Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics

ISSN: 0008-7114 (Print) 2165-5391 (Online) Journal homepage: http://www.tandfonline.com/loi/tcar20

Chromosomal Analysis of Eighteen Species of Anura

Maria Luiza Beçak

To cite this article: Maria Luiza Beçak (1968) Chromosomal Analysis of Eighteen Species of Anura, Caryologia, 21:3, 191-208, DOI: 10.1080/00087114.1968.10796299

To link to this article: http://dx.doi.org/10.1080/00087114.1968.10796299

CARYOLOGIA

VOLUME 21 LUGLIO-SETTEMBRE 1968 NUMERO 3

CHROMOSOMAL ANALYSIS OF EIGHTEEN SPECIES OF *ANURA**

MARIA LUIZA BEÇAK

Secção de Genética, Instituto Butantan, São Paulo, Brazil

Received: 12th January 1968

INTRODUCTION

Anurans karyotypes differ remarkably by chromosome number and shape and the chromosomal alterations that occured during evolution of this group are still not completely understood. Centric fusion has been considered the main occurence during phylogenesis of Anura (WICKBOM 1945).

The present paper describes a kariotype study of eighteen species of Brazilian anurans belonging to four families, Bufonidae, Leptodactylidae, Hylidae and Pseudidae (Procoela). Some considerations regarding chromosomal evolution and mechanism of sex determination are tentatively based on the investigation of chromosome number, morphology and meiotic behavior.

MATERIAL AND METHOD

The following species of Anura are described in the present paper:

Bufo paracnemis A. Lutz, 1925. Thirteen specimens were analysed from the following localities: Cabuçu, Bahia (3 ♀, 1 ♂); Cáceres and Aquidauana, Mato Grosso (1 ♀, 2 ♂); São Paulo, São Paulo, (1 ♀, 2 ♂); unknown origin (1 ♀, 2 ♂).

Bufo ictericus Spix, 1824. Sixteen animals were analysed from: Curitiba, Paranà (2 ♀, 2 ♂); Mafra, Santa Catarina (1 ♀); São Paulo, São Paulo (4 ♀, 4 ♂), unknown origin (2 ♀, 1 ♂).

Bufo crucifer Wied, 1821. Four specimens (1 ♀, 3 ♂) from São Paulo, São Paulo were analysed.

Leptodactylus ocellatus (Linnaeus), 1758. Five animals were studied from the following localities: São José do Rio Preto, São Paulo (1 ♀); São Paulo, São Paulo (3 ♂); unknown origin (1 ♂).

Eupemphix nattereri Steindachner, 1863. Three specimens (2 ♀, 1 ♂) from São José do Rio Preto, São Paulo were analysed.

* This work was supported by Public Health Service Research Grant N° GM-14577-02 from the National Institute of General Medical Sciences, and by the Fundo de Pesquisas do Instituto Butantan.

Eleutherodactylus guentheri (Steindachner), 1864. Three specimens (1 ♀, 2 ♂) from Campos de Jordão, São Paulo were studied.

Crossodactylus grandis B. Lutz, 1951. Four specimens (1 ♀, 3 ♂) from Campos de Jordão, São Paulo were analysed.

Pseudopaludicola ameghini (Cope), 1887. Four animals (2 ♀, 2 ♂) from São José do Rio Preto, São Paulo were analysed.

Pseudopaludicola falcipes (Henzel), 1867. Fourteen specimens (2 ♀, 12 ♂) from São José do Rio Preto, São Paulo were analysed.

Hyla pulchella prasina Burmeister, 1856. Ten animals (1 ♀, 9 ♂) from Campos de Jordão and São Paulo, São Paulo were studied.

Hyla hayi Barbour, 1909. Eight specimens (5 ♀, 3 ♂) from Campos de Jordão and Itapecerica de Serra, São Paulo were analysed.

Hyla faber Wied, 1821. Two animals were analysed from Terezópolis, Rio de Janeiro (1 ♂) and São Roque, São Paulo (1 ♀).

Hyla multilineata A. Lutz and B. Lutz, 1939. One female from Itapecerica da Serra, São Paulo, was analysed.

Hyla albomarginata Spix, 1824. Six specimens (1 ♀, 5 ♂) from Praia Grande, São Paulo and Paranapiacaba, São Paulo were studied.

Hyla albopunctata Spix, 1824. One male from São Paulo, São Paulo, was analysed.

Hyla microps Peters, 1872. Four specimens (1 ♀, 3 ♂) from Campos de Jordão, São Paulo were analysed.

Fritziana goeldii Boulenger, 1894. Six animals (3 ♀, 3 ♂) from Campos de Jordão, São Paulo were studied.

Pseudis paradoxus platensis Gallardo, 1961. Seven specimens (4 ♀, 3 ♂) from São José do Rio Preto, São Paulo were analysed.

The karyotypes were analysed from preparations obtained by the squash technique. The animals were treated two hours prior to sacrifice with a 0,5% solution of colchicine in the dosage of 0,02 ml/g. After hypotonical treatment for 15 minutes in cold distilled water pieces of spleen, liver, gut and gonads were fixed in a 50% solution of glacial acetic acid for 15 minutes. The fragments were then squashed and the coverslips removed with liquid CO_2. After hydrolysis in N HCl at 60°C for 10 minutes, the preparations were stained with Giemsa for 10 minutes and mounted. Short term cultures of whole blood, microtechnique (BEÇAK *et al.* 1964) were also used in some cases.

Three morphological criteria, relative length, arm-ratio and centromeric index, were used for the identification of each pair of chromosomes as well as the presence of satellites and of secondary constrictions.

RESULTS

The family Bufonidae presented very similar karyotypes in the species analysed. *Bufo ictericus* (Figs. 1, 2 and 3), *B. paracnemis* (Figs. 4 and 5)

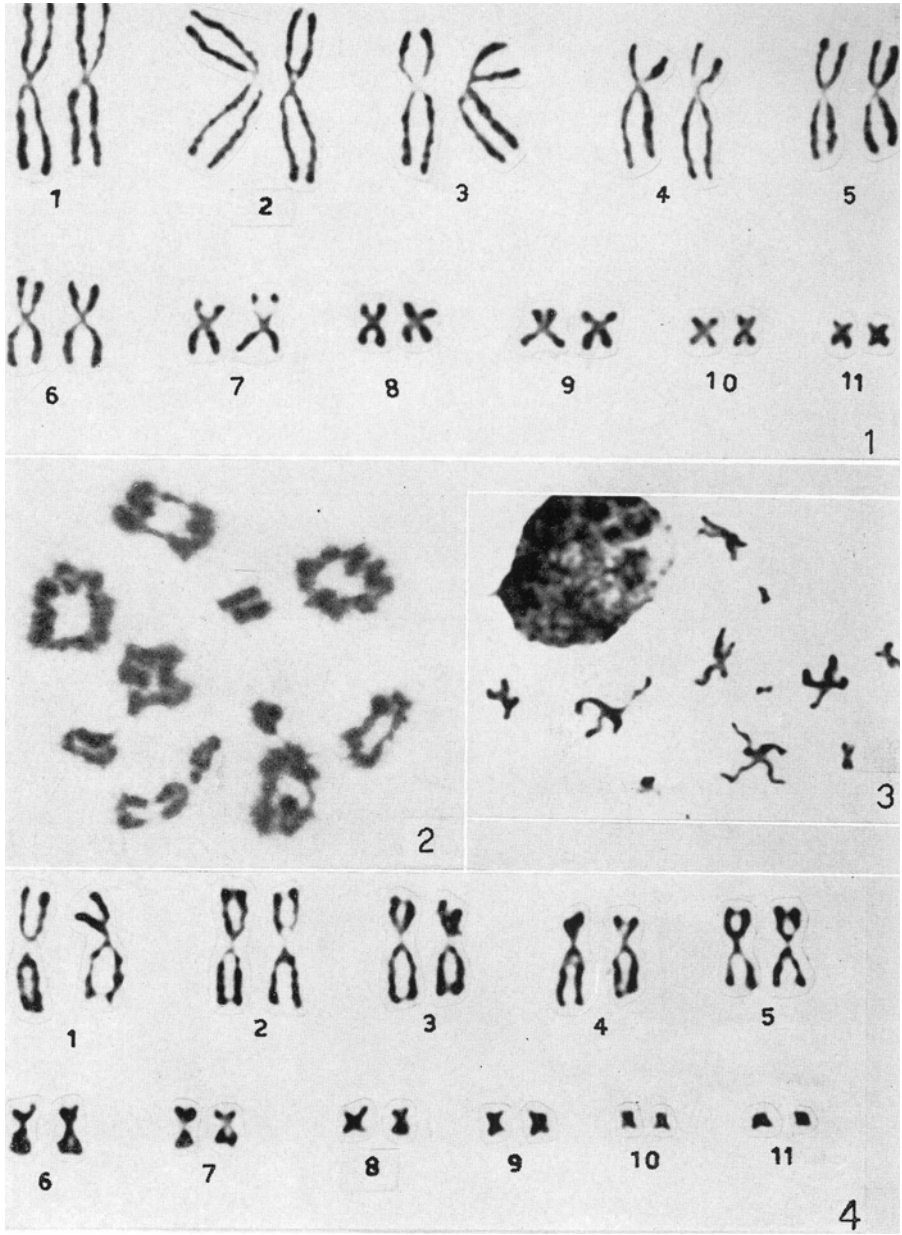

Fig. 1. — *Bufo ictericus*, female karyotype from spleen cell (1800×).
Fig. 2. — *Bufo ictericus*, male diakinesis (2100×).
Fig. 3. — *Bufo ictericus*, male metaphase II (2550×).
Fig. 4. — *Bufo paracnemis*, female karyotype from spleen cell (1950×).

and *B. crucifer* (Figs. 6 and 7) exhibit the same diploid number ($2n=22$, $n=11$) and the same chromosomal morphology (Tables I, II). With exception of the submetacentric pairs 3, 4, and 6, all are metacentrics. Pair 7 has a satellite at the short arm. Male and female have indistinct karyotypes. Male meiosis is similar in the three species. The homologues joint only by the ends which are polarized in a nuclear region resembling a « bouquet » configuration. Spermatocytes I have 11 ring shaped bivalents and metaphases II show 11 dyads. No chromosomal dimorphism in the somatic complements of both sexes has been observed nor any bivalent in male, which aspect or special behavior could be considered as characteristic of a sex chromosome pair.

In specimens of *Bufo paracnemis* and *B. ictericus* sometimes pairing of pair 4 was difficulted by the occasional difference of size and position of

TABLE I

Number of chromosomes in somatic and germ cells in 18 species of amphibians.

Family	Species	Number of Animals ♂	Number of Animals ♀	2n	n (♂)	Total of Cells
Bufonidae	*Bufo paracnemis*	7	6	22	11	218
	Bufo ictericus	7	9	22	11	267
	Bufo crucifer	3	1	22	11	87
Leptodactylidae	*Leptodactylus ocellatus*	4	1	22	11	226
	Eupemphix nattereri	1	2	22	11	86
	Eleutherodactylus guentheri	2	1	22	11	128
	Crossodactylus grandis	3	1	26	13	236
	Pseudopaludicola ameghini	2	2	20	10	174
	Pseudopaludicola falcipes	12	2	18	9	365
Hylidae	*Hyla pulchella prasina*	9	1	24	12	350
	Hyla hayi	3	5	24	12	394
	Hyla faber	1	1	24	12	268
	Hyla multilineata	—	1	24	12	11
	Hyla albomarginata	5	1	24	12	221
	Hyla albopunctata	1	—	22	11	62
	Hyla microps	3	1	30	15	193
	Fritziana goeldii	3	3	26	13	132
Pseudidae	*Pseudis paradoxus platensis*	3	4	24	12	163

TABLE II

Chromosome measurements of Bufonidae, based on ten karyotypes of each species.

	Species	1	2	3	4	5	6	7	8	9	10	11
Relative length	Bufo ictericus	17	16	14	12	11	8	6	5	4	3	2
	Bufo paracnemis	19	17	14	11	11	8	6	4	4	3	2
	Bufo crucifer	18	16	13	12	12	8	6	5	4	3	2
Arm ratio	Bufo ictericus	1.0	1.3	1.5	2.2	1.0	1.5	1.3	1.0	1.4	1.0	1.1
	Bufo paracnemis	1.0	1.3	1.5	2.3	1.0	1.5	1.3	1.0	1.3	1.0	1.0
	Bufo crucifer	1.0	1.3	1.5	2.2	1.0	1.5	1.4	1.1	1.4	1.0	1.1
Centromere index	Bufo ictericus	.49	.43	.40	.32	.48	.40	.44	.49	.41	.50	.49
	Bufo paracnemis	.50	.43	.40	.30	.50	.40	.43	.50	.43	.50	.50
	Bufo crucifer	.49	.43	.40	.31	.50	.40	.41	.48	.41	.50	.48

centromere in the homologues. One of them was eventually smaller presenting the centromere slightly more terminal. Yet, this condition appeared indistinctly in both sexes and therefore was not correlated to sexual heteromorphism.

In the Leptodactylidae the six species showed variations, $2n=18$, $2n=20$, $2n=22$ and $2n=26$ chromosomes (Tables I, III). Male and female karyotypes are indistinct. Meiotic aspects of male are similar to those described in Bufonidae. *Leptodactylus ocellatus,* however, exhibited complete pairing of homologues in pachytene and interstitial chiasmata in diplotene. No sexual heterochromosomes were recognized at mitotic or meiotic level in the Leptodactylidae.

Karyotypical analysis of *Leptodactylus ocellatus* ($2n=22$, $n=11$) showed that the pairs 1, 5, 6, 8, 9 and 10 are metacentric, the pairs 2, 3, 7 and 11 are submetacentric and pair 4 is acrocentric (Figs. 8, 9 and 10). In the species *Eupemphix nattereri* ($2n=22$, $n=11$) the pairs 1, 5, 6 and 8 are metacentric, the pairs 2, 3, 4, 7, 9 and 10 are submetacentric and pair 11 is acrocentric, with satellites on the larger arm (Figs. 11 and 12). In *Eleutherodactylus guentheri* ($2n=22$, $n=11$) the pairs, 1, 4, 6, 8, 10 and 11 are metacentric and the pairs 2, 3, 5, 7 and 9 are submetacentric. The pairs 4 and 6 have a secondary constriction on the short arm. The submetacentric pair 5 presents a secondary constriction in the middle of the larger arm (Figs. 13 and 14). In *Crossodactylus grandis* ($2n=26$, $n=13$) the pairs 1, 3, 10, 11, 12 and 13 are metacentric, the pairs 2, 6, 7, 8 and 9 are submetacentric and the pairs 4 and 5 are acrocentric. Pair 7 presents a se-

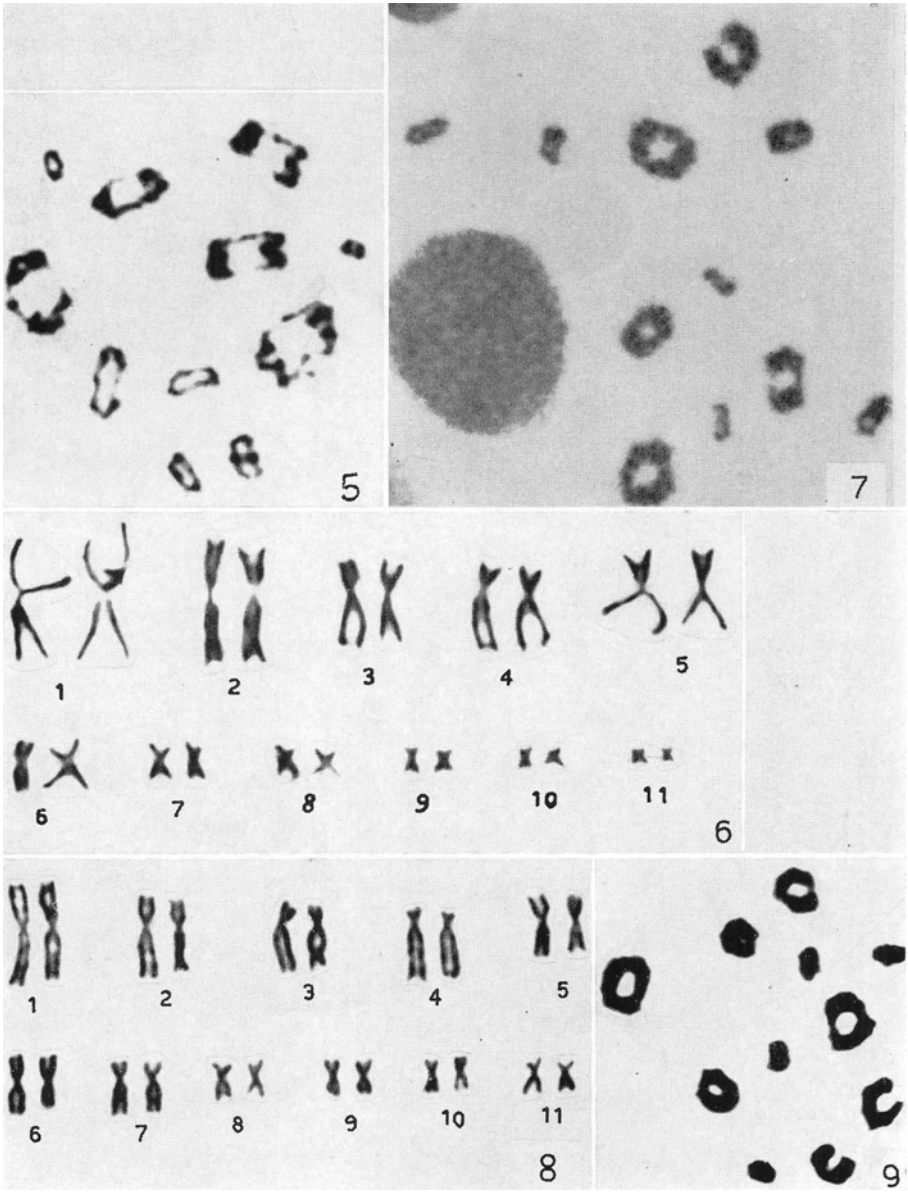

Fig. 5. — *Bufo paracnemis,* male diakinesis (1550×).
Fig. 6. — *Bufo crucifer*, male karyotype from spleen cell (2000×).
Fig. 7. — *Bufo crucifer*, male diakinesis (2150×).
Fig. 8. — *Leptodactylus ocellatus,* female karyotype from whole blood short term culture metaphase (1600×).
Fig. 9. — *Leptodactylus ocellatus*, male metaphase I (2150).

condary constriction from the centromere to the middle of the larger arm (Figs. 15, 16 and 17). In *Pseudopaludicola ameghini* ($2n=20$, $n=10$) the pairs 1, 2, 5, 6 and 7 are metacentric, the pairs 3, 4, 9 and 10 are submetacentric and the pair 8 is acrocentric (Figs. 18, 19 and 20). In *Pseudopalu-*

TABLE III

Chromosome measurements of Leptodactylidae based on ten karyotypes of each species.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
Relative length	Leptodactylus ocellatus	17	13	11	11	10	9	8	6	5	5	5		
	Eupemphix nattereri	16	13	12	11	10	10	8	6	5	5	4		
	Eleutherodactylus guentheri	16	13	12	12	11	9	7	6	5	5	4		
	Crossodactylus grandis	18	14	11	11	10	6	6	5	5	4	4	3	3
	Pseudopaludicola ameghini	17	14	13	13	10	9	7	7	5	3			
	Pseudopaludicola falcipes	19	15	12	11	10	10	10	7	6				
Arm ratio	Leptodactylus ocellatus	1.1	1.6	2.4	4.2	1.3	1.0	2.9	1.3	1.0	1.2	2.0		
	Eupemphix nattereri	1.1	1.6	2.4	4.0	1.3	1.2	2.2	1.2	1.5	1.5	9.2		
	Eleutherodactylus guentheri	1.2	1.8	1.7	1.2	4.0	1.0	4.0	1.2	1.5	1.4	1.1		
	Crossodactylus grandis	1.2	1.9	1.4	5.3	4.6	4.0	2.5	2.5	2.6	1.0	1.0	1.0	1.0
	Pseudopaludicola ameghini	1.0	1.0	4.0	2.8	1.4	1.0	1.0	4.9	3.5	2.0			
	Pseudopaludicola falcipes	1.1	1.4	4.0	2.2	1.3	2.0	10.0	15.0	2.0				
Centromere index	Leptodactylus ocellatus	.47	.39	.29	.19	.44	.49	.26	.44	.50	.47	.33		
	Eupemphix nattereri	.47	.39	.30	.20	.44	.45	.31	.45	.40	.40	.09		
	Eleutherodactylus guentheri	.45	.35	.37	.45	.20	.49	.20	.45	.40	.41	.47		
	Crossodactylus grandis	.45	.35	.41	.16	.18	.20	.29	.29	.28	.50	.50	.50	.50
	Pseudopaludicola ameghini	.50	.50	.20	26	.41	.50	.50	.17	.22	.33			
	Pseudopaludicola falcipes	.48	.41	.20	.31	.44	.30	.09	.06	.33				

dicola falcipes ($2n=18$, $n=9$) the pairs 1, 2 and 5 are metacentric, the pairs 3, 4, 6 and 9 are submetacentric and the pairs 7 and 8 are acrocentric (Figs. 21, 22 and 23).

In the family Hylidae the diploid number of eight species is heterogeneous, $2n=22$, $2n=24$, $2n=26$ and $2n=30$. The study of the karyotypes showed that besides differences in number, the complements differ too by chromosomal morphology (Tables I, IV). In *Hyla pulchella prasina* ($2n=24$, $n=12$) the metacentric pairs are the 1, 8, 11 and 12; the submetacentric pairs are the 2, 3, 4, 5, 7, 9 and 10 and the pair 6 is acrocentric. The pair

9 shows a secondary constriction at the distal part of the larger arm (Figs. 24, 25 and 26). In *Hyla hayi* ($2n=24$, $n=12$) the pairs 1, 7, 8, 9, 10, 11

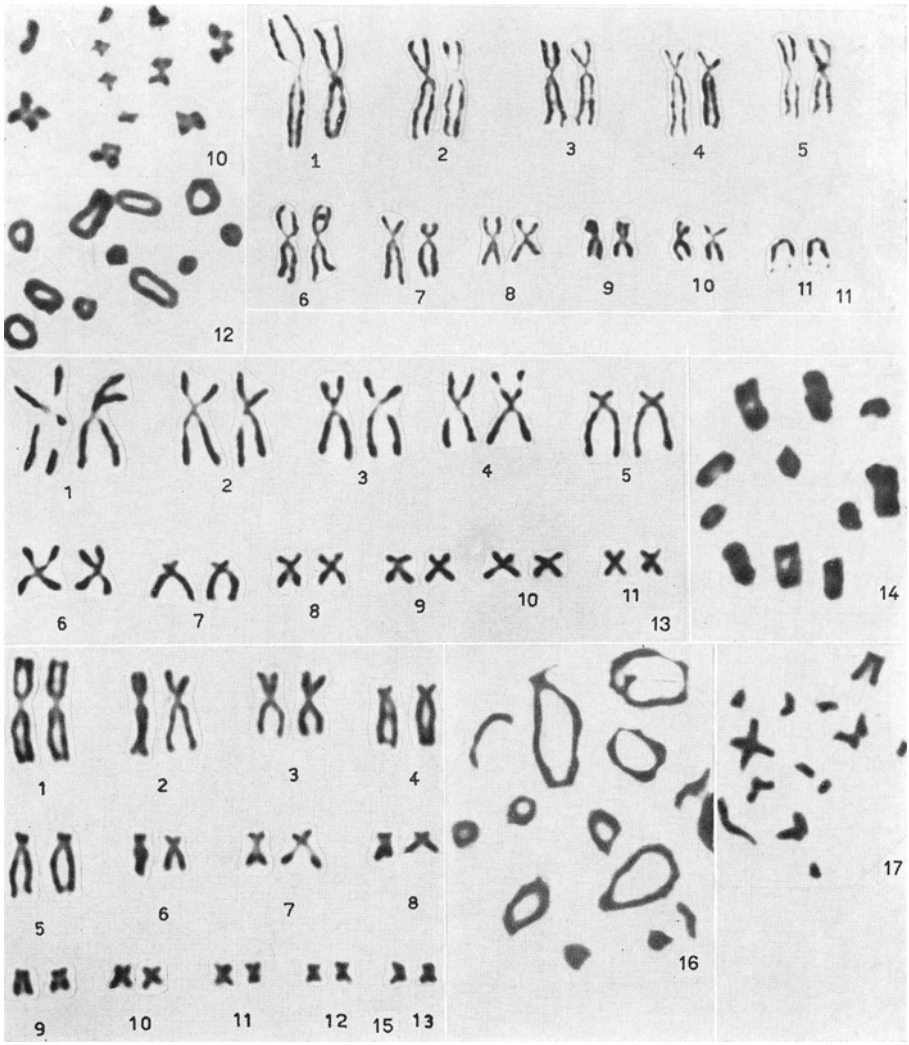

Fig. 10. — *Leptodactylus ocellatus*, male metaphase II (1650×).
Fig. 11. — *Eupemphix nattereri*, male karyotype from spleen cell (1800×).
Fig. 12. — *Eupemphix nattereri*, male metaphase I (1650×).
Fig. 13. — *Eleutherodactylus guentheri*, karyotype from testicle cell (1600×).
Fig. 14. — *Eleutherodoctylus guentheri*, male metaphase I (1500×).
Fig. 15. — *Crossodactylus grandis*, male karyotype from spleen cell (1800×).
Fig. 16. — *Crossodactylus grandis*, male metaphase I (2100×).
Fig. 17. — *Crossodactylus grandis*, male metaphase II (2100×).

and 12 are metacentric all others being submetacentric (Figs. 27 and 28). In *Hyla faber* ($2n=24$, $n=12$) the pairs, 1, 9 and 10 are metacentric, the pairs 2, 3, 4, 5, 8, 11 and 12 are submetacentric and the pairs 6 and 7 are acrocentric (Figs. 29 and 30). *Hyla multilineata* ($2n=24$) shows the pairs 1, 9,

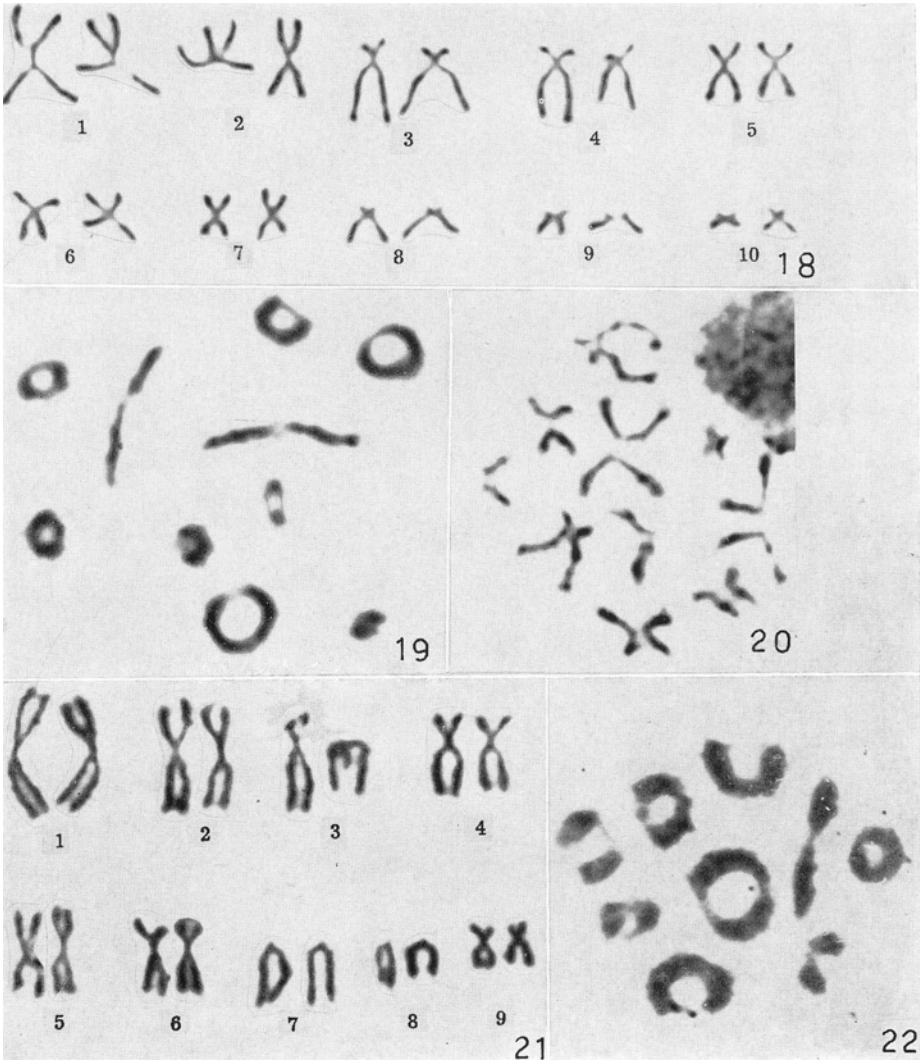

Fig. 18. — *Pseudopaludicola ameghini*, karyotype from testicle cell (2000×).
Fig. 19. — *Pseudopaludicola ameghini*, male metaphase I (2200×).
Fig. 20. — *Pseudopaludicola ameghini*, male mataphase II (1950×).
Fig. 21. — *Pseudopaludicola falcipes*, male karyotype from gut cell (1700×).
Fig. 22. — *Pseudopaludicola falcipes*, male metaphase I (1550×).

TABLE IV

Chromosome measurements of Hylidae based on ten karyotypes of each species.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Relative length	Hyla pulchella prasina	18	14	12	11	10	8	7	5	5	4	4	2			
	Hyla hayi	17	14	12	10	8	8	6	6	5	5	5	4			
	Hyla faber	16	14	13	12	11	7	6	5	5	5	3	2			
	Hyla multilineata	20	17	12	12	10	7	5	4	4	3	3	2			
	Hyla albomarginata	18	15	12	13	10	8	5	5	4	4	2	2			
	Hyla albopunctata	18	14	13	11	10	8	6	5	5	5	4				
	Hyla microps	12	11	10	9	8	7	6	6	6	5	5	4	4	4	3
	Fritziana goeldii	17	15	14	12	11	5	5	5	4	4	3	3	2		
Arm ratio	Hyla pulchella prasina	1.0	1.6	4.0	2.3	2.6	5.3	3.2	1.0	2.8	2.0	1.0	1.0			
	Hyla hayi	1.0	1.6	3.2	2.7	2.9	2.7	1.4	1.3	1.3	1.0	1.2	1.4			
	Hyla faber	1.1	1.7	3.8	2.3	2.0	5.7	5.7	2.7	1.1	1.2	1.9	1.6			
	Hyla multilineata	1.2	1.6	3.8	2.1	2.6	3.8	2.3	2.3	1.2	1.4	1.1	1.3			
	Hyla albomarginata	1.0	2.0	2.1	1.9	3.0	4.6	1.9	1.9	1.0	1.0	1.3	1.0			
	Hyla albopunctata	1.0	2.1	1.8	3.5	2.7	3.5	1.2	1.8	1.1	1.1	1.8				
	Hyla microps	3.5	2.8	1.8	3.8	20.0	19.0	16.0	2.1	1.8	1.4	1.3	1.3	1.0	1.3	10.0
	Fritziana goeldii	1.2	1.7	2.1	2.9	1.4	1.0	8.0	1.2	1.2	1.3	1.0	6.0	6.0		
Centromere index	Hyla pulchella prasina	.50	.39	.20	.31	.28	.16	.24	.50	.26	.33	.50	.50			
	Hyla hayi	.50	.38	.24	.27	.26	.27	.41	.44	.44	.50	.46	.42			
	Hyla faber	.48	.37	.21	.30	.33	.15	.15	.27	.49	.45	.34	.39			
	Hyla multilineata	.46	.38	.21	.32	.28	.21	.30	.31	.45	.42	.48	.44			
	Hyla albomarginata	.50	.33	.32	.34	.25	.18	.34	.34	.50	.50	.44	.50			
	Hyla albopunctata	.50	.32	.36	.22	.27	.22	.45	.36	.48	.48	.36				
	Hyla microps	.22	.27	.35	.21	.04	.05	.06	.32	.35	.41	.44	.44	.50	.43	.09
	Fritziana goeldii	.45	.36	.32	.25	.41	.50	.11	.47	.46	.43	.50	.14	.14		

10, 11 and 12 as metacentric, all others being submetacentric (Fig. 31). In *Hyla albomarginata* ($2n=24$, $n=12$) the pairs 1, 9, 10, 11 and 12 are metacentric, the pairs 2, 3, 4, 5, 7 and 8 are submetacentric and the pair 6 is acrocentric (Figs. 32 and 33). In *Hyla albopunctata* ($2n=22$, $n=11$) the pairs 1, 7, 9 and 10 are metacentric and all others are submetacentric (Figs. 34, 35 and 36). In *Hyla microps* ($2n=30$, $n=15$) the pairs 10, 11, 12, 13 and 14 are metacentric, the pairs 1, 2, 3, 4, 8 and 9 are submetacentric and the pairs 5, 6, 7 and 15 are acrocentric (Figs. 37, 38 and 39). In *Fritziana*

goeldii ($2n=26$, $n=13$) the pairs 1, 5, 6, 8, 9, 10 and 11 are metacentric; the pairs 2, 3 and 4 are submetacentric, and the pairs 7, 12 and 13 are acrocentric (Figs. 40 and 41).

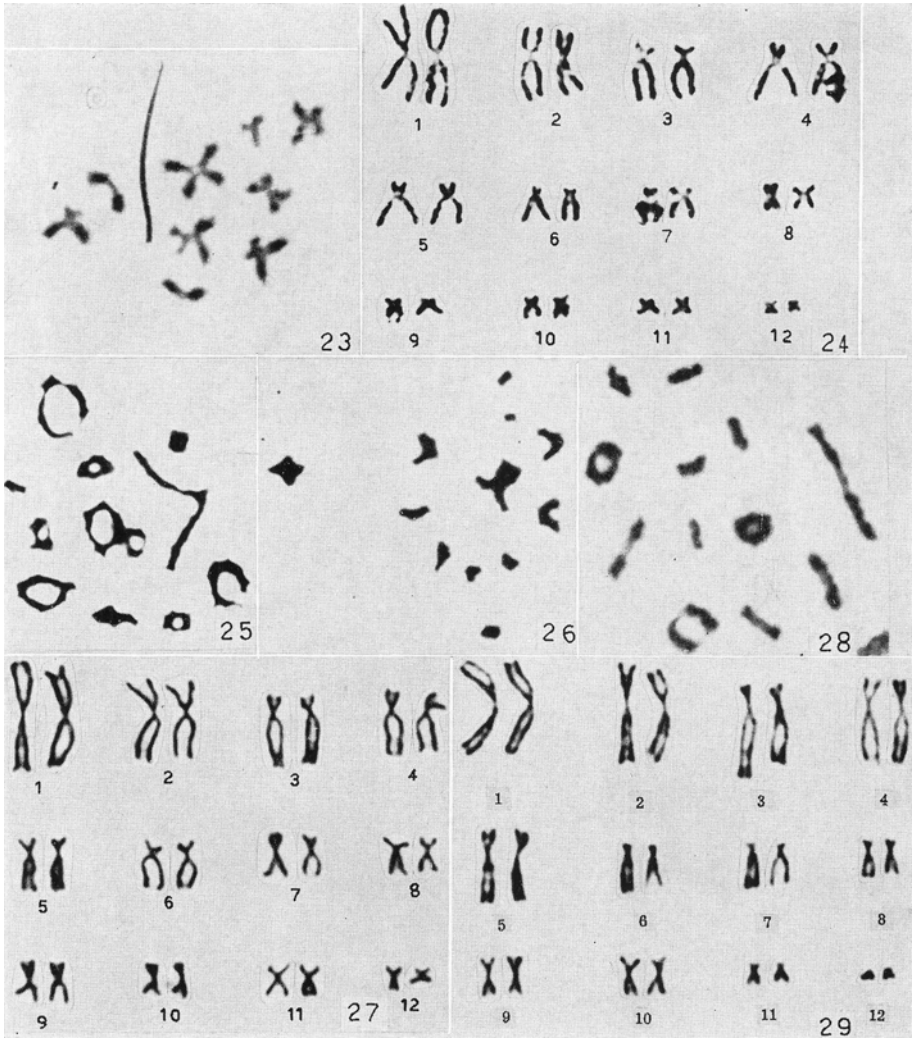

Fig. 23. — *Pseudopaludicola falcipes*, male metaphase II (1550×).
Fig. 24. — *Hyla pulchella prasina*, male karyotype from spleen cell (1200×).
Fig. 25. — *Hyla pulchella prasina*, male metaphase I (1550×).
Fig. 26. — *Hyla pulchella prasina*, male metaphase II (3000×).
Fig. 27. — *Hyla hayi*, karyotype from ovary cell (1950×).
Fig. 28. — *Hyla hayi*, male metaphase I (1700×).
Fig. 29. — *Hyla faber*, karyotype from testicle cell (1700×).

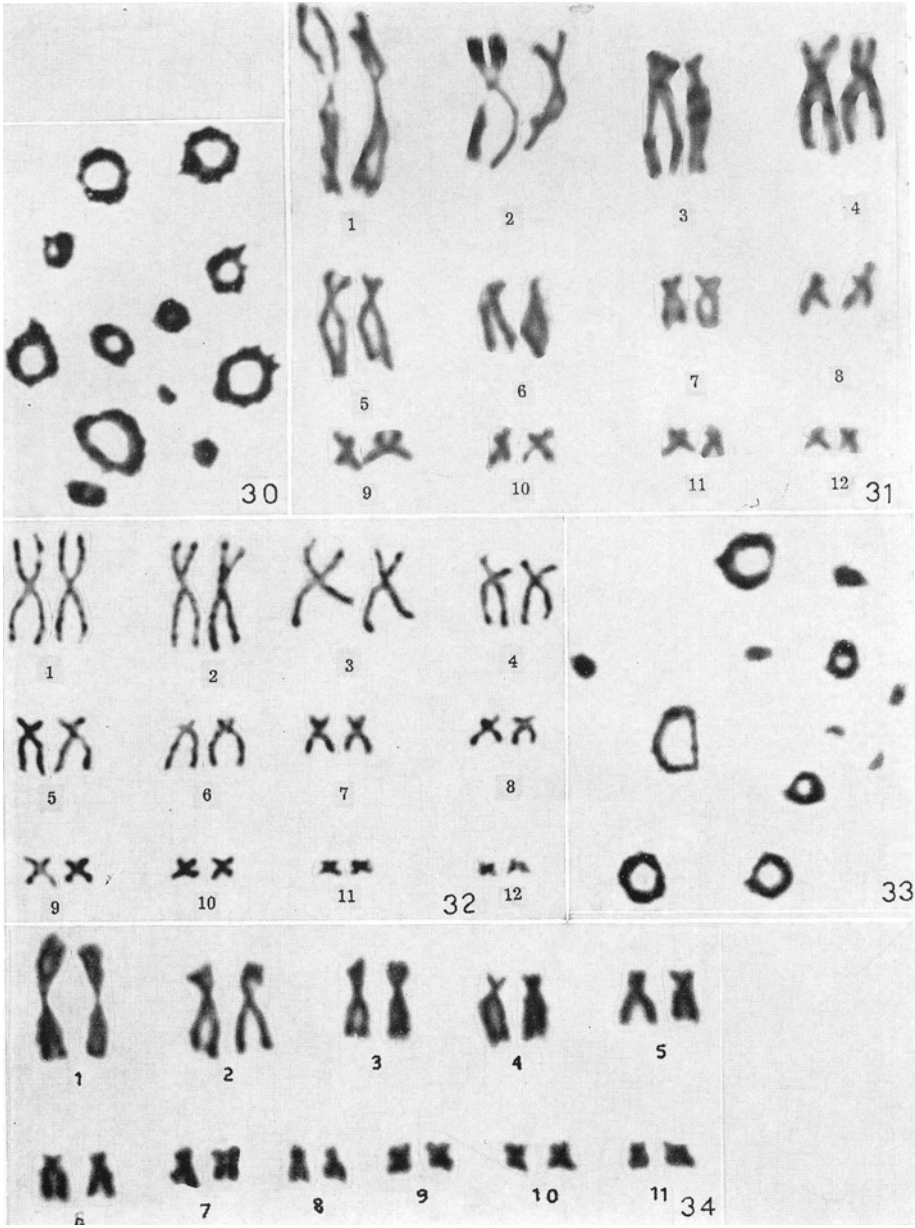

Fig. 30. — *Hyla faber*, male metaphase I (1650×).
Fig. 31. — *Hyla multilineata*, karyotype from ovary cell (2550×).
Fig. 32. — *Hyla albomarginata*, male karyotype from spleen cell (1550×).
Fig. 33. — *Hyla albomarginata*, male metaphase I (1350×).
Fig. 34. — *Hyla albopunctata*, karyotype from testicle cell (2650×).

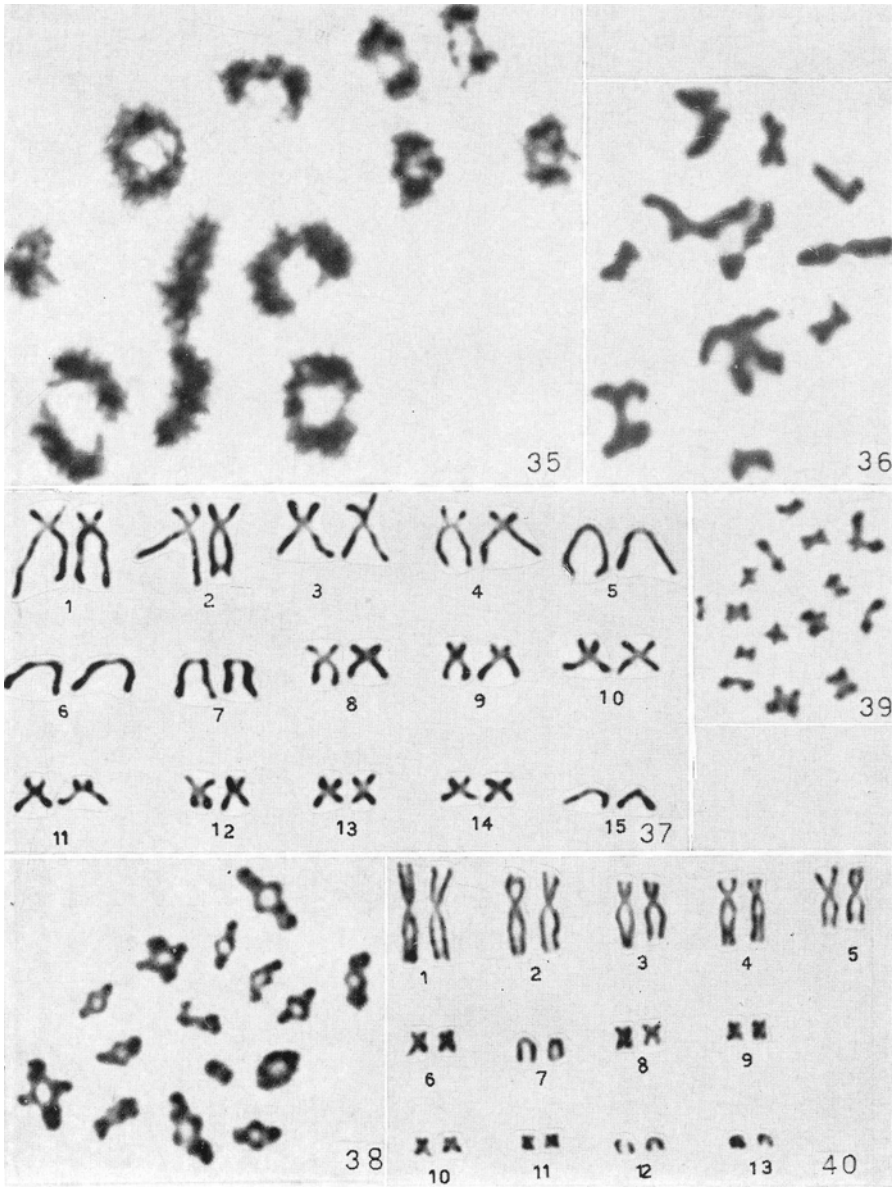

Fig. 35. — *Hyla albopunctata*, male metaphase I (2000×).
Fig. 36. — *Hyla albopunctata*, male metaplase II (2450×).
Fig. 37. — *Hyla albopunctata*, male metaphase II (1950×).
Fig. 37. — *Hyla microps*, female karyotype from spleen cell (1900×).
Fig. 38. — *Hyla microps*, male metaphase I (2150×).
Fig. 39. — *Hyla microps*, male metaphase II (1600×).
Fig. 40. — *Fritziana goeldii*, karyotype from testicle cell (2150×).

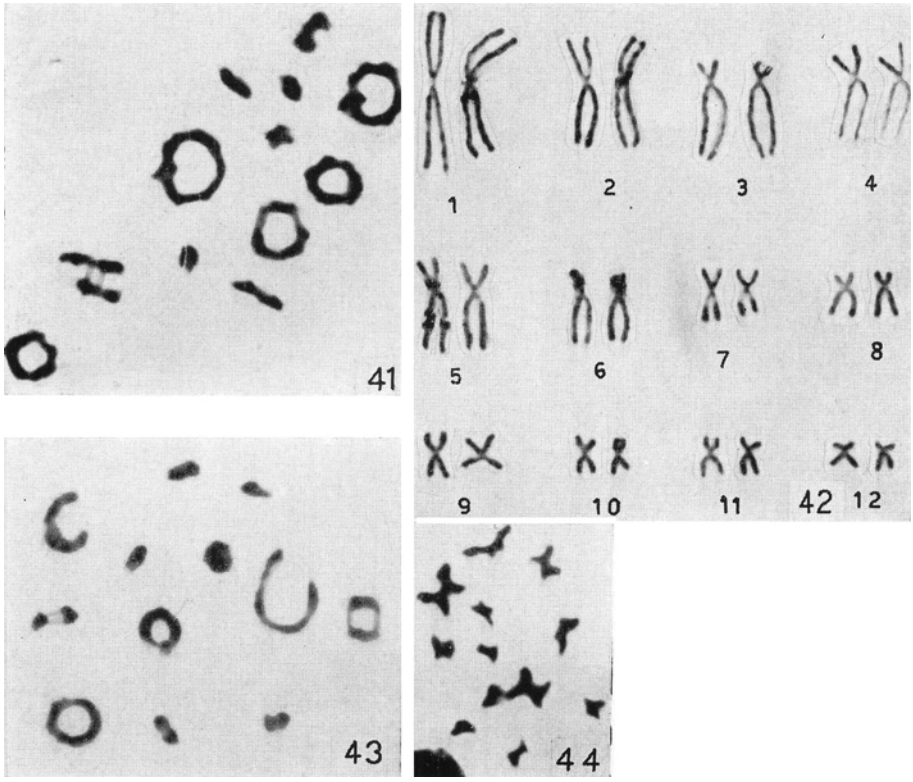

Fig. 41. — *Fritziana goeldii*, male metaphase I (2150×).
Fig. 42. — *Pseudis paradoxus platensis*, male karyotype from whole blood short term culture metaphase (1350×).
Fig. 43. — *Pseudis paradoxus platensis*, male metaphase I (1850×).
Fig. 44. — *Pseudis paradoxus platensis*, male metaphase II (2600×).

Male meiosis showed the same aspects described for the Bufonidae and Leptodactylidae, no sex heterochromosomes being identified. Concerning the ring shaped form of the bivalents, two exceptions were found. In *Hyla pulchella prasina* and *H. albopunctata* one of the bivalents showed an open ring form in nearly all metaphases I (Figs. 25 and 35).

In the family Pseudidae we examined the chromosomes of the species *Pseudis paradoxus platensis* ($2n=24$, $n=12$) (Tables I, V). Sex heterochromosomes were not identified in mitotic cells of either sex nor in meiosis of the male. The pairs 1, 7, 9 and 10 are metacentric, all others being submetacentric. The pair 7 has a secondary constriction near the centromere of the larger arm. During male meiosis complete pairing of homologues in pachytene

TABLE V

Chromosome measurements of Pseudis paradoxus platensis, Pseudidae, based on ten karyotypes.

	1	2	3	4	5	6	7	8	9	10	11	12
Relative length	16	12	11	11	10	8	6	6	6	5	5	4
Arm ratio	1.1	1.6	3.8	2.0	2.0	3.5	1.2	1.9	1.3	1.3	1.5	1.7
Centromere index	.47	.38	.21	.33	.33	.22	.45	.34	.43	.44	.40	.38

and interstitial chiasmata in diplotene have been observed (Figs. 42, 43 and 44).

DISCUSSION

Evolution of karyotype.

Our observations indicate that in the suborder Procoela, chromosome number is very heterogeneous. In Leptodactylidae the numbers found are $2n=18$, $2n=20$, $2n=22$ and $2n=26$. In Hylidae we found $2n=22$, $2n=24$, $2n=26$ and $2n=30$ and in Ceratophrydidae BEÇAK *et al.* (1966, 1967) described 22, 44 and 104 chromosomes. Bufonidae have a uniform diploid number of 22 chromosomes.

In the Hylidae and Leptodactylidae variation of diploid number is followed by morphological alterations of the karyotypes. Comparing the karyotypes of both families we found that in general the smaller the chromosome number of the complement the smaller the number of acrocentrics. Thus, among the Hylidae, *Hyla microps* ($2n=30$) and *Fritziana goeldii* ($2n=26$) showed respectively, 4 and 3 acrocentric pairs. The number of acrocentric pairs of the four species with $2n=24$ is variable, occurring two pairs in *H. faber,* one pair in *H. pulchella prasina* and *H. albomarginata* and none in *H. hayi.* The karyotype of *H. albopunctata* ($2n=22$) did not present acrocentrics. In the Leptodactylidae, *Crossodactylus grandis* ($2n=26$) has two pairs of acrocentrics, *Pseudopaludicola ameghini* ($2n=20$) has one pair of acrocentrics, *Leptodactylus ocellatus* ($2n=22$) and *Eupemphix nattereri* ($2n=22$) have one pair of acrocentrics and *Eleuterodactylus guentheri* ($2n=22$) did not present any one. Apparently centric fusions have occured in species of these families, the decrease of chromosome number being sometimes parallel to the one of acrocentrics. However, in certain species this phenomenon is not

evident, suggesting that further alterations, as pericentric inversions or other types of translocations concealed the perception of centric fusion mechanism. *Pseudopaludicola falcipes* for example which showed the smallest diploid number ($2n=18$) has two acrocentric pairs of chromosomes. In the Bufonidae we did not find any evidence of centric fusion. Uniformity of diploid number and morphology in the karyotypes of the three species was found. The somatic number is of 22 chromosomes, all of them metacentrics and submetacentrics, none acrocentric.

According to WICKBOM (1945) centric fusion has occured with relative frequency during phylogenetic development of Anura. This conclusion is based on the fact that a higher diploid number was found in the more primitive forms, when comparing the Anura of different suborders. Thus, the author compares the karyotypes with 36, 32, 28 and 24 chromosomes of the families Discoglossidae and Pipidae, suborder Opistocoela, to the karyotypes with 22 chromosomes of Bufonidae and to the ones with only 24 chromosomes of Hylidae.

Centric fusion could also be detected in some species studied by us. However good evidences are only at the level of some related species within determined families.

The morphological differentiation of anurans karyotypes and the great variability of the chromosome number from the lowest diploid number known, $2n=18$ in *Pseudopaludicola falcipes* to the highest one of 104 in *Ceratophrys dorsata* suggest that besides centric fusions, other mechanisms of chromosomal modifications have occurred during the evolution of this group. Chromosomal studies on the Ceratophrydidae (BEÇAK *et al.* 1966, 1967) demonstrated that polyploidy played an important role on evolutive differentiation, in this family.

Sex chromosomes.

The occurrence of sex chromosomes in Amphibian is discussed by many authors. Some, assume male heterogamety in Anura (WITSCHI 1924, 1933; YOSIDA 1957). Female digamety was supported by IRIKI 1930, 1932; MINOUCHI and IRIKI 1931; SATO 1934, 1936; BUSHNELL 1939; CALLAN and LLOYD 1960. Yet, others do not accept sex chromosome heteromorphism in this group (STOHLER 1928; GALGANO 1933, 1941; SAEZ *et al.* 1935; WICKBOM 1945; SAEZ and BRUM 1960).

Our observations in the families Bufonidae, Leptodactylidae, Hylidae and Pseudidae showed absence of heteromorphic sex chromosomes in this group of vertebrates. Sexual bivalents described in males of Bufonidae (MINOUCHI and IRIKI 1931; WITSCHI 1933; SATO 1936) were not observed in the three species studied.

Among the Hylidae, the species *Hyla pulchella prasina* and *Hyla albopunctata* showed an open ring bivalent in male meiosis. It seems to us that this aspect is due to occasional absence of a distal chiasma. In some spermatocytes I, two distal chiasmata could be observed in every bivalent. In some specimens of the two species most nuclei have only one V-shaped bivalent, but some showed two or more large bivalents with this particular aspect. This configuration is mostly found in large bivalents, therefore it is not specific of a sex chromosome pair.

Sex chromosomes of Amphibia probably are in such a primitive stage of differentiation, as to hinder a cytological identification based on heteromorphism. As shown by MATTHEY (1949), in lower vertebrates, sex chromosomes morphologically recognizable are inexistent. An exception to this rule is the existence of heteromorphic sex chromosomes in female snakes of the families Crotalidae and Colubridae (BEÇAK *et al.* 1962; BEÇAK 1965, 1966).

Aknowledgements. — The author is indebted to Dr. W. BEÇAK for his helpful suggestions during the course of this work, to W. BOCKERMAN and Dr. L. D. VIZOTTO who generously supplied the classified animals used in this investigation, to N. RABELLO and W. JORGE for their efficient assistance, to S. HELLER for her editorial aid, and to M. KUCHENBUCH for typewriting.

REFERENCES

BEÇAK M. L., BEÇAK W. and RABELLO M. N., 1966. — *Cytological evidence of constant tetraploidy in the bisexual South American frog Odontophrynus americanus.* Chromosoma (Berl.), **19**: 188-193.

—, 1967. — *Further studies on polyploid amphibians (Ceratophrydidae). I Mitotic and meiotic aspects.* Chromosoma (Berl.), **22**: 192-201.

BEÇAK W., 1965. — *Constituição cromossômica e mecanismo de determinação do sexo em ofídios sul-americanos. I. Aspectos cariotípicos.* Mem. Inst. Butantan, **32**: 37-78.

—, 1966. —*Constituição cromossômica e mecanismo de determinação do sexo em ofídios sul-americanos. II. Cromossomos sexuais e evolução do cariótipo.* Mem. Inst. Butantan, **33**: 775-798.

BEÇAK W., BEÇAK M. L. and NAZARETH H. R. S., 1962. —*Karyotypic studies of two species of South American snakes (Boa constrictor amarali* and *Bothrops jararaca).* Cytogenetics, **1**: 305-313.

BEÇAK W., BEÇAK M. L., NAZARETH H. R. S. and PECCININI D., 1964. — *Chromosomes of cold-blood animals from whole short- term cultures. Microtechnique.* Mamm. Chrom. Newsletter, **14**: 55-56.

BEÇAK W., BEÇAK M. L., LAVALLE D. and SCHREIBER G., 1957. — *DNA content and nuclear volume of polyploid species of Ceratophrydidae (Amphibia-Anura).* Chromosoma (Berl.), **23**: 14-23.

BUSHNELL R. J., BUSHNELL S. P. and PARKER M. V., 1939. — *A chromosome study of five members of the family Hylidae.* J. Tenn. Acad. Sc., **14**: 209-215. (Biol. Abstr., 1939, 10722).

CALLAN H. G.: and LLOYD L., 1960. — *Lampbrush chromosomes, in* Walker P. M. B. New Approaches in Cell Biology, Academic Press, pp. 23-46.

GALGANO M., 1963. — *Evoluzione degli spermatociti di I ordine e cromosomi pseudosessuali in alcune specie di anfibi.* Arch. Ital. Anat. Embriol., **32**: 171-200.

GALGANO M., 1941. — *La variazione dei chiasmi nei maschi e negli intersessuati di Rana esculenta* L. Arch. Ital. Anat. Embriol., **56**: 127-165.

IRIKI S., 1930. — *Studies on Amphibian chromosomes I. On the chromosomes of Hyla arborea japonica Guenther.* Mem. Coll. Sci. Kyotto Imp. Univ. Ser. B., **5**: 1-17.

—, 1932. — *Studies on Amphibian chromosomes. IV. On the chromosomes of Rana rugosa and Rana nigromaculata.* Sci. Rep. Tokyo Burn. Daig. Sec. B., **1**: 61-72.

MATTHEY R., 1949. — *Les chromosomes de vertébrés.* F. Rouge-Lausanne, p. 356.

MINOUCHI O. and IRIKI S., 1931. — *Studies on Amphibian chromosomes. II. On the chromosomes of Bufo bufo japonicus Schelegelii.* Mem. Coll. Sci. Kyotto Imp. Univ. Serv. B., **6**: 39-50.

SAEZ F. A., ROJAS P. and DE ROBERTIS E., 1935. — *El problema de los cromosomas sexuales en los anfibios.* Rev. Soc. argent. Biol., **11**: 173-176.

SAEZ F. A. and BRUM N., 1960. — *Chromosomes of South American Amphibians.* Nature (Lond.), **185**: 945.

SATO I., 1934. — *Preliminary notes on the chromosomes of Rana limnocharis Wiegman.* Proc. Imp. Acad. (Tokyo), **9**: 8.

—, 1936. — *Notes on the chromosomes of Cacopoides tornieri and Bufo radderi.* Zool. Mag., **48**: 958-960.

STOHLER R., 1928. — *Cytologische Untersuchungen an den Keimdrüsen Mitteleuropäischer Kröten (Bufo viridis Laur., B. calamita Laur. und B. vulgaris Laur.).* Z. Zellf. mikr. Ant. Gewebelehre, **7**: 400-475.

WICKBOM T., 1945. — *Cytological studies on Dipnoi, Urodela, Anura and Emys.* Hereditas, **31**: 241-346.

WITSCHI E., 1924. — *Die Entwicklung der Keimzellen bei Rana temporaria L. - I Urkeimzellen und Spermatogenese.* Z. Zellf. mikr. Ant. Gewebelehre, **1**: 523-361.

—, 1933. — *Contribution of the cytology of Amphibian germ cells. I. Chromosomes in the spermatocyte division of five North American species of toads.* Cytologia (Tokyo), **4**: 174-181.

YOSIDA T. H., 1957. — *Sex chromosomes of the tree frog, Hyla arborea japonica.* J. Fac. Sci. Hokkaido Univ. Serv. VI Zoology, **13**: 352-358.

SUMMARY

Karyotypes of eighteen species of Anura from Brazil have been studied (Bufonidae, Leptodactylidae, Hylidae, and Pseudidae).

Leptodactylidae show a variation of chromosomal number from $2n = 18$ to $2n = 26$. Hylidae presents also variations from $2n = 22$ to $2n = 30$. These differences were correlated to morphological alterations of the karyotypes. Bufonidae, however, disclosed a great chromosomal uniformity, showing the same diploid number, $2n = 22$. In Pseudidae one species showed $2n = 24$ chromosomes. *Pseudopaludicola falcipes* has $2n = 18$. It seems to be the lowest chromosome number described in Anura.

Variation of number and shape of chromosomes in Hylidae and Leptodactylidae suggests that the mechanism of centric fusion played a role in karyotype differentiation. This mechanism could not be evidenced in the Bufonidae.

Centric fusion however is not the only mechanism in amphibian evolution. Indeed, the occurence of natural bisexual polyploid species in the family Ceratophrydidae (BEÇAK M. L. *et al.* 1966, 1967; BEÇAK W. *et al.* 1967), shows that duplication of the whole genome is another mechanism of differentiation in Anura.

Heteromorphic sex chromosomes, were not observed in any species, and no bivalents with characteristic morphology and behavior of sex bivalent during male meiosis was observed.