Chromosomal diversity in mole-rats of the genus *Cryptomys* (Rodentia: Bathyergidae) from the Zambezian region: with descriptions of new karyotypes

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Abstract

The genus *Cryptomys* contains a number of social, subterranean rodents that are widely distributed throughout sub-Saharan Africa. Specimens of *Cryptomys* from 23 localities in south-west Zambia were karyotyped using a standard staining protocol. A minimum of five metaphases per specimen was scored for 2n and the fundamental number (NF) was determined in females. Nine new karyotypes, which may represent several new species, were identified: (1) 2n = 42, NF = 78 (Dongo, Southern Province); (2) 2n = 44, NF = 76 (Salujinga, North-western Province); (3) 2n = 45, NF = 78 (Lochinvar, Southern Province); (4) 2n = 52, NF = 86 (Chinyingi, North-western Province); (5) 2n = 54, NF = 78 (Monze, Southern Province); (6) 2n = 56, NF = 76 (Watopa, North-western Province); (7) 2n = 58, NF = 80 (Livingstone, Southern Province); (8) 2n = 58, NF = 86 (Senanga, Western Province); (9) 2n = 60, NF = 82 (Kataba, Western Province; type locality of *C. damarensis micklemi*). Contrary to previous reports, the specimens from Kataba and Senanga on the left bank of the Zambezi do not correspond to *C. damarensis* and should be considered a separate species: *C. micklemi* (as confirmed by molecular analyses; Ingram, Burda & Honeycutt, 2004). According to the karyotype, *C. damarensis* occurs only on the right bank of the Zambezi River in the Western Province. In contrast to the high karyotypic variability on the right bank of the Kafue River, it was found that *C. anselli* (2n = 68) is widely distributed throughout the Central province on the left bank of the Kafue River. The resulting pattern of occurrence of the different karyotypes correlates well with the extant river system configuration that separates most karyotypes. We hypothesize that geomorphological changes and in particular river system dynamics in recent geological times have played an important role in the chromosomal diversification and may have provided opportunities for speciation to occur.

Key words: *Cryptomys*, Zambia, karyotype, chromosomal diversity, geomorphological barrier

INTRODUCTION

*Cryptomys*, Gray 1864 (Bathyergidae, mole-rats) is a very speciose genus endemic to sub-Saharan Africa. Molecular phylogenetic studies (looking both at mitochondrial and nuclear loci) have revealed that this genus comprises two major clades, which may represent two different genera that diverged between 11 and 18 Ma (Faulkes, Verheyen et al., 2004; Ingram, Burda & Honeycutt, 2004). A southern clade has a limited distribution in South Africa, Mozambique and southern Zimbabwe. The northern clade is distributed throughout the rest of sub-Saharan Africa, where it occupies a wide range of grassland and woodland habitats. Hitherto, classical morpho-taxonomic traits (body size, pelage coloration, craniometry) have not sufficed to diagnose the different species (e.g. Rosevear, 1969; Honeycutt, Edwards et al., 1987). Therefore, taxonomic treatment of the genus remains a challenge, which is further complicated by high genetic variation in some taxa (Faulkes, Bennett et al., 1997; Bennett & Faulkes, 2000). Accordingly there has been great discrepancy in the number of species (1–49) recognized by different authors (Allen, 1939; Ellerman, Hayman & Holt, 1940; Ansell, 1978; Nowak, 1991; Wilson & Reeder, 1993). In a landmark study, Honeycutt, Allard et al. (1991) recognized seven species: *Cryptomys bocagei* (De Winton, 1897), *C. damarensis* (Ogilby, 1838), *C. foxi* (Thomas, 1911), *C. hottentotus* (Lesson, 1826), *C. mechowi* (Peters, 1881), *C. ochraceocinereus* (Heuglin, 1864), and *C. zechi* (Matschie, 1900). Subsequent allozyme and karyological studies demonstrated the specific status of *C. darlingi* (Aguilar, 1993), *C. amatus* (Macholan, Scharff et al., 1998), *C. kafuensis* (Filippucci et al., 1994; Burda...
Fig. 1. Sampling localities of Cryptomys.

et al., 1999), C. anselli (Filippucci et al., 1994; Burda et al., 1999) and C. whytei (Chitaikali, Burda & Kock, 2001). Two other karyotypes were described from Kasama, Zambia (Kawalika, Burda & Brüggert, 2001) and Kalomo, Zambia (Faulkes, Bennett et al., 1997). The latter karyotype was referred to as ‘Choma’ by Faulkes, Bennett et al. (1997) and by Bennett & Faulkes (2000).

These data suggest that diversity may be particularly high in the Zambezian region (Burda et al., 1999; Burda, 2001; Kawalika et al., 2001). The aim of the current study is to further detail chromosomal variation in the Zambezian region and examine if geomorphological changes since the Miocene may have driven evolution in Cryptomys. The current data and discussion are focused primarily on Zambia.

MATERIAL AND METHODS

Mole-rats were captured at 23 localities in the central, western and southern parts of Zambia (Fig. 1) during 6 expeditions between 1999 and 2003. The sampling area is characterized by substantial habitat and climatic diversity (as indicated by differences in precipitation and vegetation; Table 1). Trapping was conducted using a traditional method (cf. Jarvis, 1991). Voucher specimens are deposited at the Department of Vertebrate Morphology, University of Ghent and the Department of General Zoology, University of Duisburg-Essen.

Karyotyping was performed both in the field and the laboratory. Mitotic metaphases were obtained directly from the bone marrow of long bones, using a standard protocol (Lee, 1969; Hillis, Moritz & Mable, 1996). Slides were conventionally stained with 4% Giemsa-solution. Well-spread metaphases were recorded using a Colorview 8 (SIS) CCD camera attached to a Polyvar microscope. Karyotypes were prepared from the best metaphases. Chromosomes were paired by eye, using the position of the centromere and chromosome size. The diploid number (2n), the fundamental number (NF) and where possible the autosomal fundamental number (NFa) were scored in at least five metaphases per specimen. To allow comparison between the different karyotypes, the NF and the NFa that are listed refer to the female karyotype. With the exception of the karyogram of the Monze female, all the depicted karyograms originate from the chromosomal complement of a single cell.

RESULTS

Diagnostic features for the different karyotypes are described below and summarized in Table 2.
Chromosomal diversity in *Cryptomys* 319

Table 1. Number of colonies sampled (N1) and number of animals karyotyped (N2). Habitat type (adapted from White, 1983) and the mean annual precipitation (P) (calculated from data from Global Historical Climatological Network database: <http://www.ncdc.noaa.gov/ol/climate/research/ghcn/ghcn.html>.)

<table>
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<th>No.</th>
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<th>N2</th>
<th>Habitat</th>
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Table 2. Chromosomal data for the genus *Cryptomys* in Zambia. 2n: diploid number; M/SM: number of pairs of metacentric and submetacentric chromosomes; A/ST: number of pairs of acrocentric and subtelocentric chromosomes; NF(a): (autosomal) fundamental number (female)

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<tr>
<th>Taxon or locality</th>
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<th>M/SM</th>
<th>A/ST</th>
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<th>Y</th>
<th>NFa</th>
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<td>A</td>
<td>92</td>
<td>96</td>
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<td>28</td>
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<td>A</td>
<td>76</td>
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<td>dot</td>
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<td>M/SM</td>
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<td>–</td>
<td>86</td>
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<td>A</td>
<td>dot</td>
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<td>Chromosome morphology undetermined</td>
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</tr>
</tbody>
</table>

1–7. Kaindu, Nalubanda, Moono, Mumbwa (karyotype not depicted)

Central Province: left bank of the Kafue River

All specimens had a 2n = 68 as in *C. anselli*. Pelage colour varied from blackish in the smallest animals to dark brown in sub-adults and brown in the heaviest specimens. The shape, size and demarcation of headspots were variable both within and between colonies (cf. Burda et al., 1999).

8–9. Monze (Fig. 2)

Southern Province: right bank of the Kafue River

The two females were characterized by a 2n = 54 and NF = 78. They live in sympathy or parapaternity with the next
form (10). Headspots varied from large and symmetrical to absent. Pelage colour was variable as in C. anselli.

10. Lochinvar (Fig. 3)
Southern Province: right bank of the Kafue River
The single female from the eastern edge of the Kafue floodplain system had a \(2n = 45\), NF = 78 including a metacentric chromosome without homologue. The animal had a blackish-brown pelage coloration and a small triangular headspot.

11. Dongo (Fig. 4)
Southern Province: right bank of the Kalomo River
Mole-rats from this area had a \(2n = 42\) and NF = 78. The X chromosome was a medium-sized metacentric while the Y was dot-like (male karyogram not depicted). Fourteen animals out of 21 caught from different colonies in the area were characterized by a remarkable asymmetry in the headspot. Pelage colour ranged from blackish-brown to rufous brown and was variable within age classes.

12. Itezhi-Itezhi (karyotype not depicted)
Southern Province: right bank of the Kafue River
The karyotyped specimen from west of the Kafue floodplain system on the Kafue River showed the same karyotype as C. kafuensis (\(2n = 58\), NF = 82). The record constitutes a small range extension for this species (cf. Burda et al., 1999). Pelage colour and headspot were variable as in C. anselli.

13–16. Livingstone & Sekute (Fig. 5)
Southern Province: left bank of the Zambezi River
The karyotype of specimens from both study populations was characterized by a \(2n = 56\) and NF = 80. The X chromosome was a medium-sized metacentric, while the Y was dot-like. Pelage colour as well as the shape and size of the headspot were highly variable.

17–18. West Zambezi and Sioma Ngwezi (not depicted)
Western Province: right bank of the Zambezi River, south of the Lungwabungu River
Chromosomal diversity in *Cryptomys*

Fig. 4. Standard karyotype of *Cryptomys* sp. from Dongo (female), with $2n = 42$. (Female karyotype is depicted because a complete chromosomal complement for the one male specimen is not available.)

In both the specimens from the southern locality at Sioma Ngwezi and the west Zambezi locality $2n = 78$ as in *C. damarensis* (Nevo et al., 1986). However, in the individual from West Zambezi NFa = 116 as opposed to NFa = 92 in *C. damarensis* of Namibia. Morphology of all chromosomes of the Sioma Ngwezi specimen could not be determined with certainty, but NFa is different from both other forms. Three black mole-rats were caught at Sioma Ngwezi: two young animals had very asymmetric headspots, while an adult had a clearly defined, symmetrical headspot.

19. Chinyingi (Fig. 6)
North-western Province: right bank of the Zambezi River, north of the Lungwebungu River
The karyotype consisted of a $2n = 52$ and NF = 86. Chromosome pairs 25 and 26 were very small and are classified tentatively as acrocentric. The only female, which was karyotyped, was fawn-grey and had a very small headspot.

20. Kataba (Fig. 7)
Western Province: left bank of the Zambezi River
The specimens from Kataba are from the type locality of *C. damarensis micklemi* (Chubb, 1908; cf. Ansell, 1978). They have a $2n = 60$ and NF = 82. All the animals were black with well-demarcated headspots and in most cases clear dorsal lines.

21. Senanga (not depicted)
Western Province: left bank of the Zambezi River
At Senanga, one specimen with $2n = 58$ and NF = 86 was found (J. L. Meier, pers. obs.). The single male from Senanga was grey-brown with a small headspot.

22. Watopa (Fig. 8)
North-western Province: South of the Kabompo River
In Watopa we found $2n = 56$ and NF = 76. Headspots in these animals were very small. This form lives in sympathy with *C. mechowi* ($2n = 40$). Here, they seem to be segregated by habitat. *Cryptomys mechowi* was collected in the dry evergreen Marquesia woodland while the other form was found on the edge of the adjacent dambos (water-logged grassland).

23. Salujinga (Fig. 9)
North-western Province: Salujinga
The single female which was karyotyped had $2n = 44$, NF = 76. All specimens from that area have a solid
body shape with a reddish pelage colour. Headsspots were very small or absent. This form too is sympatric with C. mechowi. Habitat segregation at this particular location seems to be similar as above, with C. mechowi in the miombo woodland and the smaller, new form occupying the grassland–woodland ecotone.

**DISCUSSION**

**Chromosomal variation in Zambian Cryptomys**

The karyotypic data confirm that Cryptomys belonging to the Zambezian lineages of the northern clade is characterized by an extraordinary large chromosomal diversity (Table 2). The karyotypic extent parallels that in other subterranean rodents (spalacids, ctenomyids and geo- myids; cf. Nevo, 1999). The present study extends the list of karyotypes of Cryptomys known at present in Zambia with nine new karyotypes from the Zambezian region.

The data show that Cryptomys anselli (2n = 68) has a broad distribution on the left bank of the Kafue River. In contrast, the pattern around the Kafue floodplain system is more complex. Mole-rats with two distinct karyotypes (2n = 45 and 2n = 54) occur in sympatry in the Monze area and it is likely that at least a third one (no conclusive metaphases were available) may occur just south of that area (2n between 45 and 54) (P. Van Daele, pers. obs.).

The karyotype of the mole-rats from Dongo (2n = 42) represents a cytogenetically distinct taxon, with a high percentage of metacentric chromosomes. The sampled population was characterized by a notably high frequency of mole-rats (young and adult) with very asymmetric headspots. The ‘Dongo’ population is separated by the Kalomo River from the ‘Kalomo’ population (2n = 50). Only 200 km south-west, the karyotype of animals from Livingstone/Sekute (2n = 56) is found. This population is genetically closely related to C. kafuensis (Ingram et al., 2004). The karyotype of the population south of the Kabompo River at Watopa (2n = 56) is also similar to the karyotype of C. kafuensis (2n = 58). The tiny headspots seem to be characteristic in the former, though the diagnostic value of this feature has yet to be tested.
Chromosomal diversity in Cryptomys

Fig. 8. Standard karyotype of Cryptomys sp. from Watopa (male), with $2n = 56$.

Mole-rats from Salujinga, in the Mwinilunga high rainfall area, are distinct in external morphology (pelage colour, lack of headspots) and karyotype ($2n = 44$). Note, however, that the karyotype of *C. bocagei* ($2n = 58$, NF = 98; Aguillar, pers. comm.) although expected in northwest Zambia (Honeycutt, Allard *et al.*, 1991; Bennett & Faulkes, 2000; Cotterill, 2002) was not found in our samples and we consider the species extralimital to Zambia.

Cytogenetic results indicate that the mole-rats from the south-west, on the right bank of the Zambezi River, are closely related if not identical to *C. damarensis*, which was confirmed for the west Zambezi samples by a molecular genetic analysis (Ingram *et al.*, 2004). In contrast, on the other side of the Zambezi, the topotypical population of *C. damarensis micklemi* ($2n = 60$) is cytogenetically very distinct. Two independent molecular genetic analyses further showed that the karyotypes of *C. damarensis micklemi* and *Cryptomys* from Senanga ($2n = 58$) are closely related and belong to a sister clade of *C. kafuensis* (Ingram *et al.*, 2004; P. Van Daele, pers. obs.). It is therefore necessary to abandon the traditional view that *C. micklemi* is a subspecies of *C. damarensis*. It seems justified to consider *Cryptomys micklemi* as a separate species.

Fig. 9. Standard karyotype of Cryptomys sp. from Salujinga (female), with $2n = 44$.

While the chromosomal diversity in Zambian Cryptomys is, as shown by our data, enormous, it is difficult at this point to decide whether or not a particular karyotype represents a separate species or just reflects intraspecific chromosomal variation. The impact that chromosomal change has on speciation is still a matter of debate. However, there is little doubt that some kinds of chromosomal rearrangements can severely reduce the viability and/or fitness of chromosomal hybrids, thus acting as a possible first step in population divergence (for a review see King, 1993). Furthermore olfactory experiments with *C. anselli, C. kafuensis* and *C. mechowi* have shown that at least these (karyotypically distinct) species treat anogenital odours of genetically closer heterospecifics as more similar to conspecifics’ odours than to odours of less closely related heterospecifics (Heth, Todrank & Burda, 2002). Particular preference for individuals with similar odours could then serve as a possible pre-mating isolating mechanism during speciation (Todrank & Heth, 2003). Therefore, although it is not known if hybrids between the new chromosomal forms exist, several of the new karyotypes may represent separate species living in allopatry, parapatry or even sympatry.
Fig. 10. Occurrence of known karyotypes of Cryptomys in Zambia (with indication of 2n).

Chromosomal variation and the role of palaeogeography

As opposed to the karyological stability (2n = 54) in the southern clade of Cryptomys (Nevo et al., 1986; Bennett & Faulkes, 2000; Faulkes, Verheyen et al., 2004; N. C. Bennett, pers. comm.), our data confirm that Zambezian mole-rats evolved into an array of cytogenetically different forms. This radiation may be correlated with subtle geomorphological heterogeneity, resulting from geomorphological evolution in recent geological times, with an emphasis on the dynamics of drainage systems in the Zambezian region. The available data suggest that the current configuration of major drainage systems constitute major barriers to dispersal of many of these karyotypically distinct mole-rat populations (Fig. 10). We hypothesize that the joining and fragmentation of precursors of extant rivers driven by crustal flexion (both down warping and uplifting) and climatic shifts may have influenced diversification in Zambian mole-rats.

Moore & Larkin (2001; and references therein) provide evidence of the principal events in drainage system transformation in south-central Africa (summarized in Moore & Larkin, 2001: fig. 14a–d). Cotterill (2003) proposed a model which explains Plio-Pleistocene speciation of particular mammals in the region. His model did not account for Cryptomys, but suggested that geomorphological changes may have played a role. In the following phylogeographic scenario, a model is proposed structured on drainage evolution to explain how among other things geomorphic changes may have controlled diversification of the northern clade. Important geomorphological events are summarized in Fig. 11a–d.

1. Through the late Neogene, the palaeo-Chambeshi River was part of a major endorheic system that drained into the palaeo-lake Makgadikgadi. This barrier would have separated ancestral eastern and western populations Cryptomys on the Zambian plateau. An ancestor of the widely distributed C. mechowi probably spread along the Zambezi watershed, a route that was open for more than a million years.

2. In the next stage, the Luapula captured the Chambeshi south-west of the Bangweulu Swamps, resulting in the palaeo-Kafue as a new barrier. Thus, populations in north-east Zambia (ancestors of a clade containing C. amatus, Cryptomys from ‘Kasama’ and C. whytei) were then isolated. As the headwaters of the Luangwa River were located south of their current position, the ancestor(s) of C. whytei dispersed to the Nyika plateau. Populations on the left bank of the current Zambezi
Chromosomal diversity in Cryptomys

Fig. 11. Drainage system evolution in South-Central Africa. Modified after Du Toit (1933), Moore & Larkin (2001) and Cotterill (2003). M: Palaeo-lake Makgadikgadi, B: Lake Bangweulu. (a) Pliocene; (b) Early Pleistocene; (c) Late Pleistocene; (d) Late Pleistocene/Early Holocene: configuration very similar to the modern configuration. Dashed lines, axes of crustal flexuring: OB, Okavango–Bangweulu; OKZ, Ovamboland–Kalahari–Zimbabwe.

are then still contained by the Palaeo-Kafue, while C. damarensis ancestors were contained west of the Upper Zambezi.

3. In the Late Pleistocene, C. damarensis was persistently isolated from its northern congeners after the Mid-Zambezi captured the Upper Zambezi at the Victoria Falls. This event was probably contemporaneous with headwater capture of the Upper Kafue by the Mid-Zambezi in the vicinity of Namwala (indicated by a pronounced capture elbow). This scenario envisages that the Kafue River achieved its current configuration only recently in the Late Pleistocene. Around capture-elbows, floodplains typically came into existence. In such areas, the chances for fixation of chromosomal changes would have been higher since there would be ample opportunity for allopatric and peripatric speciation around floodplains as a consequence of both undulating water levels and general geomorphological disturbance in such areas. This may well explain the high chromosomal variability that was found along a 250 km stretch at the Kafue Flats. Here at least three forms (C. kafuensis, C. ‘Lochinvar’ and C. ‘Monze’) coexist.

4. In the late Pleistocene, after a phase of sundering and reconnecting, the Zambezi attains its current bed. This would have allowed C. damarensis to spread into what is now Zambia, west of the Upper Zambezi.

The extant pattern of distribution in the Zambezian Cryptomys would therefore be consistent with a model of speciation through geomorphological dynamics. Interestingly, Cotterill (2003) originally proposed a similar scenario to explain vicariance in certain African mammals (antelopes and primates) in south-central Africa. Although drainage evolution has been an important determinant driving Cryptomys speciation, climatic changes (e.g. xeric conditions vs conditions during pluvials) and habitat heterogeneity (e.g. Kalahari sand vs alluvium) are other factors that could have had a synergetic effect. Finer sampling, comparisons of chromosome banding and phylogeographic analyses (using mtDNA markers) are a prerequisite to illuminate the evolutionary history in Zambian mole-rats more clearly. It is highly likely that further cryptic forms await discovery in areas (e.g. floodplain systems at capture elbows) of the Zambezian region predicted by the model.

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