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FROM FIVE SPECIES OF THE GENUS RANA

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The karyotypes of five species of ranid frogs were determined from metaphase plates of bone marrow cells. The colchicine, hypotonic-citrate, blaze-dry technique was used to obtain the plates. Morphometric data were obtained from the karyotypes and compared with other species of the genus Rana.

Like most other members of the genus Rana, the five species examined in this study have 26 chromosomes. These can be divided into a group of five large pairs and a group of eight small pairs. Relative chromosomal length is similar in all species. However, differences are apparent. Rana aurora has more metacentric chromosomes than the other species. R. cascadae has one pair of subtelocentric chromosomes and R. muscosa has three pairs of telocentric chromosomes.

Comparisons of chromosomal arm ratios confirm the present taxonomy of the species. R. cascadae is more similar to R. pretiosa

than to R. aurora. R. boylii and R. muscosa can be separated from the other three by two pairs of chromosomes.

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A Comparative Study of the Chromosomes  
from Five Species of the Genus Rana

by

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A COMPARATIVE STUDY OF THE CHROMOSOMES  
FROM FIVE SPECIES OF THE GENUS RANA

INTRODUCTION

Following the development of the colchicine-hypotonic citrate technique, there has been a number of papers dealing with somatic chromosomes of numerous species of the Order Anura. The present study was undertaken to determine the karyotype of five species of the genus Rana from the Pacific Northwest and to make karyotype comparisons which hopefully would unveil enough differences to clarify relationships at the species level.

In the anurans, the use of karyotype analysis as a taxonomic tool meets with varying degrees of success. Matthey (1949) superimposed chromosome numbers and Nombre Fondamental (N. F.) on the anuran phylogenetic scheme of Noble. He suggests that chromosomal evolution is broadly indicated by a decrease in chromosome numbers with the N. F. remaining more or less constant at 50. Wickbom (1950), after cytogenetic studies on Ascaphus truei, considers 24 macro- and 24 micro-acrocentric chromosomes to be the primitive anuran condition. He postulates that centric fusion of macro- to macrochromosomes and micro- to microchromosomes explains the karyotype of the recent anurans. Both Matthey's and Wickbom's schemes on chromosomal evolution rely on the stability of anuran chromosomes.

By chromosomal analysis, Morescalchi (1968) raises questions about the phyletic relationships between the families Ranidae, Rhacophoridae, Microhylidae, and Phrynomeridae, which Goin and Goin (1962) include in the same suborder. He advances a monophyletic theory for the origin of the Microhylidae and Ranidae which, if confirmed with more data, would shed doubt upon the polyphyletic theory of Inger (1967).

Within the single family of Leptodactylidae there is a range of chromosome number ( $2N$ ) from 20 to 26 (Bianchi and Molina, 1967; Robinson and Stephenson, 1967; Brum-Zorilla and Saez, 1968). Of the 27 leptodactylids reported, 20 have 22 chromosomes; five, 26; one, 20; and one, 24. A detailed comparison of the karyotypes of all of the species is lacking and the evolutionary importance of chromosomal rearrangements has not been outlined.

Members of the South American family Ceratophryidae show a marked contrast to the concept of the "stability of anuran chromosomes" (Bogart, 1967). Of the four species studied, Bogart considers Odontophrynus americanus,  $2N = 44$ , a tetraploid species; Ceratophrys ornata,  $2N = 104$ , an octoploid species; and Chacophrys pierotti and Leptodactylus melanonotus,  $2N = 26$ , non-polyploid species. Bogart (1967) states that "O. americanus and C. ornata are the only naturally occurring, polyploid, bisexual species known in the vertebrates." Becak and Becak (1967), by studies of comparative

DNA values also arrived at the same conclusion of polyploidy within the family.

The Family Bufonidae is more consistent; 26 species of the genus Bufo have 22 chromosomes (Makino, 1951; Goin and Goin, 1962; Bogart, 1968; Cole et al., 1968; and Volpe and Gebhardt, 1968). Bogart (1968) found exceptions to the consistency of the Bufo species number. He presented the karyotypes of six species of African toads which had a diploid number of 20. It is Bogart's opinion that the 20-chromosome toads represent a natural species grouping derived from a common ancestor having 22 chromosomes.

With a few possible exceptions among the 30<sup>+</sup> species studied thus far, members of the families Pelobatidae and Ranidae have 26 chromosomes (Wickbom, 1949; Makino, 1951; DiBerardino, 1962; Goin and Goin, 1962; Hennen, 1964; Duellman, 1967; Guillemin, 1967; Roche and Mateyko, 1967; Morescalchi, 1968; Wasserman and Bogart, 1968; Houser, Jr. and Sutton, 1969). In the family Pelobatidae it is possible to separate Scaphiopus holbrookii hurteri, S. couchii and their hybrid by a distinctive marker, different in each species, on the number two chromosome (Wasserman and Bogart, 1968). In the atlas of chromosome numbers Makino shows several species of the genus Rana which possibly deviate from having 26 chromosomes.

The species in question are R. arvalis, R. catesbeiana, R. esculenta, R. fusca, R. pipiens, and R. temporaria ornativentris. With the

exception of R. temporaria ornativentris other workers listed by Makino show these species to have 26 chromosomes (Makino, 1951). DiBerardino (1962) accepts R. t. ornativentris as  $2N = 24$  and feels that the deviations of the others are due to the technique of determining chromosome number by sectioned material, employed 50 plus years ago. More recent authors listing the chromosome number for species of the genus Rana give  $2N = 26$ ; this includes the above species in question except for R. t. ornativentris, to which no further reference is made (Wickbom, 1949; Goin and Goin, 1962; and Duellman, 1967).

However, on the basis of more recent literature, the chromosome number of R. arvalis should still be in question. The original determination was made by Wickbom in 1945 who concluded  $2N = 24$ ; in 1946 Cei concluded  $2N = 26$  (Makino, 1951). In 1949 Wickbom accepted  $2N = 26$ , but listed Cei as the observer. Fortunately, the case of R. arvalis was not laid to rest and a form of chromosomal evolution by translocation in the genus Rana is indicated. In 1967 Ullerich established that R. arvalis has 24 chromosomes. Furthermore, R. arvalis contains six large pairs of chromosomes rather than five, the usual ranid condition. In 1968 a microhylid, Breviceps gibbosus, was described which also has six large pairs and six small pairs of chromosomes and appears to be derived from the generalized karyotype of the Ranidae (Morescalchi, 1968).

Since the majority of ranids have 26 chromosomes, numerology as such shall now be dismissed and other criteria of comparison will be discussed briefly. The following methods described for chromosomal comparisons are widely used in cytogenetics. The literature cited refers only to recent authors who use these methods with ranids.

1. Idiograms present the relative length of the chromosomes, usually in relation to the total length of the haploid genome. Usually the largest chromosome is on the left and the rest positioned by their decrease in length to the shortest on the right and numbered consecutively. This allows comparison of chromosomes from different metaphase plates which have undergone different degrees of contraction or spiralization.

2. Centromeric positions are determined by dividing the length of the short arm into that of the long arm of the chromosome. In 1964 Levan et al. established definite ratio limits of 1.0-1.7, 1.7 to 3.0, 3.0 to 7.0, and 7.0 and above (Levan et al., 1964). Several authors have used these and classified the chromosomes as metacentric, m; submetacentric, sm; subtelocentric, st; and telocentric, t; (e.g. Bogart, 1967; Kiley and Wohnus, 1968). These, too, permit comparison of chromosomes from different metaphase plates.

3. The number and position of secondary constrictions is also important, and may be used for comparative purposes (DiBerardino, 1962; Hennen, 1964; Guillemin, 1967; and Kiley and Wohnus, 1968).

To date there is no conclusive proof of a dimorphic sexual pair of chromosomes among the anurans. In all the papers thus far cited, none was evident. Morescalchi, in a 1963 paper which I have not been able to obtain, argues for sex chromosomes in Xenopus laevis, but Volpe and Gebhardt (1968) find the evidence unconvincing. In a sense, the successful bi-sexual polyploid species described by Bogart would indicate the absence of a dimorphic pair (Bogart, 1967).

## METHODS AND MATERIALS

Metaphase plates were obtained from the following numbers of frogs:

1. Twenty R. cascadae, collected at Summit Springs and Bear Springs, Jefferson County, Oregon; Davis Lake, Klamath County, Oregon; Gold Lake, Lane County, Oregon; and Lava Lake, Deschutes County, Oregon.
2. Nineteen R. aurora collected near Philomath, Benton County, Oregon; 2 1/2 miles south of Hebo, Tillamook County, Oregon; Ferguson Creek, Lane County, Oregon; and 1 1/2 miles north of Winchuck River Camp, Curry County, Oregon.
3. Twenty-two R. pretiosa collected at Davis Lake, Klamath County, Oregon; Gold Lake, Lane County, Oregon; and Big Lava Lake, Deschutes County, Oregon.
4. Seventeen R. muscosa collected at French Meadow Creek, Tollhouse Lake and Coleman Lake, Nevada County, California.
5. Ten R. boylii collected along the Santiam River, Linn County, Oregon; and near Oneil Camp Ground, Siskiyou County, California.

With the exception of R. aurora, the animals were composed of about equal numbers of males, females and immatures. There were fewer males among the R. aurora. Prior to the experiment,



the animals were stored in quart jars at 4°C. The jars contained 1 1/2" of tap water and two or three animals each. The water was changed at approximately weekly intervals to avoid fouling.

Two major techniques for obtaining metaphase plates were compared. In the first, the colchicine-treated squash technique was used to obtain metaphase plates from tail fins of experimentally produced larvae, and from testicular tissue and corneal epithelium of adult frogs. The corneal epithelium technique has been outlined by Bogart (1968).

The second, more efficient, technique was a modification of a colchicine, hypotonic citrate, blaze-dry technique developed for use with mammalian bone marrow (Ford and Hamerton, 1956). The solutions used were: a 0.1% (W/V) aqueous solution of colchicine and a 1.0% (W/V) sodium citrate solution. The colchicine solution was divided into 2ml aliquots and frozen until used. The cells were fixed in Carnoy's solution (acetic-alcohol 1:3) and stained with Giemsa. The preparation of Giemsa stock and working solution followed that outlined by Humason (1962).

Mitosis in bone marrow was negligible without induction. The two methods of induction used were:

1. Use of the hemolytic agent phenylhydrazine (Flores and Frieden, 1968).
2. Removal of the right rear foot at the distal end of the tarsus to

cause bleeding.

The bleeding technique induced mitoses more consistently than did the phenylhydrazine. The largest number of mitoses were obtained when the foot was removed at 2:00 PM rather than earlier or later in the day.

The following procedure was used:

1. The frog was transferred from 4°C to room temperature, and the foot was removed.
2. After 56 hours, the frog was given an intraperitoneal injection of colchicine, using 1 cc colchicine/20g of frog.
3. After eight hours the frog was pithed and the right femur removed. The epiphyses were removed from the femur and the marrow was blown into 4ml of hypotonic sodium citrate. The cells were suspended in the hypotonic solution by aspirating with a pasteur pipette and allowed to stand for 30 minutes.
4. The cells were collected by centrifuging at 1000 rpm for seven minutes. All but 0.5ml of supernatant was carefully aspirated off and the button of cells was resuspended in 4ml of Carnoy's solution and allowed to stand for 20 minutes.
5. Step #4 was repeated.
6. The solution was again centrifuged for seven minutes. All but 0.5-1.0ml of solution was removed and the cells were resuspended in the remaining solution with a pasteur pipette.

7. A drop of the suspension was placed on a precleaned slide, allowed to spread for about ten seconds, and ignited by passing the slide through a flame.
8. The slides were then stained for ten minutes in Giemsa solution. After staining, they were bathed for 30 seconds in each of the following solutions:
  - a. Buffered water (30 seconds).
  - b. Buffered water (30 seconds).
  - c. Acetone (30 seconds).
  - d. Acetone (30 seconds).
  - e. Xylol-acetone (30 seconds).
  - f. Xylol-acetone (30 seconds).
  - g. Xylol (30 seconds).
  - h. Xylol (five minutes).
9. The slide preparations were then mounted in histoclad.

Eight hundred eighty-three metaphase plates were located on the prepared slides. These metaphase plates were divided among the five species as follows: Rana cascadae, 111; R. aurora, 209; R. pretiosa, 239; R. mucosa, 391; and R. boylii, 33. A few additional plates were found which did not have all 26 chromosomes. Presumably, these chromosomes were lost in preparation. Occasionally, isolated chromosomes were found. As many as 78 metaphase plates were examined from one individual frog. Since no

apparent differences were noted, observations were concentrated on fewer numbers of plates from greater numbers of frogs.

The metaphase plates were then photographed using a Leitz microscope and 35mm panatomic x film. Oil immersion was used for all photography. The following numbers of plates were photographed: 103 from 16 different R. cascadae, 139 from 18 different R. aurora, 100 from 23 different R. pretiosa, 100 from 17 different R. mucosa, and 33 from 16 different R. boylii.

Chromosomes were cut from the photographic prints and arranged in order of descending lengths to form karyotypes. The following numbers of karyotypes were formed: R. cascadae, 39; R. aurora, 55; R. pretiosa, 51; R. mucosa, 51; and R. boylii, 15. The karyotypes were formed visually and difficulties in arrangement were resolved by intraspecies comparisons.

Measurements were taken from selected photographically enlarged karyotypes. From these measurements an idiogram (Figure 1) was constructed showing relative lengths, centromere positions, and prominent secondary constrictions. Six karyotypes were used to construct the idiogram for R. cascadae, ten for R. aurora, seven for R. pretiosa, nine for R. muscosa, and three for R. boylii.

## RESULTS

### Karyotype Comparison

The karyotypes of the five species (Plate I) are very similar, but a few differences are apparent, and it is possible to separate the species on the basis of the karyotypes. The chromosomes of Rana aurora (A) are most metacentric throughout the karyotype. The number 7 chromosome of R. cascadae is subtelocentric and the number 13 chromosome has a secondary constriction at the end of the long arm. The number 10 chromosome of R. pretiosa has a secondary constriction on the long arm. The karyotype of R. muscosa is the most unique; the last three pairs of chromosomes are telocentric. The karyotype of R. boylei is quite similar to R. aurora, but chromosome number 13 is submetacentric in R. boylei.

### Morphometric Data

Measurements made from the selected karyotypes are summarized in Table 1. The range of relative lengths shows a sharp break between chromosomes number 5 and 6 for all species. In other words, there are five large chromosomes and eight smaller chromosomes. Chromosome 1 overlaps chromosome 2 in length in only one species, R. muscosa. Chromosome 2 overlaps chromosome 3 in all species

Plate I    The karyotypes of Rana aurora (A), Rana cascadae  
(C), Rana pretiosa (P), Rana muscosa (M), and  
Rana boylii.



Table 1. A morphometric summary of chromosomes from five species of the genus Rana.

Chromosome no.	Range of Relative Length					Average Relative Length					Arm ratio					Chromosome type*				
	A**	C	P	M	B	A	C	P	M	B	A	C	P	M	B	A	C	P	M	B
1	13.8-16.5	15.0-17.3	15.2-18.4	14.1-17.4	15.2-16.3	14.7	15.9	17.0	15.5	15.6	1.2	1.2	1.2	1.2	1.3	m	m	m	m	m
2	11.4-13.3	12.6-13.2	12.0-13.7	12.6-14.4	12.0-12.7	12.3	13.1	13.0	13.5	12.5	1.8	1.9	1.9	1.9	1.9	sm	sm	sm	sm	sm
3	11.3-12.8	11.6-12.4	11.6-13.5	11.6-13.2	12.0-12.7	11.7	12.0	12.8	12.3	12.4	2.1	1.8	1.5	2.1	1.4	sm	sm	m	sm	m
4	10.4-11.5	11.0-12.3	10.3-11.7	10.4-12.6	11.2-11.7	10.8	11.5	11.0	11.3	11.4	1.6	1.9	2.0	1.5	1.8	m	sm	sm	m	sm
5	9.0-10.6	9.0-10.4	9.2-10.4	9.2-11.5	7.9-10.4	9.5	9.3	9.7	10.0	9.5	1.4	1.6	1.5	1.3	1.4	m	m	m	m	m
6	5.4-7.5	5.9-6.7	5.2-5.9	5.4-6.9	6.0-6.1	6.2	6.2	5.6	6.1	6.1	1.5	1.5	1.6	2.0	2.0	m	m	m	sm	sm
7	5.4-6.1	5.1-6.4	4.9-5.8	4.8-6.0	5.6-6.0	5.8	5.6	5.2	5.4	5.8	1.8	4.2	2.8	1.4	1.3	sm	st	sm	m	m
8	5.0-6.2	4.8-5.7	4.8-5.5	5.1-5.8	5.3-5.4	5.6	5.5	5.1	5.4	5.4	1.6	1.9	1.8	1.5	1.8	m	sm	sm	m	sm
9	5.0-5.7	4.7-7.7	4.4-5.1	4.8-5.8	4.5-5.2	5.3	5.6	4.8	5.2	4.9	1.5	1.8	2.7	1.4	2.0	m	sm	sm	m	sm
10	4.4-5.3	4.0-4.8	4.2-4.8	4.7-5.6	4.1-5.0	4.9	4.3	4.5	4.8	4.7	1.5	1.4	1.6	1.2	1.5	m	m	m	m	m
11	3.9-5.2	3.3-4.3	4.0-4.9	3.7-4.5	4.0-4.3	4.6	3.9	4.3	4.2	4.1	1.4	2.0	1.8	>7.0	1.3	m	sm	sm	t	m
12	3.9-4.6	3.3-4.4	3.2-4.4	3.0-3.9	3.9-4.3	4.3	3.9	3.8	3.3	4.1	1.5	1.4	1.6	>7.0	1.3	m	m	m	t	m
13	3.5-4.0	3.1-4.7	1.8-3.9	2.3-3.3	3.3-4.2	3.7	3.6	3.1	2.8	3.6	1.4	1.3	1.9	>7.0	1.7	m	m	sm	t	sm

\*m = metacentric (arm ratio 1.0-1.7)  
 sm = submetacentric (arm ratio 1.7-3.0)  
 st = subtelocentric (arm ratio 3.0-7.0)  
 t = telocentric (arm ratio >7.0)

\*\*A = R. aurora  
 C = R. cascadae  
 P = R. pretiosa  
 M = R. muscosa  
 B = R. boylei



except R. cascadae. Chromosome 3 overlaps chromosome 4 in all species except R. boylei. Chromosome 4 overlaps chromosome 5 in all but R. boylei and R. cascadae. In only one case does an overlap occur between three chromosomes; chromosomes 2 through 4 of R. aurora.

The relatively small amount of overlap between the first five chromosomes makes it possible to separate them on the basis of relative length. However, overlap occurs very extensively in chromosome 8 through 13 in all species, and separation on the basis of length alone is impossible. In these chromosomes, identification of homologous chromosomes has to be based to a large extent on chromosome arm ratios.

#### Composite Idiogram

The composite idiogram (Figure 1) makes possible further comparison between different species. It reflects the similarity in appearance of the karyotypes (Plate I). In addition, it is apparent that the relative lengths of the chromosomes are very similar.

The number 1 chromosome of Rana pretiosa is the longest shown for all species, whereas chromosomes 6, 7, 8, and 9 of R. pretiosa are relatively shorter. Whether this phenomenon is indicative of translocation or fortuitous because of the way the

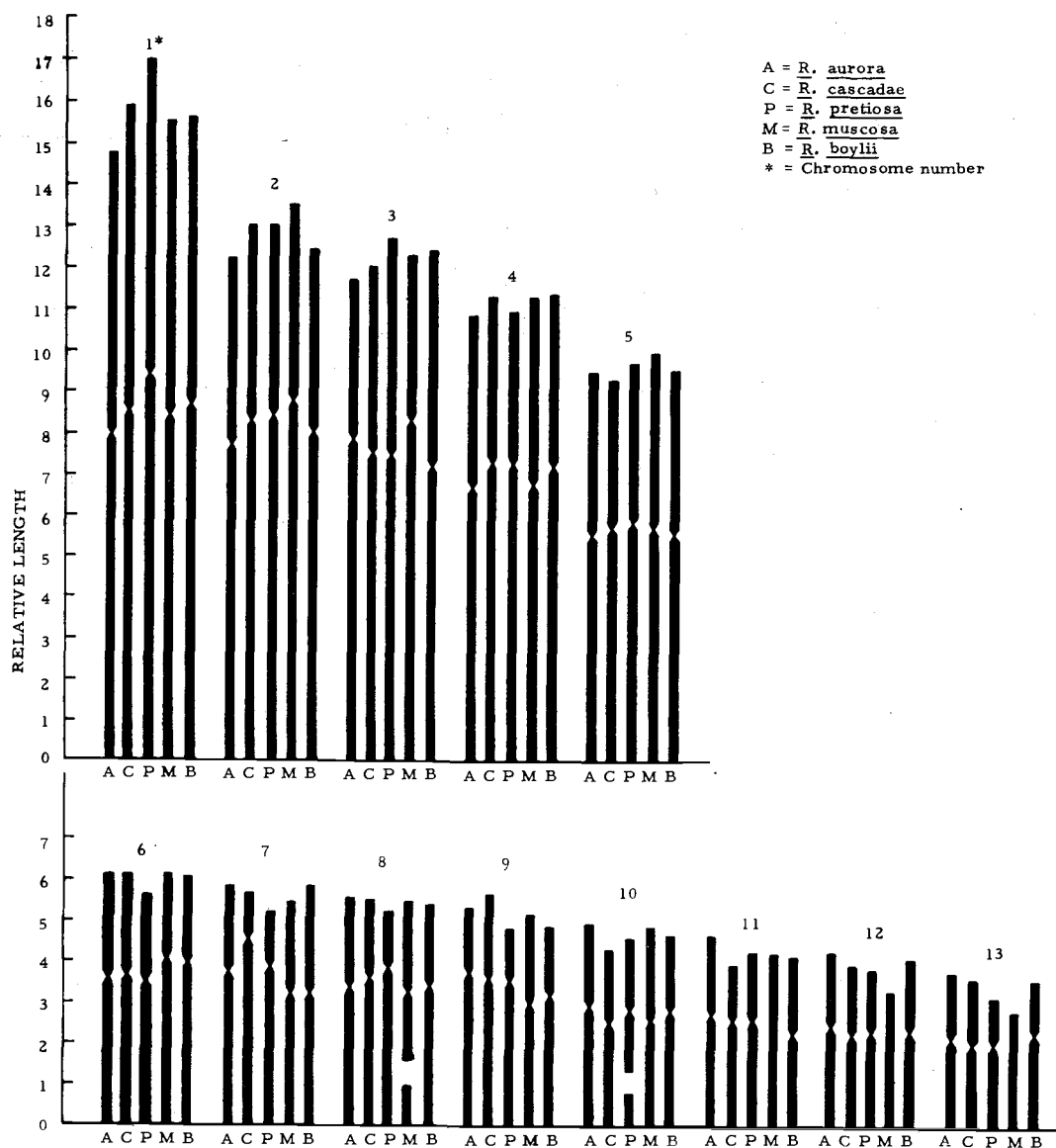


Figure 1. Idiogram of five species of the genus *Rana* based on measurements of metaphase plates from bone marrow cells.

chromosomes were paired is difficult to determine in the absence of definite chromosome markers. Chromosomes 12 and 13 of Rana muscosa are also relatively shorter than those in the other species. Since these chromosomes represent the first telocentric chromosomes found in the genus Rana, they may represent an evolutionary process. If the idiogram is a true genetic picture, i.e., if the length of the chromosome shown is representative of the actual length, the relative shortness would indicate that the formation of the telocentric chromosomes is due to loss of genetic material rather than pericentric inversion.

#### Comparative Arm Ratios

Figure 2 gives a graphic comparison of the arm ratios of the chromosomes of five species of the genus Rana. The arm ratios indicate a similarity between R. cascadae and R. pretiosa in chromosomes 1, 2, 3, 4, 5, 7, and 11. Other species similarities are not equally evident.

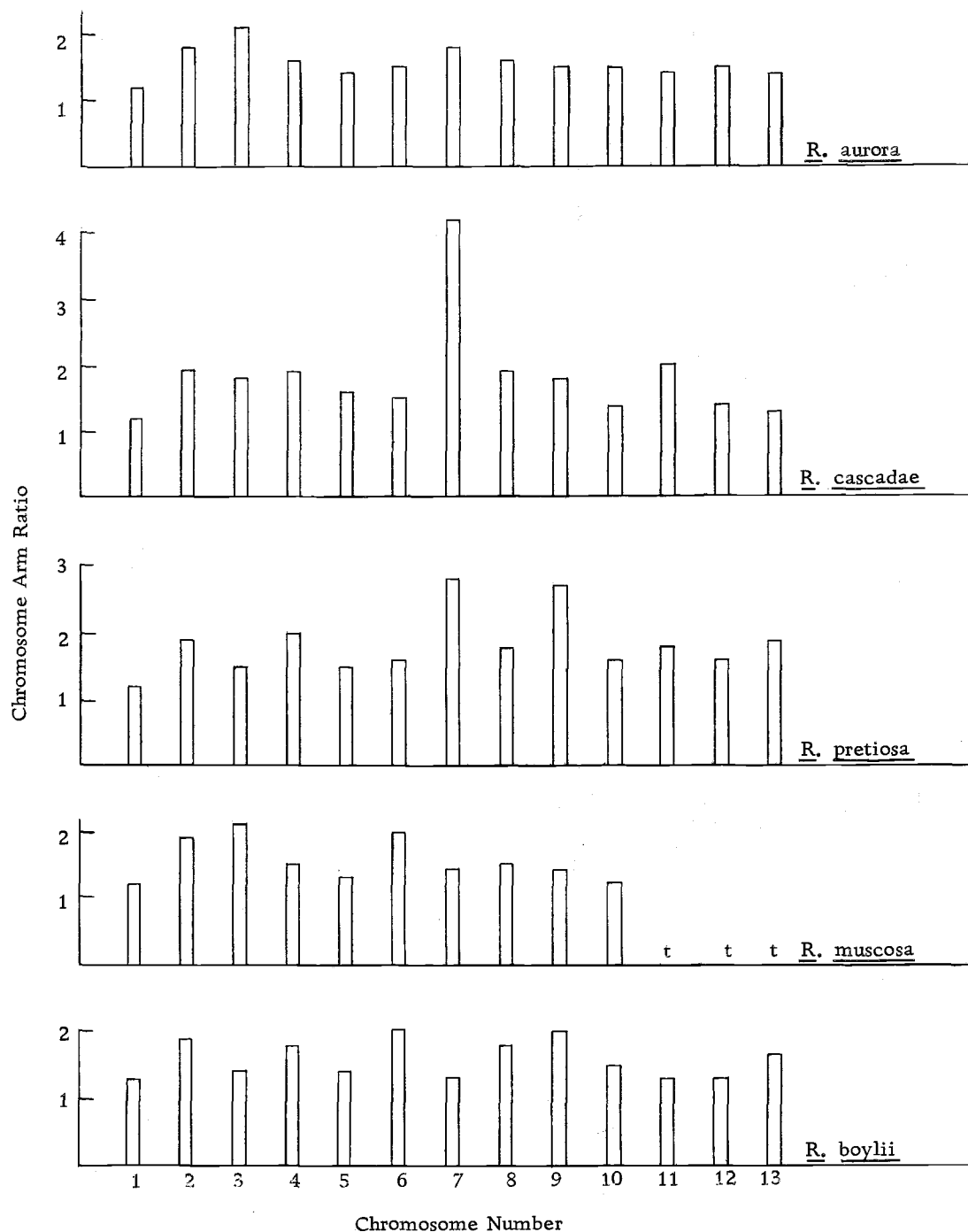


Figure 2. A graphic representation of arm ratios for the  $n$  number of chromosomes from five species of the genus *Rana*. (t = telocentric chromosomes.)

## DISCUSSION

Secondary Constrictions

Secondary constrictions represent one possible means of separation of the different karyotypes. Unfortunately, constrictions were not present in every metaphase plate. They did appear in 74.4% of the R. muscosa and 73.4% of the R. pretiosa metaphase plates. In both cases they were on a long arm of a submetacentric chromosome.

A similar chromosome pair is found in R. pipiens according to DiBerardino (1962) but not according to Kiley and Wohnus (1968), in R. temporaria (Guillemin, 1967), and in R. esculenta (Ullerich, 1967). The number 7 chromosomes of R. sylvatica have prominent secondary constrictions above and below the centromere (Hennen, 1964). In all anuran cytotaxonomic studies secondary constrictions are present on one chromosome or another. Wasserman and Bogart (1968) used them to separate species of Scaphiopus and Robinson and Stephenson (1967) used them as a marker in a leptodactylid. However, the occurrence and position is frequently nebulous. For example, secondary constrictions occurred in 53% of the metaphase plates of R. aurora, but their occurrence was without regularity in respect to position or chromosome. In this

respect, R. aurora is similar to R. dalmatina (Guilleman, 1967).

In the Order Carnivora, the so called "carnivore chromosomes" are identified by secondary constrictions in the short arm of a medium-sized submetacentric chromosome, but the importance in showing relationships within the order is undetermined (Wurster and Benirschke, 1968). In sheep, Bruere and McLaren (1967) found the highest incidence of secondary constrictions on six large metacentric chromosomes, which suggests sites of regular occurrence. They also noticed an increase of occurrence when hypotonic citrate was used in the preparation rather than Hanks balanced salt solution. Bianchi and Molina (1967), in studying a leptodactylid, found that secondary constrictions were late replicating areas. However, they also found other late replicating areas which did not form secondary constrictions.

Most authors suggest that secondary constrictions are nucleolus organizers, but conclusive evidence is needed. Hsu et al. (1967) studied the behavior of the nucleolus organizers in mammalian cells, and proved secondary constrictions to be nucleolus organizers in a number of mammals. The expression of the secondary constriction was variable from cell to cell and tissue to tissue. Electron micrographs indicated that the area of the secondary constriction was not a constriction but was of the same width as the rest of the arm. It was composed of smaller filaments (50-70Å) as opposed to the

filaments (150-200 Å) in the other part of the arm. Since the behavior of anuran secondary constrictions seems to be similar to that of mammalian secondary constrictions, they, too, are probably nucleolus organizers.

When the secondary constrictions are as regular in appearance and on similar sized chromosomes as in R. pipiens, R. temporaria, R. esculenta, R. pretiosa, and R. muscosa, their use as a marker is reliable.

However, when secondary constrictions are less regular in occurrence and position as in R. aurora, they cannot be used as chromosome markers. R. cascadae showed a terminal constriction on the 13th chromosome in 43% of the plates, but this frequency was too low to be considered useful.

#### Morphometric Data

Morphometric data is given in the literature for R. pipiens, R. temporaria, and R. dalmatina (DiBerardino, 1962; Guillemin, 1967); the similarities between these and those of the present study are striking. There is general agreement for the relative lengths of all chromosomes. The centromere position is similar in the first five chromosomes: Chromosome 1 is metacentric in all species, chromosome 2 is submetacentric in all but one species, chromosome 3 is submetacentric in five species and metacentric in three,

chromosome 4 is metacentric in five and submetacentric in three, and chromosome 5 is metacentric in all species.

The centromere positions of the small eight chromosomes show dissimilarities between the species. R. aurora has only one submetacentric among them, all others have a minimum of four. R. cascadae and R. pretiosa show similarity between seven of the eight chromosomes, and they and R. pipiens show similarity between five of the eight chromosomes. R. cascadae, R. pretiosa, and R. temporaria show similarity between three of the eight chromosomes, and R. cascadae, R. pretiosa, and R. dalmatina show similarity between six of the eight chromosomes. R. boylii and R. muscosa are similar in chromosomes 6 and 7 and differ from all the other species with respect to these chromosomes. R. muscosa is unique with its telocentric chromosomes.

#### Cytotaxonomy

The karyotype of the frog family Ranidae seems to be conservative. Karyotype data on 15 genera of lizards in the family Iguanidae also appear conservative (Gorman et al, 1969). Nevertheless, Gorman would agree that inference concerning chromosomal evolution can be made when chromosomal data is compared to well detailed morphological taxonomic data.

One of the most inclusive studies made concerning the



relationships of R. aurora, R. cascadae and R. pretiosa is that by Dumas (1966). He used physiological data, dehydration-hydration ratios, immunological data, precipitin ring tests, electrophoretic separation of blood protein, experimental hybridization, and morphological data to conclude that R. cascadae was a definite species and that it was more closely related to R. pretiosa than to R. aurora. Altig (1969), on the basis of developmental cranial osteology, agrees that R. cascadae should hold specific status and that it is a pretiosa type.

The chromosomal arm ratios (Figure 2) support the above relationship. The distribution of submetacentric chromosomes in R. cascadae is similar to R. pretiosa and markedly dissimilar to R. aurora. The similarity of chromosomes 6 and 7 between R. boylii and R. muscosa which are in turn very dissimilar to chromosomes 6 and 7 of R. pretiosa, R. cascadae, and R. aurora also suggests a relationship. Prior to the work of Zweifel (1955), R. boylii and R. muscosa were considered a polytypic species.

Further detailed studies giving morphometric data are needed before the value of cytotaxonomy at the species level within the genus Rana can be determined. Autoradiographs of metaphase chromosomes showing patterns of labeling with  $H^3$  thymidine, as done with snakes (Bianchi et al., 1969) would aid in pair determination and complement the present study.

### Chromosomal Rearrangements

Within the genus Rana the subtelocentric chromosome of R. cascadae (chromosome number 7) and the three telocentric chromosomes of R. muscosa are unique. It is possible that these evolved through pericentric inversion. Wasserman and Bogart (1968) postulate that this occurred among the large chromosomes of a spadefoot toad. Ohno (1967) postulates that pericentric inversion on the Y or W is the first step in the evolution of heteromorphism of the sex chromosome. However, Roberts (1967) found a positive correlation between the amount of crossing over within pericentric inversions and the degree of egg hatch reduction in Drosophila females heterozygous for pericentric inversion. Also, according to White (1954) it is likely that pericentric inversions will only survive in organisms where chiasmata are strictly confined to some other part of the chromosome.

The idiogram (Figure 1) indicates that the telocentric chromosomes in R. muscosa could also have been formed by a loss of the short arm. The short arm could either have been lost to the genome or translocated to another chromosome pair.

### Sex Chromosomes

No definite heteromorphic pair of sexual chromosomes was found. This reflects the current thinking for the anurans. A

comparison of the male and female idiograms in R. muscosa indicated that the fourth pair of chromosomes was relatively longer in the female than in the male. The arm ratios are also different between male and female, the female arm ratio being lower. This might indicate heterogameity in either the third or the fourth pair of the female R. muscosa and possibly the early evolution of the ZZ/ZW type of sex chromosomes (Ohno, 1967). However, this comparison is based on idiograms formed from only three female and three male karyotypes, and the differences may be fortuitous.

## SUMMARY

The karyotypes, idiograms, and morphometric data for R. aurora, R. cascadae, R. pretiosa, R. boylii, and R. muscosa are given. A comparison of these data with similar data for R. temporaria, R. dalmatina, and R. pipiens indicates a marked similarity between the ranid chromosomes in relative lengths; five large and eight small pairs seem to be the rule. There are also some differences; R. aurora has more metacentric chromosomes than the other species. R. muscosa has three pairs of telocentric chromosomes; this is unique to the genus Rana. R. cascadae possesses one pair of subtelocentric chromosomes, and this is also unique to the genus.

A comparison of the arm ratios indicates that R. cascadae and R. pretiosa are more similar to one another than to R. aurora; this parallels recent taxonomic data for the three species. There are also minor differences which separate R. boylii and R. muscosa from the other ranid species, again a parallel to taxonomic characteristics.

Heteromorphic sex chromosomes were not conclusively demonstrated, although a heteromorphic pair of chromosomes in female R. muscosa may be indicated.

## BIBLIOGRAPHY

- Altig, Ronald Gail. 1969. Developmental cranial osteology of three species of Rana (Anura). Ph.D. thesis. Corvallis, Oregon State University. 81 numb. leaves.
- Becak, Willy and Maria Luiza Becak. 1967. Further studies on polyploid amphibians (Ceratophrydidae). II. DNA content and nuclear volume. *Chromosoma* (Berlin) 23:14-23.
- Bianchi, N. O. and J. O. Molina. 1967. DNA replication patterns in somatic chromosomes of Leptodactylus ocellatus (Amphibia, Anura). *Chromosoma* (Berlin) 22:391-400.
- Bianchi, N. O. et al. 1969. Chromosome replication in four species of snakes. *Chromosoma* (Berlin) 26:188-200.
- Bogart, James P. 1967. Chromosomes of the South American amphibian family Ceratophrydidae with a reconsideration of the taxonomic status of Odontophrynus americanus. *Canadian Journal of Genetics and Cytology* 9:531-542.
- \_\_\_\_\_. 1968. Chromosome number difference in the amphibian genus Bufo: the Bufo regularis species group. *Evolution* 22:42-45.
- Bruere, A. N. and R. D. McLaren. 1967. The idiogram of the sheep with particular reference to secondary constrictions. *Canadian Journal of Genetics and Cytology* 9:543-553.
- Brum-Zorrilla, N. and F. A. Saez. 1968. Chromosomes of Leptodactylidae (Amphibia, Anura). *Experientia* 24:969.
- Cole, C. J., C. H. Lowe and J. W. Wright. 1968. Karyotypes of eight species of toads (genus Bufo) in North America. *Copeia*, 1968, p. 96-100.
- DiBerardino, Marie A. 1962. The karyotype of Rana pipiens and investigation of its stability during embryonic differentiation. *Developmental Biology* 5:101-126.
- Duellman, William E. 1967. Additional studies of chromosomes of anuran amphibians. *Systematic Zoology* 16:38-43.

- Dumas, Philip C. 1966. Studies of the Rana species complex in the Pacific Northwest. *Copeia*, 1966, p. 60-74.
- Flores, Gustavo and Earl Frieden. 1968. Induction and survival of hemoglobin-less and erythrocyte-less tadpoles and young bullfrogs. *Science* 159:101-103.
- Ford, C. E. and J. L. Hamerton. 1956. A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. *Stain Technology* 31:247-251.
- Goin, Coleman J. and Olive B. Goin. 1962. Introduction to herpetology. San Francisco, Freeman. 341 p.
- Gorman, G. C., L. Atkins, and T. Holzinger. 1967. New karyotype data on 15 genera of lizards in the family Iguanidae, with a discussion of taxonomic and cytological implications. *Cytogenetics* 6:286-299.
- Guillemin, Catherine. 1967. Caryotypes de Rana temporaria (L.) et de Rana dalmatina (Bonaparte). *Chromosoma* (Berlin) 21: 189-207.
- Hennen, Sally. 1964. The karyotype of Rana sylvatica and its comparison with the karyotype of Rana pipiens. *Journal of Heredity* 55:124-128.
- Houser, Harold, Jr. and Dallas A. Sutton. 1969. Morphologic and karyotypic differentiations of the California frogs Rana muscous and R. boylei. *Copeia*, 1969, p. 184-188.
- Hsu, T. C., B. R. Brinkley and F. E. Arrighi. 1967. The structure and behavior of the nucleolus organizers in mammalian cells. *Chromosoma* (Berlin) 23:137-153.
- Humason, Gretchen L. 1962. Animal tissue techniques. San Francisco, Freeman. 468 p.
- Inger, R. F. 1967. The development of a phylogeny of frogs. *Evolution* 21:369-384.
- Kiley, Sheila and J. F. Wohnus. 1968. Chromosomal analysis of Rana pipiens and Bufo americanus and their hybrid. *Cytogenetics* 7(1):78-90.