Genetic variation in *Arvicanthis* (Rodentia: Muridae) from Sudan: an analysis based on cytosystematic and PCR-RAPD data

E.H. ABDEL-RAHMAN AHMED1,2, J. M. LAMB1, G. CONTRAFATTO1 & P.J. TAYLOR3,*

1 School of Biology and Conservation Sciences, George Campbell, Building, University of KwaZulu-Natal, Durban 4041, South Africa
2 Sudan Natural History Museum, University of Khartoum, P.O. Box 321, Khartoum, Sudan 11115
3 Durban Natural Science Museum, P.O. Box 4085, Durban 4000, South Africa

* Author for correspondence: e-mail: taylorpeter@durban.gov.za

Summary

Abdel-Rahman Ahmed, E.H., Lamb, J.M., Contrafatto, G. & Taylor, P.J. 2007. Genetic variation in *Arvicanthis* (Rodentia: Muridae) from Sudan: an analysis based on cytosystematic and PCR-RAPD data. *Durban Museum Novitates* 32: 49-59. The genus *Arvicanthis* Lesson, 1842 (Rodentia; Muridae; Murinae) comprises herbivorous unstriped grass rats widespread in the Nile Valley and West, Central and East Africa. Two species (*A. niloticus* and *A. testicularis*) have previously been reported to occur in the Nile Valley but with many taxonomic uncertainties. This investigation was aimed at clarifying the systematic position of these two putative species in Sudan by using cytogenetic data and random amplification of polymorphic DNA (PCR-RAPDs) fragments. Karyological comparisons of the two putative species, captured in six different regions of Sudan, revealed the existence of one species, *A. niloticus*, with a diploid number of 2n = 62 and N.F.A = 62, identical to karyotyped populations from northeastern Africa (Egypt and Ethiopia), which belong to the known cytotype ANI-1a. The PCR-RAPD protocol used in this study was shown to be consistent, reproducible and highly informative. Analysis of the genetic profiles of samples showed no separation of *Arvicanthis* individuals into “species” or populations (divergence range = 0.05 - 0.19%). Furthermore, t-tests revealed that intra-population divergences were significantly higher than inter-population values (two-tailed t-test for unequal variances; 0.001 < P < 0.05), indicating that genetic variation is not structured geographically. The data obtained from the two systematic methods suggest the absence of reproductive isolation and it therefore appears more appropriate to consider *Arvicanthis* from Sudan to represent a single species, namely *A. niloticus*.

KEYWORDS: Arvicanthis, cytosystematics, DNA fingerprinting, molecular taxonomy, Muridae, RAPD-PCR, Rodentia, Sudan.

Introduction

The taxonomy of the genus *Arvicanthis* Lesson, 1842 (Rodentia; Muridae; Murinae) has remained highly unstable throughout much of the 20th and into the 21st century (Dollman 1911; Thomas 1916; Allen 1939; Ellerman 1941; Setzer 1956; Misonne 1974; Delany 1975; Yalden et al. 1976; Corbet & Hill 1991; Walker 1991; Musser & Carleton 1993, 2005).

Kingdon (1974) recognised two distinct and sympatric East African species of *Arvicanthis*, *A. niloticus* (Geoffroy, 1803) and *A. lacernatus* (although the latter name was later shown to be an example of the gerbil *Meriones*). Delany (1975) reported on two species of *Arvicanthis* (*A. niloticus* and *A. testicularis* (Sundevall, 1843)) in East Africa and along the Nile Valley, that differed in tail length, tooth-row length and either the presence or absence of a white belly. His identification key to differentiate between *A. niloticus* and *A. testicularis* is limited, as there are overlaps in the ranges of each of these measurements and the designation of female specimens is difficult. Afework (1983) reported a difference in maxillary tooth-row crown length between *A. niloticus* and *A. testicularis*. *Arvicanthis testicularis* was later considered to be synonymous with *A. dembeensis* (Ruppell, 1842) by Corbet & Hill (1991), who did not detect any differences between these two alleged forms. Musser & Carleton (1993) regarded *A. dembeensis* and *A. testicularis* to be synonymous with *A. niloticus*. Nevertheless, the occurrence of two species, *A. niloticus* and *A. testicularis*, in the Nile valley was later supported by Fadda & Corti (1998) on craniometric grounds. Protein electrophoretic data (Philippi, 1994) and an integrated morphometric and molecular study (Abdel-Rahman 2005, Abdel-Rahman Ahmed et al. 2008) suggested the presence of only one species from the Nile Valley, namely *A. niloticus*. In the light of these contradictory data, the existence of two species (*A. niloticus* and *A. testicularis*) in the Nile Valley, and in Sudan in particular, is generally considered doubtful (Musser & Carleton 1993, 2005; Philippi 1994; Abdel-Rahman 2005, Abdel-Rahman Ahmed 2008) but requires rigorous testing.
Apart from the North African taxonomic impasse mentioned above, a number of species, such as *A. niloticus sensu lato*, *A. nairobae* Allen, 1909, *A. somalicus* Thomas, 1903, *A. dembeensis* and *A. testicularis*, remain controversial or poorly defined on morphological grounds. Corti & Fadda (1996) examined geometric morphometrics of *Arvicanthis* endemic to the Horn of Africa (*A. abyssinicus*, Ruppel, 1842), *A. blicki* Frick, 1914, *A. dembeensis* and *A. somalicus*) and the population of *A. niloticus* from West Africa. These authors concluded that morphometric divergence neither reflects cladogenetic sequences nor any association with altitude, but rather adaptive patterns that are unique to each species.

On cytogenetic and molecular grounds, Ducroz et al. (1997, 1998) and Volobouev et al. (2002a), recognised *A. niloticus sensu lato* from West Africa to comprise a complex of species including *A. niloticus sensu stricto* (2n = 62, F.N.A = 62 or 64), *A. ansorgei* Thomas, 1910 (2n = 62, F.N.A = 74-76) and *A. rufina* Temminck, 1853 (2n = 62, F.N.A = 76). Also on cytogenetic grounds, Corti et al. (1996) distinguished three East African species: *A. abyssinicus* (2n = 62, F.N.A = 68; endemic to the Ethiopian Plateau), *A. blicki* (2n = 48, F.N.A = 68; endemic to the Ethiopian Plateau) and the widespread *A. niloticus sensu stricto* (which they called *dembeensis*). Castiglio et al. (2003) further distinguished the karyotypes of East African endemics, *A. nairobae* (2n = 62; F.N.A = 78) and *A. neumanni* Matschie, 1894 (2n = 62, F.N.A = 66 or 67 on Ethiopia, in 2n = 53-54; F.N.A = 62 for Tanzania). Musser & Carleton (2005) recognised all of the above species, seven in total: *abyssinicus*, *ansorgei*, *blicki*, *nairobae*, *neumanni*, *niloticus* and *rufina*. Based on uncertainty and absence of critical data from North Africa, they recognised only one species, *A. niloticus* from the Nile Valley (including as synomonyms both *dembeensis* and *testicularis*).

Coming back to the Nile Valley and based on the above, a study including more specimens and more sensitive comparative techniques was needed to resolve the taxonomy of *Arvicanthis* in this region. In particular, since karyotypes have been the key to resolving the taxonomy of this group in West and East Africa, it was critical to obtain karyotypes from across the North African range of *niloticus* and *testicularis*, and thus to test the specific status of *testicularis*. With this goal in mind, our study represents the first attempt to differentiate the two putative species *A. niloticus* and *A. testicularis* from Sudan, using both karyotype analysis and a confirmatory molecular technique, Randomly Amplified Polymorphic DNA (PCR-RAPD). This latter technique is a simple, relatively inexpensive but powerful polymerase chain reaction (PCR)-based technique that requires only small amounts of template DNA (~20 ng) and can be used to detect DNA polymorphism without prior knowledge of the nucleotide sequence (Williams et al. 1990). Further molecular analyses of Sudanese samples involving cytochrome-*b* sequencing are reported elsewhere (Abdel-Rahman Ahmed et al. 2008). Our results, taken with other recently published studies, unequivocally establish the presence of just one species of *Arvicanthis* (*A. niloticus*) in the Nile Valley.

### Materials and Methods

For the purpose of identification, Delany’s (1975) key was used (see Introduction). Although this key is apparently useful in identifying males, it is not reliable for the identification of female specimens due to substantial character overlap. Thus, all females were labelled as *A. sp.*

A total of 34 *Arvicanthis* specimens were karyotyped, including ten male *A. niloticus*, eight male *A. testicularis* and 16 unidentified females from three Sudanese localities (Dongola, Khartoum and Suki, Fig. 1 and Table 1) were determined.

The PCR-RAPD analysis was based on 32 specimens from six localities (Table 2, Fig. 1) that included: 12 male *A. testicularis*, eight male *A. niloticus* and 12 unidentified females. The geographic origin, sample size and the sex of the specimens included in this analysis are presented in Table 1.

### Cytogenetics

Metaphase chromosome preparations were obtained from the bone marrow of 34 specimens (Fig. 1) using the classic technique originally described by Hsu & Patton (1969) and modified by Sumner (1972). Live animals were injected intraperitoneally with a 0.002% colchicine solution (Merck, Germany) in sterile distilled water at a dosage of 0.1 ml/10 g body weight. After 20-40 minutes, the treated animals were

### Table 1. List of sampling localities of *Arvicanthis* used in this study, sample sizes for PCR-RAPD analysis, geographical coordinates, altitude and habitat type. Species identification was based on the key of Delany (1975).

<table>
<thead>
<tr>
<th>Sampling locality</th>
<th>Species(sex)*</th>
<th>Sample size for PCR-RAPDs</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude</th>
<th>Ecological zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suki</td>
<td><em>A. testicularis</em> (M)</td>
<td>1</td>
<td>13° 19' N</td>
<td>33° 54' E</td>
<td>440 m</td>
<td>Savannah</td>
</tr>
<tr>
<td></td>
<td><em>A. sp. (F)</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medani</td>
<td><em>A. testicularis</em> (M)</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. niloticus</em> (M)</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. sp. (F)</em></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khartoum</td>
<td><em>A. testicularis</em> (M)</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. niloticus</em> (M)</td>
<td>1</td>
<td>15° 40' N</td>
<td>33° 25' E</td>
<td>380 m</td>
<td>Semi-desert</td>
</tr>
<tr>
<td></td>
<td><em>A. sp. (F)</em></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>El-Sabaloga</td>
<td><em>A. testicularis</em> (M)</td>
<td>5</td>
<td>17° 34' N</td>
<td>33° 26' E</td>
<td>350 m</td>
<td>Semi-desert</td>
</tr>
<tr>
<td></td>
<td><em>A. niloticus</em> (M)</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. sp. (F)</em></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shendi</td>
<td><em>A. testicularis</em> (M)</td>
<td>3</td>
<td>16° 42' N</td>
<td>33° 29' E</td>
<td>360 m</td>
<td>Semi-desert</td>
</tr>
<tr>
<td></td>
<td><em>A. sp. (F)</em></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dongola</td>
<td><em>A. testicularis</em> (M)</td>
<td>1</td>
<td>19° 10' N</td>
<td>30° 29' E</td>
<td>228 m</td>
<td>Desert</td>
</tr>
<tr>
<td></td>
<td><em>A. sp. (F)</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*M* = male; *F* = female; *k* = specimen karyotyped and also used for PCR-RAPDs. Additional specimens not shown in this table were also karyotyped (2n=34; see Materials and Methods)
Table 2. Intra-population nucleotide sequence diversity of *Arvicanthis* populations from Sudan.

<table>
<thead>
<tr>
<th>Population</th>
<th>ND ± SD x 10^-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dongola</td>
<td>1.67 ± 2.5</td>
</tr>
<tr>
<td>Shandi</td>
<td>2.09 ± 2.6</td>
</tr>
<tr>
<td>Sabaloga</td>
<td>2.14 ± 2.2</td>
</tr>
<tr>
<td>Khartoum</td>
<td>1.71 ± 2.2</td>
</tr>
<tr>
<td>Madeni</td>
<td>2.34 ± 2.3</td>
</tr>
<tr>
<td>Suki</td>
<td>1.32 ± 1.9</td>
</tr>
</tbody>
</table>

SD = Standard Error and ND = nucleotide diversity

Table 3. Nucleotide sequence divergences between *Arvicanthis* populations from Sudan.

<table>
<thead>
<tr>
<th>Location</th>
<th>Suki</th>
<th>Shendi</th>
<th>Sabaloga</th>
<th>Khartoum</th>
<th>Madeni</th>
<th>Dongola</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suki</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shendi</td>
<td>0.0186</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sabaloga</td>
<td>0.0154</td>
<td>0.0076</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khartoum</td>
<td>0.0188</td>
<td>0.0086</td>
<td>0.0052</td>
<td>0.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Madeni</td>
<td>0.0142</td>
<td>0.0071</td>
<td>0.0078</td>
<td>0.0076</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>Dongola</td>
<td>0.0129</td>
<td>0.0129</td>
<td>0.0108</td>
<td>0.0129</td>
<td>0.0111</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Diploid numbers were obtained by establishing the modal number of chromosomes from chromosome counts of no less than 20 metaphase spreads per specimen, using Gimp version 1.2.3. These were arranged according to size and morphology (see Massarini et al. 1991) following the arrangement of published *Arvicanthis* karyograms (Volobouev et al. 2002a).

PCR-RAPDs

DNA was isolated from either liver, heart, kidney or muscle tissues preserved in 80% ethanol by the CTAB method of Winnepenninckx et al. (1993). DNA concentrations and purities were measured and assessed spectrophotometrically following Sambrook et al. (1989).

The concentrations of the components of the PCR-RAPD reactions were optimised prior to sample analysis as the quality of amplification is a function of the concentration of parameters such as template, primer, enzyme, dNTP and MgCl₂ (Carlson et al. 1991; Devos & Gale 1992). Four separate amplifications were performed using the same DNA samples in order to check for repeatability. RAPD reactions were set up in sterile 200 μl thin-walled tubes by addition of 8 μl of diluted DNA template (20 ng) and 16 μl of master mix to attain a final volume of 24 μl. Each reaction contained primers (Operon Technologies, Kit A, numbers 1 - 20) (0.4 μM), deoxynucleoside-triphosphate mixture (0.2 mM), MgCl₂ (4 mM), 1X Stoffel reaction buffer and 1.6 Units of Taq DNA polymerase (Stoffel Fragment). The following thermal cycling
Genetic Variation in *Arvicanthis* from Sudan

Cytogenetics

All male specimens identified as *A. niloticus* and *A. testicularis* using the identification key of Delany (1975) and all unidentified females, had an indistinguishable karyotype as illustrated in Figure 2. These Sudan populations of *Arvicanthis* showed a diploid number of 2n = 62 and a fundamental number (N.F.A) of 62. The karyotype comprises 29 acrocentric pairs and one metacentric pair (pair 25) of autosomes. One large, submetacentric chromosome was assumed to represent the X chromosome while the Y gonosome was tentatively identified as a small acrocentric. There were no differences in chromosome morphology in any of the karyograms examined, nor were heterozygosities detected.

The C-banding patterns of this karyotype of *Arvicanthis* from Sudan are shown in Figure 3 where a prominent, centromeric block of heterochromatin is evident in all autosomal pairs. Additionally, telomeric heterochromatin was apparent in some of the largest pairs. The X chromosome showed a wholly heterochromatic short arm while the Y chromosome did not display any peculiar pattern that could be used for identification.

Chromosome measurements were not useful in the identification of chromosome pairs because length variation between similar-sized autosomes was, on average, two percent of chromosome length (one or two pixels). Such variation was

Results

Genetic similarity was calculated following a formula modified from Nei & Li (1979) The phenogram obtained was based on the clustering of genetic similarity using the Unweighted Pair Group Method using Arithmetic Averages (UPGMA). The Sequential, Agglomerative, Hierarchical and Nested (SAHN) algorithm was used for cluster analysis. Nucleotide divergences between *Arvicanthis* populations and nucleotide diversity within each population were computed by using the presence/absence data matrix as input to the programme RAPDIP (Clark & Lamigian 1993). A triangular matrix was created from this output and diversities of inter-population relationships summarised using SAHN and a UPGMA cluster analysis. A cophenetic correlation coefficient was used to determine the goodness of fit of the phenogram to the original data.

The degree of nodal support in the UPGMA phenogram was estimated by bootstrap re-sampling (1000 replicates) using the program RAPDDIST (Blank & Antolin 1997). The 1000 matrices generated by RAPDDIST were used as input data to NEIGHBOR program in PHYLIP3.5-pc (Felsenstein 1993) to create a set of 1000 ‘pseudo-replicated’ dendrograms. From these, a consensus tree was generated by the CONSENSE algorithm in PHYLIP3.5-pc. To test the significance of population divergence, the mean of the pairwise divergences between each population and all other populations were computed and the values obtained tested for statistical significance using two-tailed t-tests.
study, therefore, is based exclusively on the results from C-banded and non-differentially stained chromosomes.

**PCR-RAPDs**

All DNA samples yielded a discrete high molecular weight band when subjected to agarose gel electrophoresis. The average A260/A280 ratio of the DNA was 1.65 ± 0.09. A concentration of 20 ng of DNA per reaction, 0.4 μM primers, 4.0 μM Mg²⁺ ions, and 0.23 mM dNTPs produced consistent high-level amplification patterns. Furthermore, repeated amplifications using the same DNA samples showed very little or no variation.

Of the 237 RAPD fragments analysed, 21 (11%) were monomorphic. Nine bands were exclusive to the Khartoum population, ten bands to Sabaloga, eight bands to Shendi, 12 bands to Dongola, ten bands to Medani and 20 bands to Suki. However, no "species-specific" amplification bands were detected for *A. niloticus* and/or *A. testicularis*.

A UPGMA phenogram generated by clustering of similarities (SAHN option of NTSYS-pc; Rohlf, 1998) between all 32 individuals (Fig. 4) shows genetic similarities of between 59.2% and 89.9%. The phenogram did not reveal a clear and meaningful separation with reference to the putative species (*A. niloticus* and *A. testicularis*) and/or geographically distinct populations, with specimens from the various localities being randomly placed in the phenogram.

A cluster analysis of divergences among six *A. niloticus* populations (estimated using the programme RAPDDIP; Fig. 5) revealed very low inter-population divergence (high similarity), indicating an absence of gene-flow restriction between populations and/or individuals (Figs 4 & 5; Tables 2 and 3). The first major dichotomy was represented by the separation of the Suki population (irrigated clay environment) from all other population. Specimens other than those from Suki were grouped into a cluster that combined the semi-desert populations (Shendi, Sabaloga, Khartoum and Medani) and desert specimens (Dongola population) (Fig. 5).

The among-populations mean divergence was 1.06%, and the within-population mean divergence was 1.88%. A *t*-test for unequal variances (two-tailed) showed that divergence between...
populations did not differ significantly from the divergence within populations ($P > 0.05$). This suggests that genetic variation in *Arvicanthis* from Sudan is not geographically structured.

**Discussion**

Cytogenetic features can be used as an aid in establishing taxonomic and phylogenetic relationships among species and genera (Shellhammer 1967, 1969; Carleton & Myers 1979; Robbins & Baker 1980; Engstrom et al. 1981; Arnold et al. 1983; Contrafatto et al. 1992a, 1992b, 1996; Ducroz et al. 1997, 1998; Volobouev et al. 2002a, b). Traditionally, analyses of karyotypes have included the study of the number, size and morphology of chromosomes. These are considered important markers of karyotypic evolution in different taxa and have been helpful in systematic and phylogenetic investigations (Bender & Chu 1963; Matthey 1965; Patton 1967; Visser & Robinson 1986; Robinson & Elder 1987; Baker et al. 1988; Capanna & Civitelli 1988; Volobouev et al. 1988, 2002a; Contrafatto et al. 1992a; Aguilar 1993; Hogan et al. 1993; Civitelli et al. 1995; Ducroz et al. 1997, 1998).

The present study showed that the diploid and fundamental numbers, as well as the chromosome morphology, of *Arvicanthis* (*A. niloticus* and *A. cf. testicularis*) from Sudan (2n = 62 and NFa = 62) are similar to those found in *A. niloticus* from Egypt and Ethiopia and to those described for *A. dembeensis* from Ethiopia (Corti et al. 1996; Volobouev et al. 1988). Following Ducroz et al. (1997) and Volobouev et al. (1988, 2002a), *Arvicanthis* from Sudan correspond to the ANI-1a karyotype.

The lack of G-banding patterns in this study prevented the unequivocal identification of homologies between these published karyotypes and those from the present study. For the same reason, it is not possible to identify cryptic rearrangements that could differentiate specimens of the putative *A. niloticus* and *A. testicularis* examined in the present investigation. As discussed below, however, this may not be a major constraint for reasons relating to gross chromosome morphology and the mechanics of meiotic malsegregation.

From the perspective of the biological species concept (BSC), the occurrence of reproductive isolation between two more-or-less differentiated forms can be taken as an indication of valid species. Reproductive isolation can be mediated by chromosomal differences and such cases have been reported (King 1993). As argued by King (1993) and Contrafatto (1996), however, not all chromosome rearrangements are equal in this respect and only those rearrangements that can cause meiotic malsegregation have the potential to mediate reproductive isolation. Reciprocal translocations are listed among these, but the most frequently reported seem to be tandem fusions and Robertsonian translocations. Inversions are also considered to be potentially disruptive of meiotic pairing and segregation, because of loop formation at pachytene. (King 1993; White 1973).

However, King (1993; pp 80-84) argued for the occurrence of corrective meiotic mechanisms of synaptonemal errors, while cases of pericentric inversions persisting as balanced polymorphisms in natural populations have been described (Contrafatto et al., 1992b; Greenbaum et al. 1978; Warner 1976). With the exception of reciprocal translocations involving chromosome portions of similar lengths (balanced translocations), all these rearrangements would result in gross changes in chromosome morphology that can be detected without the aid of differential staining. However, no such morphological changes were observed in the present investigation. Hence, the only reproducitively significant chromosome changes that could have gone undetected in the present study would be balanced translocations, but these are not well-documented in rodents.

On the evidence presented here therefore it is highly likely that there is no difference between the karyotypes of specimens identified on morphological grounds as *A. cf. niloticus* and *A. cf. testicularis*. Hence, there are no cytogenetic grounds to suspect reproductive isolation and these specimens can be considered conspecific, a conclusion supported by both morphometric and molecular sequence data (Abdel-Rahman 2005; Abdel-Rahman Ahmed et al. 2008).

All specimens karyotyped in this study (n = 34) conformed to the ANI-1a cytotype (Volobouev et al. 2002a). Although cases of recognized small mammal species with similar, or indistinguishable, karyotypes are known (Nadler et al. 1973, 1975, 1984; Robbins & Baker 1980; Volobouev 2002b), chromosomal similarities are considered to be exclusively the result of common ancestry (Simon 1991; Contrafatto et al. 1992a, 1992c; Meester et al. 1992; Smith & Patton 1993; Myers et al. 1995; Contrafatto 1996; Verheyen et al. 1996; Ducroz et al. 1998; Fadda et al. 2001). Therefore, although the lack of chromosomal differences between the two putative species of *Arvicanthis* does not, in itself, prove conspecificity, taken with the RAPD and other data reported by Abdel-Rahman (2005), this finding provides strong evidence for the existence of a single species in Sudan, namely, *A. niloticus*.

The results in this study are consistent with the generic classification of *Arvicanthis* based on classical systematics (Musser & Carleton 1993, 2005; Abdel-Rahman 2005) and protein electrophoresis (Philippi 1994). Although *A. niloticus* and *A. cf. testicularis* from Sudan are considered to be different species by some authors (Delany, 1975; Afework et al., 1983; Fadda & Corti 1998), the results in this study indicate that they are closely related, and it may be more appropriate to consider them as a single species, *A. niloticus*.

**Application of PCR-RAPDs**

Many of the constraints of phenotype-based methods are mitigated by assaying the genotype with a DNA-based diagnostic technique such as PCR-RAPDs (Burr et al. 1983; Beckman & Soller 1986). RAPDs have proven useful in the identification and characterization of plant materials such as rice (Welsh & McClelland 1994), soybean (Caetano-Anolles et al. 1991a, b), barley (Weining & Langride 1991) and apples (Mulcahy et al. 1993), as well as in the determination of the genetic diversity of various other crops (Vierling & Nguyen, 1992; Hosaka & Hanneman 1994). In addition, RAPDs have extensively been applied to the study of genetic variation in...
animal genomes (Chapco et al. 1992; Hunt & Page 1992; Cheung et al. 1993; Dinesh et al. 1993; Figueroa et al. 1993; Gokool et al. 1993; Bardakci & Skinbinski 1994; Johnson & Fernando 1995; Temate et al. 1995; Martinez et al. 1997). Furthermore, the RAPD approach is technically feasible in cases where little is known about the genome of the species being investigated. The present study represents the first attempt to differentiate the two putative species, *A. niloticus* and *A. testicularis* by PCR-RAPD fingerprinting.

Nevertheless, since it was first described by Williams et al. (1990), this technique has not been free from criticism, which is mostly focused on a lack of reproducibility (Martinez et al. 1994, 1997), sensitivity to quality and concentration of various master mixture components (Saiki 1989; Sambrook et al. 1989; Carlson et al. 1991; Chou et al. 1992; Devos & Gale 1992; Fritsch & Rieseberg 1992) and to PCR conditions (Meunier & Grimont 1993). However, the PCR-RAPD protocol used in the present study was shown to be consistent, reproducible and highly informative, a finding consistent with those reported by other studies (Multani & Lyon 1995; Perron et al. 1995; Parker et al. 1998; Ponsuksili et al. 1998). In the present study, this was achieved by controlling the factors that influence PCR-RAPD performance.

The reliability of PCR-RAPD fingerprinting for the purpose of measuring genetic divergence and genetic variability due to origin and breeding has been previously demonstrated (Tibayrenc et al. 1993; Plotisky et al. 1995; Ponsuksili et al. 1998). Relationships among *Arvicanthis* individuals were expressed in terms of similarity coefficients (Table 2) and estimates of sequence divergence. Genetic similarity and variability of *Arvicanthis* populations from Sudan were determined by calculating the frequency of bands shared between the individuals analysed. The frequency of these bands may be considered equivalent to allele frequency, hence RAPD bands may be considered as genetic markers (Ponsuksili et al. 1998). Allelic variation is represented by the presence or absence of a particular DNA segment as amplified by PCR-RAPDs and visualized by gel electrophoresis (Parker et al. 1998).

As reported in the results of the present study, 217 bands were polymorphic (91.56% of total number of bands), with an average of 24 polymorphic loci per primer. Such polymorphisms have been used in other studies to distinguish between genera, species, varieties and clones (Williams et al. 1990; Anolles et al. 1991; Caetano-Anolles et al. 1991b; Hoelzel & Dover 1991; Hu & Quiros 1991; Welsh et al. 1991; Herder et al. 1994; Ho et al. 1995). There was a high degree of genetic similarity between *Arvicanthis* specimens from Sudan and a very low level of divergence (0.39%-0.98%) between the two putative species (*A. cf. niloticus* and *A. cf. testicularis*). This genetic distance, which is less than 2.0%, is at the level of an intra-specific distance (see Bradley & Baker, 2001). Furthermore, there was no statistically significant difference in the number of polymorphic and monomorphic loci between the two putative species, *A. niloticus* and *A. cf. testicularis* (t-test for unequal variances, *P* > 0.05).

It appears that all 32 genomes analysed were not significantly different, suggesting the presence of a single species, *A. niloticus*. These results confirm those from a multidisciplinary study by Abdel-Rahman (2005) that showed no variation between *A. cf. niloticus* and *A. cf. testicularis*.

The degree of similarity between individuals from different populations was often greater than for individuals obtained from the same population (Table 2). In addition, within-population diversity was significantly greater than the mean divergence between each population and the others (t-test for unequal variances, 0.001 < *P* < 0.05), which indicates that genetic variation is not partitioned geographically and is suggestive of panmixia. This indicates that the differences among the 32 *Arvicanthis* specimens are not substantial enough to justify their inclusion into different species.

Virtual panmixia, in *Arvicanthis* inhabiting different ecological zones (desert, semi-desert and savannah; Kingdon 1974), can be explained in terms of the wet grassy habitats in the riparian fringe of the Nile River providing a continuous corridor for genetic exchange across diverse ecological zones. Such a population structure would not favour reproductive isolation and, hence, would prevent the establishment of separate species.

Further studies involving cytochrome b DNA sequencing (Abdel-Rahman Ahmed et al. 2008) are in progress to corroborate the taxonomic status of *A. niloticus* in Sudan.

Acknowledgements

The National Research Foundation is thanked for funding to PJT and JML.

References


MITCHELL, L.G., BODENTEICH, A. & MERRIL, C.R. (eds), Basic DNA and RNA protocols, 58: 97-103. Humana Press Inc.: Totowa, N.J.


