Ecological constraints drive social evolution in the African mole-rats

C. G. FAULKES[1*], N. C. BENNETT[2], M. W. BRUFORD[1], H. P. O'BRIEN[1], G. H. AGUILAR[3] AND J. U. M. JARVIS[3]

[1]*Conservation Genetics Group, Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK*
[2]*Department of Zoology & Entomology, University of Pretoria, Pretoria 0002, South Africa*
[3]*Department of Zoology, University of Cape Town, Rondebosch 7700, Cape Town, South Africa*

SUMMARY

The African mole-rats (family Bathyergidae) are subterranean hystricomorph rodents occurring in a variety of habitats and displaying levels of sociality which range from solitary to eusocial, making them a unique mammalian taxonomic group to test ecological influences on sociality. Here, we use an extensive DNA-based phylogeny and comparative analysis to investigate the relationship between ecology, sociality and evolution within the family. Mitochondrial cytochrome-*b* and 12s rRNA trees reveal that the solitary species are monophyletic when compared to the social species. The naked mole-rat (*Heterocephalus glaber*) is ancestral and divergent from the Damaraland mole-rat (*Cryptomys damarensis*), supporting previous findings that have suggested the multiple evolution of eusociality within the family. The *Cryptomys* genus is species-rich and contains taxa exhibiting different levels of sociality, which can be divided into two distinct clades. A total of seven independent comparisons were generated within the phylogeny, and three ecological variables were significantly correlated with social group size: geophyte density ($p < 0.05$), mean months per year of rainfall greater than 25 mm ($p < 0.001$), and the coefficient of rainfall variation ($p = 0.001$). These results support the food–aridity hypothesis for the evolution of highly social cooperative behaviour in the Bathyergidae, and are consistent with the current theoretical framework for skew theory.

1. INTRODUCTION

The Bathyergidae (African mole-rats) is a unique group of subterranean hystricomorph rodents, ranging in sociality from solitary dwelling species through to two eusocial species, the Damaraland mole-rat, *Cryptomys damarensis*, and the naked mole-rat, *Heterocephalus glaber* (Jarvis 1981; Bennett & Jarvis 1988; Jarvis & Bennett 1993). In these latter species, groups of cooperatively breeding conspecifics exhibit a reproductive division of labour, cooperative care of offspring, and overlapping generations, defined by Wilson (1971) as the criteria for eusociality. The multiple occurrence of highly social behaviour makes the bathyergid rodents a powerful model to examine the phylogenetic, genetic, and ecological factors involved in the evolution of such traits.

The Bathyergidae are endemic to sub-Saharan Africa, and under the current scheme of classification five genera and 12 species are recognized. The monotypic genera *Heterocephalus* and *Heliophobius* are restricted to East Africa, with the latter also extending into eastern Zaire and Malawi. The genera *Bathyergus* and *Georychus*, currently thought to contain three species, are restricted to parts of South Africa and Namibia. The most specious genus, *Cryptomys*, is also the most widely distributed with species occurring from Ghana, Nigeria, Cameroon and the Central African Republic through to the extreme south-western tip of South Africa (for a review, see Honeycutt *et al.* 1991). The relationships within the *Cryptomys* genus are particularly unclear. While Honeycutt *et al.* (1991) recognize seven species (based on morphological characteristics), in the past, 49 forms have been named. Furthermore, populations in Zambia are known to be divergent (Filippucci *et al.* 1994). *Cryptomys* is of particular interest because the species inhabit a wide range of habitats from mesic (*C. h. natalensis*) to arid (*C. damarensis*), and exhibit varying degrees of sociality from the relatively small groups of around eight individuals in *C. h. natalensis*, to the eusocial *C. damarensis* which can occur in colonies of up to 41 individuals (Jarvis *et al.* 1994).

Previous phylogenetic studies based on mitochondrial DNA restriction fragment analysis have placed the two eusocial species *H. glaber* and *C. damarensis* as sister groups (Honeycutt *et al.* 1991), however, a molecular phylogeny based on sequence differences of the mitochondrial 12S rRNA gene (Allard & Honeycutt 1992) and control region (Faulkes *et al.* 1997) suggests that the two species are evolutionarily very divergent.

A current hypothesis regarding the evolution of cooperatively breeding societies with a high reproductive skew

*Author for correspondence (c.faulkes@ucl.ac.uk).

in the Bathyergidae correlates sociality with habitat aridity and food distribution (Lovegrove & Wissel 1988; Lovegrove 1991; Jarvis 1985; Jarvis *et al*. 1994). Solitary species tend to inhabit mesic areas, while the two eusocial species are found in arid regions with unpredictable rainfall (Jarvis *et al*. 1994). In the latter kind of habitat, reduced rainfall generally leads to harder soil and elevates the energetic cost of burrowing. Patchy food resources in the form of underground roots and the swollen tubers of arid-adapted plants lead to an increased risk of unsuccessful foraging (Bennett 1988; Jarvis *et al*. 1994; Lovegrove 1991). Consequently, selection for group living, cooperative foraging and communal care of offspring might be expected because of the high cost of dispersal for most individuals. To date, these trends in social group size, rainfall, and food distribution have not been quantified and analysed with respect to a comprehensive phylogeny for the family.

The aim of this study was to use mitochondrial DNA sequence analysis and comparative analysis by independent contrasts (CAIC; Purvis & Rambaut 1995) to (i) investigate the relationship between phylogeny, ecology and sociality within the Bathyergidae, and thereby test the food–aridity hypothesis, and (ii) clarify the phylogenetic relationships within the *Cryptomys* genus.

2. MATERIALS AND METHODS

(*a*) *Sample collection and DNA preparation*

Samples were obtained from mole-rats captured in a number of geographical locations in sub-Saharan Africa (table 1). Tissue was collected from the animals either immediately post-mortem, or from toe clips taken during mark–recapture studies. Samples were then frozen in liquid nitrogen and transferred to a freezer at −20 °C, or preserved in a solution of 25% dimethyl sulphoxide in saturated NaCl, then transferred to a freezer at −20 °C. DNA was extracted from the tissue samples using a standard protocol as previously described by Faulkes *et al*. (1990, 1997).

(*b*) *Mitochondrial DNA analysis*

Double stranded amplifications of genomic DNA using PCR, and sequencing by chain termination, were carried out with a standard protocol as previously described for mole-rats (Faulkes *et al*. 1997). Primers used for both PCR amplification and sequencing of the 12S rRNA and cytochrome-*b* gene of the mitochondrial DNA were as follows: 'A','B','C','D' for 12S rRNA (Allard & Honeycutt 1992), and L14724 and H15915 for cytochrome-*b* (Irwin *et al*. 1991). In addition, nine additional mole-rat-specific cytochrome-*b* primers were used to obtain overlapping sequence as follows, with numbers referring to the position of the 3′ end of the primer relative to the human mitochondrial DNA map (Anderson *et al*. 1981): L14960: 5'-TAGCACACATCTGCCGAG-3′ (all mole-rat species except *C. mechowi*); L14960: 5'-TGATACACATCTGCCGGG-3' (*C. mechowi*); L15184: 5'-TGAGGTGCTACCGTAATC-3' (*Cryptomys* spp. except *C. hottentotus*); L15184: 5'-TGAGGAGCTACTGTGATT-3' (*H. argentiocinereus*); L15184: 5'-TGAGGTGCGACAGTCATC-3' (*H. glaber*); L15184: 5'-TGGGGAGCCACCGTAATT-3' (*G. capensis*); L15184: 5'-TGAGGGGCTACCGTAATA-3' (*B. suillus*); L15184: 5'-TGAGGTGCTACCGTAATT-3' (*C. hottentotus*); L15420: 5'-CATTCCACCCCTACTACTC-3' (*H. glaber*).

Table 1. *Number of individuals sequenced (cytochrome-b) for each species with their approximate geographical locations*

(An asterisk denotes that 12s rRNA was also sequenced for this sample.)

species	no. of individuals sequenced	country and location
H. glaber	1	Ethiopia, Dembalawachu (4°N, 38°E)
	3	Kenya, Lerata (1°N, 38°E)
	1	Kenya, Mtito Andei (3°S, 38°E)
C. damarensis	4	Namibia, Dordabis (24°S, 18°E)
	4	Botswana, Okavango Delta (19°S, 23°E) Botswana, Maun (20°S, 23°E)
C. h. hottentotus	1	South Africa, Steinkopf (29°S, 18°E)
	1	South Africa, Klawer (32°S, 18°E)
	1	South Africa, Somerset West (34°S, 18°E)
C. h. 'pretoria'	1*	South Africa, Pretoria (26°S, 28°E)
C. h. nimrodi	1*	Zimbabwe, Hillside (20°S, 29°E)
C. h. natalensis	1	South Africa, Kokstad (31°S, 29°E)
C. h. amatus	1*	Zambia, Lusaka (15°S, 28°E)
C. mechowi	1*	Zambia, Chingola (12°S, 28°E)
	1	Zaire, Kinshasa (4°S, 16°E)
C. bocagei	1*	Angola, Lubango (15°S, 13°E)
C. darlingi	1*	Zimbabwe, Goromonzi (17°S, 30°E)
C. h. 'choma'	1*	Zambia, Choma (17°S, 27°E)
G. capensis	1	South Africa, Cape Town (34°S, 18°E)
B. suillus	1	South Africa, Stilbaai (34°S, 22°E)
B. janetta	1	South Africa, De Riet (30°S, 17°E)
H. argenteocinereus	1	Kenya, Athi Plains (1°S, 37°E)

(*c*) *Analysis of mitochondrial DNA sequences*

Sequences were aligned manually and the number of substitutions, transition/transversion ratios, and the amino acid sequence for the cytochrome-*b* region were determined using MacClade v. 3 (Madison & Madison 1992). As an outgroup for the phylogenetic analysis, we obtained the published sequence for another old-world hystricomorph rodent, the Cape porcupine (*Hystrix africaeaustralis*; Nedbal *et al*. 1994; Ma *et al*. 1993). Relationships among haplotypes were then analysed by a number of phylogenetic algorithms, including maximum parsimony using bootstrap replications of the branch-and-bound search option in PAUP v. 3.1 for the

Table 2. *Summary of species, group sizes and ecological factors for the family Bathyergidae*

(Numbers in brackets after the mean geophyte densities refer to the area (m2) that was surveyed. For mean annual rainfall values are grand means ±s.e.m., numbers in brackets refer to the number of weather stations sampled for each species.)

species	max group size	mean body mass (g)	mean gestation (days)	mean geophyte density (no. m^{-2})	mean digestible energy (kj m^{-2})	mean annual rainfall (mm yr^{-1})	mean no months of >25 mm rainfall (months yr^{-1})	rainfall coefficient of variation
Heterocephalus glaber	295[1]	34[3]	72[3]	0.08±0.02 (3600)[1]	204.5	362.1±43.6 (9)	3.95±0.30	79.2±6.1
Cryptomys damarensis	41[2]	104[3]	85[3]	3.7±2.4 (9300)[5,6,†]	179.1	389.7±66.6 (6)	4.29±0.55	59.2±7.3
Cryptomys h. hottentotus	16[†]	77[3]	63[3]	278±1.71 (14)[†]	1342.7	538.2±57.9 (3)	6.43±0.98	33.5±5.64
Cryptomys h. 'pretoria'	12[†]	—	—	—	—	703.6±101.4 (1)	7.08±0.86	27.0
Cryptomys h. amatus	10[†]	67[†]	100[7]	—	—	889.6±38.8 (3)	5.58±0.27	37.6±2.8
Cryptomys h. darlingi	9[†]	65[†]	59[8]	37.2±6.6 (10)[†]	1654.1	769.3±49.0 (3)	5.76±0.23	44.6±2.0
Cryptomys mechowi	8[†4]	233†	104[9]	173.0±17.1 (20)[†]	2786.6	1129.2±33.1 (4)	6.37±0.29	44.5±5.3
Cryptomys h. natalensis	8[†]	106[3]	—	—	—	871.6±108.6 (3)	8.58±0.52	47.1±7.1
Cryptomys h. nimrodi	15[†]	—	—	—	—	—	—	—
Cryptomys h. 'choma'	4[†]	—	—	—	—	—	—	—
Cryptomys bocagei	4[†]	—	—	44.4±20.1 (5)[†]	520.5	840.1±20.3 (3)	6.3& ±0.29	87.9±38.7
Georychus capensis	1[3]	181[3]	46[3]	278.8±1.71 (14)[†]	1342.7	556.2±46.6 (8)	7.57±0.49	29.6±2.3
Bathyergus suillus	1[3]	933[3]	52[3]	278.8±1.71 (14)[†]	1342.7	550.2±71.3 (5)	7.46±0.72	29.6±3.5
Bathyergus janetta	1[3]	451[3]	—	18.8±6.8 (318)[†]	376.8	81.8±24.6 (3)	1.09±0.50	23.5±7.4
Heliophobius argenteocinereus	1[3]	160[3]	87[3]	—	—	912.6±60.8 (4)	7.31±0.48	81.8±14.0

[†]C. G. Faulkes, N. C. Bennett, J. U. M. Jarvis, A. Spinks and G. H. Aguilar unpublished results.
[1]Brett 1991*a*,*b*.
[2]Jarvis & Bennett 1993.
[3]Jarvis & Bennett 1991.
[4]Burda, 1993.
[5]Bennett 1988.
[6]Lovegrove & Knight-Eloff 1988.
[7]Burda 1990.
[8]Bennett *et al*. 1994.
[9]Bennett & Aguilar 1995.

Apple Macintosh (Swofford 1993), and bootstrap replications of the maximum parsimony by exhaustive search, maximum likelihood, UPGMA and neighbour joining methods in PHYLIP v. 3.57c for UNIX (Felsenstein 1989). Parsimony analysis was performed with and without weighting of codon positions with empirically derived values as follows: codon position 1: 2.9; position 2: 10.0; position 3: 1.0. Kimura two-parameter genetic distances were calculated with the dnadist program in PHYLIP.

Sequences have been deposited in NCBI with accession numbers U87521–U87527 and AF012213–AF012243.

(*d*) *Comparative analysis*

Ecological factors thought to be important in the food–aridity hypothesis were selected (table 2). The phylogenetic trees constructed following sequence analysis, together with the ecological data, were used to generate phylogenetically independent contrasts (Purvis & Rambaut 1995), enabling us to correlate sociality with rainfall and food distribution parameters.

As a measure of sociality, we chose maximum group size, a parameter for which we have the most complete data set, although group sizes for *C. h.* '*choma*', *C. bocagei* and *C. mechowi* are based on limited data for 3–6 colonies. Mean body mass values used were for males where sexual dimorphism occurred.

Rainfall data were obtained from the Global Historical Climatological Network database (`www.ncdc.noaa.gov/ghcn/ghcn.html`). A total of 49 representative weather stations were chosen across the known ranges for the mole-rat species studied. Where possible these were close to field sites where colonies had been trapped. The monthly rainfall data available for each station ranged over 22–124 years (mean ± s.e.m. was 67.7±3.2 years). The mean number of months per year having more than 25 mm of rain gives an approximation of the time available for major burrowing

activity. The figure of 25 mm is the estimated amount of rainfall needed to penetrate and soften the soil at the depth of most foraging tunnels (Jarvis *et al.* 1994). A measure of the unpredictability of rainfall is given by the coefficient of variation, obtained by dividing the mean annual rainfall by its variance.

Food distribution parameters were obtained mainly from the literature, and from unpublished ecological surveys (N. C. Bennett, C. G. Faulkes, J. U. M. Jarvis and A. C. Spinks, unpublished data). Only geophytes eaten by the mole-rats were quantified. For geophyte density, mean values together with the s.e.m. are given where available. These were obtained either by taking quadrat samples (20–340 replicates of $0.5\,m^2$–$1.0\,m^2$ quadrats) or conducting complete surveys of an area. The digestible energy available per unit area was calculated from the geophyte density values using published nutritional information (Bennett & Jarvis 1995). Geophyte data for *C. h. hottentotus*, *G. capensis* and *B. suillus* were gathered from an area where all three species occur sympatrically. For *H. glaber*, density values included all tubers except *Macrotyloma* spp. For the latter, which form less than 5% of the total available biomass (Brett 1991*a*), it was not possible to quantify the density data due to their highly patchy distribution. Rainfall and geophyte data for *C. h. hottentotus* are for mesic habitats, although their range does also extend into arid areas (mean annual rainfall for three weather stations $= 145.0 \pm 39.3\,mm\,yr^{-1}$), geophyte densities ($78.8 \pm 8.1\,m^2$) are relatively high but group sizes (2–14) are similar to mesic adapted hottentotus (A. C. Spinks, N. C. Bennett and J. U. M. Jarvis, unpublished data).

The standardized linear contrasts produced by CAIC were tested for statistical significance by regression through the origin using log maximum group size as the dependent variable.

3. RESULTS

(*a*) *Sequencing*

For 12S rRNA, a total of 835 base pairs (bp) were sequenced for six species and compared with previously published sequences for nine other taxa (Allard & Honeycutt 1992). A 22 bp conserved section of the gene, not sequenced by Allard & Honeycutt, was omitted from our data for the analysis, to allow direct comparison of 813 bases (Allard & Honeycutt 1992). Out of the sequence of 813 bp, there were 325 informative sites, and the ratio of transition to transversion substitutions was 2.09. For cytochrome-*b*, a total of 1140 bp of sequence (corresponding to 379 amino acids) comprising the entire gene were obtained. There were 557 informative sites, and the transition-transversion ratio was 2.57.

Each of the 31 samples (including intraspecific comparisons) that were sequenced produced a distinct haplotype, and a distance matrix quantifying the differences between the taxa is shown in table 3. Patterns of divergence at cytochrome-*b* were greater than the 12s rRNA region, with mean percentage sequence differences being approximately twice that of 12S.

Mean percentage within-species differences were quantified for cytochrome-*b* within and between geographical locations for three species, as follows. Southern and northern Kenyan, and Ethiopian populations of *H. glaber* ($n = 5$ individuals): within, 1.50; between, 4.44 (range 2.00–5.80; Faulkes *et al.* 1997); *Cryptomys h. hottentotus* from South African populations having 200–400 km separation ($n = 3$): between, 3.00 (range 2.27–4.28); *Cryptomys damarensis* from Namibia and Botswana ($n = 10$): within, 0.86; between, 0.78 (range 0.09–1.47). The ten *C. damarensis* individuals analysed were breeding malefemale pairs from five different colonies from three different geographical locations. Each of these individuals had a different cytochrome-*b* haplotype, indicating that these breeding pairs were from different maternal lineages, and confirming observations of obligate outbreeding in wild colonies (Jarvis & Bennett 1993).

DNA sequences from each of the two loci were analysed independently and combined to give a total of 1951 bp. All phylogenetic analyses produced trees having the same overall topology, with slight differences only in the grouping of the solitary species relative to one another, and the placement of *C. damarensis* and *C. darlingi* relative to one another. Representative trees from three analyses are shown in figure 1*a*–*c*.

Results indicate that a revision of the classification of *Cryptomys* is required. The genus divided into two subclades within the tree, having minimum genetic distances between them comparable to inter-generic distances within the family. For example, for cytochrome-*b*, the genetic distance of *C. h. hottentotus* from *C. bocagei* was 20.62%, which compares with a distance of 21.6% between *B. janetta* and *G. capensis*. Two species currently thought to be subspecies of *C. hottentotus*, *C. h. amatus* and *C. h. darlingi*, were found to be divergent. Phylogenetic analysis placed both the latter species in a different subclade to *C. hottentotus* (figure 1 and table 3), supporting a previous suggestion, based on karyotypic differences in the fundemental number, that *C. h. darlingi* should be regarded as a separate species (Aguilar 1993). *C. h. amatus* also had a different karyotype and diploid number to *C. h. hottentotus* (figure 1*b*; G. H. Aguilar, unpublished data). Two other taxa, *C. hottentotus* captured near Pretoria and near Choma, Zambia, currently of unclear taxonomic status were also identified. Again, the latter was clearly divergent and karyotypically different from *C. h. hottentotus*. Three other allopatric species of *Cryptomys* from west and central Africa, *C. foxi*, *C. zechi* and *C. ochraeceocinereus*, were not investigated in this study and their phylogenetic relationships with the other *Cryptomys* species remains unknown.

The data in table 2, excluding gestation length, were used together with each of the three trees in figure 1 to perform a series of three comparative analyses (CAIC), each of which produced seven phylogenetically independent contrasts at the nodes designated A to G in figure 1, between ecological factors and social group size (table 3). Because the phylograms in figure 1*a*,*b* are consensus trees with no available branch-length data, the option in CAIC to assume equal branch

Table 3. *Estimates of pairwise genetic distances*

(Values are expressed as percentages, calculated from Kimura's two-parameter model in PHYLIP. (i) Above diagonal: between 12S rRNA haplotypes. (ii) Below diagonal: between cytochrome-*b* haplotypes. *H. africaeaustralis* was the outgroup used to root trees in the phylogenetic analysis.)

	1	2	3	4	5	6	7	8
1. *C. damarensis*	—	5.18	5.40	3.87	5.10	3.55	11.17	10.94
2. *C. bocagei*	11.21	—	3.92	4.79	6.56	5.50	11.60	11.45
3. *C. mechowi*	11.91	8.37	—	4.66	5.58	5.55	12.20	10.88
4. *C. h. darlingi*	7.19	11.97	11.01	—	3.61	3.89	11.36	10.23
5. *C. h. amatus*	6.11	10.77	12.14	7.31	—	2.44	12.32	10.39
6. *C. h.* '*choma*'	7.76	12.66	12.62	8.39	3.42	—	11.31	10.52
7. *C. h. natalensis*	19.05	19.96	22.22	21.94	21.55	23.15	—	3.93
8. *C. h.* '*pretoria*'	19.80	20.29	23.44	21.65	21.25	22.21	8.45	—
9. *C. h. nimrodi*	20.01	19.81	22.52	20.98	21.75	22.19	11.31	10.44
10. *C. h. hottentotus*	21.27	20.62	24.24	22.90	22.77	23.74	12.63	11.40
11. *B. janetta*	20.49	20.78	22.49	20.97	21.29	23.37	21.55	22.56
12. *B. suillus*	21.13	21.20	22.56	21.18	21.96	23.75	20.93	22.08
13. *G. capensis*	22.77	21.80	25.99	23.16	22.82	24.15	22.08	22.32
14. *H. argenteocinereus*	22.70	23.66	24.56	23.62	22.99	24.70	26.61	26.04
15. *H. glaber*	28.99	27.44	29.23	28.59	29.46	31.98	28.11	27.20
16. *H. africaeaustralis*	25.05	26.77	26.78	26.76	26.08	26.82	28.13	27.64

	9	10	11	12	13	14	15	16
1. *C. damarensis*	12.26	11.29	14.48	14.66	13.15	19.46	22.81	25.49
2. *C. bocagei*	12.10	12.36	14.14	14.85	12.85	19.72	21.68	25.31
3. *C. mechowi*	12.17	11.46	16.61	16.84	13.92	19.35	22.04	27.76
4. *C. h. darlingi*	11.16	11.30	15.13	15.35	13.03	19.71	22.57	27.08
5. *C. h. amatus*	11.52	12.94	16.11	16.31	14.73	21.30	23.76	27.60
6. *C. h.* '*choma*'	11.42	11.89	14.54	14.36	13.42	20.10	23.42	26.41
7. *C. h. natalensis*	3.96	6.51	15.39	16.23	14.11	16.54	22.93	21.34
8. *C. h.* '*pretoria*'	3.99	5.79	14.89	15.23	13.41	18.15	22.17	22.21
9. *C. h. nimrodi*	—	4.80	15.06	15.93	12.67	17.73	21.67	22.22
10. *C. h. hottentotus*	11.51	—	15.40	16.35	14.11	17.19	21.12	23.29
11. *B. janetta*	23.55	24.83	—	3.41	11.84	16.88	22.09	23.37
12. *B. suillus*	22.64	24.81	5.46	—	12.96	17.91	22.48	23.20
13. *G. capensis*	21.29	21.74	21.90	21.61	—	15.49	21.53	22.96
14. *H. argenteocinereus*	25.43	27.18	24.33	25.17	26.25	—	22.99	23.65
15. *H. glaber*	28.24	30.99	26.69	28.18	31.41	33.33	—	24.24
16. *H. africaeaustralis*	29.98	29.77	29.08	28.63	30.37	28.71	28.01	—

lengths was used. In figure 1*c*, the empirical branch-length data was used in CAIC, thus accounting for the amount of genetic difference between taxa as well as their phylogenetic relationships.

In all three of these analyses (table 4), log maximum group size showed a negative correlation with log mean geophyte density ($r = -0.778$ to -0.822; $p < 0.05$), and was positively correlated with the rainfall coefficient of variation ($r = 0.882$–0.915; $p < 0.004$). Digestible energy available per unit area did not correlate significantly with group size ($p < 0.179$).

A further analysis, using the tree topology in figure 1*a* included gestation length as an independent variable and produced six independent contrasts, as gestation length was not known for *B. janetta* (at nodes A to G, excluding E in figure 1*a*; table 4, 1a(ii)). In addition to significant correlations between log maximum group size and log mean geophyte density ($p = 0.008$), and rainfall coefficient of variation ($p = 0.003$), the loss of the contrast at node E (figure 1) resulted in a third independent variable, the mean nunber of months per year of over 25 mm of rainfall, becoming highly negatively correlated with group size ($p = 0.0009$). This arises because the excluded node E included rainfall data from *B. janetta*, which is an arid-adapted solitary species (see §4). Although *H. glaber* has the largest group sizes and is also the smallest in body size, there were no clear trends in body mass ($r = 0.201$–0.576; $p = 0.175$–0.633) or gestation length ($r = 0.522$; $p = 0.229$).

4. DISCUSSION

A previous molecular phylogeny of nine species of African mole-rats (Allard & Honeycutt 1992) revealed the independent grouping of the solitary species (figure 1) with respect to *H. glaber* and the *Cryptomys* genus, suggesting at least two convergent evolutionary events

Table 4. *Summary of regression statistics generated from independent standard linear contrasts between group size and ecological factors for the family Bathyergidae*

(Values were calculated following CAIC analysis of the three phylogenetic trees displayed in figure 1*a*, *b* and *c*; For analysis of the trees 1*a* and 1*b*, equal branch lengths were assumed in the CAIC program (branch length data are not generated for consensus trees), while analysis of 1*c* used empirical branch lengths from parsimony analysis. The nodes A–G at which the independent contrasts were calculated by CAIC are also labelled on figure 1. Gestation length was included as an independent variable in 1*a* (ii) only, with the loss of one contrast at node E (figure 1*a*). Statistics were obtained by regression (through the origin) of each parameter (independent variable) onto the log maximum group size (dependent) variable.)

tree	statistic	log mean geophyte density	log mean digestible energy	log mean annual rainfall	mean no months of >25 mm rainfall	rainfall coefficient of variation	mean body mass	gestation length
1*a* (i)	*r*	−0.778	−0.527	0.003	−0.223	0.915	−0.469	—
	$F(1,6)$	9.223	2.313	0.0001	0.315	31.050	1.690	—
	p	0.022*	0.179	0.993	0.595	0.001***	0.241	—
1*a* (ii)	*r*	−0.884	−0.690	−0.476	−0.952	0.919	−0.576	0.522
	$F(1,5)$	17.965	4.554	1.463	49.02	27.310	2.485	1.877
	p	0.008**	0.086	0.280	0.0009***	0.003**	0.175	0.229
1*b*	*r*	−0.822	−0.358	−0.092	−0.243	0.902	−0.328	—
	$F(1,6)$	12.484	0.880	0.051	0.376	26.135	0.723	—
	p	0.012*	0.384	0.828	0.562	0.002**	0.428	—
1*c*	*r*	−0.787	−0.513	−0.190	−0.243	0.882	−0.201	—
	$F(1,6)$	9.748	2.149	0.224	0.375	20.98	0.253	—
	p	0.021*	0.193	0.652	0.562	0.004**	0.633	—

leading to eusociality. These groupings and this conclusion are also supported by our analysis of the mitochondrial cytochrome-*b* gene. Further analysis of 12S rRNA in six additional cryptomids, and cytochrome-*b* analysis of 15 bathyergid taxa, together with intraspecific comparisons, have enabled us to clarify relationships within the *Cryptomys* genus. It is clear from the results of this sequence analysis and karyotype data that the genus is more specious than suggested by Honeycutt *et al.*'s (1991) reassesment of the morphological data, although probably not to the extent of there being 49 forms (Ellerman 1940).

Although there are no established criteria for the assignment of subspecies, species, or genera from molecular data, we can make valid comparisons of sequence divergence within the family. What constitutes a species is dependent on which definition is applied, and this has been the subject of much debate. The biological species concept (Mayr 1942) states that species are reproductively isolated populations, while the wider ranging phylogenetic species concept (Cracraft 1983) defines a species as a group whose members contain one diagnostic character that is common to all, but absent in close relatives of the group. While a fully integrated approach including morphometric and behavioural characters is beyond the scope of this paper, some conclusions can be drawn from the molecular phylogeny. From the genetic distances displayed in table 3, the smallest distances between currently recognized species were 8.37 for *C. mechowi* and *C. bocagei*, and 5.46 for *B. suillus* and *B. janetta* (cytochrome-*b*). Intraspecific distances ranged from 0.09 to 5.80. Using these values as an approximate index, and with reference to the phylogentic relationships displayed in figure 1, we suggest that *C. h. amatus* from Lusaka and *C. h. darlingi* from Harare are divergent from *C. h. hottentotus*, and should be classed as separate species. Secondly, the species of *Cryptomys* caught near Choma in Zambia, which grouped with *C. amatus*, but which were karyotypically quite different (figure 1*b*) and had 3.42% difference at cytochrome-*b* (within our observed intraspecific range), should possibly be considered as a subspecies of *C. amatus*. Other populations of Zambian mole-rats are also known to be divergent (Filipucci *et al.* 1994), and further work needs to be done on these populations. Within the *C. hottentotus* subclade (figure 1), *C. h. nimrodi* from Zimbabwe, *C. h. natalensis* from Natal, and *C. hottentotus* captured near Pretoria, were divergent with minimum cytochome-*b* distances of 8.45% between them. They were also a minimum distance of 11.45% away from *C. h. hottentotus*, and could perhaps be considered as separate species, rather than as subspecies.

The division of the *Cryptomys* genus into two distinct and divergent subclades within the tree, having genetic distances between them of the same magnitude as inter-generic distances within the family, was unexpected. Whether these clades really constitute different genera can probably only be established by further analysis of morphological data in the light of these molecular results. Division of some *Cryptomys* species into two groups based on skull morphology has been suggested previously (e.g. Honeycutt *et al.* 1991), and such partitioning is consistent with the molecular phylogeny.

Comparative analysis using CAIC produced results in support of the food–aridity hypothesis (Jarvis *et al.*

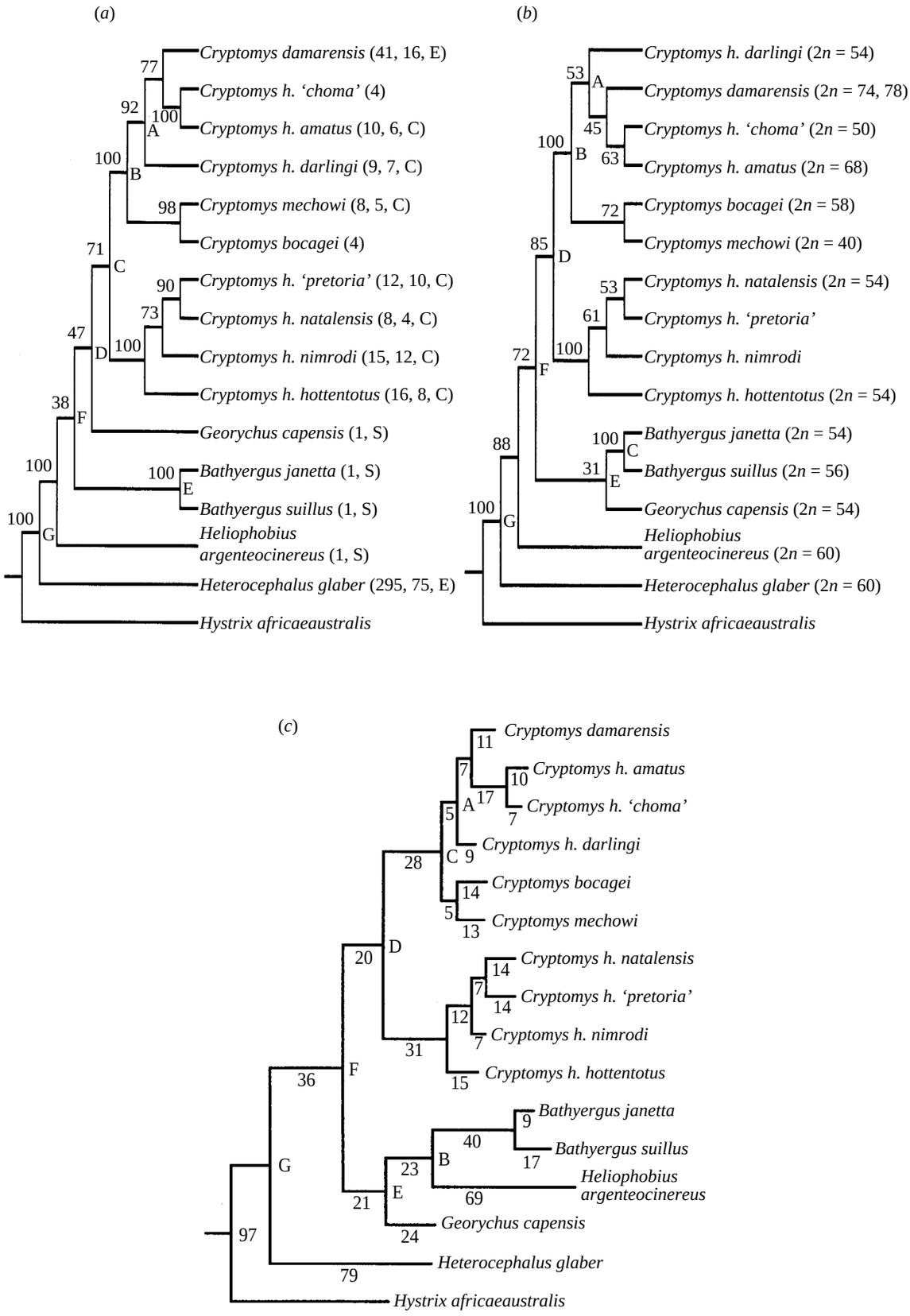

Figure 1. Phylogentic relationships of 15 African mole-rat haplotypes and one outgroup species (*H. africaeaustralis*) based on (*a*) parsimony analysis (PHYLIP) of combined 12S rRNA and cytochrome-*b* sequences. This is a consensus of two trees which differed only in the branching order of *Bathyergus* and *Heliophobius*, having a tree length of 2123 and a consistency index of 0.57. Maximum social group sizes and, where known, mean group sizes and social system (S = solitary, C = colonial with usually one breeding pair per colony, E = eusocial), are shown in parentheses. (*b*) Consensus tree following parsimony

1994). Maximum group size (log) showed a negative correlation with log mean geophyte density, and was positively correlated with the rainfall coefficient of variation. Digestible energy available per unit area did not differ significantly with group size. Thus, in the habitats that support the more social species, available energy in the form of geophytes is less densely distributed, a fact clearly shown in other studies that have calculated measures of geophyte clumping and patchiness for *H. glaber* and *C. damarensis* (Bennett 1988; Brett 1991*a*; Lovegrove & Knight-Eloff 1988). While mean annual rainfall was lower in the habitats of the most social species, *H. glaber* and *C. damarensis*, there was no significant correlation. A confounding factor may have been inclusion of the data for *B. janetta*, an arid-adapted solitary species that inhabits extremely dry coastal regions, in apparent contradiction to the foodaridity hypothesis. However, in these areas the soil may be wetter than the rainfall data indicates, as coastal fogs and seepage areas provide additional unquantified precipitation and moistening of the soil (Jarvis, unpublished data). This may also explain why geophyte density and availible energy were high relative to the amount of rainfall in these areas. The analysis in table 4 that excluded the contrast at node E (figure 1*a*), resulted in another ecologically important rainfall variable for mole-rats, the mean number of months per year having more than 25 mm of rain (the amount of rain needed to penetrate the soil to a depth of most foraging burrows; Jarvis *et al*. 1994), showing a significant negative correlation with increasing group size. Although *H. glaber* has the largest group sizes and is also the smallest in body size, there were no clear trends between either body mass or gestation length and sociality, as has previously been suggested for the Bathyergidae (Burda 1990).

Maximum group size was chosen as our measure of sociality as it reflects contraints on dispersal. Ideally, we would have included the degree of reproductive skew in our comparative analysis. However, apart from the solitary species where we could assume skew to be zero (all have equal chances of reproduction), estimates of reproductive skew have only been made for two social species. In these cases, high skew is associated with increased group size: the number of individuals within a colony that never attained breeding status were 99.9% for the naked mole-rat ($n > 4000$ animals), and 92% for the Damaraland mole-rat ($n > 403$ animals; Jarvis *et al*. 1994).

In contrast to the other genera within the family, *Cryptomys* was relatively species-rich, with the ten taxa analysed here exhibiting a variety of levels of sociality. Given that all the Bathyergidae are subject to high population viscosity and reproductive isolation due to the subterranean niche, why has speciation not occurred to the same extent in the other genera? In the fire ant, *Solenopsis invicta*, it has been suggested that speciation may be driven by reproductive isolation and barriers to gene flow resulting from the development of alternative social organizations (DeWayne Shoemaker & Ross 1996). While this hypothesis could explain the radiation of the social mole-rats, it may not explain species richness within *Cryptomys* (all taxa appear to be social, albeit to different degrees), and the lack of speciation in *Heterocephalus* (Faulkes *et al*. 1997). A possible explanation for the latter could be that *H. glaber* is highly adapted to a very uniform and narrow ecological niche, whereas *Cryptomys* occurs over a much wider range of habitats.

The reduced genetic variation in natural populations of *H. glaber*, a facultative inbreeder with only rare dispersal events (Faulkes *et al*. 1990, 1997; Reeve *et al*. 1990; O'Riain *et al*. 1996), is also in complete contrast to the eusocial *C. damarensis* where inbreeding is avoided and the chances of dispersal and outbreeding are greater (Jarvis & Bennett 1993; Jarvis *et al*. 1994). Field observations of outbreeding were confirmed genetically, as all five breeding pairs of *C. damarensis* analysed in this study had different mitochondrial haplotypes, and were therefore derived from different maternal lineages. The importance of intra-group relatedness and the relative costs and benefits of altruistic behaviour in cooperative breeders was first quantified by Hamilton (1964) in social insects and has developed into modern skew theory (Vehrencamp 1983; Keller & Reeve 1994). We are now able for the first time to consider phylogeny, ecology, and relatedness together in analysing the causes of social evolution in the Bathyergidae, and show the importance of environmental constraints as a determining factor. It would now seem that inbreeding is a derived trait peculiar to naked mole-rats, and studies in the Damaraland mole-rat suggest that given a high enough level of ecological constraint, cooperative care of outbred siblings is sufficient for high skew, eusocial societies to evolve.

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Figure 1. (*continued*) analysis (PHYLIP) of 12S rRNA sequences, having a tree length of 662 and a consistency index of 0.66. Diploid numbers ($2n$) are shown in parentheses (references as cited in Jarvis & Bennett (1991), Aguilar (1993) and G. H. Aguilar, unpublished data, and Macholan *et al*. (1993)). (c) One of three trees generated by parsimony analysis (PAUP) of 12S rRNA sequences, having a tree length of 661 and a consistency index of 0.66. Numbers above branches in (*a*) and (*b*) are percentage bootstrap values following 100 replications, while numbers below branches in (*c*) are branch lengths. Letters A–G designate the seven internal nodes at which independent contrasts were calculated by CAIC.

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