Cytosystematics of the South African *Aethomys* (Rodentia: Muridae)

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The G- and C-band chromosome patterns and the location of the nucleolus organizer regions (NORs) are presented for *A. namaquensis* (2n = 24), *A. granti* (2n = 32) and *A. chrysophilus* (2n = 44; 2n = 50). The presence of two distinct cytotypes in what is conventionally recognized as *A. chrysophilus* is indicative of the presence of two discrete species which, karyology apart, appear to be indistinguishable using existing identification keys. The chromosomal relationships of the South African species and the taxonomic implications of these data are discussed.
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Die G- en C-bandchromosoompatrone en ligging van die nukleolus organiserende areas van *A. namaquensis* (2n = 24), *A. granti* (2n = 32) en *A. chrysophilus* (2n = 44; 2n = 50) word beskryf. Die teenwoordigheid van twee afsonderlike sitotipes in wat konvensioneel beskou word as *A. chrysophilus*, is aanduidend van die bestaan van twee verskillende spesies wat, buiten kariologie, ononderskeibaar met behulp van bestaande identifikasiesleutels blyk te wees. Die chromosomale verwantskappe van die Suid-Afrikaanse spesies en die taksonomiese implikasies van die data word bespreek.
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Rodents of the genus *Aethomys* are morphologically similar and yet form a karyotypically diverse group. As is presently understood, the genus comprises three South African species of which two, *A. namaquensis* and *A. granti* are placed in the subgenus *Michaelamys* and the third, *A. chrysophilus*, in the subgenus *Aethomys*. Because of the morphological similarity of the species, the lack of intensive studies employing modern techniques and the relative rarity of some species (*A. granti*), there has been a degree of uncertainty regarding the taxonomic relationships in this genus (Davis 1975). The present investigation examines, through the use of chromosome-banding data, the various karyotypic changes that have accompanied the evolutionary divergence of the South African *Aethomys* species. These data provide an unequivocal means for the delimitation of the taxa and contribute to the development of a reliable identification scheme for these species.

Material and Methods

Fibroblast cultures were established from skin or tail biopsies, or disaggregated kidney tissue, using standard procedures (Paul 1975). Trypsin G-banding and barium hydroxide C-banding were performed according to the methods of Wang & Fedoroff (1972) and Sumner (1972) respectively. Nucleolus organizer regions were silver-stained following Bloom & Goodpasture (1976). Collection localities and the number of specimens utilized for this study are presented below:

A. namaquensis (*n* = 24): Pretoria (25°40′S/28°20′E) 1♂ + 2♀♀; Springbok (29°40′S/17°52′E) 2♂♂ + 2♀♀; Deelfontein (30°59′S/23°48′E) 2♂♂ + 2♀♀; Calvinia (31°28′S/19°50′E) 1♂ + 1♀; Karoo National Park (32°22′S/22°44′E) 1♂ + 2♀♀; Thabazimbi (24°38′S/27°25′E) 1♂ + 1♀; Hutchinson (31°30′S/23°11′E) 2♂♂ + 2♀♀; Alldays (22°43′S/29°10′E) 1♂ + 1♀.

A. granti (*n* = 10): Sutherland (32°23′S/20°40′E) 4♂♂ + 6♀♀.

A. chrysophilus 2n = 44 (*n* = 16): Vaalkopdam (25°18′S/27°25′E) 6♂♂ + 5♀♀; Pilanesberg National Park (25°12′S/27°15′E) 1♂; Durban (29°55′S/30°55′E) 2♀♀; Satara Camp (24°21′S/31°46′E) 1♂ + 1♀.

A. chrysophilus 2n = 50 (*n* = 22): Messina (22°21′S/30°03′E) 1♂ + 1♀; Letsitele (23°50′S/30°18′E) 2♂♂ + 2♀♀; Boshoek (25°28′S/27°09′E) 1♂ + 1♀; Pilanesberg National Park (25°12′S/27°15′E) 2♂♂ + 1♀; Rooibokkraal (24°15′S/26°50′E) 1♂ + 1♀; Thabazimbi (24°38′S/27°25′E) 3♂♂ + 1♀; Brits (25°34′S/27°45′E) 2♂♂ + 1♀.

Animals examined in this study are available as voucher specimens in the mammal collections of the Transvaal Museum, Pretoria, Republic of South Africa.

Results

Namaqua rock mouse, *A. namaquensis* (2n = 24)

The G-banded karyotype of a male Namaqua rock mouse is shown in Figure 1A. The autosomes can be arranged into three distinct groups on the basis of centromere position with the karyotype comprising four pairs of large metacentric chromosomes (pairs 1 – 4), one pair of large submetacentrics (pair 5) and six pairs of small acrocentric chromosomes (pairs 6 – 11). The X is the largest acrocentric chromosome in the genome and the Y is similar in size to the larger acrocentric chromosomes (6 – 8). The morphology of all bi-armed autosomes is distinctive, but the gradation in size of the small acrocentrics necessitates the use of G-banding in the pairing of homologues.

The C-banded chromosomes of *A. namaquensis* are shown in Figure 1B. Interstitial constitutive heterochromatin was found in the proximal portion of the long arms of chromosome pair 3 (arrows). The largest amount of heterochromatin is pericentromeric in distribution and is located on pairs 6 and 9, while the smallest autosomal chromosomes (pair 11) appear to be almost totally heterochromatic (Figure 1B arrow heads).

A partial silver-stained metaphase cell of a male is shown in Figure 1C. The nucleolar organizer regions were detected at the telomeric ends of the short arms of eight of the acrocentric chromosomes. Satellite association was infrequently observed between NOR bearing chromosomes (Figure 1C arrow).

Grant's rock mouse, *A. granti* (2n = 32)

The G-banded karyotype of a male Grant's rock mouse is shown in Figure 2A. The diploid number confirms Matthey's (1964) report. Three distinct chromosomal categories are evident based on morphology with the karyotype comprising four pairs of large metacentric autosomes (pairs 1 – 4), one pair of medium acrocentrics (pair 5) and ten pairs of small acrocentrics (pairs 6 – 15). The X chromosome is the largest acrocentric in the genome and the Y, also acrocentric, is similar in size to pair 5.

The C-banded chromosomes of *A. granti* are illustrated in Figure 2B. A single band of interstitial heterochromatin can be seen in the proximal region of the long arms of pair 3 (Figure 2B arrows) mirroring the situation in *A. namaquensis*. These shared interstitial C-bands are absent in *A. chrysophilus* (see below) and this provides supportive evidence for the close relatedness of the former species. The largest amount of autosomal heterochromatin is concentrated in pairs 12 – 15 which probably contributes to the poor G-band resolution of these autosomes. Telomeric heterochromatin was detected in the distal ends of pairs 1 and 2 (Figure 2B arrow heads). The Y chromosome can be easily identified as it is entirely heteropycnotic while the distal half of the large acrocentric X chromosome is C-band positive; the centromeric region of this chromosome also stains darkly. In some metaphases a small euchromatic region was frequently visible at the extreme distal end of the long arm of the X (Figure 2B open arrow).

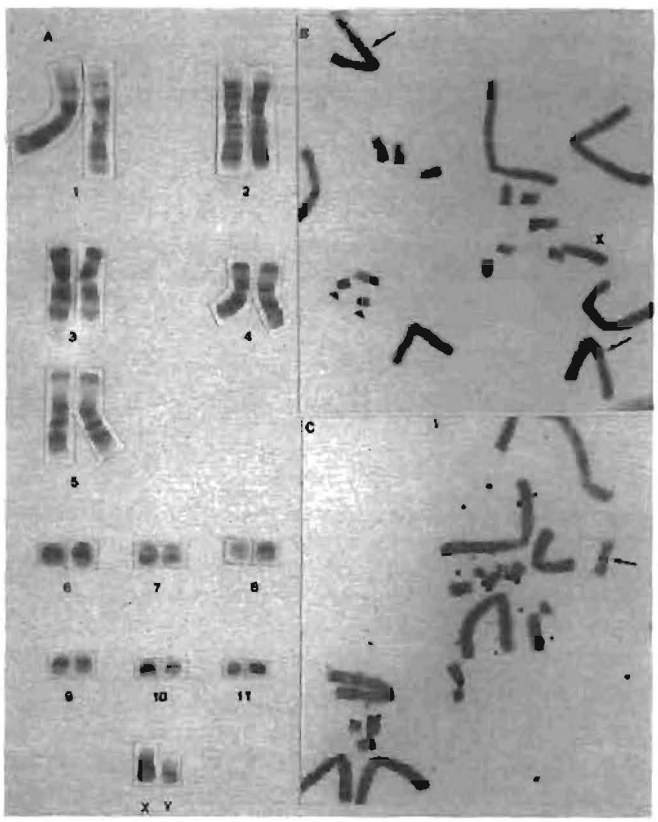

Figure 1 Karyotype of a male Namaqua rock mouse, *Aethomys namaquensis* (2n = 24): (A) G-banding; (B) C-banding. The arrows indicate a band of interstitial heterochromatin on the long arms of pair 3. Arrow heads illustrate the acrocentric chromosomes (pair 11) that are almost totally heterochromatic. (C) Silver-stained metaphase cell showing the presence of eight NOR-bearing chromosomes. The arrow indicates the satellite association sometimes observed between NOR-bearing chromosomes.

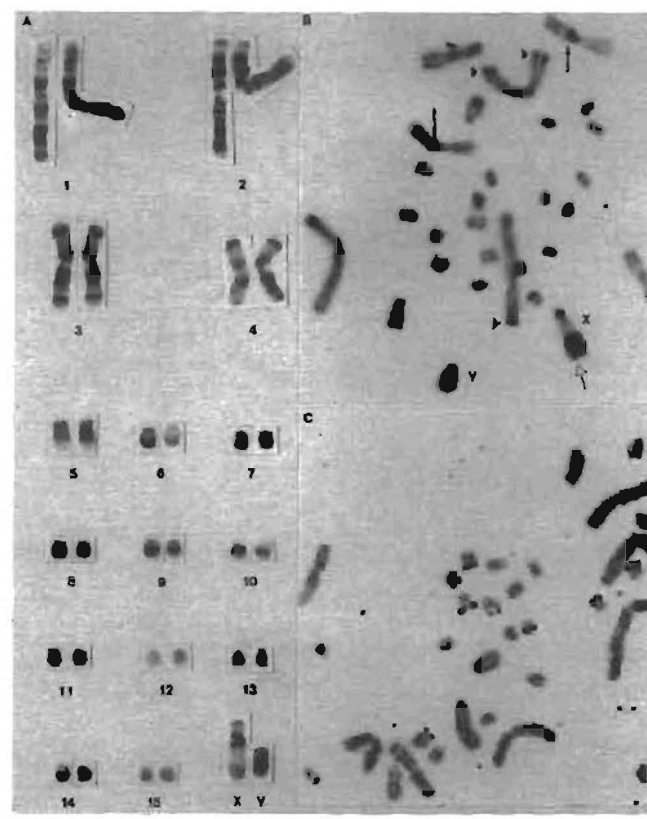

Figure 2 Karyotype of a male Grant's rock mouse, *Aethomys granti* (2n = 32): (A) G-banding; (B) C-banding. The arrows indicate a band of interstitial heterochromatin in the long arms of pair 3. The locations of telomeric heterochromatin on pairs 1 and 2 are shown by arrow heads while the small euchromatic section visible at the distal end of the X chromosome's long arm (open arrow) is also indicated. (C) A partial silver-stained metaphase cell showing 10 NOR-bearing chromosomes.

A partial silver-stained metaphase cell from a male Grant's rock mouse is presented in Figure 2C. The nucleolar organizer regions are situated at the telomeric ends of the short arms of five small acrocentric chromosome pairs.

Red bush rat, *A. chrysophilus*

Two distinct cytotypes, 2n = 44 and 2n = 50, were found in the *A. chrysophilus* test material. The chromosomes of two males, representative of the respective cytotypes, are presented in Figures 3 and 4 respectively.

The G-banded karyotype of *A. chrysophilus* (2n = 44) is shown in Figure 3A. As with the preceding species the autosomes can be arranged into three groups according to their size and the positions of their centromeres. Eight pairs of metacentric chromosomes are present with an abrupt size differential occurring between pairs 3 and 4. Thirteen pairs of acrocentric chromosomes, showing a gradation in size, form the remainder of the autosomal complement. The sex chromosomes can easily be identified on the basis of their morphology and banding patterns with the X chromosome being the largest acrocentric in the genome and the Y the only submetacentric element present.

The sex chromosomes are also readily identifiable following C-banding (Figure 3B). Although not as darkly stained as the centromeric regions of many of the autosomes, both sex chromosomes are characteristically dark following this technique. In addition, small amounts of heterochromatin are visible at the centromeres of most autosomes as well as the X. No interstitial C-bands were visible in any of the preparations examined.

A partial silver-stained metaphase cell of *A. chrysophilus* (2n = 44) is shown in Figure 3C. Four NORs were found in this cytotype which were situated on the short arms of two of the small metacentric pairs and correspond to the satellites visible on the G-banded chromosomes, pairs 4 and 5 (Figure 3A).

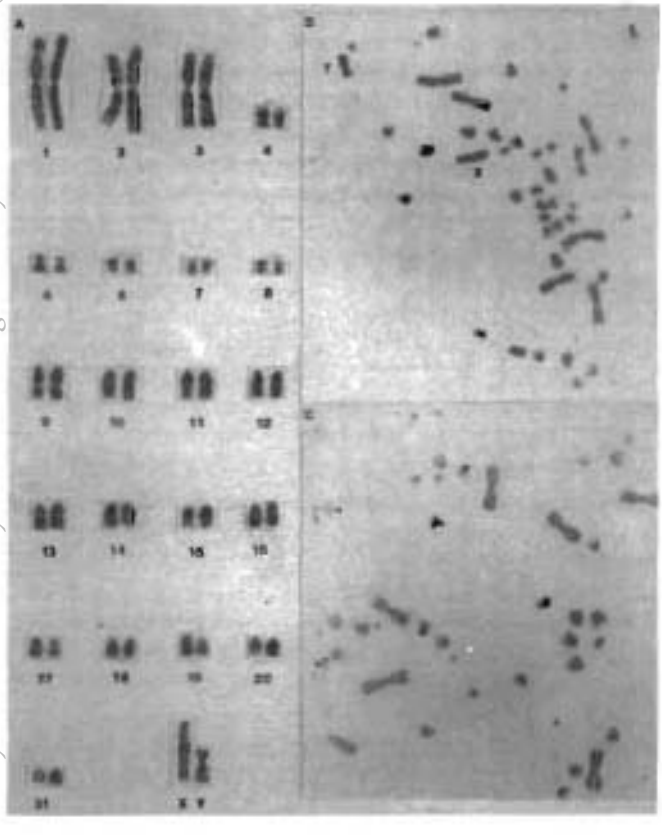

Figure 3 Karyotype of a male red veld rat, *Aethomys chrysophilus* (2n = 44): (A) G-banding; (B) C-banding; (C) A silver-stained metaphase cell showing the presence of four NOR-bearing chromosomes.

The G-banded karyotype of *A. chrysophilus* (2n = 50) is presented in Figure 4A. Two groups of autosomes can be distinguished using centromere position. The first group comprises 19 pairs of acrocentric chromosomes (pairs 1 – 19), followed by five pairs of small metacentric chromosomes (pairs 20 – 24).

A typical C-banded preparation of *A. chrysophilus* (2n = 50) is shown in Figure 4B. While the heteropycnotic Y is clearly visible, the X chromosome does not contain large amounts of C-band material and consequently could not be distinguished from the autosomes using this banding technique.

A partial silver-stained metaphase cell of a male *A. chrysophilus* (2n = 50) is illustrated in Figure 4C. The six nucleolar organizing regions found in this cytotype are located on three pairs of the small metacentric chromosomes (pairs 21 – 23).

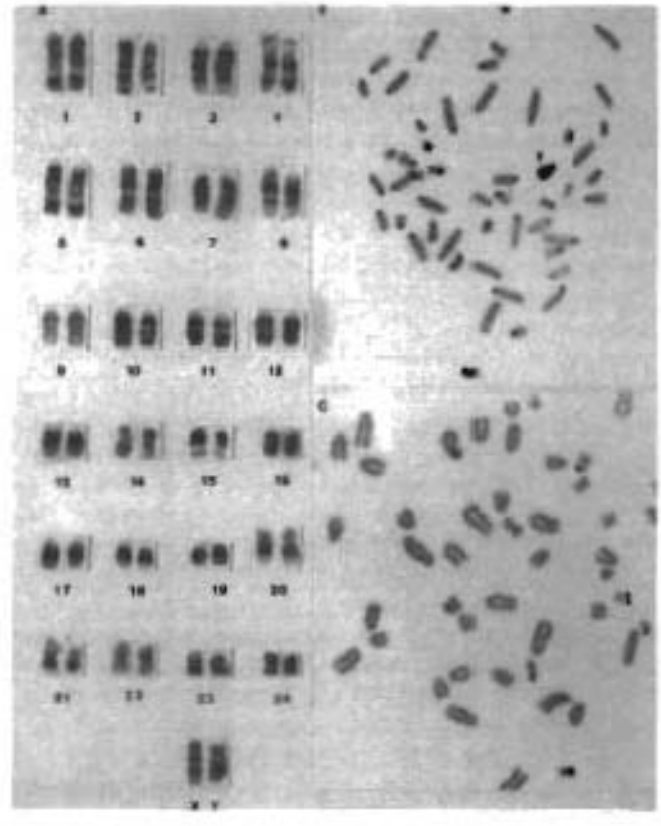

Figure 4 Karyotype of a male representative of the 2n = 50 cytotype of *Aethomys chrysophilus*: (A) G-banding; (B) C-banding; (C) A partial silver-stained metaphase cell showing the presence of six NOR-bearing chromosomes displayed by this *A. chrysophilus* cytotype.

Comparison of karyotypes

Our interpretation of G-band homologies between the two cytotypes of *A. chrysophilus* is presented in Figure 5. The difference in diploid number is attributable to the presence of three fusion products in the 2n = 44 cytotype (chromosomes 1 – 3) which correspond to the unfused acrocentric elements 1/2, 3/4 and 5/8 present in the 2n = 50 specimens. With the exception of chromosome 4 in the 2n = 44 cytotype, and chromosome 20 in the 2n = 50 cytotype (Figure 5 box), the remaining autosomes all have banding equivalents in their respective genomes; it is not clear what mechanism is responsible for the differing morphologies and unique band pattern

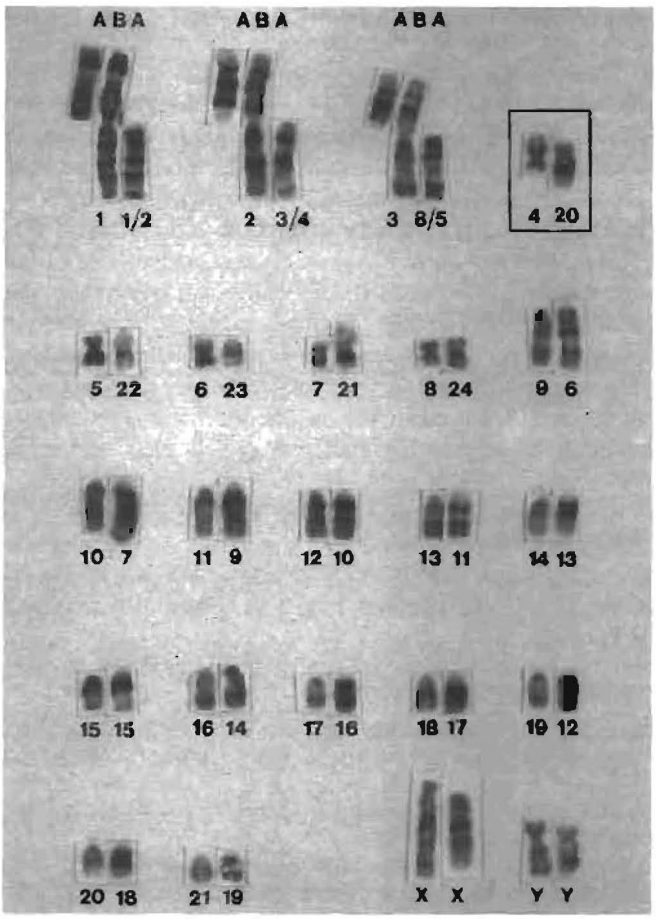

Figure 5 Haploid composite illustrating the proposed G-band homologies between the 2n = 44 and 2n = 50 *Aethomys chrysophilus* cytotypes (indicated with the symbols B and A respectively). Chromosome identification numbers correspond to those of their respective karyotypes. The first chromosome in each pair is that of *A. chrysophilus* (2n = 44) unless otherwise indicated. The box contains the unmatched small metacentric chromosomes remaining from each genome.

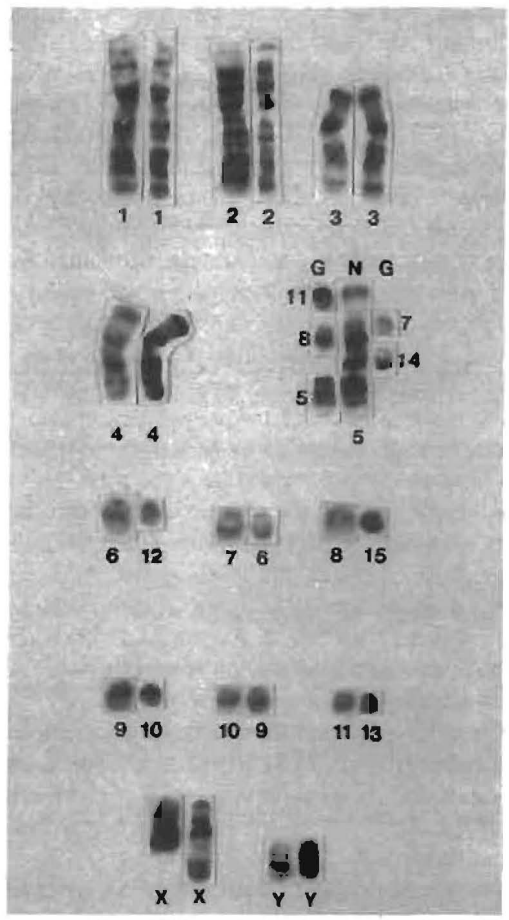

Figure 6 Comparison of half karyotypes showing proposed G-band homologies between *Aethomys namaquensis* (2n = 24; N) and *Aethomys granti* (2n = 32; G). Chromosome identification numbers correspond to those of their respective karyotypes. The first chromosome in each pair is that of *A. namaquensis* unless otherwise indicated.

sequences of these small metacentric chromosomes. In addition, noticeable differences were also apparent in the G-band patterns of both the X and the Y chromosomes.

Half karyotypes of *A. namaquensis* (2n = 24) and *A. granti* (2n = 32) are compared in Figure 6. The banding patterns of the four large metacentric pairs (1 – 4) of these species show good concordance. However, owing to the indistinctive G-bands and similarity in size of the acrocentrics, some difficulty was experienced in matching the homologous chromosomes. The submetacentric chromosome pair 5 present in *A. namaquensis* is thought to have originated through a series of tandem fusions, involving four pairs of the small *A. granti* acrocentrics (numbers 5, 14, 8 and 7), and one centric fusion involving chromosome 11. The euchromatic portions of the X chromosomes of *A. namaquensis* and *A. granti* are similar. The discrepancy in size of the X chromosomes of these species is attributable to the presence of a large block of heterochromatin in the X chromosome of *A. granti*. No apparent band homology was evident in the comparisons of the Y chromosomes.

Only a very small portion of the genomes of *A. namaquensis* and *A. granti* on one hand, and the two *A. chrysophilus* cytotypes on the other, are directly homologous in band sequence. The long arm of chromosome 3 in both *A. namaquensis* and *A. granti* have good banding homology with the long arms of pair 3 in the 2n = 44 *A. chrysophilus* cytotype and the corresponding chromosome 5 in the 2n = 50 cytotype. Other matches involving repeated tandem fusions could be made but the homologous segments could, at best, only be regarded as tentative. It is interesting to note that the X chromosomes of all four taxa show conservation of the primitive X chromosome banding pattern (Pathak & Stock 1974).

Discussion

The unbanded chromosomes of *A. granti*, *A. namaquensis* and *A. chrysophilus* were first reported by Matthey (1958, 1964). The diploid numbers for *A. namaquensis* (2n = 24) and *A. granti* (2n = 32) are confirmed in the present investigation. More recently, Gordon and Rautenbach (1980) provided cytogenetic data on *A. chrysophilus* from Zimbabwe which indicate the presence of two cytotypes (2n = 44, 2n = 50) in this region. Although sympatric populations were identified, no evidence of hybridization was found and they suggested that the two forms represent different species. This observation is confirmed and extended by the present investigation. The distinctness of the karyotypes of the two *A. chrysophilus* cytotypes, the absence of hybrids in areas of sympatry as well as pronounced differences in the morphology of their spermatozoa (Gordon & Watson 1986) provide clear evidence of the absence of gene flow between the two groups and support the recognition of the cytotypes as two distinct sibling species. Importantly in the absence of diagnostic criteria other than the chromosomes, only the *A. chrysophilus*

2n = 50 cytotype occurs at Mazoe (Gordon, D.H. 1986, pers. comm.), the type locality of this species.

Matthey (1964) proposed that *A. namaquensis* and *A. granti* are closely related. In his comparison of the karyotypes of these two species, he suggested that Robertsonian translocations (or centric fusions) and pericentric inversions could have played a significant role in their chromosomal evolution. Although a close chromosomal relationship between these species is indicated by our data the difference in diploid number was traced to tandem fusions and a single centric fusion, with no evidence of pericentric inversions being found.

Gordon & Rautenbach (1980) refer to a report by Matthey (1954) of a submetacentric X chromosome in *A. chrysophilus* (2n = 44), which is in accordance with their own findings. However, in a subsequent paper Matthey (1964) documented the X chromosome as being a large acrocentric. This morphology is confirmed by the present results. Unfortunately Matthey's original report (Matthey 1954) and somewhat surprisingly, Gordon & Rautenbach (1980), provide only schematic representations of the species chromosomes thereby confounding comparisons of the data sets. The acrocentric X chromosome evident in the test material could have arisen through a pericentric inversion, or alternatively, the metacentric morphology reported by Gordon & Rautenbach (1980) and the earlier Matthey report (1954) may have resulted from the addition of heterochromatin to the short arms of a previously acrocentric chromosome. Since banded karyotypes were not presented by these authors, it is impossible to determine which of these mechanisms were responsible for this structural change. However, the X chromosomes of the two *A. chrysophilus* cytotypes appear to be homologous. Furthermore, the X chromosomes of all four taxa retained the two major bands representing the primitive G-banding pattern characteristic of most mammals (Figure 7; Pathak & Stock 1974).

Of particular interest is the contrast in modes of karyotypic change followed by the species of each subgenus. Representatives of the subgenus *Aethomys, A. bocagei, A. kaiseri* and *A. chrysophilus* are all characterized by a diploid number of 2n = 50 (Matthey 1954), the 2n = 44 cytotype of *A. chrysophilus* being the only exception. Should the chromosomal constitutions of the species comprising this subgenus closely reflect the ancestral condition for the two subgenera then it is not unreasonable to argue that the constituent species of the subgenus *Michaelamys* have undergone what amounts to a rapid and extensive reorganization of their genomes since diverging from a common ancestor. An obvious question that arises is what factors may have contributed to the differential rates of karyotypic change between the two subgenera? In this respect it is interesting to note that both *A. namaquensis* and *A. granti* inhabit rocky and mountainous situations whose disjunct distribution may facilitate the fixation of structural rearrangements through fragmentation of the species into isolated demes. *A. chrysophilus*, on the other hand, occurs in open savanna and may be less prone to isolation.

The taxonomy of the genus *Aethomys* has in the past been the subject of much debate (De Graaff 1981). Of relevence here is that Davis (1965) treated *Aethomys* as a full genus which included the subgenera *Stochomys* and *Michaelamys*. He later removed *Stochomys* from the genus and divided it into the subgenera *Aethomys* and *Michaelamys* (Davis 1975). This treatment is currently generally accepted although some uncertainty still exists as to the status of some species and their taxonomic affinities. In this respect, the present results provide supportive evidence for Davis's later classification. There are evidently closer karyological affinities between *A. granti* and *A. namaquensis* on the one hand, and the two *A. chrysophilus* cytotypes on the other, than between the two subgenera.

Acknowledgements

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