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Allozyme differentiation and systematic relationship of Zambian Giant mole-rats, *Cryptomys mechowi* (Bathyergidae, Rodentia)

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Abstract

Allozymic variation encoded by 31 gene loci was studied in giant mole-rats (*Cryptomys mechowi*) and two species of common mole-rats from Zambia, and allozymic diversity encoded by 22 loci was re-analysed in *Cryptomys damarensis*, *C. h. hottentotus*, and *C. h. natalensis* from South Africa. The Zambian common mole-rat of the karyotype 2n = 68 is more closely related to the giant mole-rat than to the common mole-rat of the karyotype 2n = 58. There is a clear dichotomy between the three Zambian taxa and *C. damarensis*, on the one hand, and *C. h. hottentotus* and *C. h. natalensis*, on the other hand. The relationship (based upon the allozymic variation) does not correspond to the current geographical and ecological distribution or to morphological (particularly coloration and size) differentiation.

Introduction

The family Bathyergidae includes five genera of subterranean hystricognathous rodents endemic to Africa. While four genera are represented by only a single or two species with restricted distribution, one genus – *Cryptomys* – is remarkably polyspecific and occurs from semi-arid to mesic habitats in different soil types over a wide geographical range from Ghana to the Cape Province in South Africa. Extreme variation in many morphological traits makes taxonomic treatment of this genus very difficult. While up to 44 and 49 species of *Cryptomys* have been listed by Allen (1939) and Ellermann (1940), respectively, only three to seven species were recognized by later authors (Nowak and Paradiso 1983 and Honeycutt et al. 1991, respectively). More recently, Filippucci et al. (1994) have examined mole-rats of two populations in Zambia and having identified them as new (formally not yet named) species clearly distinct from those considered by Honeycutt et al. (1991), thus, we have suggested that the number of species of the genus should be higher than seven.

There is a general agreement (for further citations, see Filippucci et al. 1994 and Macholan et al. 1993) that because of extreme morphological variation, systematics of *Cryptomys* has to be based on (or at least should involve) karyology, serology, and molecular genetics. Nevertheless, all students of bathyergid taxonomy also agree that giant mole-rats (*Cryptomys mechowi*) which are morphologically distinct (particularly as far as their body size is concerned) from other *Cryptomys* mole-rats, should be considered a separate species.

Cryptomys mechowi occurs in relatively mesic habitats (annual rainfall over 1,000 mm) in Angola, Zaire, Zambia, Malawi, and Tanzania (e. g., Ansell and Dowsett 1988). For a long time, the biology of giant mole-rats has been virtually unknown to zoologists. Only recently we have demonstrated their facultative carnivory and have shown that in contrast to predictions of the "aridity-hypothesis" of eusociality, giant mole-rats are social (Burda and Kawalika 1993). Giant mole-rats have a body weight at least four-times that of the common mole-rats. The white head spot, which is characteristic of the common mole-rats, is completely missing in most individuals of giant mole-rats. Furthermore, we have shown (Macholan et al. 1993) that *C. mechowi* is clearly distinct also in karyotype, having the lowest number of chromosomes (2 n = 40) found among *Cryptomys* so far. It was therefore of interest to examine the taxonomic status of *C. mechowi* also by means of other methods; particularly allozyme analysis which has been employed successfully in the study of the bathyergid taxonomy previously (Nevo et al. 1987; Janecek et al. 1992; Filippucci et al. 1994).

Material and methods

Electrophoretic analysis was carried out on four specimens of the giant mole-rat, *Cryptomys mechowi*, collected at the Ndola town periphery (Copperbelt Province, Zambia) and on 18 specimens belonging to two karyotypically distinct forms of common mole-rats, *Cryptomys* sp., from Zambia, characterized by different chromosomal sets: 2 n = 68 (population Lusaka; 15 specimens examined) and 2 n = 58 (population Itezhi-Tezhi – Hot-Springs; 3 specimens). For comparison, samples of *C. h. hottentotus* (6 animals), *C. h. natalensis* (4 animals), and *C. damarensis* (1 specimen) from South Africa, previously analysed and described by Nevo et al. (1987), were used.

Tissues of each specimen were preserved in the laboratory at –80 °C until processed. Homogenates for electrophoresis were obtained from portions of muscle and kidney tissues crushed in distilled water. Genic variation was assessed using standard horizontal starch-gel electrophoresis of enzymes coded for by 31 presumptive loci. All gels were prepared using an 11%-suspension of Connaught hydrolysed starch.

Homogenates obtained from muscle were processed for the following enzymatic proteins: α-glycerophosphate dehydrogenase (E.C. 1.1.1.8; αGpdh), lactate dehydrogenase (E.C. 1.1.1.27; Ldh-1 and Ldh-2), malate dehydrogenase (E.C. 1.1.1.37; Mdh-1 and Mdh-2), malic enzyme (E.C. 1.1.1.40; Me-1 and Me-2), isocitrate dehydrogenase (E.C. 1.1.1.42; Idh-1 and Idh-2), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44; 6-Pgdh), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49; G-6-pdh), indophenol oxidase (E.C. 1.15.1.1; Ipo-1 and Ipo-2), nucleoside phosphorylase (E.C. 2.4.2.1; Np), glutamate-oxalacetate transaminase (E.C. 2.6.2.1; Got-1 and Got-2), creatine kinase (E.C. 2.7.3.2; Ck), adenylate kinase (E.C. 2.7.4.3; Adk), phosphoglucomutase (E.C. 2.5.7.1; Pgm-1 and Pgm-2), esterases (E.C. 3.1.1.1; Est-1,Est-2 and Est-3), acid phosphatase (E.C. 3.1.3.2; Acph), aminopeptidase (E.C. 3.4.11; Ap-2), adenosine deaminase (E.C. 3.5.4.4; Ada), fumarase (E.C. 4.2.1.2; Fum), and phosphoglucose isomerase (E.C. 5.3.1.9; Pgi).

Homogenates obtained from kidney were processed for: alcohol dehydrogenase (E.C. 1.1.1.1; Adh), sorbitol dehydrogenase (E.C. 1.1.1.14; Sdh), and xanthine dehydrogenase (E.C. 1.2.3.2; Xdh).

The employed procedures were described by Nevo et al. (1987) and Filippucci et al. (1988). Isozymes were numbered in order of decreasing mobility from the most anodal one. Allozymes were designated numerically according to their mobility, relative to the most common allele (=100; <100 = slower mobility; >100 = faster mobility) in *C. h. hottentotus* from South Africa.

Allozyme data were analysed with the BIOSYS-1 program of Swofford and Selander (1981). Intrapopulational genetic variation was estimated by the following genetic indices: the mean observed (Ho) and expected (He) heterozygosity per locus, the proportion of polymorphic loci in the population under the 1% criterion (i. e., a locus is considered polymorphic if the frequency of the most commmon allele does not exceed 0.99), and the mean number of alleles per locus (A). The amount of genetic divergence between populations was estimated with both Nei's standard and unbiased indices of genetic identity I, and distance D (Nei 1972, 1978). A dendrogram of the genetic relationships among populations was obtained using the UPGMA clustering method (Sokal and Sneath 1963).

Results and discussion

Pattern of variation

Fifteen out of the thirty-one loci scored were monomorphic and fixed for the same allele in the three Zambian *Cryptomys* taxa (i. e. Lusaka, Itezhi-Tezhi, and *C. mechowi*). These were in turn: Adh, Sdh, Ldh-1, Ldh-2, Mdh-2, Me-2, Idh-1, Idh-2, Ipo-1, Ipo-2, Got-2, Adk, Pgm-2, Ap-2, Est-2. The allele frequencies of the polymorphic and/or diagnostic loci in the three Zambian *Cryptomys* populations under study are given in table 1. For detailed allele frequencies in South African populations see, Filippucci et al. (1994).

The population from Lusaka, characterized by $2n = 68$, displayed polymorphism at the following loci: αGpdh, Me-1, G6pdh, Got-1, Pgm-1, Ada, Pgi, Acph, Xdh, and Est-3. The Itezhi-Tezhi population, characterized by $2n = 58$, was polymorphic at the following loci: αGpdh, Mdh-1, Me-1, 6Pgdh, Pgi, Pgm-l, and Est-1. Finally, *C. mechowi* was polymorphic at Mdh-1, Np, Got-1, Ck, Pgm-1, and Fum.

Genetic summary

The mean value of expected and observed heterozygosity, proportion of polymorphic loci, and the mean number of alleles per locus are shown in table 2. In the Lusaka sample, the observed heterozygosity (Ho) corroborated well with the value expected under the Hardy-Weinberg equilibrium (He). The obvious discrepancy between He and Ho in the Itezhi-Tezhi population was most probably caused by a bias due to small sample size (cf., also He and Ho in *C. mechowi*). Yet, according to Gorman and Renzi (1979), the effect of a small sample size upon the heterozygosity should be less than 2.5% as compared with a larger sample size.

The overall mean proportion of polymorphic loci (P1%) for the three populations ranged from 0.193 in *C. mechowi* to 0.323 in the Lusaka population. Instead, values of the number of alleles per locus (A) were similar in all three samples (1.193 in *C. mechowi* to 1.323 in Lusaka mole-rats).

The observed genetic variation thus corresponded to the values already observed by Nevo et al. (1987) in South African species of *Cryptomys* and was within the range reported for other rodents in general (Nevo et al. 1990).

Genetic differentiation

Two loci (6Pgdh and Acph) were found discriminant between Lusaka and Itezhi-Tezhi, displaying fixation of alternative alleles, and five loci (αGpdh, Mdh-1, Got-1, Pgi, and Est-1) partially discriminated the two populations. Furthermore, two loci were fixed for alternative alleles in the Lusaka sample and *C. mechowi* (6Phdh and Est-1) and two loci discriminated the latter species and $2n = 58$ species from Itezhi-Tezhi (Acph and Est-1), another two loci (6Pgdh and Pgi) being discriminant partially. In addition, appreciable differences in allelic frequencies were revealed in Pgm-1 between *C. mechowi* and both Zambian populations of common mole-rats.

Genetic distance

For comparison with the South African taxa, *C. h. hottentotus*, *C. h. natalensis* and *C. damarensis*, the number of loci considered had to be decreased to 22. The following loci were therefore excluded from the subsequent analysis: Sdh, Me-2, Ipo-l, Ipo-2, Ck, Adk, Ap-2, Fum, and Est-3. Since only one specimen of *C. damarensis* was included in

Table 1. Allelic frequencies observed at the polymorphic and/or discriminant loci for the analysed Zambian populations of the genus *Cryptomys*. Number of examined specimens in parentheses

Loci	Alleles	Lusaka	Itezhi-Tezhi	*C. mechowi*
αGpdh		(14)	(3)	(4)
	100	–	–	–
	106	0.93	0.50	1.00
	110	0.07	0.50	–
Mdh-1		(15)	(3)	(4)
	100	1.00	0.67	0.75
	103	–	–	0.25
	105	–	0.33	–
Me-1		(15)	(3)	(4)
	110	0.97	0.67	1.00
	113	0.03	0.33	–
6Pgdh		(15)	(3)	(4)
	100	–	0.83	–
	105	1.00	–	–
	95	–	0.17	1.00
G6pdh		(15)	(3)	(4)
	100	0.23	–	–
	95	0.77	1.00	1.00
Xdh		(13)	(3)	(4)
	100	0.89	1.00	1.00
	105	0.12	–	–
Np		(15)	(3)	(4)
	100	1.00	1.00	0.75
	95	–	–	0.25
Got-1		(15)	(3)	(4)
	100	0.30	–	0.75
	90	0.70	–	–
	105	–	1.00	0.25
Ck		(15)	(3)	(4)
	100	1.00	1.00	0.75
	105	–	–	0.25
Pqm-1		(9)	(3)	(4)
	100	0.94	0.67	–
	103	0.06	0.33	0.50
	105	–	–	0.50
Ada		(13)	(2)	(4)
	105	0.96	1.00	1.00
	109	0.04	–	–
Fum		(15)	(3)	(4)
	100	1.00	1.00	0.88
	95	–	–	0.12
Pgi		(15)	(3)	(4)
	100	0.90	0.17	1.00
	90	–	0.83	–
	96	0.10	–	–
Acph		(11)	(2)	(1)
	100	0.05	–	–
	105	0.95	–	1.00
	110	–	1.00	–
Est-1		(13)	(3)	(4)
	105	1.00	0.50	–
	108	–	0.50	–
	110	–	–	1.00
Est-3		(5)	(2)	(2)
	100	0.90	1.00	1.00
	105	0.10	–	–

Table 2. Values of expected (He) and observed (Ho) heterozygosity, percentage of polymorphic loci (P1%), and average number of alleles per locus (A) based on 31 loci

Population	N	He	Ho	P1%	A
Lusaka	15	0.058	0.057	0.323	1.323
Itezhi-Tezhi	3	0.093	0.043	0.226	1.226
C. mechowi	4	0.071	0.056	0.193	1.193

the analysis, the "biased" Nei's identity and distance indices (Nei 1972) had to be used. A UPGMA dendrogram summarizing the genetic relationships between the populations studied is given in figure 1. (Since the phenogram of the three Zambian taxa based on 31 loci conforms to the corresponding part of the tree constructed for all six taxa, only one figure is presented).

Strikingly, in spite of its conspicuous size differentiation from other *Cryptomys* species, *C. mechowi* revealed the closest relationship to 2n = 68 population of the common mole-rat from Lusaka (D = 0.116). On the other hand, the 2n = 58 species from Itezhi-Tezhi appears to be more distinctly related to both former taxa (D = 0139 and 0.163 respectively).

As already shown by Filippucci et al. (1994) for Lusaka and Itezhi-Tezhi common mole-rats, and now demonstrated also for *C. mechowi*, all three Zambian taxa group together with South African *C. damarensis*, both subspecies of *C. hottentotus* being genetically much more distinct from all other species under study. A clear separation of *C. damarensis* from *C. hottentotus* was demonstrated already in previous studies (Nevo et al. 1987; Janecek et al. 1992; Filippucci et al. 1994). Interestingly, the grouping based upon the genetic distances is reflected also in the numbers of arms of autosomes (cf., Fig. 1). It would be, however, preliminary to speculate at this point about the chromosome speciation in mole-rats. On the other hand, the systematic relationship between Zambian mole-rats and *C. damarensis* does not correspond to the actual

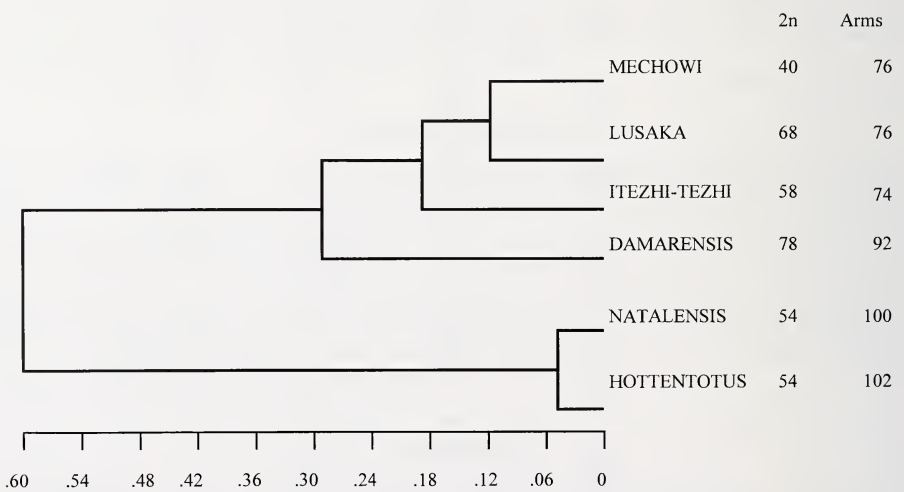

Fig. 1. UPGMA dendrogram summarizing the genetic relationship among populations of the genus *Cryptomys* from Zambia and South Africa. (Data on the number of chromosomes and arms of autosomes are taken from Macholan et al. (1993) and the literature cited therein.)

geographical and ecological distribution. The South African species (*C. h. hottentotus*, *C. h. natalensis* and *C. damarensis*) occur in dry regions (annual rainfall = 200–600 mm), while Zambian mole-rats originate from rather mesic areas (annual rainfall = 800–1,200 mm).

Zusammenfassung

Allozymdifferenzierung und systematische Beziehungen von sambischen Riesengraumullen, Cryptomys mechowi (Bathyergidae, Rodentia)

Allozymvariation (31 Genloci) wurde bei Riesengraumullen (*Cryptomys mechowi*) und zwei Taxa von Kleingraumullen aus Sambia untersucht. Zum Vergleich wurden parallel Allozyme (22 Genloci) bei *Cryptomys damarensis*, *C. h. hottentotus* und *C. h. natalensis* aus Südafrika neu analysiert. Der Kleingraumull mit dem Karyotyp 2 n = 68 zeigt eine nähere Verwandtschaftsbeziehung zum Riesengraumull als zum Kleingraumull mit dem Karyotyp 2 n = 58. Es besteht eine klare Dichotomie zwischen den drei untersuchten sambischen Taxa und *C. damarensis* einerseits und *C. h. hottentotus* und *C. h. natalensis* anderseits. Die jetzige geographische und ökologische Verbreitung und die morphologischen (insbesondere Größen- und Fellfarb-)Unterschiede der untersuchten Arten stimmen nicht mit den auf der Allozymvariation beruhenden Verwandtschaftsbeziehungen überein.

References

Allen, G. M. (1939): A checklist of African mammals. Harvard Coll., Cambridge, Mass. USA: Bull. Mus. Comp. Zool.

Ansell, W. F. H.; Dowsett, R. J. (1988): Mammals of Malawi. An annotated check list and atlas. Cornwall: Trendrine Press.

Burda, H.; Kawalika, M. (1993): Evolution of eusociality in the Bathyergidae. The case of the giant mole rats (*Cryptomys mechowi*). Naturwissenschaften **80**, 235–237.

Ellermann, J. R. (1940): The families and genera of living rodents. London: Trustees of the Brit. Mus. Nat. Hist.

Filippucci, M. G.; Burda, H.; Nevo, E.; Kocka (1994): Allozyme divergence and systematics of common mole-rats (*Cryptomys*, Bathyergidae, Rodentia) from Zambia. Z. Säugetierkunde **59**, 42–51.

Filippucci, M. G.; Rodino, E.; Nevo, E.; Capanna, E. (1988): Evolutionary genetics and systematics of the garden dormouse, *Eliomys* Wagner, 1840. 2-Allozyme diversity and differentiation of chromosomal races. Boll. Zool. **55**, 47–54.

Gorman, G. C.; Renzi, J. Jr. (1979): Genetic distance and heterozygosity estimates in electrophoretic studies. Effects of sample size. Copeia 1979, 24–249.

Honeycutt, R. L.; Allard, M. W.; Edwards, S. V.; Schlitter, D. A. (1991): Systematics and evolution of the family Bathyergidae. In: The Biology of the Naked Mole-Rat. Ed. by P. W. Sherman, J. U. M. Jarvis, and R. D. Alexander. Princeton, New Jersey: Princeton Univ. Press. Pp. 66–96.

Janecek, L. L.; Honeycutt, R. L.; Rautenbach, I. L.; Erasmus, B. H.; Reig, S.; Schlitter, D. A. (1992): Allozyme variation and systematics of African mole-rats (Rodentia: Bathyergidae). Biochem. Syst. Ecol. **20**, 401–416.

Macholan, M.; Burda, H.; Zima, J.; Misek, I.; Kawalika, M. (1993): Karyotype of the giant mole-rat, *Cryptomys mechowi* (Rodentia, Bathyergidae). Cytogenet. Cell Genet. **64**, 261–263.

Nei, M. (1972): Genetic distance between populations. Am. Nat. **106**, 283–292.

Nei, M. (1978): Molecular Evolutionary Genetics. New York: Columbia Univ. Press.

Nevo, E.; Ben-Shlomo, R.; Beiles, A.; Jarvis, J. U. M.; Hickman, G. C. (1987): Allozyme differentiation and systematics of the endemic subterranean mole rats of South Africa. Biochem. Syst. Ecol. **15**, 489–502.

Nevo, E.; Filippucci, M. G.; Beiles, A. (1990): Genetic diversity and ist ecological correlates in nature: Comparison between subterranean, fossorial and aboveground small mammals. In: Evolution of Subterranean Mammals at the Organismal and Molecular Levels. Ed. by E. Nevo and A. O. Reig. New York: Allan R. Liss. Pp. 347–366.

Nowak, R. M.; Paradiso, J. L. (1983): Walker's Mammals of the World. 4th ed. Baltimore, London: The John Hopkins Univ. Press.

Sokal, R. R.; Sneath, P. N. A. (1963): Principles of Numerical Taxonomy. San Francisco: W. H. Freeman.

Swofford, D. L.; Selander, R. B. (1981): BIOSYS-1: A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. **72**, 281–283.

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