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Cytogenetics of the genus *Arvicanthis* (Rodentia, Muridae). 1. *Arvicanthis niloticus* from Republic of Benin (West Africa)

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Abstract

Arvicanthis niloticus is characterized, according to previous karyological studies, by a wide chromosome variability. In distinct populations of its wide area of distribution, the diploid number ranges from between 2 n = 44 and 2 n = 62. The present study analysed the karyotype of 13 *Arvicanthis niloticus* from South Benin, Guinea Gulf (West Africa). The constitutive heterochromatin pattern was evidenced by C-banding and the location of Nucleolar Organiser Regions was assessed by $AgNO_3$ plus G-banding reactions. The G-banding pattern was also performed both on cultured fibroblasts and bone marrow preparations. The 2 n = 62 and the aFN = 74 characterize the South Benin cytotype which was found to be identical to that described for Southern Senegal specimens. A few chromosomal rearrangements (3 pericentric inversions) are enough to transform the Benin cytotype into the ANI-3 cytotype described in animals from Mali and Burkina Faso. A polymorphism of the X chromosome is described; three different forms (i. e. metacentric, submetacentric, and subtelocentric) were observed both in homomorphic and heteromorphic conditions. The problem of intraspecies chromosomal variability in *Arvicanthis niloticus* is discussed.

Introduction

The genus *Arvicanthis* Lesson, 1842 includes numerous species of African grass rats. The systematic relationships within this genus have been a matter of debate since the first revisions by Dollman (1911) and Allen (1939). More recent taxonomical revisions (Rosevear 1969; Dorst 1972; Yalden et al. 1976; Corbet and Hill 1980; Rousseau 1983) disagreed with the opinion of Allen (1939) and proposed a reduction of the number of valid species. Musser and Carleton (1993) consider only five species as valid for the genus *Arvicanthis*, i. e. *A. abyssinicus* (Rüppel, 1842), *A. blicki* Frick, 1914, *A. nairobae* J. A. Allen, 1909, *A. niloticus* (Desmarest, 1822), *A. somalicus* Thomas, 1903. All systematic revisions recognized *Arvicanthis niloticus* as a valid species. Systematic debate has recently been reopened (Volobouev et al. 1988; Granjon et al. 1992) concerning precisely *Arvicanthis niloticus* on the basis of genetic and cytogenetic evidence diversifying the genetic structure of different African populations ascribed to *Arvicanthis niloticus* on the basis of their morphological characters. The diploid number (2 n) and the autosomal fundamental number (aFN) actually vary widely within the species: i. e. 2 n from 62 to 44 and a FN from 62 to 76 (Tab. 1).

The dramatic karyological differences characterizing the Somalian population (2 n = 44; FN = 72) and that of the Central African Republic (2 n = 56; aFN = 60) clearly differen-

tiate them from the others and could justify assigning them to different species. The voucher specimen from Somalia we studied (CAPANNA and CIVITELLI 1988) is deposited in the collection of the Muséum National d'Histoire Naturelle in Paris (no. 1983–840) and can be re-examined. Furthermore, also some of the populations with 2 n = 62 display a different fundamental number after substantial structural modifications of the genome, which also in this case could be an indication of genetic differentiation. This opinion is supported by VOLOBOUEV et al. (1988) who identify the three West African populations they analysed as separate species and have named them with the provisional acronyms ANI-1 (2 n = 62; aFN = 62), ANI-2 (2 n = 58; aFN = 70), and ANI-3 (2 n = 62; aFN = 76). GRANJON et al. (1992) are correctly more cautious in this regard. They do not exclude the possibility that in some cases the difference in the aFN may identify a new species, but consider that more biological evidence in support needs to be collected. The evolutionary status of *Arvicanthis niloticus* seems to be that of a complex of cryptic species.

In such an uncertain context, we deemed it opportune to analyse the chromosome assessment of populations of *Arvicanthis niloticus* from a region facing the Gulf of Guinea, in order to add further evidence to the complex problem of chromosomal variability in *A. niloticus* and to verify the spreading of different cytotypes of the species towards the southernmost part of West Africa.

Material and methods

The 13 animals (4 males and 9 females) studied came from three localities of South Benin: Attogon (cultivated fields, 2° 10′ 03″ E; 6° 43′ 01″ N), Lokossa (open savanna, 1° 37′ 10″ E; 6° 43′ 42″ N), and Toffo (forest, 2° 05′ 58″ E; 6° 49′ 30″ N).

The specimens are held in the collections of the Museum of Comparative Anatomy of the University of Rome "La Sapienza" under the numbers B.0014.92; B.0021.92; B.0024.92; B.0034.92; B.0036.92; B.0037.92; B.0038.92; B.0039.92; B.0042.92; B.0044.92; B.0045.92; B.0047.92; B.0048.92.

Somatic metaphases were obtained both from bone marrow, by means of the method of HSU and PATTON (1969), and from in vitro cultured fibroblasts derived from ear pinna biopsies according to STANYON and GALLENI (1991). Male meiotic patterns were studied in testis preparations according to EVANS et al. (1964). Standard staining of the metaphases was performed using Giemsa 4% in phosphate buffer pH7. Differential staining was also carried out. G-bands were obtained according to SEABRIGHT's (1971) method, and C-bands according to BICKMAN (1979). Nucleolus Organizer Regions (NORs) were enhanced by means of the silver reaction according to HOWELL and BLACK (1980). In order to identify the chromosomes carrying NORs, the silver-stained metaphases were treated also with trypsin to highlight the G-banding.

Table 1. Karyological data on *Arvicanthis niloticus*

Locality	2 n	aFN	References
Egypt	62	62	VIEGAS-PÉQUIGNOT et al. (1983)
Centro African Rep.	56	60	MATTHEY (1965)
Centro African Rep.	58	70	VOLOBOUEV et al. (1987)
Burkina Faso	62	76	VOLOBOUEV et al. (1988)
Mali	62	76	VOLOBOUEV et al. (1988)
Senegal	62	62	VOLOBOUEV et al. (1988)
Senegal (West)	62	64	GRANJON et al. (1992)
Senegal (South West)	62	74	GRANJON et al. (1992)
Senegal (South East)	62	66	GRANJON et al. (1992)
Niger	62	64	GRANJON et al. (1992)
Somalia	44	72*	CAPANNA and CIVITELLI (1988)

* The FN is reported in this case because females only were studied and heterochromosomes were not identified

Results

The autosomal set

The diploid number of *Arvicanthis niloticus* from Benin is 2 n = 62 in all animals studied, and the structure of the karyotype is identical as far as the autosomal set is concerned. Chromosomal polymorphism was found concerning the X chromosome.

The two largest autosomal pairs are composed of large subtelocentric chromosomes, and the third pair by submetacentric chromosomes. The subsequent 23 pairs (from no. 4

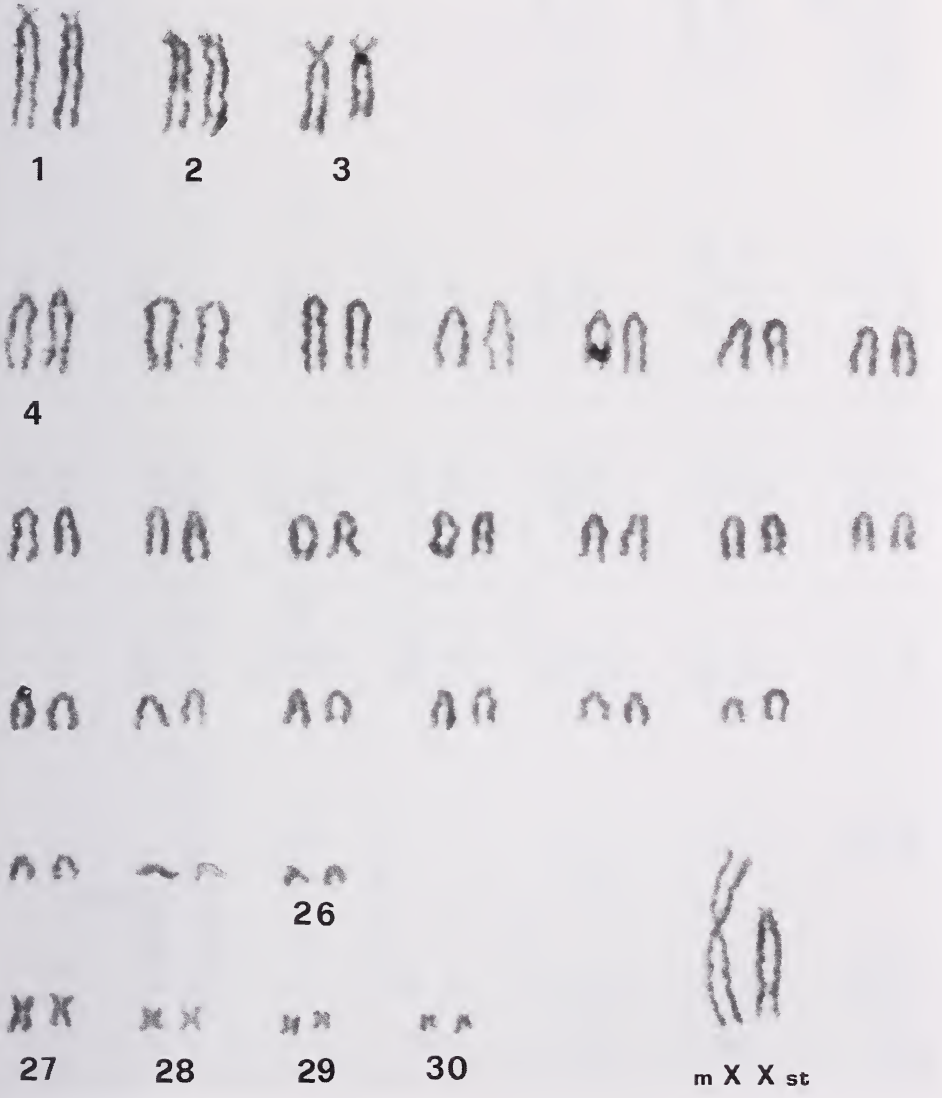

Fig. 1. Giemsa stained karyotype from a female of *A. niloticus*. X chromosomes are in a heteromorphic state [metacentric (m), subtelocentric (st)].

to 26) are acrocentrics, the size of which gradually decreases from medium sized to very small. The last four pairs (from no. 27 to 30) are composed of small metacentrics (Fig. 1). The resulting aFN is 74.

The G-band pattern (Fig. 2) allows accurate pairing of the acrocentric autosomes and assures correct comparison with different R-band patterns suggested by VOLOBOUEV et al. (1988) for the cytotypes they proposed for the West African *Arvicanthis niloticus*.

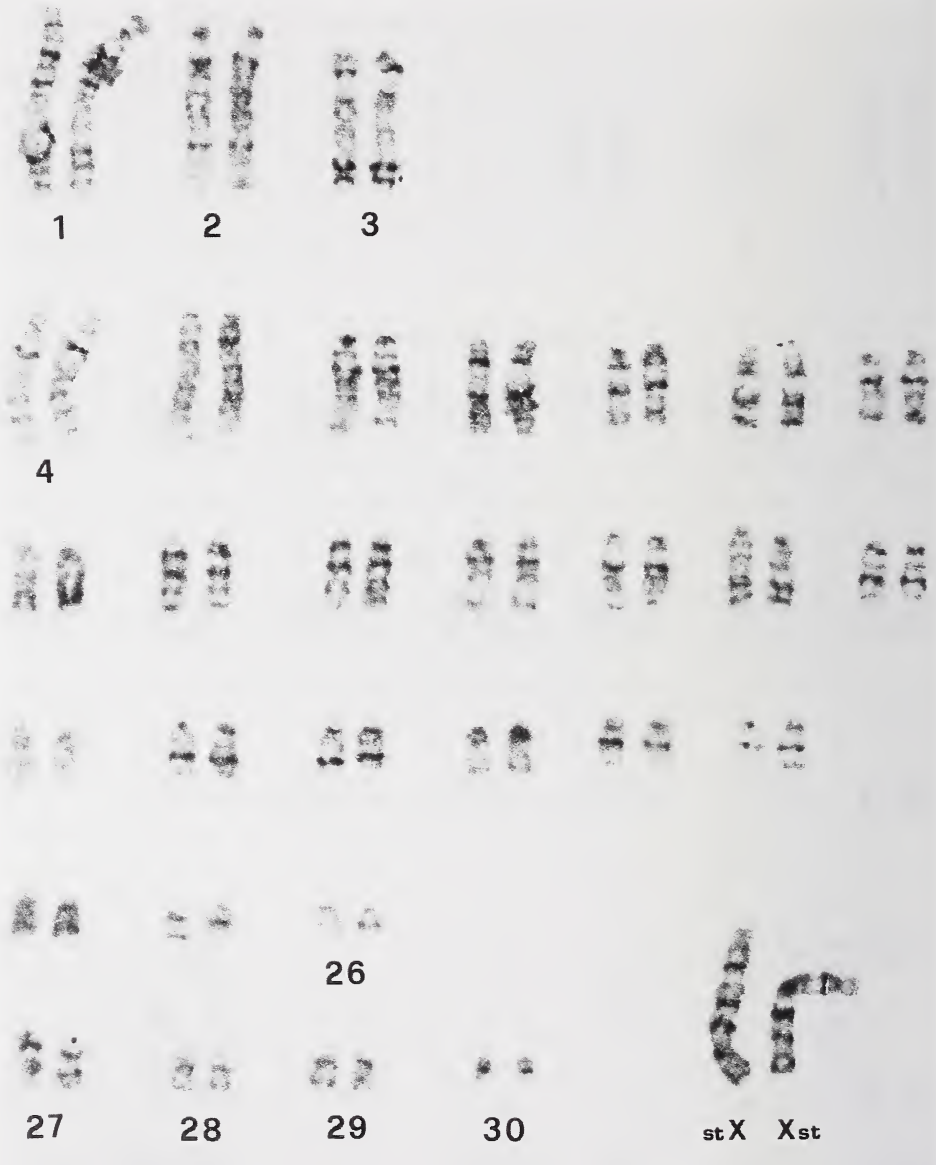

Fig. 2. G-banded karyotype from a female of *A. niloticus*. Both X chromosomes are subtelocentric (st).

Heterochromosomes and X chromosome polymorphism.

The X chromosome appears in three different forms (Fig. 3): metacentric, submetacentric, and subtelocentric. The analysis of the G-bands and the allocation of the constitutive heterochromatin (C-bands) is particularly important in ascertaining the homology of the three forms of the X chromosome. The long arms of the metacentric and submetacentric forms show an identical G-band pattern. Likewise, the pattern of the short arm of the submetacentric X chromosome G-bands corresponds to the closest to centromere part of the short arm of the metacentric form. The short arms of these types of X chromosomes are entirely heterochromatic. Most of the long arm of the subtelocentric form shows the

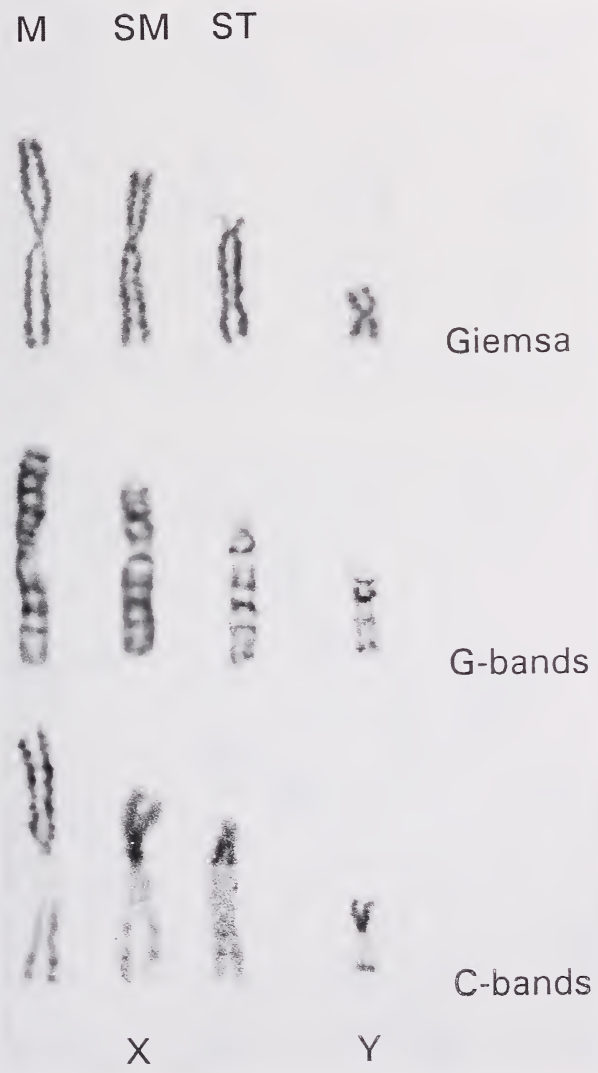

Fig. 3. *A. niloticus* sex chromosomes stained using different methods. M, SM, and ST indicate the different types of X-chromosomes, respectively metacentric, submetacentric, and subtelocentric.

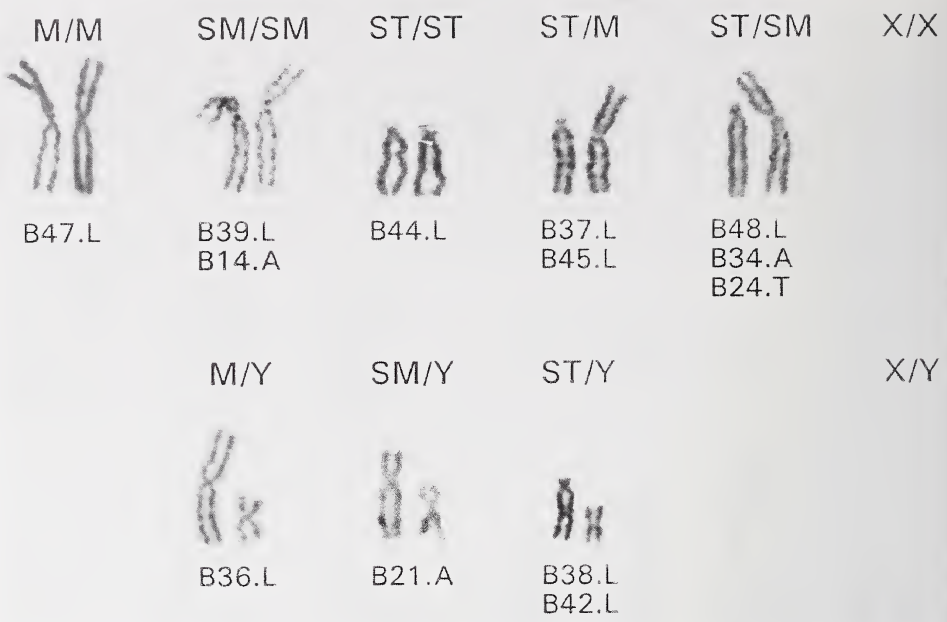

Fig. 4. Sex complements of the specimens studied. For each specimen the acronym and the collecting site is indicated: A – Attogon, L –Lokossa, and T. – Toffo.

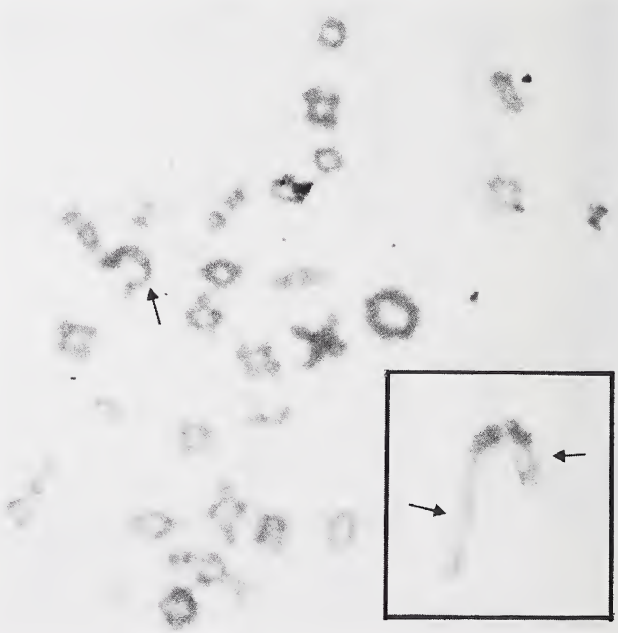

Fig. 5. Meiotic late diakinesis of B21, a Xsm/Y male. The sex bivalent shows one uncoiled tail (arrow). At the diplotene stage (inside the box) the sex bivalent shows two tails (arrows).

same G-band pattern as the long arm of the metacentric and submetacentric form. The rest of the long arm and the short arm are completely heterochromatic.

The small number – i. e. only 13 – of animals analysed does not allow any within-population statistical evaluation of the frequencies of each form. Nonetheless, no form seems to be more extensively represented than any other. In females no greater frequency of the homomorphic state was observed than the heteromorphic one. In males all three forms of X chro-

Fig. 6. C-banded karyotype of a Xst/Y male of *A. niloticus*. Arrow indicate a thin C-band on the distal part of the long arm of the X-chromosome.

mosomes were observed to be paired with a medium size metacentric Y chromosome (Fig. 4). The distal part of the Y chromosome long arm shows a G-band corresponding to the distal part of the long arm of the X-chromosomes. The short arm of the Y chromosome is entirely heterochromatic. An evident C-positive band is located on the distal part of the long arm of the Y chromosome (Fig. 3). At the different stages of the male meiotic process the sex bivalent shows one (at diakinesis) or two (at pachytene-diplotene) unpaired uncoiled tails probably due to the heterochromatic region of the heterochromosomes (Fig. 5).

Heterochromatin pattern and Nucleolar Organizers.

C-banding (Fig. 6) reveals heterochromatic centromeric spots in all autosomes; larger heterochromatic blocks are pericentromerically located on the chromosomes of the three first pairs of biarmed chromosomes, and on two pairs of small metacentrics (pairs 27 and 28).

Fig. 7. Ag-NOR location on autosomes of *A. niloticus*. First row: NOR-bearing chromosomes are identified by combined $AgNO_3$/G-band staining. Numbers indicate the autosomal pairs.

Ag-NORs (Fig. 7) are located on four different pairs of autosomes, i. e. the two largest subtelocentrics (pairs no. 1 and 2), the larger of the acrocentric pair (pair no. 4) and the smaller one of the acrocentric pair (pair no. 20). Pairs no. 1 and no. 20 bear NORs on the short arm, near the centromere, whilst pairs no. 2 and no. 4 show terminal NORs on the long arm.

Discussion

As far as the 2n and aFN are concerned, the karyotype we describe for South Benin animals corresponds exactly to that described by Granjon et al. (1992) for *Arvicanthis niloticus* from a Senegal population located south of the River Gambia. The karyotype coded ANI-3 by Volobouev et al. (1988) found in animals from Mali and Burkina Faso (2 n = 62; aFN = 76) shows remarkable morphological similarity, above all as far as the larger elements, i. e. chromosome pairs no. 1, 2, and 3, are concerned. Nevertheless, these three large chromosomes of the Burkina Faso karyotype display no centromeric heterochromatin, which is instead observed in the corresponding elements of the Benin karyotype. G-band comparison allows the pericentric inversions responsible for the reciprocal changes of the two karyotypes involving chromosomes no. 8, no. 14 and one among the smallest metacentric chromosomes to be identified. Granjon et al. (1992) consider ANI-3 as an intraspecies variant of the karyotype described by them for Southern Senegal animals. This hypothesis is supported by biochemical data provided by Kaminski et al. (1987) concerning genetic affinities between South Senegal and Burkina Faso populations of *Arvicanthis niloticus*. Conversely, the Senegal population, located north of the Gambia River (2 n = 62; aFN = 64–66) could be included in the polymorphous complex ANI-1 (2 n = 62; aFN = 62) of Volobouev et al. (1988). The small numbers of animals analysed by the above authors – only one or two in each locality – do not allow the current existence of these supposed polymorphisms to be ascertained, or to establish whether the chromosomal variants coexist in a Hardy-Weinberg equilibrium. The same limitation on the number of animals studied also ruled out identification of the X chromosome polymorphism. Consequently, the differences in the X chromosome morphology were misinterpreted as a stable element of the karyotype diversity between cytotypes. Both karyotypes described by Granjon et al. (1992) for Senegal *Arvicanthis niloticus* display a submetacentric X chromosome, while all forms of X chromosomes are found in the karyotypes described by Volobouev et al. (1988); i. e. metacentric in ANI-1, submetacentric in ANI-2; and subtelocentric in ANI-3. Our observations, based on the analysis of a relatively large number of animals, demonstrate that the morphological variants of the X chromosomes are maintained in a balanced state in the South Benin population.

No hypothesis can be put forward at present concerning either the direction of the evolutionary process or which form is to be considered primitive. Nonetheless, the chromosomal rearrangement responsible for the change in the three X chromosomes forms appears to be a pericentric inversion of the apical heterochromatic segment of the subtelocentric form that originally produces the submetacentric form. Further addition of heterochromatin can change the submetacentric into metacentrics.

In accordance with the premises set out in the introduction, we have clarified the karyological situation of the *A. niloticus* population of Benin, on the one hand, and, on the other, we have shown how a cytotype of *A. niloticus*, identical to the one described in Southern Senegal, extends as far as the Gulf of Guinea. As far as the premises are concerned, the finding of a self-maintained polymorphism of the X chromosome was an unexpected result.

Nevertheless, the problem of karyotype variability in *Arvicanthis niloticus* in West Africa still appears unresolved. New problems are posed by the extended distribution

from Gambia River to the Guinea Gulf: is this wide distribution continuous? Does a retained chromosomal polymorphism actually exist in this area? Is the ANI-3 cytotype also present in this area? It is not possible to answer these questions without a detailed cytogenetic analysis of the *Arvicanthis niloticus* populations from Burkina Faso to South Benin.

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Zusammenfassung

Zytogenetik der Gattung Arvicanthis (Rodentia, Muridae). 1. Arvicanthis nilotlcus aus der Republik Benin (Westafrika)

Karyotypen von 13 *Arvicanthis niloticus* (4♂, 9♀) aus dem Süden Benins (Westafrika, Golf von Guinea) wurden studiert. Das konstitutive Heterochromatin wurde durch C-Banden und die Lage der NORs durch Silbernitratreaktion und G-Banden sichtbar gemacht. G-Bandenmuster wurden an Fibroblastenkulturen und Knochenmarkspräparaten studiert. Der Karyotyp der untersuchten Population aus dem südlichen Benin ist mit 2n = 62 und aFN = 74 identisch mit dem von Granjon et al. (1992) beschriebenen Karyotyp aus Süd-Senegal. Mit wenigen Schritten (3 perizentrische Inversionen) läßt sich der Benin-Karyotyp überführen in den von Volobouev et al. (1988) als "ANI-3" bezeichneten Karyotyp von Grasratten aus Mali und Burkina Faso. Das X-Chromosom ist polymorph. Es wurden meta-, submeta- und subtelozentrische Formen in homo- und heteromorpher Verbindung vorgefunden. Das Problem intraspezifischer Chromosomenvariabilität bei *Arvicanthis niloticus* wird diskutiert.

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