showing differences in relative chromosome length among five species and one subspecies of pond frogs.

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.

subspecies are shown in Figs. 8 and 9. The result of examinations by the method of HUBBS and HUBBS (1953) on the existence of significant differences was in complete agreement with that by t-test.

1. *Rana brevipoda* and the others

a. Difference in relative chromosome length

The relative chromosome lengths of *Rana brevipoda* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana nigromaculata* in six chromosomes, Nos. 3, 6, 9, 10, 12 and 13 ($t=2.13 \sim 4.95$, $P=5\% \sim 0\%$), from those of *Rana plancyi*

TABLE 7

Numbers of chromosome pairs which significantly differ in relative length among five species and one subspecies of pond frogs. Parentheses indicate a difference with a high significance

Species	*nig.*	*cho.*	*fuk.*	*les.*	*pip.*
Rana brevipoda	6 (2)	5 (3)	8 (4)	8 (1)	11 (8)
Rana nigromaculata		4 (1)	5 (3)	2 (2)	8 (8)
Rana plancyi chosenica			6 (2)	7 (4)	6 (5)
Rana plancyi fukienensis				6 (6)	12 (7)
Rana lessonae					10 (9)

chosenica in five chromosomes, Nos. 2, 4, 6, 10 and 12 (t=2.58~4.83, P=2%~0%), from those of *Rana plancyi fukienensis* in eight chromosomes, Nos. 3, 4, 5, 7, 8, 9, 10 and 12 (t=2.30~11.03, P=5%~0%), from those of *Rana lessonae* in eight chromosomes, Nos. 1, 5, 6, 7, 9, 11, 12 and 13 (t=2.06~7.84, P=5%~0%) and from those of *Rana pipiens* in 11 chromosomes, Nos. 1, 2, 3, 4, 6, 7, 9, 10, 11, 12 and 13 (t=2.65~9.76, P=2%~0%) (Fig. 8; Table 7).

The relative chromosome lengths of *Rana brevipoda* differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana nigromaculata* in two chromosomes, No. 10 (t=4.95) and No. 12 (t=3.88), from those of *Rana plancyi chosenica* in three chromosomes, No. 2 (t=4.72), No. 10 (t=4.83) and No. 12 (t=4.10), from those of *Rana plancyi fukienensis* in four chromosomes, No. 3 (t=4.74), No. 4 (t=5.92), No. 10 (t=11.03) and No. 12 (t=4.50), from those of *Rana lessonae* in chromosome No. 11 (t=7.84) and from those of *Rana pipiens* in eight chromosomes, No. 1 (t=8.06), No. 2 (t=6.72), No. 4 (t=4.35), No. 6 (t=9.05), No. 7 (t=6.50), No. 9 (t=9.62), No. 10 (t=3.83) and No. 12 (t=9.76) (Fig. 8; Table 7).

b. Difference in centromere position

The centromere positions of the chromosomes of *Rana brevipoda* differed with a statistical significance (t≧2.01, P≦5%) from those of *Rana nigromaculata* in seven chromosomes, Nos. 2, 5, 7, 8, 9, 12 and 13 (t=2.90~5.06, P=1%~0%), from those of *Rana plancyi chosenica* in three chromosomes, Nos. 2, 4 and 5 (t=2.65~5.96,

TABLE 8

Numbers of chromosome pairs which significantly differ in centromere position among five species and one subspecies of pond frogs. Parentheses indicate a difference with a high significance

Species	*nig.*	*cho.*	*fuk.*	*les.*	*pip.*
Rana brevipoda	7 (4)	3 (2)	7 (5)	8 (7)	11 (8)
Rana nigromaculata		5 (2)	11 (9)	11 (6)	13 (10)
Rana plancyi chosenica			7 (6)	6 (4)	9 (9)
Rana plancyi fukienensis				9 (7)	10 (8)
Rana lessonae					10 (9)

P=2%~0%), from those of *Rana plancyi fukienensis* in seven chromosomes, Nos. 1, 2, 3, 4, 9, 11 and 13 (t=2.88~13.29, P=1%~0%), from those of *Rana lessonae* in eight chromosomes, Nos. 1, 2, 4, 5, 6, 7, 11 and 13 (t=2.86~12.95, P=1%~0%) and from those of *Rana pipiens* in 11 chromosomes, Nos. 1, 3, 4, 5, 6, 7, 8, 9, 11, 12 and 13 (t=2.21~16.83, P=5%~0%) (Fig. 9; Table 8).

The centromere positions of the chromosomes of *Rana brevipoda* differed with a high statistical significance (t>3.50, P<0.1%) from those of *Rana nigromaculata* in four chromosomes, No. 2 (t=4.61), No. 5 (t=4.08), No. 7 (t=5.06) and No. 12 (t=4.89), from those of *Rana plancyi chosenica* in two chromosomes, No. 2 (t=5.96) and No. 5 (t=5.33), from those of *Rana plancyi fukienensis* in five chromosomes, No. 1 (t=5.85), No. 2 (t=13.29), No. 3 (t=4.22), No. 4 (t=3.91) and No. 9 (t=4.70),

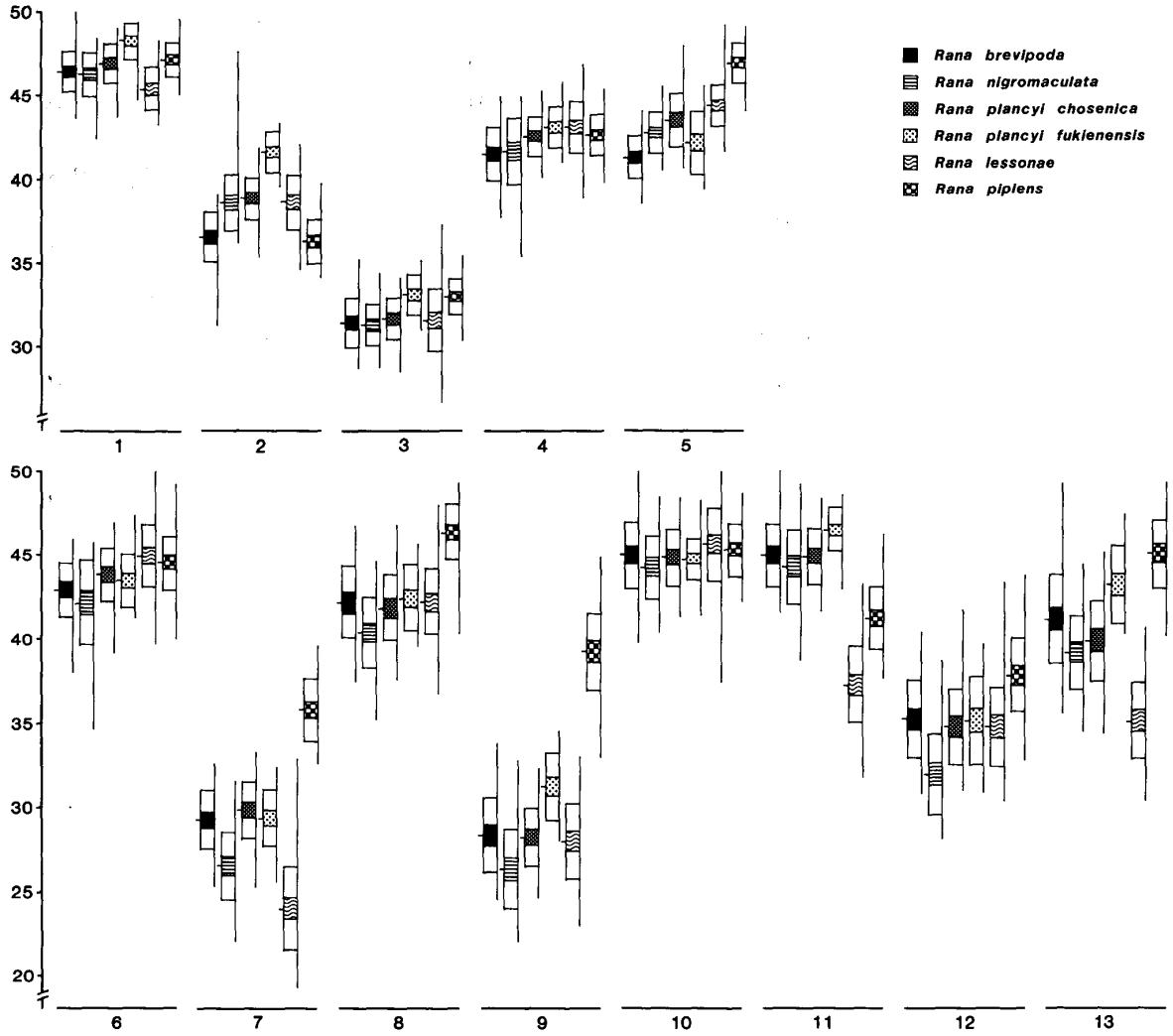

Fig. 9. Graphs showing differences in centromere position among five species and one subspecies of pond frogs.

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.

from those of *Rana lessonae* in seven chromosomes, No. 2 (t=4.66), No. 4 (t=3.61), No. 5 (t=8.53), No. 6 (t=4.01), No. 7 (t=8.70), No. 11 (t=12.95) and No. 13 (t=8.64) and from those of *Rana pipiens* in eight chromosomes, No. 3 (t=4.27), No. 5 (t=16.05), No. 7 (t=12.68), No. 8 (t=7.54), No. 9 (t=16.83), No. 11 (t=6.89), No. 12 (t=4.04) and No. 13 (t=5.78) (Fig. 9; Table 8).

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

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

TABLE 9
Total numbers of chromosome pairs which significantly differ in either relative chromosome length or centromere position among five species and one subspecies of pond frogs. Parentheses indicate a difference with a high significance

Species	*nig.*	*cho.*	*fuk.*	*les.*	*pip.*
Rana brevipoda	13 (6)	8 (5)	15 (9)	16 (8)	22 (16)
Rana nigromaculata		9 (3)	16 (12)	13 (8)	21 (18)
Rana plancyi chosenica			13 (8)	13 (8)	15 (14)
Rana plancyi fukienensis				15 (13)	22 (15)
Rana lessonae					20 (18)

in RL and seven in NVC, from that of *Rana plancyi chosenica* in a total of eight chromosomes including five in RL and three in NVC, from that of *Rana plancyi fukienensis* in a total of 15 chromosomes including eight in RL and seven in NVC, from that of *Rana lessonae* in a total of 16 chromosomes including eight in RL and eight in NVC and from that of *Rana pipiens* in a total of 22 chromosomes including 11 in RL and 11 in NVC (Table 9).

The karyotype of *Rana brevipoda* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana nigromaculata, Rana plancyi chosenica, Rana plancyi fukienensis, Rana lessonae* and *Rana pipiens* in relative chromosome length or centromere position of six, five, nine, eight and 16 chromosomes in total, respectively (Table 9).

2. *Rana nigromaculata* and the others

a. Difference in relative chromosome length

The relative chromosome lengths of *Rana nigromaculata* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda* in six chromosomes, as described above. They also differed with the same significance from those of *Rana plancyi chosenica* in four chromosomes, Nos. 2, 4, 7 and 13 ($t=2.40\sim4.24$, $P=5\%\sim0\%$), from those of *Rana plancyi fukienensis* in five chromosomes, Nos. 3, 4, 7, 10 and 13 ($t=2.45\sim5.66$, $P=5\%\sim0\%$), from those of *Rana lessonae* in two chromosomes, Nos. 10 and 11 ($t=4.88\sim6.40$, $P=0.1\%\sim0\%$) and from those of *Rana pipiens* in eight chromosomes, Nos. 1, 2, 4, 6, 7, 9, 12 and 13 ($t=4.27\sim7.50$, $P=0.1\%\sim0\%$) (Fig. 8; Table 7).

The relative chromosome lengths of *Rana nigromaculata* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana plancyi chosenica* in chromosome No. 13 ($t=4.24$), from those of *Rana plancyi fukienensis* in three chromosomes, No. 4 ($t=5.66$), No. 7 ($t=3.64$) and No. 10 ($t=5.38$), from those of *Rana lessonae* in two chromosomes, No. 10 ($t=4.88$) and No. 11 ($t=6.40$) and from those of *Rana pipiens* in eight chromosomes, No. 1 ($t=6.29$), No. 2 ($t=4.71$), No. 4 ($t=4.27$), No. 6 ($t=6.36$), No. 7 ($t=7.50$), No. 9 ($t=7.21$), No. 12 ($t=5.37$) and No. 13 ($t=6.51$) (Fig. 8; Table 7).

b. Difference in centromere position

The centromere positions of the chromosomes of *Rana nigromaculata* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana brevipoda* in seven chromosomes, as stated above. They also differed with the same statistical significance from those of *Rana plancyi chosenica* in five chromosomes, Nos. 6, 7, 8, 9 and 12 ($t=2.55\sim6.27$, $P=2\%\sim0\%$), from those of *Rana plancyi fukienensis* in 11 chromosomes, Nos. 1, 2, 3, 4, 6, 7, 8, 9, 11, 12 and 13 ($t=2.15\sim7.77$, $P=5\%\sim0\%$), from those of *Rana lessonae* in 11 chromosomes, Nos. 1, 4, 5, 6, 7, 8, 9, 10, 11 12 and 13 ($t=2.32\sim10.95$, $P=5\%\sim0\%$) and from those of *Rana pipiens* in all the 13 chromosomes ($t=2.01\sim19.37$, $P=5\%\sim0\%$) (Fig. 9; Table 8).

The centromere positions of the chromosomes of *Rana nigromaculata* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana plancyi chosenica* in two chromosomes, No. 7 ($t=6.27$) and No. 12 ($t=4.27$), from those of *Rana plancyi fukienensis* in nine chromosomes, No. 1 ($t=5.92$), No. 2 ($t=7.21$), No. 3 ($t=5.00$), No. 7 ($t=5.37$), No. 8 ($t=3.52$), No. 9 ($t=7.77$), No. 11 ($t=4.20$), No. 12 ($t=4.50$) and No. 13 ($t=6.25$), from those of *Rana lessonae* in six chromosomes, No. 5 ($t=4.68$), No. 6 ($t=4.40$), No. 7 ($t=3.97$), No. 11 ($t=10.95$), No. 12 ($t=4.16$) and No. 13 ($t=6.37$) and from those of *Rana pipiens* in 10 chromosomes, No. 2 ($t=5.36$), No. 3 ($t=5.16$), No. 5 ($t=12.32$), No. 6 ($t=3.91$), No. 7 ($t=16.67$), No. 8 ($t=11.02$), No. 9 ($t=19.37$), No. 11 ($t=5.17$), No. 12 ($t=9.02$) and No. 13 ($t=9.69$) (Fig. 9; Table 8).

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

The karyotype of *Rana nigromaculata* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from that of *Rana brevipoda* in the relative chromosome lengths or centromere positions of 13 chromosomes in total, as stated above. It also differed with the same statistical significance from that of *Rana plancyi chosenica* in a total of nine chromosomes including four in RL and five in NVC, from that of *Rana plancyi fukienensis* in a total of 16 chromosomes including five in RL and 11 in NVC, from that of *Rana lessonae* in a total of 13 chromosomes including two in RL and 11 in NVC, and from that of *Rana pipiens* in a total of 21 chromosomes including eight in RL and 13 in NVC (Table 9).

The karyotype of *Rana nigromaculata* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana plancyi chosenica, Rana plancyi fukienensis, Rana lessonae* and *Rana pipiens* in the relative chromosome lengths or centromere positions of three, 12, eight and 18 chromosomes in total, respectively (Table 9).

3. *Rana plancyi chosenica* and the others

a. Difference in relative chromosome length

The relative chromosome lengths of *Rana plancyi chosenica* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana brevipoda* and *Rana nigromaculata* in five and four chromosomes, respectively, as stated above. They also differed with the same statistical significance from those of *Rana plancyi*

fukienensis in six chromosomes, Nos. 1, 2, 3, 4, 10 and 11 (t=2.17~6.93, P=5%~0%), from those of *Rana lessonae* in seven chromosomes, Nos. 1, 2, 4, 7, 10, 11 and 13 (t=2.03~7.03, P=5%~0%) and from those of *Rana pipiens* in six chromosomes, Nos. 1, 6, 7, 9, 12 and 13 (t=2.67~9.83, P=2%~0%) (Fig. 8; Table 7).

The relative chromosome lengths of *Rana plancyi chosenica* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana plancyi fukienensis* in two chromosomes, No. 2 (t=3.99) and No. 10 (t=6.93), from those of *Rana lessonae* in four chromosomes, No. 2 (t=3.77), No. 10 (t=4.67), No. 11 (t=7.03) and No. 13 (t=4.53) and from those of *Rana pipiens* in five chromosomes, No. 1 (t=9.09), No. 6 (t=5.94), No. 7 (t=6.00), No. 9 (t=9.83) and No. 12 (t=6.67) (Fig. 8; Table 7).

b. Difference in centromere position

The centromere positions of the chromosomes of *Rana plancyi chosenica* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda* and *Rana nigromaculata* in three and five chromosomes, respectively, as stated above. They also differed with the same statistical significance from those of *Rana plancyi fukienensis* in seven chromosomes, Nos. 1, 2, 3, 5, 9, 11 and 13 (t=2.75~7.85, P=2%~0%), from those of *Rana lessonae* in six chromosomes, Nos. 1, 5, 6, 7, 11 and 13 (t=2.11~13.42, P=5%~0%) and from those of *Rana pipiens* in nine chromosomes, Nos. 2, 3, 5, 7, 8, 9, 11, 12 and 13 (t=4.01~18.94, P=0.1%~0%) (Fig. 9; Table 8).

The centromere positions of the chromosomes of *Rana plancyi chosenica* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana plancyi fukienensis* in six chromosomes, No. 1 (t=4.39), No. 2 (t=7.85), No. 3 (t=3.94), No. 9 (t=5.58), No. 11 (t=3.77) and No. 13 (t=4.97), from those of *Rana lessonae* in four chromosomes, No. 1 (t=4.19), No. 7 (t=9.81), No. 11 (t=13.42) and No. 13 (t=7.09), and from those of *Rana pipiens* in nine chromosomes, No. 2 (t=6.94), No. 3 (t=4.01), No. 5 (t=8.46), No. 7 (t=11.74), No. 8 (t=8.65), No. 9 (t=18.94), No. 11 (t=7.12), No. 12 (t=4.84) and No. 13 (t=8.15) (Fig. 9; Table 8).

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

The karyotype of *Rana plancyi chosenica* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda* and *Rana nigromaculata* in the relative chromosome lengths or centromere positions of eight and nine chromosomes in total, respectively, as stated above. It also differed with the same statistical significance from that of *Rana plancyi fukienensis* in a total of 13 chromosomes including six in RL and seven in NVC, from that of *Rana lessonae* in a total of 13 chromosomes including seven in RL and six in NVC, and from that of *Rana pipiens* in a total of 15 chromosomes including six in RL and nine in NVC (Table 9).

The karyotype of *Rana plancyi chosenica* differed with a high statistical significance

($t>3.50$, $P<0.1\%$) from those of *Rana plancyi fukienensis, Rana lessonae* and *Rana pipiens* in relative chromosome lengths or centromere positions of eight, eight and 14 chromosomes in total, respectively (Table 9).

4. *Rana plancyi fukienensis* and the others

a. Difference in relative chromosome length

The relative chromosome lengths of *Rana plancyi fukienensis* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda, Rana nigromaculata* and *Rana plancyi chosenica* in eight, five and six chromosomes, respectively, as stated above. They also differed with the same statistical significance from those of *Rana lessonae* in six chromosomes, Nos. 3, 4, 7, 10, 11 and 13 ($t=3.54\sim10.25$, $P=1\%\sim0\%$) and from those of *Rana pipiens* in all the chromosomes ($t=2.08\sim8.00$, $P=5\%\sim0\%$) except No. 8 (Fig. 8; Table 7).

The relative chromosome lengths of *Rana plancyi fukienensis* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana lessonae* in six chromosomes, No. 3 ($t=3.96$), No. 4 ($t=4.64$), No. 7 ($t=3.75$), No. 10 ($t=10.25$), No. 11 ($t=8.61$) and No. 13 ($t=3.54$) and from those of *Rana pipiens* in seven chromosomes, No. 1 ($t=8.00$), No. 2 ($t=5.94$), No. 6 ($t=6.31$), No. 9 ($t=6.79$), No. 10 ($t=6.38$), No. 12 ($t=4.67$) and No. 13 ($t=3.83$) (Fig. 8; Table 7).

b. Difference in centromere position

The centromere positions of the chromosomes of *Rana plancyi fukienensis* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda, Rana nigromaculata* and *Rana plancyi chosenica* in seven, 11 and seven chromosomes, respectively, as stated above. They also differed with the same statistical significance from those of *Rana lessonae* in nine chromosomes, Nos. 1, 2, 3, 5, 6, 7, 9, 11 and 13 ($t=3.09\sim17.52$, $P=1\%\sim0\%$) and from those of *Rana pipiens* in 10 chromosomes, Nos. 1, 2, 5, 6, 7, 8, 9, 11, 12 and 13 ($t=2.40\sim14.76$, $P=5\%\sim0\%$) (Fig. 9; Table 8).

The centromere positions of the chromosomes of *Rana plancyi fukienensis* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana lessonae* in seven chromosomes, No. 1 ($t=8.43$), No. 2 ($t=7.16$), No. 5 ($t=4.96$), No. 7 ($t=9.01$), No. 9 ($t=5.40$), No. 11 ($t=17.52$) and No. 13 ($t=12.24$) and from those of *Rana pipiens* in eight chromosomes, No. 1 ($t=3.93$), No. 2($t=14.76$), No. 5 ($t=10.86$), No. 7 ($t=12.68$), No. 8 ($t=7.57$), No. 9 ($t=12.99$), No. 11 ($t=11.29$) and No. 12 ($t=3.91$) (Fig. 9; Table 8).

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

The karyotype of *Rana plancyi fukienensis* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda, Rana nigromaculata* and *Rana plancyi chosenica* in the relative chromosome lengths or centromere positions of 15, 16 and 13 chromosomes in total, respectively, as stated above. It also statistically differed from that of *Rana lessonae* in a total of 15 chromosomes including six in RL

and nine in NVC, and from that of *Rana pipiens* in a total of 22 chromosomes including 12 in RL and 10 in NVC (Table 9).

The karyotype of *Rana plancyi fukienensis* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana lessonae* and *Rana pipiens* in the relative chromosome lengths or centromere positions of 13 and 15 chromosomes in total, respectively (Table 9).

5. *Rana lessonae* and the others

a. Difference in relative chromosome length

The relative chromosome lengths of *Rana lessonae* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda, Rana nigromaculata, Rana plancyi chosenica* and *Rana plancyi fukienensis* in eight, two, seven and six chromosomes, respectively, as stated above. They also differed with the same statistical significance from those of *Rana pipiens* in 10 chromosomes, Nos. 1, 2, 4, 6, 7, 9, 10, 11, 12 and 13 ($t=3.30\sim7.64$, $P=1\%\sim0\%$) (Fig. 8; Table 7).

The relative chromosome lengths of *Rana lessonae* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana pipiens* in nine chromosomes, No. 1 ($t=4.02$), No. 2 ($t=5.40$), No. 6 ($t=5.34$), No. 7 ($t=7.64$), No. 9 ($t=6.93$), No. 10 ($t=3.88$), No. 11 ($t=4.70$), No. 12 ($t=6.36$) and No. 13 ($t=6.79$) (Fig. 8; Table 7).

b. Difference in centromere position

The centromere positions of the chromosomes of *Rana lessonae* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda, Rana nigromaculata, Rana plancyi chosenica* and *Rana plancyi fukienensis* in eight, 11, six and nine chromosomes, respectively, as stated above. They also differed with the same statistical significance from those of *Rana pipiens* in 10 chromosomes, Nos. 1, 2, 3, 5, 7, 8, 9, 11, 12 and 13 ($t=3.32\sim19.02$, $P=1\%\sim0\%$) (Fig. 9; Table 8).

The centromere positions of the chromosomes of *Rana lessonae* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from those of *Rana pipiens* in nine chromosomes, No. 1 ($t=5.08$), No. 2 ($t=5.41$), No. 5 ($t=7.28$), No. 7 ($t=19.02$), No. 8 ($t=7.98$), No. 9 ($t=17.66$), No. 11 ($t=6.67$), No. 12 ($t=4.67$) and No. 13 ($t=15.93$) (Fig. 9; Table 8).

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

The karyotype of *Rana lessonae* differed with a statistical significance ($t\geqq2.01$, $P\leqq5\%$) from those of *Rana brevipoda, Rana nigromaculata, Rana plancyi chosenica* and *Rana plancyi fukienensis* in the relative chromosome lengths or centromere positions of 16, 13, 13 and 15 chromosomes in total, respectively, as stated above. It also differed with the same statistical significance from that of *Rana pipiens* in a total of 20 chromosomes including 10 in RL and 10 in NVC (Table 9).

The karyotype of *Rana lessonae* differed with a high statistical significance ($t>3.50$, $P<0.1\%$) from that of *Rana pipiens* in a total of 18 chromosomes

including nine in RL and nine in NVC (Table 9).

6. *Rana pipiens* and the others

a. Difference in relative chromosome length

The relative chromosome lengths of *Rana pipiens* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana brevipoda, Rana nigromaculata, Rana plancyi chosenica, Rana plancyi fukienensis* and *Rana lessonae* in 11, eight, six, 12 and 10 chromosomes, respectively, as stated above. They also differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of the five species or subspecies in eight, eight, five, seven and nine chromosomes, respectively (Fig. 8; Table 7).

b. Difference in centromere position

The centromere positions of the chromosomes of *Rana pipiens* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana brevipoda, Rana nigromaculata, Rana plancyi chosenica, Rana plancyi fukienensis* and *Rana lessonae* in 11, 13, 9, 10 and 10 chromosomes, respectively, as stated above. They also differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of the five species or subspecies in eight, ten, nine, eight and nine chromosomes, respectively (Fig. 9; Table 8).

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

The karyotype of *Rana pipiens* differed with a statistical significance ($t \geqq 2.01$, $P \leqq 5\%$) from those of *Rana brevipoda, Rana nigromaculata, Rana plancyi chosenica, Rana plancyi fukienensis* and *Rana lessonae* in the relative chromosome lengths or centromere positions of 22, 21, 15, 22 and 20 chromosomes in total, respectively, as stated above. It also differed with a high statistical significance ($t > 3.50$, $P < 0.1\%$) from those of the five species or subspecies in the relative chromosome lengths or centromere positions of 16, 18, 14, 15 and 18 chromosomes in total, respectively (Table 9).

DISCUSSION

1. Comparison of karyotypes

GALGANO (1933a, b), WICKBOM (1945) and MORESCALCHI (1962) have reported that *Rana esculenta* has $2n=26$ chromosomes which are divided into five pairs of large chromosomes and eight pairs of small chromosomes. As *Rana lessonae* had been regarded as a synonym of *Rana esculenta* in taxonomy until about twenty years ago, it is very probable that the animals used by these investigators really belonged to *Rana lessonae,* because populations of the latter species are widely distributed in Italy and Sweden, where their laboratories were located. EBENDAL (1977) has observed the chromosomes of *Rana lessonae* obtained from an isolated

population in Central Sweden and confirmed that the karyotype consists of five pairs of large chromosomes and eight pairs of small chromosomes, as found by previous investigators. According to him, chromosome No. 10 has a secondary constriction in the long arm.

In Japanese pond frogs, SETO (1965) has reported that the karyotype of *Rana nigromaculata* also consists of five pairs of large chromosomes and eight pairs of small chromosomes. He arranged and numbered the 13 pairs of chromosomes in order of their lengths. Of the large chromosomes, Nos. 1, 4 and 5 are metacentric and Nos. 2 and 3 are submetacentric. Of the small chromosomes, Nos. 6, 7, 8, 10, 12 and 13 are submetacentric and Nos. 9 and 11 are submetacentric or subtelocentric. Chromosome No. 7 has a secondary constriction in the long arm. NISHIOKA (1972) has compared the karyotypes of two Japanese pond frog species, *Rana nigromaculata* and *Rana brevipoda,* with each other by using tadpoles collected from the field and those of reciprocal diploid and triploid hybrids produced in the laboratory. She has analyzed 407 metaphase spreads of 105 tadpoles and 393 metaphase spreads of 100 tadpoles obtained from five females of *Rana nigromaculata* and five females of *Rana brevipoda,* respectively. While Nos. 1, 4 and 5 of the five pairs of large chromosomes are of the median type, as found by SETO, No. 2 is of the median type near the submedian and No. 3 is of the submedian type in *Rana nigromaculata.* Of the eight pairs of small chromosomes, Nos. 6, 8, 10 and 11 are of the median, No. 7 is of the subterminal type, No. 9 is of the submedian or subterminal and Nos. 12 and 13 are of the submedian type. In *Rana brevipoda,* Nos. 1, 4 and 5 of the five pairs of large chromosomes are of the median type as those of *Rana nigromaculata,* No. 2 is of the median or submedian type and No. 3 is of the submedian type. Of the eight pairs of small chromosomes, Nos. 6, 8, 9, 10, 11 and 12 are of the same type as those of *Rana nigromaculata,* while No. 7 is of the submedian type and No. 13 is of the median or submedian type. In both species, chromosome No. 11 has a secondary constriction in the long arm.

The karyotype of *Rana plancyi chosenica,* a Korean subspecies of Chinese *Rana plancyi,* was reported for the first time by NISHIOKA (1983) and NISHIOKA and OKUMOTO (1983). Although the details of the karyotype have not yet been described, it is evident from the microphotographs included in their paper that the diploid chromosomes consist of five pairs of large chromosomes and eight pairs of small chromosomes and that chromosome No. 11 has a secondary constriction in the long arm, as found in *Rana nigromaculata* and *Rana brevipoda.*

The karyotype of *Rana plancyi fukienensis,* a Taiwanese subspecies of *Rana plancyi,* has been reported by LIN and HUANG (1979). This subspecies has also 2n=26 chromosomes which are divided into five pairs of large chromosomes and eight pairs of small chromosomes. Of the large chromosomes, Nos. 1, 2, 4 and 5 are of the median type and No. 3 is of the submedian type. Of the small chromosomes, Nos. 6, 8, 10, 11 and 13 are of the median type and Nos. 7, 9 and 12 are of the submedian type. Chromosome No. 11 has a secondary constriction in the long arm.

The karyotype of *Rana pipiens* has been observed by DIBERARDINO (1962) and

HENNEN (1964). According to DIBERARDINO, this Nearctic species has also 2n=26 chromosomes which consist of 12 of the median type and 14 of the submedian type. Chromosome No. 10 has a secondary constriction in the long arm. HENNEN has reported that the 26 chromosomes are divided into five pairs of large chromosomes and eight pairs of small chromosomes, being similar to the foregoing Palearctic *Rana* species.

In the present study, the karyotypes of *Rana brevipoda, Rana nigromaculata, Rana plancyi chosenica, Rana plancyi fukienensis, Rana lessonae* and *Rana pipiens* were compared with one another by counting the numbers of chromosome pairs which significantly differ in relative length (Table 7) and centromere position (Table 8). Total numbers of chromosome pairs which significantly differ in either relative length or centromere position among the foregoing five species and one subspecies are presented in Table 9. Of these numbers, a smaller one seems to indicate a closer similarity between the karyotypes of two species or subspecies. The smallest number is eight found between *Rana brevipoda* and *Rana plancyi chosenica* and the second number is nine found between *Rana nigromaculata* and *Rana plancyi chosenica.* The third number is 13 found between *Rana brevipoda* and *Rana nigromaculata,* between *Rana plancyi chosenica* and *Rana plancyi fukienensis* and between *Rana nigromaculata* or *Rana plancyi chosenica* and *Rana lessonae.* The fourth number is 15 found between *Rana brevipoda* and *Rana plancyi fukienensis,* between *Rana plancyi fukienensis* and *Rana lessonae* and between *Rana plancyi chosenica* and *Rana pipiens.* The fifth number is 16 found between *Rana nigromaculata* and *Rana plancyi fukienensis* and between *Rana brevipoda* and *Rana lessonae.* The sixth numbers are 20~22 found between *Rana pipiens* and *Rana brevipoda, Rana nigromaculata, Rana plancyi fukienensis* or *Rana lessonae.*

2. Karyotypic difference, genetic distance and reproductive isolation

It seems interesting to compare the orders of differences in karyotype with the kinds and degrees of reproductive isolating mechanisms and genetic distances calculated on the basis of electrophoretic patterns of various enzymes and blood proteins. The genetic distances among *Rana brevipoda, Rana nigromaculata, Rana plancyi chosenica, Rana plancyi fukienensis* and *Rana lessonae* have been calculated by NISHIOKA, SUMIDA and WU (1984) on the basis of electrophoretic patterns of 18 enzymes and two blood proteins obtained from these frogs. While the smallest genetic distance, 0.2900, is found between *Rana brevipoda* and *Rana plancyi fukienensis,* those between *Rana nigromaculata* and *Rana brevipoda* or *Rana plancyi fukienensis* and between *Rana plancyi chosenica* and *Rana plancyi fukienensis* are 0.4796~0.5713. The genetic distance between *Rana nigromaculata* or *Rana brevipoda* and *Rana plancyi chosenica* is 0.8378 or 0.7499. In contrast, the genetic distances between *Rana lessonae* and *Rana nigromaculata, Rana brevipoda, Rana plancyi chosenica* or *Rana plancyi fukienensis* are 1.6825~1.7932.

The reproductive isolating mechanisms among the five species and one subspecies have been reported by KAWAMURA and NISHIOKA (1979). The single Nearctic species, *Rana pipiens,* is most remotely isolated from the Palearctic species

and subspecies, as all the hybrids between them cannot live beyond the late blastula or early gastrula stage. Although European *Rana lessonae* is usually isolated from each of the Far Eastern species or subspecies by hybrid inviability to some extent, this isolating mechanism is incomplete and produces some viable hybrids. However, both males and females of these hybrids are completely or almost completely sterile.

In contrast to these situations, the hybrids among species or subspecies distributed in the Far East are viable in most cases, although the hybrids between the warm-adapted *Rana plancyi fukienensis* and the other species or between the somewhat warm-adapted *Rana brevipoda* and the somewhat cold-adapted *Rana plancyi chosenica* are completely or incompletely inviable. On the other hand, the female hybrids between *Rana nigromaculata* and *Rana brevipoda, Rana plancyi chosenica* or *Rana plancyi fukienensis* and between *Rana plancyi chosenica* and *Rana plancyi fukienensis* are almost completely fertile, while female hybrids between *Rana brevipoda* and *Rana plancyi chosenica* or *Rana plancyi fukienensis* are incompletely fertile. The male hybrids between *Rana nigromaculata* and *Rana brevipoda, Rana plancyi chosenica* or *Rana plancyi fukienensis* are almost completely sterile, while the male hybrids between *Rana brevipoda* and *Rana plancyi chosenica* or *Rana plancyi fukienensis* and between *Rana plancyi chosenica* and *Rana plancyi fukienensis* are incompletely sterile. These findings seem to show that the above three species and one subspecies are reproductively isolated from one another to nearly the same degree.

It seems noteworthy that similarity in karyotypes does not always reflect weakness in reproductive isolation nor shortness in genetic distance based on electrophoretic patterns of various kinds of proteins, although there is an agreement to some extent between these affairs. *Rana plancyi chosenica* and *Rana brevipoda* or *Rana nigromaculata* resemble most closely each other in karyotype among the Far Eastern species and subspecies, while they are more remotely separated from each other in genetic distance than any other combination of these frogs. The karyotype of *Rana lessonae* is similar to those of *Rana nigromaculata* and *Rana plancyi chosenica* in the same degree as found between those of *Rana brevipoda* and *Rana nigromaculata,* and between those of *Rana plancyi chosenica* and *Rana plancyi fukienensis,* although *Rana lessonae* is remotely separated from the Far Eastern frogs in genetic distance and by reproductive isolation. While *Rana brevipoda* and *Rana plancyi fukienensis* are the shortest in genetic distance among the species and subspecies used in the present study, they are not so similar to each other in karyotype. They are nearly of the same degree in similarity of karyotypes as found between *Rana lessonae* and *Rana plancyi fukienensis* and between *Rana pipiens* and *Rana plancyi chosenica.* North American *Rana pipiens* remarkably differs in karyotype from the other species and subspecies.

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LITERATURE

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