

Research article

Isozyme and allozyme markers distinguishing two morphologically similar, medically important *Mastomys* species (Rodentia: Muridae) Andre A Smit and Herman FH Van der Bank*

Address: Dept. of Zoology, Rand Afrikaans University, PO Box 524, Auckland Park, 2006, South Africa

E-mail: Andre A Smit - andrekarl@yahoo.com; Herman FH Van der Bank* - fhvdb@na.rau.ac.za

*Corresponding author

Published: 21 September 2001

Received: 27 June 2001

BMC Genetics 2001, **2**:15

Accepted: 21 September 2001

This article is available from: <http://www.biomedcentral.com/1471-2156/2/15>

© 2001 Smit and Van der Bank; licensee BioMed Central Ltd. Verbatim copying and redistribution of this article are permitted in any medium for any non-commercial purpose, provided this notice is preserved along with the article's original URL. For commercial use, contact info@biomedcentral.com

Abstract

Background: Two common southern African mice species, *Mastomys coucha* and *M. natalensis*, are widely distributed throughout the subregion and overlap in many areas. They also share a high degree of morphological similarity, making them impossible to distinguish in the field at present. These multimammate mice are documented carriers of serious disease vectors causing Lassa fever, plague and encephalomyocarditis, which coupled to their cohabitation with humans in many areas, could pose a significant health risk. A preliminary study reported the presence of isozyme markers at three loci (**GPI-2**, **PT-2**, **-3**) in one population each of *M. coucha* and *M. natalensis*. Two additional populations (from the Vaal Dam and Richards Bay) were sampled to determine the reliability of these markers, and to seek additional genetic markers.

Results: Fifteen proteins or enzymes provided interpretable results at a total of 39 loci. Additional fixed allele differences between the species were detected at **AAT-1**, **ADH**, **EST-1**, **PGD-1**, **Hb-1** and **-2**. Average heterozygosities for *M. coucha* and *M. natalensis* were calculated as 0.018 and 0.032 respectively, with a mean genetic distance between the species of 0.26.

Conclusions: The confirmation of the isozyme and the detection of the additional allozyme markers are important contributions to the identification of these two medical and agricultural pest species.

Background

The *Praomys/Mastomys* species complex is a group of morphologically similar species that occur in very diverse habitats throughout sub-Saharan Africa [1]. The taxonomy of this group has been clouded by a diversity of opinions and this confusion has been further compounded by many external and cranial similarities between species, and thus the systematics of this group remains poorly understood, with many species yet to be described [1]. The genus *Mastomys* is well represented in southern Africa, especially by the ubiquitous multimammate mice,

Mastomys coucha (Smith, 1836) and *M. natalensis* (Smith, 1834). The distribution of both species is only provisional at this stage [2], and the species are known to be sympatric in some areas, and allopatric in others. These common agricultural pests are also commensal with man, often sheltering in houses in order to safely rear their young. In African kraals they occur in very large numbers, living in the fabric and thatch of pole and mud huts [2].

Because these mice carry important diseases, the medical implications of this cohabitation with man are obvious. *Mastomys coucha* acts as a reservoir for the Rickettsian *Yersinia pestis*, the organism causing plague [3]. The three forms of plague are bubonic, primary pneumonic and primary septicaemic. Bubonic plague, which is the most common type in epidemics, is fatal in about 25% to 50% of untreated cases. Pneumonic plague, a highly contagious (airborne) form, and septicaemic plague, a generalised blood infection, are more rare forms, and usually fatal [4]. Plague still exists as a threat in southern Africa, with much current concern over the possibility of outbreaks in rat infested inner cities.

Both species are also carriers of the Banzi and Witwatersrand viruses [3] and *M. natalensis* is a natural reservoir for the arenavirus causing Lassa fever. This infection, occurring predominantly in West Africa up until now, has a high mortality rate, especially amongst pregnant women and even in patients with modern health care. The Mopeia virus is another similar virus carried by *M. natalensis*, but the effect thereof on man is as yet unknown [5]. *Mastomys* rodents were also linked to an outbreak of encephalomyocarditis (EMC) among African elephants in the Kruger National Park in the last decade [6]. However, due to the fact that both species occur within the boundaries of the park, it is uncertain as to whether only one or both of these species may carry the EMC virus.

The differences between the two species are thus neither ecologically nor economically trivial. This illustrates the need for research into their relatedness, especially of a genetic nature, as this could yield insights as to why only certain species carry the above mentioned diseases, especially as these two sibling species are impossible to distinguish on the basis of external morphology alone. For this reason, both *M. coucha* and *M. natalensis* were de-

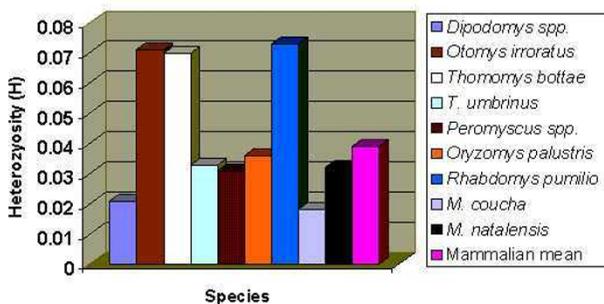


Figure 1
Comparative heterozygosities (H) for various species of Rodentia, along with the mammalian mean.

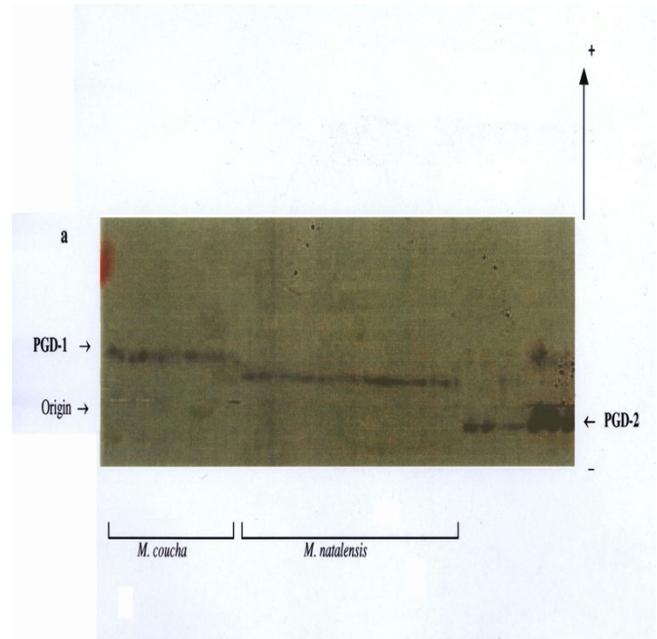


Figure 2
Fixed allele differences between *Mastomys coucha* and *M. natalensis* at **PGD-1**.

scribed as one species, that of *M. natalensis*[7]. However, these species do differ in chromosome number, with *M. coucha* having $2n = 36$ and *M. natalensis* having $2n = 32$ [8]. Differences in behaviour, urethral lappet and spermatozoal morphology and haemoglobin mobility in electrophoresis were also reported by [8]. In contrast, more recent research into spermatozoal morphology of these two species led Breed [9] to report that they were extremely similar, if not identical in this respect. Dippenaar *et al.*[3] used multivariate analysis of the cranial characteristics of each species in order to identify them. However, there were discrepancies in identification of 50% of the specimens collected at a sympatric locality according to this method and that of haemoglobin mobility. The preliminary report of Smit *et al.*[10] showed isozyme differences at three loci, **GPI-2** in liver, and **PT-2** and **-3** in muscle tissue. This study also revealed through iso-electric focusing that the haemoglobin components present in each species were unique from the other. In the present study, we have used electrophoretic techniques in an attempt to confirm the isozyme markers (locus differences) reported above in two additional allopatric populations of *M. coucha* and *M. natalensis*, as well as to identify additional genetic markers.

Results and Discussion

All 15 of the proteins stained for provided adequate resolution or activity to be interpreted. Locus abbreviations and enzyme commission numbers along with the buffers

Table 1: Locus abbreviations, enzymes stained for, enzyme commission numbers (E. C. no.) as well as the tissues and buffers giving optimal results (buffer descriptions are given in Materials and Methods).

Protein	Locus	E.C. No.	Buffer	Tissue
1. Aspartate aminotransferase	AAT-1, -2	2.6.1.1	P	Bl
2. Alcohol dehydrogenase	ADH	1.1.1.1	TC	L
3. Creatine kinase	CK-1 to -3	2.7.3.2	TC	M
4. Esterases	EST-1 to -3	3.1.1.-	RW, MF	M, L
5. General proteins	PT-1 to -5		PAGE	M
6. Glucose-6-phosphate isomerase	GPI-1, -2	3.5.1.9	RW, P	L
7. Glyceraldehyde-3-phosphate dehydrogenase	GAP	1.2.1.12	TC	M, L
8. Glycerol-3-phosphate dehydrogenase	GPD-1 to -4	1.1.1.8	RW	M, L
9. Haemoglobin	Hb-1, -2		PAGE	Bl
10. Isocitrate dehydrogenase	IDH-1 to -3	1.1.1.42	TC	M, L
11. L-Lactate dehydrogenase	LDH-1, -2	1.1.1.27	RW	Bl, M
12. Malate dehydrogenase	MDH-1, -2	1.1.1.37	TC	M, L
13. 6-Phosphogluconate dehydrogenase	PGD-1 to -3	1.1.1.44	HC	M, L
14. Phosphoglucomutase	PGM-1 to -3	5.4.2.2	P, RW	M
14b Phosphoglucomutase	PGM-4	5.4.2.2	RW	L
15. Superoxide dismutase	SOD-1, -2	1.15.1.1	P, RW	M, L

Bl = blood, L = liver, M = Muscle

and tissues yielding the best results are given in Table 1. The following protein products migrated cathodally: AAT-2, ADH, GAP, GPI-1, -2, MDH-2 and PGD-2.

Nineteen (49%) of the 39 loci studied were polymorphic in one or more of the four populations analysed (Table 2). These were: **AAT-1, -2, ADH, EST-1, -2, GAP, GPI-2, IDH-1, -2, LDH-2, PGD-1, PGM-1 to -4, PT-2, -3, Hb-1 and Hb-2**. This was somewhat higher than the previous 33% estimate in the preliminary study, and mainly due to the presence of the additional markers detected. Fixed allele differences between *M. natalensis* and *M. coucha* were detected at **AAT-1** (blood; Fig. 2), **ADH** (liver; Fig. 3), **EST-1** (liver; Fig. 6), **PGD-1** (muscle; Fig. 4), **Hb-1** and **-2** (blood; Fig. 5). Locus differences were confirmed in the new material at **GPI-2, PT-2** and **-3**. The differences at these last three loci were considered to be isozyme rather than allozyme differences due to the large separation distance between the bands [10]. Thus these three loci remain useful in identifying individuals from either species, along with the new allozyme markers.

PGD and **Hb** were not analysed in the La Lucia and Montgomery Park populations in the initial study. However, the results obtained via PAGE for the **Hb** locus in the Vaal and Richards Bay populations, using the haemoglobin purification method described by Falk *et al.*[11], are analogous to those by Gordon [8]. Significant differences ($F_{IT} = 0.936$, $P < 0.05$) were obtained for **EST-1** in the earlier study, but the additional data from the new material and increased resolution at this locus

allowed us to re-evaluate those results, and indicate this locus is useful as another marker (Fig. 6). Results for the **ADH** locus were also re-evaluated, as analysis of the new populations was performed on different buffer systems that provided better resolution than previously obtained (Fig. 4). The most cost and labour effective analysis for the routine identification of these two species would appear to be to stain for the **GPI-2** and **EST-1** loci on an **RW** buffer system running muscle and liver samples. While PAGE may provide better resolution, it is more expensive, requires more time to run and only one protein can be stained for at a time. The other markers at **PGD-1** and **ADH-1** are on incompatible buffer systems, and the purified haemoglobin samples needed for the **AAT-2**

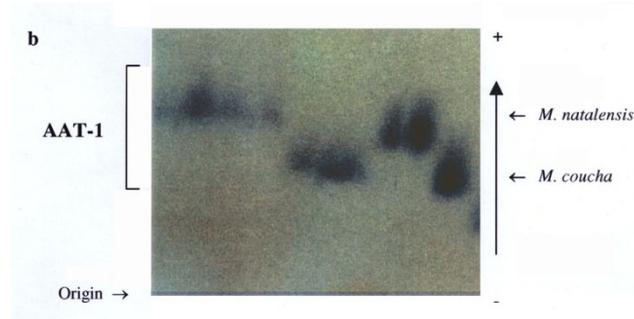


Figure 3
Fixed allele differences between *Mastomys coucha* and *M. natalensis* at **AAT-1**.

Table 2: Allele frequencies, average heterozygosity per locus (H), mean number of alleles per locus (A) and percentage of loci polymorphic (P) for all populations studied with standard errors in parentheses.

Locus	Allele	Montgomery Park	Vaal	La Lucia	Richards Bay
AAT-1	A			1.000	1.000
	B	1.000	1.000		
AAT-2	A	0.250			
	B	0.750	1.000	1.000	0.500
	C				0.500
ADH	A				0.062
	B			1.000	0.938
	C	1.000	1.000		
EST-1	A			1.000	1.000
	B	1.000	1.000		
EST-2	A	0.156			
	B	0.844	1.000	1.000	1.000
GAP	A				0.167
	B	1.000	1.000	1.000	0.833
GPI-2	A	1.000	1.000		
	B			1.000	1.000
IDH-1	A		0.944	0.375	1.000
	B	0.833	0.056	0.500	
	C	0.167		0.125	
IDH-2	A			0.091	
	B	1.000	1.000	0.909	0.933
	C				0.067
LDH-2	A	0.033	0.056		
	B	0.967	0.944	1.000	1.000
PGD-1	A	-	1.000	-	
	B	-		-	1.000
PGM-1	A		0.062		
	B	1.000	0.938	1.000	1.000
PGM-2	A		0.062		
	B	1.000	0.938	1.000	1.000
PGM-3	A		0.062		
	B	1.000	0.938	1.000	1.000
PGM-4	A		-	0.036	-
	B	1.000	-	0.964	-
PT-2	A	1.000	1.000		
	B			1.000	1.000
PT-3	A			1.000	1.000
	B	1.000	1.000		
Hb-1	A	-	1.000	-	
	B	-		-	1.000
Hb-2	A	-	1.000	-	
	B	-		-	1.000
H		0.029	0.006	0.035	0.029
		(±0.015)	(±0.004)	(±0.019)	(±0.017)
A		1.12	1.06	1.21	1.11
		(±0.06)	(±0.04)	(±0.08)	(±0.05)
P		8.80	5.71	14.71	11.43

and **Hb** loci are rendered less economic by the additional time, materials and equipment needed for preparation.

The genotypes at the **IDH-1** and **LDH-2** loci in the Vaal (*M. coucha*) population and the **PGM-1**, **-2** and **-3** loci in

the La Lucia (*M. natalensis*) population were close to the expected Hardy-Weinberg proportions for naturally occurring populations. Deficiencies of heterozygotes were observed at the following loci: **AAT-2** (Richards Bay and Montgomery Park populations), **ADH-1** (Richards Bay),

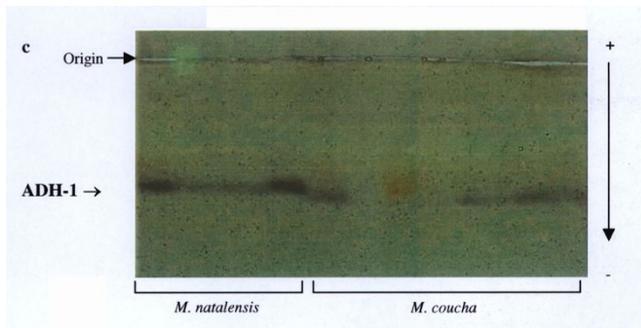


Figure 4
Fixed allele differences between *Mastomys coucha* and *M. natalensis* at **ADH-1**.

EST-2 (Montgomery Park), **GAP** (Richards Bay), **IDH-1** (Montgomery Park and La Lucia), **IDH-2** (La Lucia and Richards Bay), **LDH-2** (Montgomery Park) and **PGM-4** (La Lucia). There were no deviations of allele classes from Hardy-Weinberg proportions in the Vaal population, which is surprising as this had the smallest sample size of all four sites.

Polymorphism (**P**) was 14.7 and 11.4% in the La Lucia and Richards Bay populations of *M. natalensis*, whereas the values for Montgomery Park and Vaal populations of *M. coucha* were 8.8 and 5.7% respectively, with average values of 13.1% for *M. natalensis* and 7.3% for *M. coucha* (Table 2). These are comparable to values reported for other rodent species. Johnson and Selander [12] obtained 7.9% in the Heteromyid *Dipodomys*, 17.4% for *Otomys irroratus*[13], 16.1% for *Rhabdomys pumilio*[14], and 6.7% for *Oryzomys palustris*[15]. The values for *M. coucha* are lower than those for *M. natalensis*, and although the lowest value of 5.7% for the Vaal population could be attributed to small sample size, one could expect that the much larger geographic range of *M. natalensis* (from southern Africa right through to west Africa) and the greater diversity of biome type would give this species a somewhat larger degree of genetic variation.

Numbers of alleles per locus (**A**) values were similar, ranging from 1.06 for the Vaal population to 1.21 for the La Lucia population, whereas the Richards Bay and Montgomery Park populations were calculated to be 1.11 and 1.12. Individual heterozygosity (**h**) values for the Montgomery Park population ranged from 0.064 (**LDH-2**) to 0.278 (**IDH-1**), for the Vaal population, 0.105 (**LDH-2** was the only heterozygous locus within this population), La Lucia from 0.069 (**PGM-4**) to 0.594 (**IDH-1**) and from 0.124 (**IDH-2**) to 0.500 (**AAT-2**). The mean **H** value for *M. coucha* was 0.018, and 0.032 for *M. natalensis*. These values put these two species in

the lower range of rodent variation (Figure 1). A much higher mean heterozygosity (0.073) was obtained by Mahida *et al.*[14] in their genetic assay of the grassland specialist, *Rhabdomys pumilio*. This is interesting, as *Rhabdomys* is a species that prefers a more pristine habitat than the opportunistic and pioneering multimammate mice. Indeed, *M. coucha* and *M. natalensis* tend to be replaced over time by *Rhabdomys* and *Otomys* species in areas that are recovering from habitat damage of some sort [7]. **H** values determined by Taylor *et al.*[13] for *O. irroratus* were also high (mean = 0.071). One would expect that generalist species like *M. coucha* and *M. natalensis* might possess more variation in the genome to deal with more challenging types of habitats, especially human-disturbed, but it would appear that this is not the case. **H** for *M. natalensis* was calculated to be nearly double that of *M. coucha*, but the low value for the Vaal population was probably due to the small sample size, which resulted in that species' average being brought down. On the whole, *M. natalensis* does appear to possess more genetic variation than *M. coucha*, perhaps because of the much greater range of biome types spanned by the former species, as mentioned above.

The **F** values of Wright [16] are useful to estimate the amount of genetic differentiation between species (Table 3). High values of **F_{ST}** are considered to reflect substantial differences at any given locus, and are expected when studying separate species or populations that have diverged to a certain degree. Wright [16] used the following groupings for the evaluation of **F_{ST}** values: the range 0 to 0.05 is considered to reflect little genetic differentiation; 0.05 to 0.15 is indicative of moderate differentiation; 0.15 to 0.25 indicates great genetic differentiation and values greater than 0.25 reflect very great genetic differentiation. All of the measures have increased slightly with the addition of the new material, with pair-wise **F_{ST}** values between the species of 0.882 (Vaal and Richards Bay populations) and 0.743 (La Lucia and Montgomery

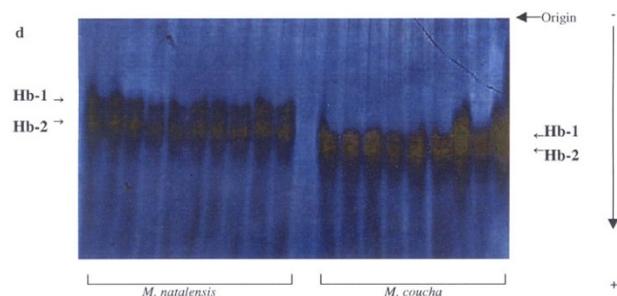


Figure 5
Fixed allele differences between *Mastomys coucha* and *M. natalensis* at **Hb**.

Park populations) indicating large amounts of differentiation between the two species by the abovementioned criteria (Table 3). As expected, this is higher than the F_{ST} value of 0.375 for populations of *O. irroratus*[13] and 0.459 for populations of the striped field mouse *R. pumilio*[14]. These are comparable to the F_{ST} values of 0.558 between the two *M. coucha* populations, and 0.228 between the two *M. natalensis* populations. Pair wise X^2 probability totals between both sets of populations (Vaal-Richards Bay and Montgomery Park-La Lucia) were zero (Table 3), indicating significant ($p = 0$) differentiation as expected for the two species.

Genetic distance (**D**) is a measure of the amount of genetic divergence that has occurred between two species since a hypothetical separation point sometime in their evolutionary past. The **D** value of Nei [17] is specially adapted for small sample sizes and the mean value, between *M. coucha* and *M. natalensis* was 0.26, with a **D** of 0.31 between the Richards Bay and Vaal populations and 0.21 between the La Lucia and Montgomery Park populations. The discrepancy between these values is the result of the additional allozyme markers detected in the new material. This value is obviously slightly larger than those obtained from intraspecific calculations of **D** be-

tween populations of *R. pumilio* (highest **D** = 0.189, [14]) and *O. irroratus* (highest **D** = 0.117, [13]).

Murid rodents are biologically interesting subjects for genetic analysis, due to their rapid evolutionary radiation, and cytogenetic differences are common between genera and species [1]. It would seem that the sibling species of *M. coucha* and *M. natalensis* are more examples of this phenomenon, with a chromosomal mutation within *M. natalensis* giving rise to the possibly younger *M. coucha*, which has been subsequently isolated reproductively. It is unknown as to why these two species, which have so much in common biologically, do not act as reservoirs for the same pathogens, although it is possible that differing physiological biochemistry may be the reason. Still, multimammate mice remain the most important pest species of African rodents [18] both medically and agriculturally, and the isozyme markers reported here, as well as the new allozyme markers are reliable methods of distinguishing between them. This is certainly an aid to identify various disease threats from either species in areas like the Kruger National Park, where the two species occur in sympatry, as well as assisting in determining the eventual distribution of both species.

Table 3: Summary of F-statistics between the Richards Bay and Vaal populations, the Montgomery Park and La Lucia populations (bold) at all loci analysed, as well as contingency X^2 analysis for the pooled data of the two species.

Locus	F_{IS}	F_{IT}	F_{ST}	X^2
AAT-1		1.000 (1.000)	1.000 (1.000)	0.000 (0.000)
AAT-2	1.000 (1.000)	1.000 (1.000)	0.333 (0.143)	0.040 (0.186)
ADH	1.000	1.000 (1.000)	0.889 (1.000)	0.000 (0.001)
EST-1		1.000 (1.000)	1.000 (1.000)	0.000 (0.000)
EST-2	(0.763)	(0.783)	(0.085)	(0.063)
GAP	1.000	1.000	0.091	0.085
GPI-2		1.000 (1.000)	1.000 (1.000)	0.000 (0.000)
IDH-1	-0.059 (1.000)	-0.029 (1.000)	0.029 (0.127)	0.555 (0.056)
IDH-2	1.000 (1.000)	1.000 (1.000)	0.034 (0.048)	0.263 (0.167)
LDH-2	-0.059 (-0.034)	-0.029 (-0.017)	0.029 (0.017)	0.178 (0.410)
PGD-1		1.000	1.000	0.000
PGM-1	(-0.067)	(-0.032)	(0.032)	(0.341)
PGM-2	(-0.067)	(-0.032)	(0.032)	(0.341)
PGM-3	(-0.067)	(-0.032)	(0.032)	(0.341)
PGM-4	(-0.037)	(-0.018)	(0.018)	(0.240)
Hb-1		1.000	1.000	0.000
Hb-2		1.000	1.000	0.000
PT-2		1.000 (1.000)	1.000 (1.000)	0.000 (0.000)
PT-3		1.000 (1.000)	1.000 (1.000)	0.000 (0.000)
Mean	0.819 (0.734)	0.979 (0.931)	0.882 (0.743)	
Total				0.000 (0.000)

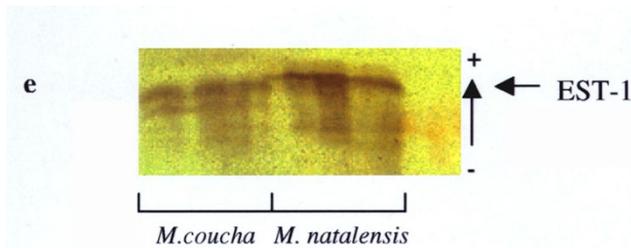


Figure 6
Fixed allele differences between *Mastomys coucha* and *M. natalensis* at **EST-I**.

Materials and Methods

The genetic analysis of an additional nine individuals of *M. coucha* caught on the shores of the Vaal Dam (26°51'49"S, 28°08'59"E), 16 individuals of *M. natalensis* from rehabilitated dunes in the Richards Bay area (28°38'48"S, 32°16'15"E) as well as a further ten individuals from Montgomery Park (previous reference site for *M. coucha*) (26°09'22"S, 27°58'58"E) and four reference samples of *M. natalensis* from La Lucia Ridge (Kwazulu-Natal) (29°44'44"S, 31°03'09"E) was done using electrophoresis. The total number of specimens from all populations was 40 for *M. natalensis* and 43 for *M. coucha*. The additional specimens were caught using live Sherman type traps and were then transported to the laboratory where samples of blood, liver, and muscle tissue were taken. All samples except blood were frozen at -20°C. Blood samples were prepared for polyacrylamide gel electrophoresis using the technique described by Falk *et al.*[11]. Whole blood samples are centrifuged at 750 × g (1800–2000 rpm) for 10 min, after which the plasma supernatant is removed with a pipette. Precooled (5°C) PBS buffer (1.815 g KH₂PO₄; 9.496 g Na₂HPO₄; 5.072 g NaCl; 0.094 g MgCl₂; 0.110 g CaCl₂; 0.125 g MnCl₂ per 1000 ml distilled water, pH 7.4) is added to the compacted erythrocyte pellet; about seven volumes of PBS to three volumes of pellet. The cells are then resuspended by gentle shaking, and the resulting suspension is centrifuged again at 750 × g for 10 min. Supernatants are again removed with a pipette. Resuspension, centrifugation, and removal of supernatant are repeated three times in total. The entire supernatant and the thin, white cloudy layer of cells above the red erythrocyte pellet must be carefully removed and discarded after each centrifugation step. After the third washing, four volumes of ice cooled distilled water are added to each volume of pellet, and mixed well; centrifuged at 2 200 × g (4000–5000 rpm) for 30 min, and the haemolysate solution (supernatant) is removed, and transferred. For stabilisation of the haemoglobin samples, 1 ml of PBS buffered glycerol (same PBS as above, but without CaCl₂, MgCl₂ or MnCl₂; 80 ml glycerol plus 20 ml PBS) is added to each millilitre

of haemolysate, and mixed well. The resulting suspension may be stored at -20°C for up to 1.5 years.

Analyses were done using 12% starch gels, as well as 7% standard polyacrylamide gels (130×135 mm)[19]. A discontinuous lithium-borate tris-citric acid buffer (**RW**; electrode: pH 8; gel: pH 8.7; [20]) and four continuous buffers: a tris-citric acid buffer system (**TC**; pH 6.9; [21]), a tris-borate-EDTA buffer (**MF**; pH 8.6; [22]), a tris-citrate system, (**P**; pH 8.6 [23]), and a histidine-citrate system (**HC**; pH 6.5; [24]) were used to study variation at a total of 32 protein coding loci on starch gels. Five loci (general protein and haemoglobin) were resolved by means of polyacrylamide gel electrophoresis (PAGE). Staining methods are as described by Shaw and Prasad [25] and Harris and Hopkinson [26], and the method of interpretation of gel-banding patterns and locus nomenclature as referred to by Van der Bank and Van der Bank [27] were used. Statistical analysis of data was executed using BIOSYS-1 [28].

Acknowledgements

We thank Sasol for funding this project. Thanks also to Shaun West, and Jaco Delpont for their assistance in the loan of traps and capture of specimens.

References

1. Qumsiyeh MB, King SW, Arroyo-Cabrales A, Aggundey IR, Schlitter DA, Baker RJ, Morrow KJ Jr: **Chromosomal and protein evolution in morphologically similar species of *Praomys sensu lato* (Rodentia, Muridae).** *J. Hered* 1990, **81**:58-65
2. Skinner JD, Smithers RHN: **The Mammals of the Southern African Subregion (second edition).** University of Pretoria, Pretoria 1990
3. Dippenaar NJ, Swanepoel P, Gordon DH: **Diagnostic morphometrics of two medically important southern African rodents, *Mastomys natalensis* and *M. coucha* (Rodentia: Muridae).** *South African Journal of Science* 1993, **89**:300-303
4. Roberts LS, Janovy J: **Foundations of Parasitology (fifth edition).** WCB Publishers, New York 1995
5. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH: **Manual of Clinical Microbiology (Sixth edition).** ASM Press, Washington, D.C 1995
6. Grobler DG, Raath JP, Braack LEO, Keet DF, Gerdes GH, Barnard BJH, Kriek NPJ, Jardine J, Swanepoel R: **An outbreak of encephalomyocarditis infection in free-ranging African elephants in the Kruger National Park.** *Onderstepoort Journal of Veterinary Research* 1995, **62**:97-108
7. De Graaff G: **The Rodents of Southern Africa.** Butterworths, Durban and Pretoria 1981
8. Gordon DH: **Evolutionary Genetics of the *Praomys* (*Mastomys*) *natalensis* species complex (Rodentia: Muridae).** PhD Thesis, Witswatersrand University 1984
9. Breed WG: **Spermatozoa of murid rodents from Africa: morphological diversity and evolutionary trends.** *J. Zool. Lond* 1995, **237**:625-651
10. Smit A, Van der Bank H, Falk T, De Castro A: **Biochemical genetic markers to identify two morphologically similar South African *Mastomys* species (Rodentia: Muridae).** *Biochemical Systematics and Ecology* 2001, **29**:21-30
11. Falk TM, Abban EK, Oberst S, Villwock W, Pullin RSV, Renwraentz L: **A biochemical laboratory manual for species characterization of some tilapia fishes.** ICLARM Education Series 17, Manila, Philippines 1996
12. Johnson WE, Selander RK: **Protein variation and systematics in kangaroo rats (Genus *Dipodomys*).** *Systematic Zoology* 1971, **20**:377-405

13. Taylor PJ, Campbell GK, Van Dyk D, Meester J, Willan K: **Genetic variation in the African vlei rat *Otomys irroratus* (Muridae: Otomyinae).***Israel Journal of Zoology* 1992, **38**:293-305
14. Mahida H, Campbell GK, Taylor PJ: **Genetic variation in *Rhabdomys pumilio* (Sparrman 1784) – an allozyme study.***South African Journal of Zoology* 1999, **34**(3):91-101
15. Loxterman JL, Moncrief ND, Dueser RD, Carlson CR, Pagels JF: **Dispersal abilities and genetic population structure of insular and mainland *Oryzomys palustris* and *Peromyscus leucopus*.***Journal of Mammalogy* 1998, **79**(1):66-77
16. Wright S: **Evolution and the Genetics of Populations, Vol. 4. Variability Within and Among Natural Populations.***University of Chicago, Chicago* 1978
17. Nei M: **Estimation of average heterozygosity and genetic distance from a small number of individuals.***Genetics* 1978, **89**:583-590
18. Van Gulck T, Stoks R, Verhagen R, Sabuni CA, Mwanjabe P, Leirs H: **Short-term effects of avian predation on population size and survival of the multimammate rat, *Mastomys natalensis* (Rodentia, Muridae).***Mammalia* 1998, **62**(3):329-339
19. Ferreira JT, Grant VVS, Avtalion RR: **Workshop on Fish Genetics.***CSIR Special Publications, Pretoria* 1984
20. Ridgway GJ, Sherbourne SW, Lewis RD: **Polymorphism in the esterases of Atlantic herring.***Trans. Am. Soc* 1970, **99**:147-151
21. Whitt GS: **Developmental genetics of the lactate dehydrogenase enzyme of fish.***J. Expl. Zool* 1970, **175**:1-35
22. Markert CL, Faulhaber I: **Lactate dehydrogenase isozyme patterns of fish.***J. Expl. Zool* 1965, **159**:319-332
23. Poulrik MD: **Starch gel electrophoresis in a discontinuous system of buffers.***Nature* 1957, **180**:1477-1479
24. Kephart SR: **Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques.***Am. J. Bot* 1990, **77**:693-712
25. Shaw CR, Prasad R: **Starch gel electrophoresis of enzymes – a compilation of recipes.***Biochem. Genet* 1970, **4**:297-320
26. Harris H, Hopkinson DA: **Handbook of Enzyme Electrophoresis in Human Genetics.***North-Holland Publishing Company, Amsterdam* 1976
27. van der Bank FH, van der Bank M: **An estimation of the amount of genetic variation in a population of the Bulldog *Marcusenius macrolepidotus* (Mormyridae).***Water S.A* 1995, **21**:265-268
28. Swofford DL, Selander RB: **BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics.***J. Hered* 1981, **72**:281-283
29. Hogan KM, Hedin MC, Sun Koh H, Davis SK, Greenbaum IF: **Systematic and taxonomic implications of karyotypic, electrophoretic, and mitochondrial-DNA variation in *Peromyscus* from the Pacific northwest.***J. Mamm* 1993, **74**(4):819-831
30. Patton JL, Selander RK, Smith MH: **Genic variation in hybridising populations of gophers (genus *Thomomys*).***Systematic Zoology* 1972, **21**:263-270

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMedcentral will be the most significant development for disseminating the results of biomedical research in our lifetime."

Paul Nurse, Director-General, Imperial Cancer Research Fund

Publish with **BMC** and your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours - you keep the copyright



Submit your manuscript here:

<http://www.biomedcentral.com/manuscript/>

editorial@biomedcentral.com