MITOTIC AND MEIOTIC CHROMOSOMES OF MACULATED TOAD, *BUFO REGULARIS*

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Abstract: An analysis of the karyotype of the maculated toad, *Bufo regularis* was based on the chromosome complement of Kidney's cells. The diploid chromosome number is 20. The chromosomes comprise a graded size series divisible into four groups: 8 large metacentrics and submetacentrics, four median length metacentrics and submetacentrics, four small metacentrics, and four small submetacentrics. There is no visible evidence of sex chromosome dimorphism. The most distinctive chromosome is No. 4, a large metacentric chromosome with a pronounced secondary constriction in its short arm. The testicular meiosis revealed 10 bivalents. Silver staining was used to reveal nucleolus organizer regions (NOR). It was located on a pair of large metacentric chromosomes.

Introduction

The analysis of chromosome morphology is fundamental to genome mapping. Amphibians are exceptionally good organisms for karyological studies because most have few and comparatively large chromosomes (Duellman and Trueb, 1994). Most of the work on amphibian cytogenetics has been with conventionally stained chromosomes which allows the determination of size, centromeric position, secondary constrictions and percentage lengths of chromosomes and their arms. Newly developed techniques have provided an insight into the chromosomal location of constitutive heterochromatin, nucleolus organizer region, and ribosomal RNA genes (Goodpasture and Bloom, 1975; Birstein, 1982).

The genus *Bufo* comprises of many species which are found on all the principal temperate and tropical land masses with the exception of Madagscar and the Australian Region (Bogart, 1968). It has generally been disputed that the diploid chromosome complement for the genus is 22 except for *Bufo regularis* group where the diploid number is 20, with some species being polyploids (Bogart, 1972).

This investigation presents the karyotype of the maculated toad, *Bufo regularis* based on a computer-based image analysis system, Micro Measure (Reeves and Tear, 2000).

Meanwhile, standard silver staining method (Gold *et al.*, 1990) was used to reveal nucleolus organizer regions (NOR) in mitotic chromosomes. Meiotic chromosomes of the species was also presented and the significance of the chromosome number 20 in the *Bufo regularis* species is discussed.

Materials and Methods

Three adult males and two adult females were collected from Assiut city and used in the present investigation. All the animals received intraperitoneal injection of a 0.3% colchicine solution (Merck) 14-16 h before they were sacrificed. The amount of the solution injected was between 0.25 and 1.5 ml, depending on the size of the specimens (3-15 cm body length). Aseptically removed kidneys were thoroughly washed with phosphate buffer saline and finally minced in watch glasses with curved-pointed scissors. They were then treated with 0.3% KCl hypotonic solution for about 45 min. Tissues were then removed from hypotonic solution and fixed in cold freshly prepared fixative 3:1 ethanol : glacial acetic acid. Two fixative changes, one hour each were applied. Slide preparations for standard karyotyping were prepared according to the method of solid tissue techniques (Kligerman and Bloom, 1977). Slides were then stained with Giemsa. Metaphases were examined using a bright field Olympus microscope. Selected karyotypes were photographed at a magnification of X 1000. Enlarged photographic prints were prepared at a total magnification of X 2,800. Twenty metaphase spreads were measured and analyzed using the computer application Micro Measure for Windows, Version 3.3 (Reeves and Tear, 2000).

The nuclear organizer regions (NORs) were revealed by standard silver staining (Gold *et al.*, 1990).

For meiotic chromosome preparations tests were dissected out from males, cut into small pieces and then fixed in acetic-ethanol mixture (1:3). A piece of the tissue was then squashed on a clean slide and stained with Giemsa.

Results

An examination of 120 metaphase plates selected at random revealed that the diploid number of this species is 20. Of these metaphases, 110 (92%) had the characteristic count of 20, four (3%) possessed 19 chromosomes and six (5%) contained 18 chromosomes. The 8% hypodiploids can be regarded as broken metaphase plates.

Karyotype analysis was carried out on twenty mitotic figures, from both sexes, in which the individual chromosomes were separate, clearly

recognizable and not exclusively contracted. A representative karyotype is shown in Fig. 1, and the mean characteristics of the twenty analyzed metaphases from both sexes are pooled together in Table 1, as the measurements of the chromosomes were similar. Chromosome measurements were obtained from enlarged photographic prints using the computer application Micro Measure for Windows, Version 3.3 (Reeves and Tear, 2000).

Table (1): Quantitative characteristics of metaphase chromosomes of *Bufo regularis*.

Chromo-some No.	Mean Total length ±S.D.	% of set[a]	Mean long arm ± S.D.	Mean short arm ± S.D.	Arm Ratio	Centro-meric Index	Chromo-some type[b]
1	29.49±0.20	8.79	15.31±0.22	14.18±0.20	1.080	0.481	mt
2	29.19±0.16	8.70	15.12±0.09	14.07±0.22	1.075	0.482	mt
3	28.86±0.32	8.60	16.56±0.26	12.30±0.18	1.346	0.426	mt
4	27.96±0.13	8.34	16.07±0.14	11.98±0.05	1.341	0.428	mt
5	24.96±0.23	7.44	15.68±0.22	9.28±0.18	1.689	0.372	sm
6	24.27±0.29	7.24	15.34±0.10	8.93±0.24	1.718	0.368	sm
7	21.34±0.15	6.36	13.68±0.20	7.66±0.23	1.786	0.359	sm
8	20.77±0.22	6.20	13.54±0.21	7.23±0.23	1.873	0.348	sm
9	17.03±0.19	5.08	8.80±0.22	8.23±0.26	1.069	0.483	mt
10	16.85±0.20	5.02	8.67±0.25	8.18±0.09	1.060	0.485	mt
11	15.31±0.21	4.56	9.72±0.20	5.59±0.25	1.739	0.365	sm
12	14.89±0.16	4.44	9.35±0.19	5.50±0.12	1.707	0.369	sm
13	10.11±0.24	3.01	5.24±0.12	4.87±0.12	1.076	0.482	mt
14	10.02±0.25	2.99	5.22±0.19	4.80±0.08	1.088	0.479	mt
15	8.43±0.18	2.51	4.50±0.13	3.53±0.15	1.145	0.466	mt
16	7.94±0.20	2.37	4.12±0.12	3.82±0.11	1.079	0.481	mt
17	7.52±0.19	2.24	4.96±0.16	2.56±0.10	1.938	0.340	sm
18	6.98±0.11	2.08	4.64±0.20	2.34±0.16	1.983	0.335	sm
19	6.83±0.12	2.04	4.18±0.09	2.65±0.14	1.577	0.388	mt
20	6.68±0.15	1.99	4.12±0.13	2.56±0.08	1.609	0.383	mt
Totals of	335.43						

[a] (length of chromosome total / length of diploid genome) x 100

[b] Chromosome type: mt, metacentric; sm, submetacentric - Based on Levan *et al.* (1964) and Green *et al.* (1980).

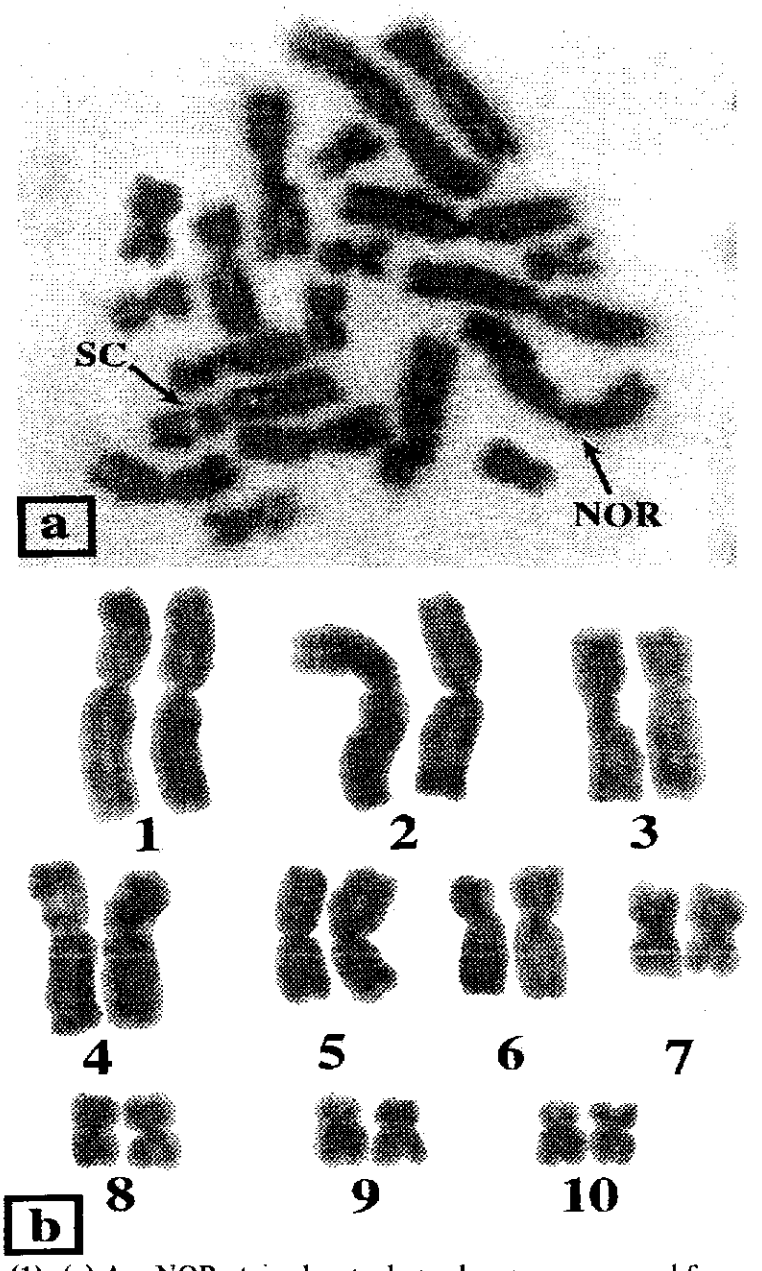

Figure (1): (a) Ag- NOR-stained metaphase chromosome spread from male *B. regularis* counterstained with Giemsa.
(b) Karyotype of *B. regularis* from chromosomes shown in (a).

Figure (2): An idiogram of *Bufo regularis* chromosomes.

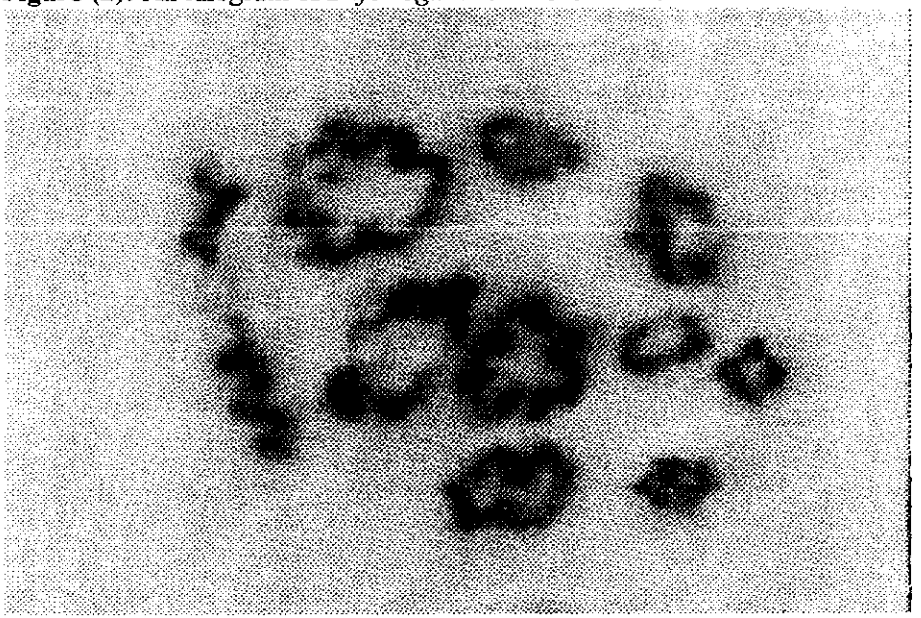

Figure (3): Diakinesis cell of *Bufo regularis* showing ten bivalents.

The chromosomes were sorted by size and centromeric index and are divided into four groups (Fig. 1). Group I consists of four pairs of relatively long chromosomes, two pairs having median centromeres, the other two pairs with submedian centromeres. Group II comprises two pairs of medium-length metacentric and submetacentric chromosomes. Group III contains two small metacentric pairs, whereas group IV constitutes two small submetacentric pairs of chromosomes. The No. 4 chromosome is unique in possessing a prominent secondary constriction in the middle portion of its short arm. The constriction takes the form of a simple, transverse, heterochromatic break in the chromosome body. There is no evidence of a heteromorphic sex pair in either the female or the male. When metaphase chromosomes from karyotypes of both sexes were compared, they do not differ in any respects.

An idiogram showing comparative lengths, positions of centromeres and the secondary constriction in the No. 4 chromosome is portrayed in Fig. 2.

The NORs were located on a pair of large-sized metacentric chromosomes (Fig. 1).

Cytological analysis of testicular meiosis confirmed the diploid count of 20. Figure 3 shows a diakinesis cell of *Bufo regularis* comprising nine closed-ring bivalents and one rod-bivalent.

Discussion

Interest in the chromosome complement as cytogenetic character had led to the investigation of chromosome numbers in about fifty species of *Bufo*. The majority of these species show a diploid number of 22 chromosomes (Bachmann, 1970). *Bufo regularis* had been assigned a chromosome number of 22 by Wickbom (1949) on the basis of examining meiotic prophases. However, new data by Bogart (1968) leave little doubt that *B. regularis* belongs to a group of African toads (Bogart lists seven species) with only ten pairs of chromosomes. At least four other species of African toads have the usual number of eleven pairs. We confirm the diploid count, 20 as revealed by somatic chromosomes prepared from the kidneys of the maculated toad, *Bufo regularis*, as well as meiotic analysis of testicular tissue.

Considering the world-wide consistency of 22 chromosomes in all the members of the genus *Bufo* so far studied, the exception of these African species is striking. It is

conceivable that there was an early dichotomy in Africa such that a change in chromosome number occurred at one time. This would suggest that the species *B. regularis* confined to Africa and possessing 20 chromosomes was derived from a common ancestor having the original number in the genus, 22 (Bogart, 1963).

Computer-assisted karyotyping, as shown in this study provides an important approach to identifying chromosomes since it can shorten errors. The chromosomes obtained by using this method were consistently identifiable. The most conspicuous chromosome is No. 3 by virtue of its pronounced secondary constriction.

The silver (Ag)-staining of the nucleolus organizer regions (NORs) is one of the methods used for demonstrating the position of the gene complex for 185 and 285 ribosomal RNA in the chromosomes (Goodpasture and Bloom, 1975). Due to its specificity, speed and simplicity, Ag-staining is increasingly being employed in cytogenetic studies of vertebrate chromosomes. The distribution and number of Ag-stained NORs were analyzed in a large number of species of the *Anura* (Schmid, 1980). It was shown that most of the primitive and highly evolved *Anura* present only one pair of NORs (Schmid, 1982). The present study revealed that NOR is located on one pair of large metacentric chromosomes of *B. regularis*.

References

Bachmann, K. 1970. Specific nuclear DNA amounts in toads of the genus *Bufo*. Chromosoma (Berl.) 29: 365-374.

Birstein, V.J. 1982. Structural characteristics of genome organization in Amphibians: differential staining of chromosomes and DNA structure. Journal of Molecular Evolution, 18: 73-91.

Bogart, J.P. 1968. Chromosome number difference in the Amphibian genus *Bufo*: the *Bufo regularis* species group. Evolution, 22: 42-45.

Bogart, J.P. 1972. Karyotypes. In "Evolution in the Genus *Bufo*" (W.F. Blais, ed.) pp. 171-195. University of Texas Press, Austin.

Duellman, W.E. and Trueb, L. 1994. Biology of Amphibians. The Johns Hopkins Univ. Press. Baltimore and London.

Gold, J.R.; Li, Y.C.; Shipley, N.S. and Powers, P.K. 1990. Improved methods for working with fish chromosomes with a review of metaphase chromosome

banding. Journal of Fish Biology, 37: 563-575.

Goodpasture, C. and Bloom, S.E. 1975. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma (Berl.), 53: 37-50.

Green, D.M.; Bogart, J.P.; Antony, E.H.; and Genner, D.L. 1980. An interactive microcomputer-based karyotype analysis, system for phylogenetic cytotaxonomy. Comput. Biol. Med. 10; 219-227.

Kligerman, A.D. and Bloom, S.E. 1977. Rapid chromosome preparations from solid tissues of fishes. J. Fish Res. Bd. Can., 34: 266-269.

Levan, A.; Fredga, D.; and Sandberg, A.A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220.

Reeves, A. and Tear, J. 2000. Micro Measure for Windows, Version 3.3. Free program distributed by the authors over the Internet from http://www.colostate.edv/depto/Biology/Micro Measure.

Schmid, M. 1980. Chromosome banding in Amphibia. Chromosoma (Berl.), 77: 83-103.

Schmid, M.; löser, C; Schmidtk, J; Engel, W. 1982. evolutionary conservation of a common pattern of activity of nucleolus organizers during spermatogenesis in vertebrates. Chromosoma (Berl.), 86: 149-179.

Wickbom, T. 1949. Cytological studies on Dipnoi, Urodela, Anura and Emys. Hereditas (Lund), 31: 241-346.

الكروموسومات الميتوزية والميوزية في الضفدعة(الرقطاء)بفو ريجولاريس

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تم إجراء هذا البحث لتحليل الهيئة الكروموسومية في الضفدعة (الرقطاء) بفو ريجولاريس وقد اعتمد التحليل على فحص الهيئة الكروموسومية في الخلايا الكلوية.

والعدد الكروموسومى لثنائي المجموعة ٢ن هو ٢٠، وتكون الكروموسومات مجاميع متدرجة الحجم يمكن تصنيفها إلى أربعة مجاميع: ثمانية كبيرة وسطية السنترومير وتحت وسطية، وأربعة ذات حجم متوسط وسطية السنترومير وتحت وسطية وأربعة صغيرة وسطية السنترومير، ثم أربعة صغيرة تحت وسطية ولا يوجد دليل مرئي للحالة الثنائية المظهر لكروموسوم الجنس وأكثر الكروموسومات تميزاً هو الكروموسوم الرابع حيث انه يمثل كرموسوم وسطى السنترومير كبير الحجم له منطقة اختناق ثانوي واضحة في ذراعه القصير. وقد بينت دراسة الانقسام الميوزى وجود ١٠ وحدات ثنائية الكروموسوم . وقد استخدم الصبغ بالفضة لإيضاح المناطق المنظمة للنوية حيث تم تحديد موقعها في زوج من الكروموسومات الكبيرة وسطية السنترومير.