

# ANALYSIS OF CHROMOSOMES IN TWO GENERA OF MICROHYLIDAE (AMPHIBIA: ANURA)

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(Communicated by B. R. Seshachar, F.N.I.)

(Received June 10, 1955 ; read January 1, 1956)

## INTRODUCTION

Microhylidae are closely related to Phrynomeridae and they have retained a few primitive features which have been lost by the Ranidae. Members of this family are cryptozoic and myrmecophagous in habit and their distribution ranges from Ceylon, Southern and Eastern India to South-East United States and Rio de la Plata (Parker, 1934). The genus *Microhyla* is common to both Asia and America. In spite of the fairly wide distribution of members of this family, the chromosome numbers of only two genera are known. Makino (1951) cites only one in his Atlas, i.e. *Cacopoides tornieri* (Sato, 1936). More recently, Gowda (1948) has described the chromosomes of *Uperodon systoma*. Two closely allied forms, *Ramanella variegata* Stoliczka and *Microhyla rubra* Jerdon, were chosen for study as they were easily available in Bangalore.

## MATERIAL AND METHODS

Specimens were collected throughout the year. The testes in both species are situated in the abdominal cavity attached to the anterior region of the kidney by peritoneal folds. They are small, white, ovoid bodies 2-3 mm. in length and are larger in size during the breeding season than in the dry months.

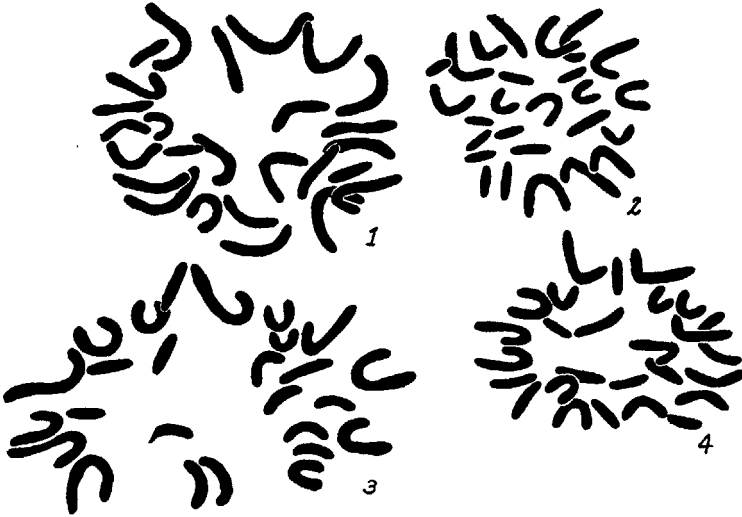
The testes were fixed in Bouin's fluid, Carnoy's fluid and Flemming with acetic acid. Paraffin sections were cut  $10\mu$  in thickness and stained in Heidenhain's haematoxylin but squashes gave better results. Testes fixed in Carnoy for twenty minutes were hydrolysed in normal hydrochloric acid for 10 minutes at  $60^{\circ}\text{C}$ . and stained in Feulgen's leucobasic fuchsin for one hour and squashes were made. Aceto-orcein squashes were also made. Observations were made using  $90\times$  and  $120\times$  Zeiss oil immersion objectives and  $10\times$  and  $20\times$  eyepieces. The lengths of the chromosomes were measured on camera lucida drawings made at a magnification of 5,700. They were reduced to half their size in reproduction. The analysis was carried out on the same basis as that by Tobias on the albino rat (1947) and *Tatera brantsii* (1952).

## OBSERVATIONS

The diploid number of chromosomes as determined in spermatogonial metaphase plates in both *Ramanella* and *Microhyla* is 26. In a number of spermatogonial prometaphase and metaphase nuclei, 26 chromosomes have been counted in both genera. There are 13 pairs of homologous chromosomes and the number was confirmed by counting 13 bivalents in diakinesis and metaphase I of meiosis. No sex chromosomes could be identified.

Figs. 1 and 2 are prometaphase and metaphase plates of spermatogonial nuclei of *Microhyla*. By studying a number of spermatogonial metaphase plates 7 pairs of metacentric chromosomes and 6 pairs of acrocentrics have been observed. The

metacentric chromosomes with equal arms have been termed V-shaped while the chromosomes with unequal arms have been called J-shaped. There are 5 pairs of V-shaped and 2 pairs of J-shaped chromosomes (Fig. 2).



FIGS. 1-4

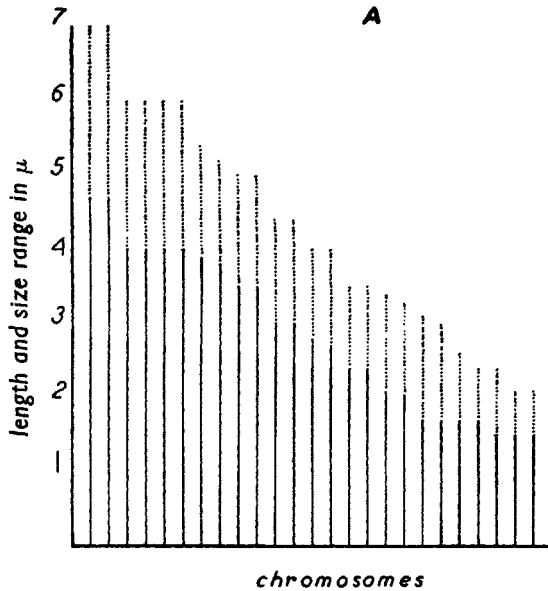
Fig. 1. Prometaphase and Fig. 2 metaphase plates of spermatogonial nuclei of *Microhyla rubra*.  $\times 2,850$ . Feulgen.  
 ,, 3. Prometaphase and Fig. 4 metaphase plates of spermatogonial nuclei of *Ramanella variegata*.  $\times 2,850$ . Feulgen.

It may be mentioned that the four J-shaped chromosomes are longer than the metacentrics with equal arms. The size range of the four chromosomes shown in Fig. 2 is  $4.0-4.7\mu$ . The lengths of the ten metacentrics with equal arms vary from  $2.4$  to  $4.0\mu$ . The six pairs of acrocentrics range from  $1.4$  to  $3.9\mu$ . Among these the chromosomes of one pair measure  $3.8$  and  $3.9\mu$  respectively. Two pairs are of the same length and measure  $1.6\mu$ . The total length of all the twenty-six chromosomes is  $72.9\mu$ .

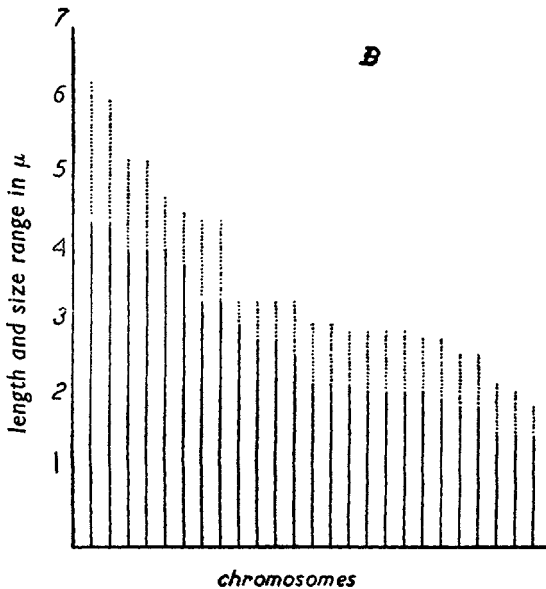
The lengths of the chromosomes in six spermatogonial metaphase plates have been measured and a composite graph A has been drawn indicating their maximum and minimum lengths. In all the six plates, the J-shaped chromosomes are longer; the size range is  $4.0$  to  $7.0\mu$ . The metacentrics with equal arms vary from  $2.4$  to  $6.0\mu$ . Two pairs of acrocentrics are of the same length and the size range for the six pairs is  $1.4$  to  $5.4\mu$ .

Figs. 3 and 4 are prometaphase and metaphase plates of spermatogonial nuclei of *Ramanella variegata*. There are eight pairs of metacentrics and five pairs of acrocentrics.

It may be noticed that the two pairs of J-shaped chromosomes are longer than the V-shaped metacentrics and measure  $4.0-4.4\mu$  but the third J-shaped pair is  $3.3\mu$  in length. The metacentrics with equal arms vary from  $2.4$  to  $4.0\mu$ . The five pairs of acrocentrics vary from  $1.4$  to  $2.2\mu$ . The total length of the 26 chromosomes in *Ramanella* is  $71.5\mu$ . The composite graph B represents the size range of the chromosomes of *Ramanella*, as seen in six spermatogonial plates. The two pairs of J-shaped chromosomes vary from  $4.0$  to  $6.3\mu$  and the third pair,  $3.3$  to  $4.4\mu$ . The V-shaped metacentrics range from  $2.1$  to  $4.7\mu$  while the acrocentrics vary from  $1.4$  to  $2.9\mu$ .



Graph A illustrates the length variations of the 26 spermatogonial chromosomes in *Microhyla rubra* in six metaphase plates examined. Scale 1 cm.— $1\mu$ .



Graph B illustrates the length variations of the 26 spermatogonial chromosomes in *Ramanella variegata* in six metaphase plates examined. Scale 1 cm.— $1\mu$ .

On comparing the lengths of the chromosomes of the two forms, it is found that the smallest acrocentric measures  $1.4\mu$  in both; but the size range of the acrocentrics is  $1.4-2.9\mu$  in *Ramanella* and  $1.4-5.4\mu$  in *Microhyla*. The number also varies; six pairs in *Microhyla* and five pairs in *Ramanella*. One pair of J-shaped

chromosomes is longer than the V-shaped metacentrics in both; 3 pairs of J-shaped chromosomes are found in *Ramanella* while only two pairs are observed in *Microhyla*.

The number of the metacentrics with equal arms is the same in both species.

Table 1 shows the size range and shapes of the 13 pairs of homologous chromosomes of *Microhyla rubra* and Table 2 shows those of *Ramanella variegata*. Table 3 summarizes the important differences between the two species.

TABLE 1

Table showing size range and shapes of the 13 pairs of homologous chromosomes of *Microhyla rubra*

<i>Microhyla rubra</i>		Diploid No. 26
Pair 1 .. ..	4.7-7.0 $\mu$	J-shaped.
Pair 2 .. ..	4.0-6.0 $\mu$	J-shaped.
Pair 3 .. ..	4.0-6.0 $\mu$	V-shaped.
Pair 4 .. ..	3.5-5.0 $\mu$	V-shaped.
Pair 5 .. ..	3.0-4.4 $\mu$	V-shaped.
Pair 6 .. ..	2.7-4.0 $\mu$	V-shaped.
Pair 7 .. ..	2.4-3.5 $\mu$	V-shaped.
Pairs 8, 9, 10, 11, 12 and 13 .. ..	1.4-5.4 $\mu$	Acrocentric.

TABLE 2

Table showing size range and shapes of the 13 pairs of homologous chromosomes of *Ramanella variegata*

<i>Ramanella variegata</i>		Diploid No. 26
Pair 1 .. ..	4.4-6.3 $\mu$	J-shaped.
Pair 2 .. ..	4.0-5.2 $\mu$	J-shaped.
Pair 3 .. ..	3.8-4.7 $\mu$	V-shaped.
Pair 4 .. ..	3.3-4.4 $\mu$	J-shaped.
Pair 5 .. ..	2.8-3.3 $\mu$	V-shaped.
Pair 6 .. ..	2.6-3.3 $\mu$	V-shaped.
Pair 7 .. ..	2.2-3.0 $\mu$	V-shaped.
Pair 8 .. ..	2.1-2.9 $\mu$	V-shaped.
Pairs 9, 10, 11, 12 and 13 .. ..	1.4-2.9 $\mu$	Acrocentric.

TABLE 3

Table showing the differences between the chromosomes of *Microhyla rubra* and *Ramanella variegata*

	<i>Ramanella variegata</i>	<i>Microhyla rubra</i>
Chromosome number ..	26	26
Number of large metacentric chromosomes .. ..	8	12
Number of small metacentric chromosomes .. ..	8	2
Number of acrocentric chromosomes .. ..	10	12
Size range, metacentrics ..	2.1-6.3 $\mu$	2.4-7.0 $\mu$
Size range, acrocentrics ..	1.4-2.9 $\mu$	1.4-5.4 $\mu$
Total size range .. ..	1.4-6.3 $\mu$	1.4-7.0 $\mu$

## SUMMARY AND CONCLUSIONS

The diploid number of chromosomes in *Ramanella variegata* and *Microhyla rubra* is 26. There are 8 pairs of metacentric chromosomes and 5 pairs of acrocentrics in *R. variegata* whereas there are 7 pairs of metacentrics and 6 pairs of acrocentrics in *M. rubra*. The chromosome lengths of the two species in mitotic metaphase have been measured.

The main differences between the chromosomes of the two species are:

(a) There is one more metacentric chromosome (J-shaped) in *R. variegata* than in *M. rubra*.

(b) The total length of the 26 chromosomes is  $72.9\mu$  in *Microhyla* and  $71.5\mu$  in *Ramanella*.

It is difficult, with the available information, to evaluate the significance of these differences or to account for them. Morphologists believe that *Microhyla* is perhaps more primitive than *Ramanella* (Noble, 1931) and chromosome analysis of more species of this family is desirable before any conclusions can be drawn.

## ACKNOWLEDGEMENT

I am thankful to Professor B. R. Seshachar for suggesting the problem and for his guidance and encouragement.

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*Issued September 14, 1956.*