# Chapter 4

# Phylogeography of *Micaelamys namaquensis* (Rodentia: Muridae) from the Eastern Kalahari Bushveld bioregion of South Africa

## Abstract

The Namaqua rock mouse *Micaelamys namaquensis* Smith, 1834 represents a species complex in southern Africa with several morphologically cryptic clades occupying distinct biomes and bioregions of southern Africa. Here I report a finer scale analysis of one of these clades, the Eastern Kalahari Bushveld bioregion of South Africa, based on mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences. Phylogeographic analysis reveals a genetic pattern of phylogenetic continuity with a lack of spatial separation. Mismatch distribution analysis suggests that the lineage has experienced recent population growth. The geographic expansion likely followed environmental changes associated with habitat modification over the past 3 000 to 10 000 years. Historical female gene flow does not appear to be equal amongst all localities and potential source and sink areas could be inferred. Metapopulation processes likely drive small mammal population dynamics in this arid region that is characterized by unpredictable climatic cycles.

# 1. Introduction

Recent mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*; Chapters 2 and 3) and Recombination activation gene (RAG1; Chapter 3) analyses revealed that the Namaqua rock mouse *Micaelamys namaquensis* Smith, 1834 represents a species complex in southern Africa. The majority of the 14 identified lineages appeared to be associated with specific southern African biomes or bioregions. In order to further explore evolutionary and ecological processes that shaped diversification in this species complex, I undertook a phylogeographic analysis of one of the phylogenetically and geographically well defined lineages from the Eastern Kalahari Bushveld bioregion of South Africa.

Phylogeography is the "mtDNA bridge between population genetics and systematics" (Avise et al., 1987). It thus focuses on processes underlying the geographic distributions of lineages (molecular variation of a species in space and time) among and within species (Avise, 2000). It also represents the interplay between vicariance and dispersal processes. Under vicariance scenarios, populations or taxa become separated when continuous ranges of ancestral forms are split by environmental changes, such as the rise of a mountain range. Under a dispersal scenario, active or passive dispersal from one or more ancestral origins, leads to the establishment of new populations. Consequently, population structure is affected by the potential of a species to disperse and successfully breed in a newly occupied area and the environmental influences that act on that potential (Avise et al., 1987). Many examples of phylogeographic studies on rodents using mtDNA data exist in the literature (e.g., Demastes *et al.,* 2002; Demboski and Sullivan, 2003; Grill *et al.,* 2009; Nicolas et al., 2008; Nicolas et al., 2009; Rajabi-Maham *et al.,* 2008; Riddle *et al.,* 2000; Yu *et al.,* 2004). These studies clearly show that phylogeographic structure within small mammals is influenced by both intrinsic (dispersal capabilities, reproductive strategies, current and historical demography and habitat specificity) and extrinsic (vegetation, geological and climatic effects) factors.

Habitat selection and inter-specific competition are amongst the most important factors that might influence the co-existence of species (Ricklefs and Schluter, 1993). The co-existence of species may therefore be explained by the amount of resources available and by the way in which species utilise these resources (Kotler and Brown, 1988). Fox (1982)

proposed a model suggesting that species enter a succession and colonise areas when habitat requirements are satisfied by changes in the environment that in turn alter the vegetation.

Limited gene flow and hence evolution in allopatry have influenced the development of specialised morphological, reproductive, and behavioural characteristics found in saxicolous (rock-dwelling) mammals (Mares and Lacher, 1987). Consequently, traits such as limited dispersal capabilities, strict habitat selection, strong territoriality, competition within and between species, patchiness of the environment and social structuring (communal versus solitary) may characterise African saxicolous mammals (Mares and Lacher, 1987). These traits would leave signatures on the structure of intraspecific genetic variation within these species, as is evident from several southern African small mammals: rock hyrax (*Procavia capensis* and *Heterohyrax brucei*; Prinsloo, 1993; Prinsloo and Robinson, 1992), rock rabbits of the genus *Pronolagus* (Matthee 1993; Matthee and Robinson, 1996), rock elephant-shrews (*Elephantulus edwardi*; Smith et al., 2007), and the Namaqua rock mouse species complex (Russo, 2003; Chapter 2).

Several biological characteristics of the Namaqua rock mouse are predicted to have an influence on its phylogeographic structure. Although the species is not a specialist, it prefers rocky habitats (Chimimba and Bennett, 2005) and is thus not continuously distributed. Small colonies live in rock crevices (Chimima and Bennett, 2005) but individuals from the Fynbos biome are believed to be solitary (T. Flemming pers. comm.). Members of the genus *Micaelamys* Ellerman, 1941, are believed to live between one to two years and have a short generation time producing as many as four litters by a single female (C.T. Chimimba pers. comm.). Thus the *M. namaquensis* population size is expected to be large due to its short generation time, with breeding occurring during nine months of the year (Smithers, 1971). The number of offspring ranges between one and seven (Rautenbach, 1978). Withers et al. (1980) reported that *M. namaquensis* tends to have unstable population cycles associated with high reproductive potential and high mortality rates. Dispersal is an important component in the regulation of populations with fluctuating sizes (Lidicker, 1975). Dispersal regulates densities below the level set by the food supply and it has been shown in voles that dispersal has the potential to alter population characteristics (Krebs 1971; Krebs et al., 1976). Likewise, dispersal is an

important factor in the determination of sociality, rates of genetic differentiation, as well as the generation and maintenance of species diversity (Lidicker 1975).

Despite dispersal being a crucial process within animal biology (Lidicker, 1975), most small mammal species appear to be organised into semi-isolated populations due to the availability and patchiness of suitable habitat (Patton et al., 1996). If the landscape between suitable habitat patches severely limits dispersal, maternal lineages should disperse more slowly and as a consequence have restricted geographic ranges (Kim et al., 1998). A habitat island of sufficient size and resources might over time reach equilibrium, even accumulating other closely related lineages (Kim et al., 1998). However, smaller habitat islands may not provide suitable resources and may be too variable so that mice living in these islands will become extinct from time to time and in turn be replaced by successful dispersers from nearby (Kim et al., 1998; Patton et al., 1996). This raises the possibility that local genetic diversity and genetic distances across geographic ranges could reflect dispersal power and demographic stability (Gaggiotti, 1996). In addition, this fine balance between habitat patchiness and dispersal ability will define the degree of population genetic structure and the level of local genetic diversity (Nunney and Campbell, 1993).

The dispersal of individuals can have drastic effects on the demographic and genetic structure of a population (Gaines and McClenaghan, 1980). Movement between populations not only facilitates gene flow but also helps maintain genetic variability (Gaines and McClenaghan, 1980). In mammals with short generation times, large and fluctuating population sizes, such as that seen in *M. namaquensis*, the new maternal lineages would disperse quickly from their points of origin creating genetic structuring in which the oldest lineages would have the broader geographic distribution (Neigel and Avise, 1993). Over time, such a species would therefore show genetic isolation by distance (Patton et al., 1996).

Extrinsic factors such as vegetation, which is directly impacted by climate variability, could have an influence in shaping phylogeographic structure within small mammals. This is clearly evident in the northern hemisphere where ice sheets directly influenced vegetation shifts and subsequent faunal responses (reviewed by Hewitt, 2000). Although only the highest mountains in southern Africa experienced periglacial conditions (Butzer,

1973), global climatic changes caused major vegetation changes in the region (Cerling et al., 1997; deMenocal, 2004). These changes likely not only influenced speciation (see Chapter 2) but also local differentiation within species. Rainfall on the other hand may increase seed production of grasses, shrubs and trees which in turn results in higher population numbers and would favour dispersal and migration would affect the genetic and phylogeographic structure of a population (Clobert et al., 2001).

The Savanna vegetation of South Africa (and Swaziland) constitutes the southern-most extension of this most widespread biome in Africa (Mucina and Rutherford, 2006). Two of the major macroclimatic elements characteristic of the Savanna biome include seasonal rainfall (alternation of wet summers and dry winters) and (sub) tropical temperatures with no or usually low incidence of frost (Mucina and Rutherford, 2006). In South Africa, savanna does not occur at high altitudes and is usually found below 1 500 m extending to about 1 800 m in parts of the highveld (Mucina and Rutherford, 2006). Savanna has an herbaceous ground layer dominated by grass species and a discontinuous to sometimes open upper layer of woody plants (Mucina and Rutherford, 2006; van Rooyen and Bredenkamp, 1996).

More specifically, the Eastern Kalahari Bushveld bioregion forms part of the greater Savanna biome. This bioregion occurs in an area where altitude ranges from sea level to about 1 800 m. It has an annual rainfall of between 235 to 1 000 millimetres (mm) and frost may occur from time to time. The region harbours almost every major geological and soil type. The average annual precipitation in the Eastern Kalahari Bushveld bioregion is 300 mm, which falls in summer and early autumn while temperatures vary between -9˚C and 42˚C. The vegetation is characterised by a well-developed tree stratum of Camel thorn and Shepherd's tree. The shrub layer is moderately developed, consisting of individuals of Black, Weeping Candle and Karoo thorn, with some grass cover depending on the amount of rainfall (van Rooyen and Bredenkamp, 1996).

This bioregion has specifically been chosen since samples were distributed over a fairly small, well-defined geographical area, localities of sympatry have been identified and this bioregion was also represented by a fairly large sample size compared to some of the other biomes/bioregions.

As molecular studies on rock-dwelling mammals have shown that population structure is often shaped by the island-like nature of their habitat (Kim et al., 1998; Patton et al., 1996), the present study reports the analysis of mtDNA cyt *b* variation within *M. namaquensis* from the Eastern Kalahari Bushveld bioregion from South Africa within a phylogeographic context. More specifically, the following research questions are addressed: 1) Is the mtDNA variation within *M. namaquensis* geographically structured?; 2) Is there gene flow between *M. namaquensis* populations from the Eastern Kalahari Bushveld bioregion?; and 3) What are the processes underlying the observed diversity?

## 2. Materials and Methods

### *2.1 Study area and sampling*

Eighty-two individuals from 10 localities from the Eastern Kalahari Bushveld bioregion in South Africa were selected for the phylogeographic analysis (Fig. 4.1; Appendix 4.1). See Chapter 2 for sampling protocols. Animals were collected under the following permits: Northern Cape Province - 040/2001 and 0545/2004; North West Province - 000027 NW-06 (see Chapter 2; Appendix 2.2 for more details).

### *2.2 DNA extraction, Polymerase Chain Reaction (PCR) amplification and sequencing*

A fragment of the mitochondrial cyt *b* gene were amplified with primers and under reaction conditions described previously (Chapter 2). Amplification and sequencing strategy followed that outlined in Chapter 2.

### *2.3 Sequencing analysis*

Unique maternal alleles (Chapter 2; GenBank accession numbers GQ472066 to GQ472077) were identified using TCS, version 1.21 (Clement et al., 2000). Frequencies and geographic distributions of different haplotypes were used to depict geographical and potential ancestor-descendant relationships.

A likelihood ratio test as implemented in Modeltest, version 3.06 (Posada and Crandall, 1998) was used to determine the model of DNA substitution that best fit the data at hand based on the Akaike Information Criterion (AIC). Parameters such as base frequencies, the shape parameter of the gamma distribution of rates among sites (Yang 1996; Yang et al.,

1994) and the proportion of invariable sites (I) were also estimated. The chosen model was subsequently used to report on sequence divergence values using PAUP, version 4.0b10 (Swofford, 2003).

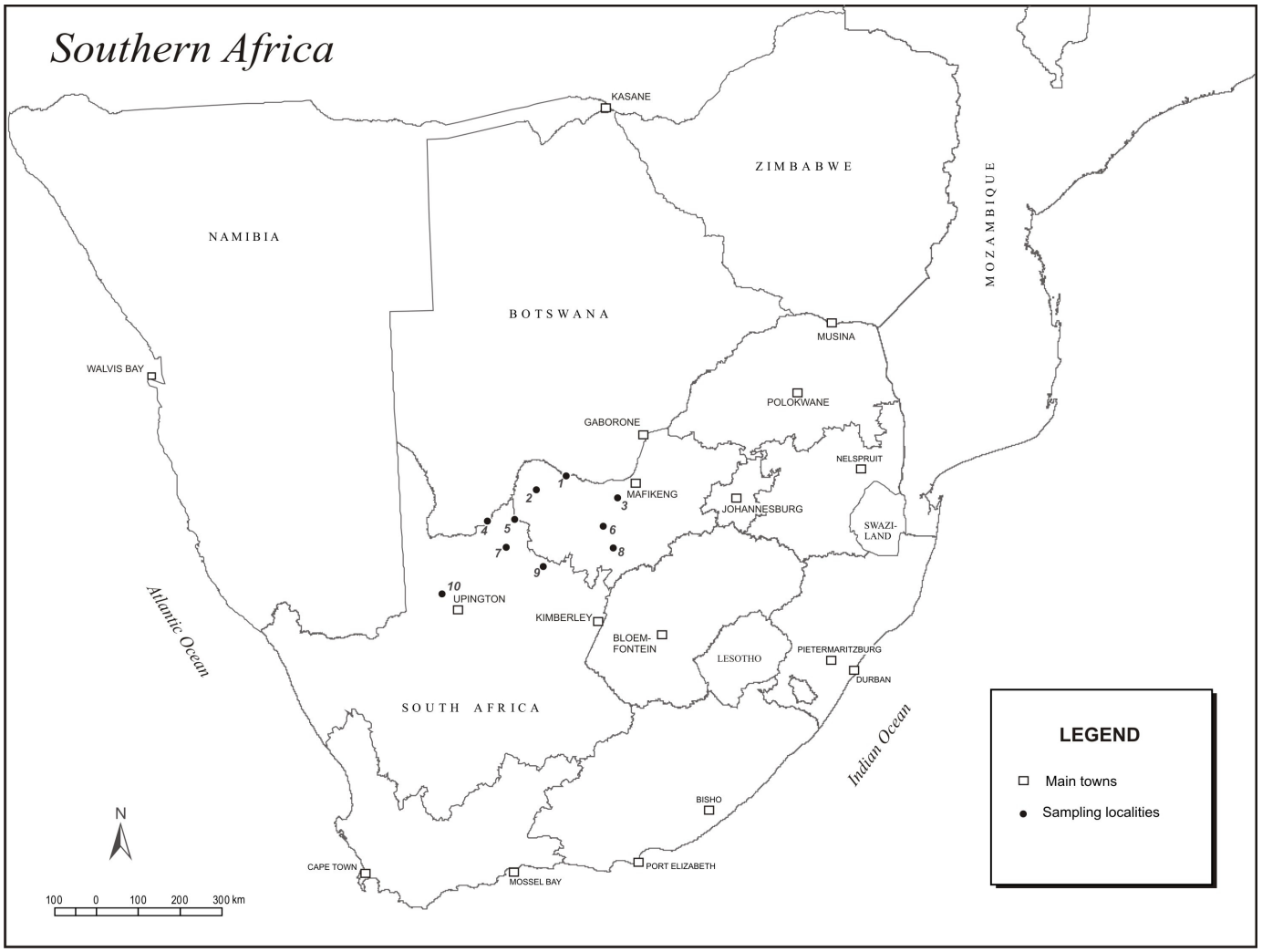


**Figure 4.1** Collecting localities of samples of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa. Collecting locality numbers correspond to those in Appendix 4.1.

*2.4 Molecular diversity and Phylogeographic analyses*

Diversity indices such as haplotype diversity (the probability that two randomly chosen mtDNA sequences in the sample are different) (Nei and Tajima, 1981) and nucleotide diversity, $\pi$ (the average number of nucleotide differences per site between two sequences) (Nei, 1987) were calculated for the entire sample using DnaSP, version 4.10.9 (Rozas et al., 2003). A Mantel test as implemented in Mantel Nonparametric Test Calculator, version 2.0 (Mantel, 1967) was used to test for isolation by distance. The test uses a permutation procedure (1 000 permutations) to determine the significance of the correlation between genetic versus geographic distances.

A spatial analysis of molecular variance (SAMOVA; Dupanloup et al., 2002) was conducted to maximise the proportion of genetic variance among *K* groups of populations. SAMOVA takes into account the geographic locations of samples and was run with different structures to determine the maximum value for $F_{CT}$ (genetic variation due to differences between groups). Using *K* number of groups of populations as defined by SAMOVA, an analysis of molecular variance (AMOVA; Excoffier et al., 1992) as implemented in Arlequin, version 3.0 (Excoffier et al., 2005) was used to assess the extent of differentiation among populations (calculating $\phi_{ST}$, $\phi_{CT}$ and $\phi_{SC}$) using all haplotypes identified. Statistical significance of the different parameters was tested based on 10 000 non-parametric permutations as implemented in Arlequin, version 3.0 (Excoffier et al., 2005). A 5% level of missing data per site was allowed in all analyses. This method builds on the analysis of variance to compute molecular variance components at three different hierarchical levels. The total variance is partitioned into covariance components due to intra- and inter-individual differences and/or inter-population differences (Excoffier et al., 1992; Weir, 1996). Different population structures were identified for both SAMOVA and AMOVA analyses.

Three hierarchical structures defined for the AMOVA analyses are as follow: 1) All populations as one group; 2); Combining individuals from Tosca (locality 1, Fig. 4.1 and Appendix 4.1) and Stella (locality 3, Fig. 4.1 and Appendix 4.1) and the rest of the localities as one group; 3) Combining individuals from Vanzylsrus (locality 4, Fig. 4.1 and Appendix 4.1) and Severn (locality 5, Fig. 4.1 and Appendix 4.1) and the rest of the localities as individual groups. The groupings of localities in the AMOVA analysis were

based on Barrier, version 2.2 (Manni et al., 2004). Barrier tests for any association between genetic and geographic distance by using spatial autocorrelation and regression methods (Manni et al., 2004). These tests suggest the possible shape of the genetic landscape (Manni et al., 2004). A Monmonier's (1973) maximum difference algorithm was used to identify genetic barriers, while a significance test was implemented in the software by means of bootstrap matrices analysis (Manni et al., 2004). By combining the results from the significance test with the molecular data superimposed on a geographic map one can attempt to identify the significance of a geographic barrier and any potential patterns of variation associated with the genetic markers (Manni et al., 2004).

### *2.5 Migrate analysis*

The program MIGRATE, version 2.4 was used to estimate effective population sizes and past migration rates between *n* number of populations assuming a migration matrix model (Beerli and Felsenstein, 2001). The 10 sampling localities were treated as independent populations. Coalescence theory based maximum likelihood estimates for the migration rates among different populations were calculated using a Markov Chain Monte Carlo approach (Hastings, 1970). MIGRATE estimates for theta (per site) were interpreted as indicators of the extant effective population size with $N_e = \theta/2\mu$, with $\mu$ as the mutation rate per site per generation (Beerli and Felsenstein, 2001). A mutation rate of $0.176 \times 10^{-7}$ was used as described in Nabholz et al. (2008). The MIGRATE analysis were run with 10 short chains (10 000 genealogies sampled, 500 recorded) and three long chains (100 000 genealogies sampled, 5 000 recorded) and a burn-in of 10 000 genealogies per chain (Beerli and Felsenstein, 2001).

### *2.6 Mismatch distribution*

Inference of population history was assessed using mismatch distribution analysis under a sudden expansion model and a spatial expansion model assuming constant deme size (Roger and Harpending, 1992) as implemented in Arlequin, version 3.0 (Excoffier et al., 2005). The expansion null hypothesis was tested using the sum of squared deviation test of significance ($P_{Ssd}$; Schneider and Excoffier, 1999) and the raggedness index of significance ($P_{Rag}$; Harpending, 1994). The fit of the observed distribution of mismatches to a sudden model of expansion was tested using 10 000 permutations. The process of habitat expansion and/or contraction often implies demographic variation (Petit et al., 1999). It is therefore important to note that episodes of population growth and decline have

a strong effect on the pattern of genetic polymorphism, leaving characteristic signatures in the distribution of nucleotide site differences between individuals (Slatkin and Hudson, 1991). The distribution is usually unimodal for lineages that have undergone a recent bottleneck or population expansions, and a multimodal distribution for populations exhibiting equilibrium (Rogers and Harpending, 1992). The model of a sudden expansion is simple - it follows a scenario of an initial population with a female effective population size of $N_0$ that rapidly grows to a new size of $N_1$ (Rogers and Harpending, 1992). Parameters were estimated as follows: $\tau = 2\mu t$, $\theta_0 = 2N_0\mu$ and $\theta_1 = 2N_1\mu$, where $\tau$ is the time to the expansion; $\mu$ is the mutation rate per generation; $t$ is the time of the expansion in generations (Harpending, 1994; Schneider and Excoffier, 1999). The effective population sizes before and after the expansion are indicated with $\theta_0$ and $\theta_1$, respectively (Harpending, 1994; Schneider and Excoffier, 1999). A generation time of 0.16 or 0.33 years were used, respectively (C.T. Chimimba pers. comm.). The raggedness statistic, $r$, which quantifies the smoothness of the observed mismatch distribution, was also estimated. A population having undergone expansion will usually generate a distribution that is smooth while populations that have remained constant in size generate distributions with very ragged peaks (Rogers and Harpending, 1992).

*2.7 Nested Clade Phylogeographic Analysis (NCPA)*

An allele network for the 82 individuals was estimated using statistical parsimony as implemented in TCS, version 1.21 (Clement et al., 2000). Since TCS excludes missing data, allele frequencies differed from those reported in Chapter 2. Nested clade phylogeographic analysis (NCPA) can discriminate between phylogeographic associations due to on-going restricted gene flow and historical events such as range expansion, fragmentation and colonisation (Templeton et al., 1995). Alleles in the derived cladogram were then grouped into hierarchical nesting levels from the tips to the interior of the cladogram following Templeton et al. (1987) and Templeton and Singh (1993). This was done by uniting haplotypes that were separated by a single mutational step (0-step clades); 0-step clade haplotypes were nested into 1-step clades. This procedure was repeated until the entire allele network was nested within a single clade. An exact contingency test was performed on each nested clade to test whether the null hypothesis of no association between clades or alleles and geographic location could be rejected. This test was performed without taking the geographic distances between localities into account (Templeton and Singh, 1993); the observed $\chi^2$ values were compared to distributions of the

values generated from 10 000 random permutations in the program GEODIS, version 2.0 (Posada et al., 2000). All the above-mentioned procedures were undertaken using algorithms in the newly developed ANeCA that fully automates the complex NCPA methodology (Panchal, 2007). The NCPA was also undertaken by hand as is traditionally the case following the procedure developed by Templeton et al. (1987) and subsequently Templeton and Singh (1993).

In addition, geographic clade distances ($D_c$), nested clade distances ($D_n$), the average interior *versus* tip clade distances ($IT_c$), and the average interior *versus* tip nested clade distances ($IT_n$) were also calculated (Templeton et al., 1995). The clade distance is indicative of how geographically widespread individuals within a particular clade are (Templeton et al., 1995). The nested clade distance is a measure of the distance of individuals in a particular clade from all individuals within the nesting clade (Templeton et al., 1995). Geographic distances were calculated from latitudinal and longitudinal coordinates measured using a Garmin eTrex Global Positioning System™ (GPS) in the field. The statistical significance for all distances measured was also determined (Templeton et al., 1995). The inference key of Templeton (2008) was used to interpret the data.

## 3. Results

### *3.1 Sequence statistics*

The pairwise GTR + Γ sequence divergence values for all individuals ranged between 0.12% and 1.71 %. The four nucleotides did not occur in equal frequencies, similar to that of other previously reported mammalian cyt *b* sequences. In addition, the first and second codon positions showed less variability than third codon positions (Irwin et al., 1991; Martin et al., 2000).

### *3.2 Molecular diversity*

Based on a 631 bp fragment of the 5' end of the cyt *b* gene, 12 unique haplotypes were identified (Table 4.1) Haplotypes NH06 ($N$ = 23) and NH08 ($N$ = 24) were the most widespread, being recorded from seven and six localities, respectively. Most of the other

haplotypes were locality-specific or were recorded from two/three localities. Two localities (Vorstershoop and Tswalu; see Table 4.1) were represented by a single allele.

Overall nucleotide diversity based on 82 individuals was estimated at 0.35% (SD = 0.03%) while the haplotype diversity value of 0.69 (SD = 0.04) was lower than that reported for other rodents (Avise et al., 1989; Fedorov and Stenseth, 2001). Haplotype diversities differed from 0.33 to 0.89 within sampling localities and nucleotide diversities ranged between 0.23% to 0.61%.

**Table 4.1** TCS based frequencies of mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) alleles among 10 sampled localities of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa examined in the present study. Abbreviations of locality names, which correspond to those in Fig. 4.1 and Appendix 4.1 are as follow: TOS = Tosca; VOR = Vorstershoop; Ste = Stella; Van = Vanzylsrus; SEV = Severn; TSW = Tswalu; VRY = Vryburg; SCH = Schweizer-Reneke; KUR = Kuruman; and UPI = Upington.

| ALLELE NUMBER | NUMBER OF INDIVIDUALS | TOS | VOR | STE | VAN | SEV | TSW | VRY | SCH | KUR | UPI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NH01 | 3 | - | - | - | - | - | - | 3 | - | - | - |
| NH02 | 1 | 1 | - | - | - | - | - | - | - | - | - |
| NH03 | 1 | - | - | - | - | - | - | - | 1 | - | - |
| NH04 | 2 | - | - | - | | 2 | - | - | - | - | - |
| NH05 | 2 | - | - | - | 1 | - | - | - | - | 1 | - |
| NH06 | 23 | - | 3 | 1 | 3 | 2 | 6 | 1 | - | - | 7 |
| NH07 | 2 | - | - | - | - | - | - | 1 | - | - | 1 |
| NH08 | 24 | - | - | 7 | 4 | 2 | - | 2 | - | 5 | 4 |
| NH09 | 6 | 4 | | 1 | | | | 1 | | | |
| NH10 | 1 | | | 1 | | | | | | | |
| NH11 | 5 | | | 2 | 1 | | | 2 | | | |
| NH12 | 12 | | | | 6 | | | | 6 | | |
| **Total** | **82** | **5** | **3** | **12** | **15** | **6** | **6** | **10** | **7** | **6** | **12** |

The Mantel nonparametric test revealed no isolation by distance between localities from the Eastern Kalahari Bushveld bioregion. The standard normal variate ($g$) of -1.1448 was smaller than the critical value of 2.575 at $P \leq 0.005$ with a correlation coefficient of -0.2029. This indicated that the null-hypothesis (no association between elements in the two matrices) could not be rejected.

Dupanloup et al. (2002) reported that the largest mean $\phi_{CT}$ value is associated with the correct number of groups, suggesting that it has some power to retrieve the unknown number of groups. Based on this information, the largest mean $\phi_{CT}$ value in the SAMOVA analyses was 0.31 and 0.32 corresponding to two and nine groups, respectively (see Table 4.2). The specified structures (as a result of the Barrier analysis) were analysed in AMOVA in order to test for statistically significant genetic structuring among the samples. The AMOVA showed weak phylogeographic structuring of haplotypes. Twenty-eight percent of the total variance was among populations when all geographic regions were considered as one group (Table 4.2). It should be noted that the structures defined in SAMOVA were different from those defined in AMOVA. SAMOVA grouped the populations from Tosca (locality 1, Fig. 4.1 and Appendix 4.1), Kuruman (locality 9, Fig. 4.1 and Appendix 4.1) and Stella (locality 3, Fig. 4.1 and Appendix 4.1) in one group and the rest of the populations in another group when two groups were specified. In contrast, only individuals from Tosca (locality 1, Fig. 4.1 and Appendix 4.1) and Stella (locality 3, Fig. 4.1 and Appendix 4.1) were grouped based on Barrier, version 2.2 (Manni et al., 2004) and the rest of the localities were grouped in another group for the AMOVA analysis (see Fig. 4.2). Vanzylsrus (locality 4, Fig. 4.1 and Appendix 4.1) and Severn (locality 5, Fig. 4.1 and Appendix 4.1) were grouped when nine barriers were defined that resulted in nine groupings with the rest of the localities as individual groups (see Fig. 4.2). Similarly, Vanzylsrus (locality 4) and Severn (locality 5) were grouped in the SAMOVA analysis when nine groups were defined with the rest of the localities as individual groups.

**Table 4.2** Hierarchical analysis of molecular variance (AMOVA) for *a priori*-defined groups and a spatial analysis of molecular variance (SAMOVA) of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa based on mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences. Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

| | AMOVA | | | | | | SAMOVA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Number of Groups** | **Statistical Estimates** | | | **Percentage Variation** | | | **Statistical Estimates** | | | **Percentage Variation** | | |
| | $\phi_{SC}$ | $\phi_{ST}$ | $\phi_{CT}$ | **Among** | | **Within Population** | $\phi_{SC}$ | $\phi_{ST}$ | $\phi_{CT}$ | **Among** | | **Within Population** |
| | | | | **Groups** | **Populations** | | | | | **Groups** | **Populations** | |
| **1** | - | 0.28*** | - | - | 27.98 | 72.02 | - | - | - | - | - | - |
| **2*** | 0.22*** | 0.36*** | 0.17 | 17.23 | 18.52 | 64.24 | 0.15*** | 0.42*** | 0.32*** | 32.04 | 10.11 | 57.85 |
| **9** | 0.19* | 0.28*** | 0.12 | 11.92 | 16.32 | 71.76 | -0.01 | 0.31*** | 0.31* | 31.32 | -0.65 | 69.33 |

### *3.3 Migration*

The θ-estimators obtained in MIGRATE were very close to the values of nucleotide diversity (π) for the individual localities thus suggesting that the populations were in genetic/demographic equilibrium (Table 4.3). Most of the immigration and emigration estimates are very low (< 1 effective female migrant per generation; Table 4.3, Fig. 4.2). Some localities display a relative balance between immigration and emigration (Vanzylsrus, Severn, Tswalu, Kuruman, Tosca and Stella). Vryburg( locality 7) and Upington (locality 10) appear to be net receiving populations while Vorstershoop (locality 2) and Schweizer-Reneke (locality 8) show net emigration. Figure 4.2 highlights all the exchanges of more than 1 female migrant per generation between localities, with Vryburg (locality 7) showing the most immigration. It can be concluded that immigration/emigration was observed over large geographic areas indicating that these small mammals can likely travel over large distances. Using the mutation rate for mtDNA based on Nabholz et al. (2008) and the θ-values from MIGRATE, the effective female population sizes for each locality was calculated (see Table 4.3 in parenthesis). The effective female population sizes differed markedly between populations; the largest value was estimated for the Vryburg population.

**Table 4.3** Estimates of migration rates in both directions among *Micaelamys namaquensis* localities from the Eastern Kalahari Bushveld bioregion in South Africa. Values in bold indicate more than one migrant per generation ($N_fm$) between populations. Migrants per generation were calculated by the following equation: Theta (θ) * M(m/mμ). + = receiving population. Locality numbers 1 - 10 correspond to those in Fig. 4.1 and Appendix 4.1. Estimated effective female population size ($N_{fE}$) is indicated in parentheses.

| LOCALITY AND ($N_{fE}$) | THETA | 1;+ | 2;+ | 3;+ | 4;+ | 5;+ | 6;+ | 7;+ | 8;+ | 9;+ | 10;+ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Tosca (82 571) | 0.00289 | - | 0.60 | 0.05 | 0.05 | 0.49 | 0.44 | 0.49 | 0.22 | 0.27 | 0.55 |
| 2. Vorstershoop (171) | 0.00000599 | 0.004 | - | 0.003 | 0.003 | 0 | 0.0006 | 0.0006 | 0.002 | 0.003 | 0.0003 |
| 3. Stella (10 857) | 0.00104 | 0.70 | 0.61 | - | 0.40 | 0.51 | 0.91 | 0.71 | 0.51 | 0.20 | 0 |
| 4. Vanzylsrus (29 714) | 0.00038 | **1.57** | 0.83 | 0.10 | - | 0 | 0.21 | 0 | **1.25** | 0.95 | 0 |
| 5. Severn (93 429) | 0.00327 | 0.08 | 0.76 | 0.26 | 0 | - | **1.19** | **1.78** | 0.34 | 0.08 | 0.08 |
| 6. Vryburg (352 571) | 0.01234 | **1.62** | **2.23** | 0.41 | 0.41 | **1.22** | - | **2.23** | 0.41 | 0.81 | 0.41 |
| 7. Tswalu (22 571) | 0.00079 | 0.19 | 0.31 | 0.31 | 0.27 | 0.66 | 0.08 | - | 0.23 | 0.35 | 0 |
| 8. Schweizer-Reneke (26 286) | 0.00092 | 0.07 | 0.09 | 0.05 | 0.09 | 0.17 | 0.03 | 0.07 | - | 0 | 0.10 |
| 9. Kuruman (40 000) | 0.00140 | 0.17 | 0.14 | 0.25 | 0.22 | 0.03 | 0.11 | 0.11 | 0.06 | - | 0 |
| 10. Upington (65 429) | 0.00229 | 0.14 | 0.20 | 0.14 | 0.14 | 0.20 | 0.07 | 0.30 | 0.20 | 0.07 | - |

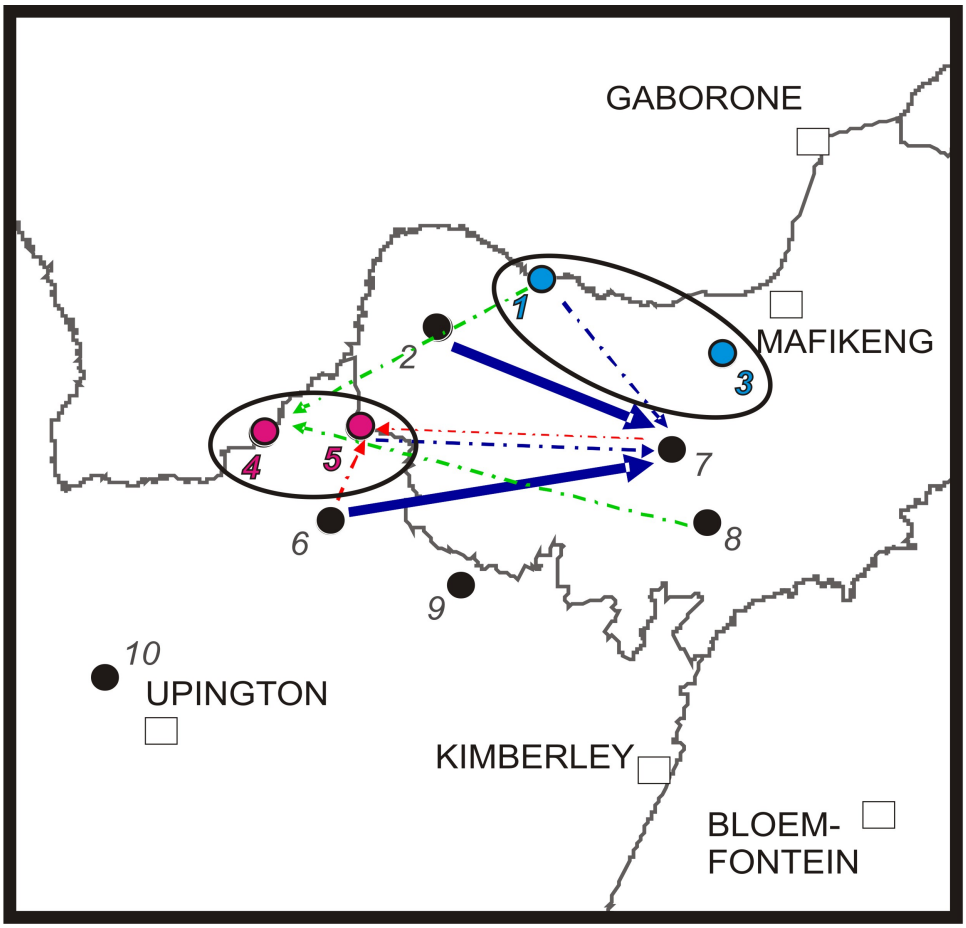


**Figure 4.2** Clustering and migration between sampling localities of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion from southern Africa. Locality numbers correspond to those in Fig. 4.1 and Appendix 4.1. Localities that are encircled were grouped according to Barrier, version 2.2 (Manni et al., 2004). Localities indicated in blue (Tosca; 1 and Stella; 3) were lumped as the first barrier when two groupings were defined. Localities in pink (Vanzylsrus; 4 and Severn; 5) were lumped when nine groupings were defined with the other localities as individual groups. Arrows indicate historical female migration between localities; solid arrows indicate more than two migrants per generation and dashed arrows more than one migrant per generation. Blue, red and green arrows indicate migration to Vryburg (7), Severn (5) and Vanzylsrus (4), respectively.

### *3.4 Mismatch distribution*

The frequency distribution of pairwise nucleotide differences is illustrated in Fig. 4.3 and parameter estimates from the mismatch analyses are indicated in Table 4.4. Although the distributions under both models were multimodel, the sum of squared deviation under the sudden expansion model (Fig. 4.3A) was not statistically significant and thus the null hypothesis of a sudden population expansion could not be rejected. Similarly, the null hypothesis of a spatial expansion assuming constant deme size (Fig. 4.3B) could not be rejected. In contrast, the Harpending's raggedness index under the sudden expansion model (Fig. 4.3A) was statistically significant. Given a mutation rate of $1.76 \times 10^{-7}$ (Nabholz *et al.,* 2008), the mutation rate per generation per haplotype for 631 bp of the mtDNA cyt *b* was estimated to be $1.1106 \times 10^{-4}$. This mutation rate was used to solve the equation $\tau = 2\mu t$ and the time of the expansion in generations, $t = \tau/2\mu$. The expansion time was estimated at 27 787 generations ago. This would therefore translate to 4 446 and 9 170 years ago if a generation time of 0.16 or 0.33 years (C.T. Chimimba pers. comm.) was used, respectively. Likewise, time to the spatial expansion assuming a constant deme size was estimated at 3 357 and 6 925 years ago depending on the use of a generation time of 0.16 or 0.33 years, respectively. Population size after the sudden expansion event was estimated at 26 459.

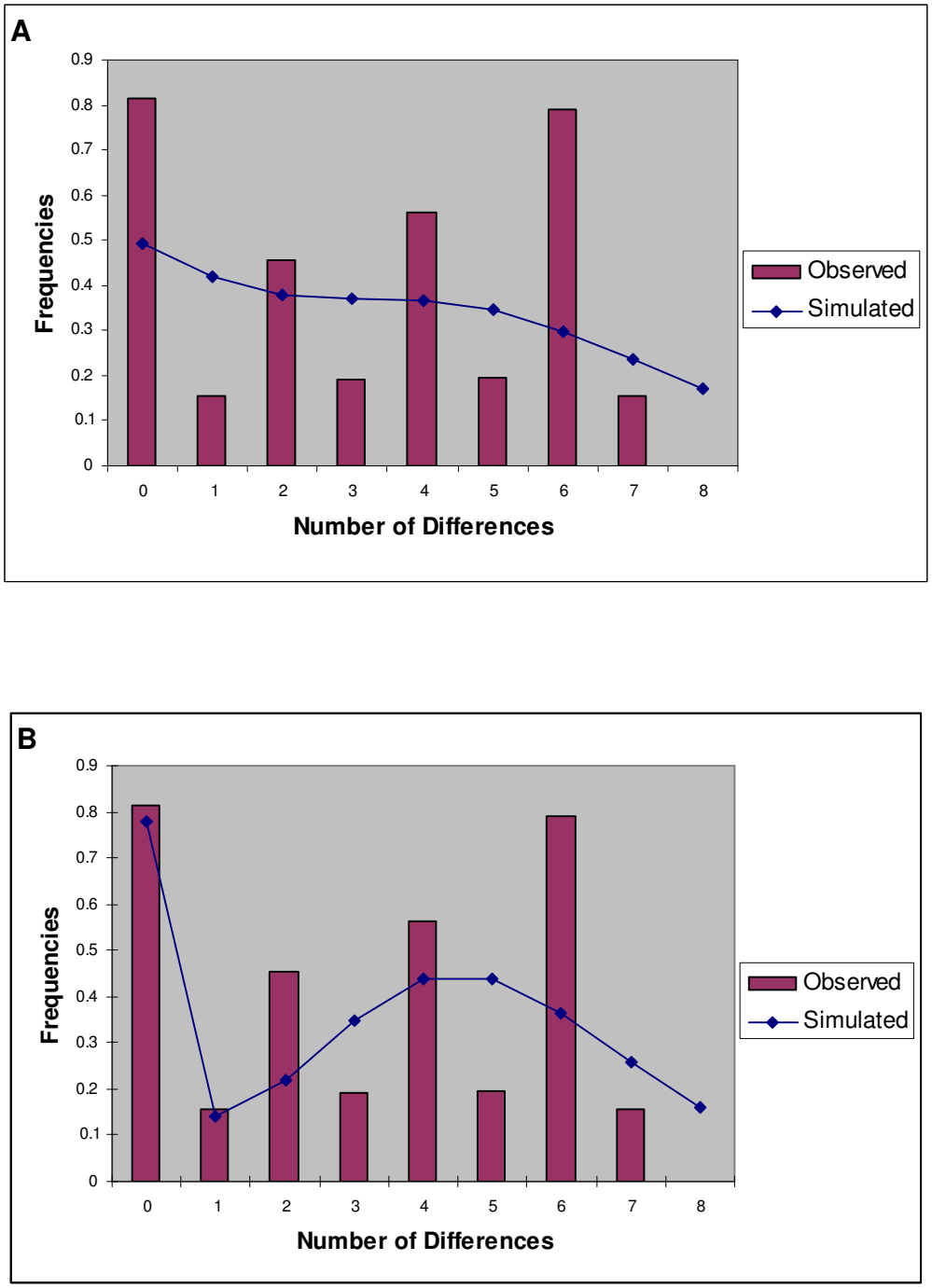


**Figure 4.3** Frequency distributions of pairwise nucleotide differences between individuals of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa with (A) parameters estimated under a sudden expansion model (Sum of squared deviation = 0.05; $P = 0.07$; Harpending's raggedness index = 0.15; $P = 0.05$) and (B) parameters estimated under a spatial expansion model assuming constant deme size (Sum of squared deviation = 0.03; $P = 0.38$; Harpending's raggedness index = 0.15; $P = 0.45$).

**Table 4.4** Mismatch distribution parameter results for *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa based on mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences. Mismatch distribution analyses were performed in two ways: 1) under a sudden expansion model and 2) under a spatial expansion model assuming constant deme size. Demographic expansion parameters are expressed in units of mutational time.

| TYPE OF ANALYSIS | OBS. MEAN* | *TAU* | THETA | $THETA_0$ | $THETA_1$ |
|---|---|---|---|---|---|
| Under sudden expansion | 3.221 (5.781) | 6.172 | - | 0.000 | 5.877 |
| Under spatial expansion | 3.221 (5.781) | 4.661 | 0.344 | - | - |

*Mismatch observed variance given in parenthesis

*3.5 Nested Clade Phylogeographic Analysis (NCPA)*

Figure 4.4 depicts the nested design for the mtDNA haplotypes found within *M. namaquensis* from the Eastern Kalahari Bushveld bioregion following the rules of Templeton et al. (1987) and Templeton and Singh (1993) (see also Table 4.1 for allele frequencies). One ambiguous branch (between allele NH01 and NH12) was broken in order to keep branches elsewhere in the cladogram that connected mtDNA alleles with the least number of mutational steps (Fig. 4.4). The maximum number of mutational steps that were confirmed to be parsimonious with a probability of $P > 0.95$ was 10. Clades that only represented one locality were not tested for association between clades and their geographic distances.

Statistically significant associations between clades and the geographic locations were revealed at all clade levels that were tested in ANeCA (see Table 4.5; probability values in bold). This test indicated strong associations between clades and sampling localities for one 1-step clade (1-1; Table 4.5), one 2-step clade (2-2; Table 4.5) and one 3-step clade (3-1; Table 4.5).

The following clades were therefore tested: Clades 1-1, 1-5, 1-8, 2-2, 2-3 and 3-1. Results from the automated ANeCA program are shown in Table 4.6. Statistically significant associations between clades and sampling locations were revealed at all clade levels and evolutionary processes inferred from the NCPA inference key of Templeton (2008) are

shown in Table 4.6. Most of the clades had an inconclusive outcome due to inadequate geographical sampling. The evolutionary process for the total cladogram (clade 3-1) showed either fragmentation or isolation by distance.

The NCPA was also undertaken by hand following the rules of Templeton et al. (1987) and Templeton and Singh (1993) (results not shown). In this analysis, a statistically significant association between clade 2-1 and geographic distance was also tested. The evolutionary processes for most clades resulted in an inconclusive outcome. Clade 2-1 showed restricted gene flow/dispersal with some long distance dispersal and restricted gene flow with some isolation by distance was inferred for the total cladogram (3-1).

The inconclusive outcomes observed for most of the cladograms (Table 4.6) may be as a result of the limitations associated with this method. It is clear, based on the evolutionary processes inferred that more analyses using larger samples sizes in combination with other markers are required to investigate the genetic structure of this species in more detail.

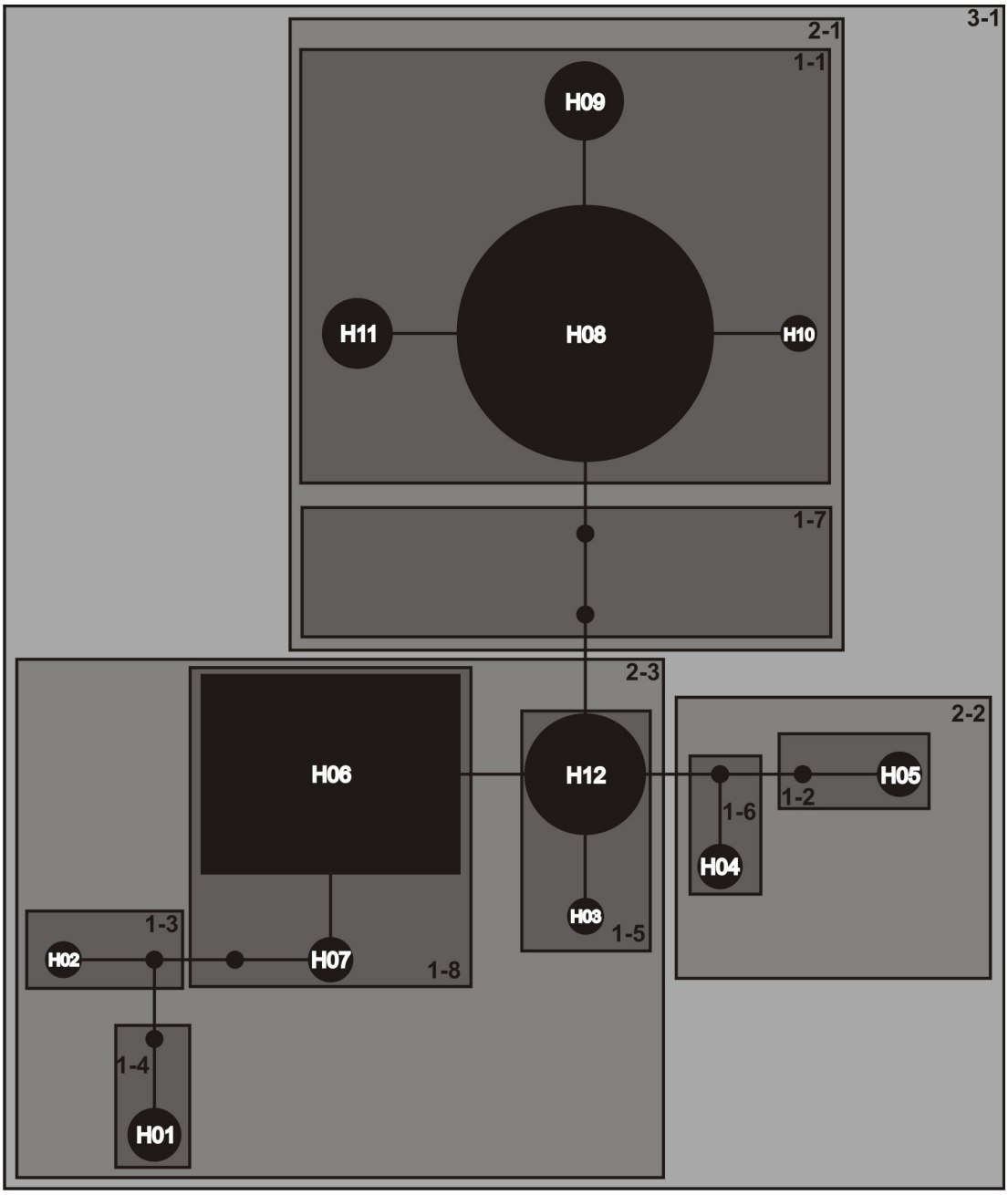


**Figure 4.4** Automated nested clade design for 82 individuals of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa based on 631 bp of the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene region. The haplotype network was constructed with TCS, version 1.21 (Clement et al., 2000) as defined by a 95 % confidence limit. A square denotes an ancestral allele (NH06) as suggested by TCS. The size of circles indicates the frequency of the alleles (also see Table 4.1). Smaller circles that are not numbered indicate missing (unsampled/extinct) alleles.

**Table 4.5** Nested contingency analysis of geographic associations between clades and sampling localities. Only clades with geographic variation are testable. The permutational probabilities were calculated by 10 000 random permutations. Statistically significant probabilities are indicated in bold.

| CLADE | 0BSERVED CHI-SQUARE STATISTICS | PROBABILITY |
|---|---|---|
| 1-1 | 32.02 | **0.022** |
| 1-5 | 0.93 | 1.000 |
| 1-8 | 6.32 | 0.470 |
| 2-2 | 4.00 | 0.331 |
| 2-3 | 97.36 | **0.000** |
| 3-1 | 49.17 | **0.000** |

**Table 4.6** Evolutionary processes as inferred from the inference key of Templeton (2008) as implemented in ANeCA. Clade designations are as derived from the automated nested clade phylogeographic analysis (ANeCA; see Fig. 4.4). Locality names are as indicated in Fig. 4.1 and Appendix 4.1. "No" in the table denotes the final step in the inference chain and leads to the conclusion of the evolutionary process.

| CLADE | POPULATIONS | INFERENCE CHAIN | PROCESSES |
|---|---|---|---|
| 1-1 | Tosca, Stella, Vanzylsrus, Severn, Vryburg, Kuruman, Upington | 1, 2, 3, 4, No | Inconclusive outcome |
| 1-5 | Vanzylsrus, Schweizer-Reneke | 1, 2, 11, 17, No | Inconclusive outcome |
| 1-8 | Vorstershoop, Stella, Vanzylsrus, Severn, Tswalu, Vryburg, Upington | 1, 2, No | Inconclusive outcome |
| 2-2 | Vanzylsrus, Severn, Kuruman | 1, 19, 20, No | **Inadequate geographical sampling** |
| 2-3 | Tosca, Vorstershoop, Stella, Vanzylsrus, Severn, Tswalu, Vryburg, Schweizer-Reneke, Upington | 1, 2, 11, 17, No | Inconclusive outcome |
| 3-1 | Tosca, Vorstershoop, Stella, Vanzylsrus, Severn, Tswalu, Vryburg, Schweizer-Reneke, Kuruman, Upington | 1, 2, 3, 4, 9, 10, No | **Geographical sampling scheme inadequate to discriminate either fragmentation or isolation by distance** |

## 4. Discussion

The *M. namaquensis* lineage from the Eastern Kalahari Bushveld bioregion is characterised by shallow phylogeographic structure. The latter is evident from several lines of analytical evidence based on mtDNA cyt *b* sequences, including low diversity indices, a star-like allele network and apparent demographic changes over recent evolutionary time. However, historical female migration levels are generally low and there are indications of potential metapopulation dynamics across the region.

The lineage has a low nucleotide diversity (0.35%), which corresponds to other studies done on small mammals; estimates of between 0.33% and 1.45% have been reported for the Norwegian lemming (Fedorov and Stenseth, 2001), while 0.54% and 1.5% have been reported for the Yellow-necked field mouse (Michaux et al., 2004). The haplotype diversity value of 0.69 is lower than the average reported for other rodents (Avise et al., 1989; Fedorov and Stenseth, 2001), probably as a result of the low incidence of locality-specific haplotypes (Avise, 2000). Most alleles are shared amongst two to five localities (see Table 4.1 and Fig. 4.4). This could either indicate a shared ancestry between these populations or on-going gene flow.

The lack of a statistically significant correlation between pairwise estimates of gene flow and geographic distance clearly indicate the absence of a pattern of isolation by distance. In addition, the AMOVA performed on localities assembled into groups according to the Barrier results did not show statistically significant apportionment of the genetic variance among regional groups (i.e., no apparent extrinsic barriers). Much of the genetic structuring observed could be explained through differentiation within localities.

Despite the apparent continuous pattern of genetic variation, the overall $\phi_{ST}$ value for *M. namaquensis* of 0.28 implies a moderate level of genetic heterogeneity among populations, suggesting a certain degree of isolation among samples (Apfelbaum et al., 1991). This value corresponds to an *Nm* larger than one which is above the minimum number of migrants per generation needed to minimise the chances of substantial local differentiation due to genetic drift ($Nm > 1$; Griswold and Baker, 2002; Hartl, 1980; Hutchison and Templeton, 1999; Slatkin, 1987), indicating either historical gene flow or recent

connectivity. The former is confirmed by the MIGRATE analysis which indicates that most populations were/are connected to each other via low to intermediate levels of female gene flow.

Evidence for both isolation and migration processes among the *M. namaquensis* localities from the Eastern Kalahari Bushveld bioregion suggest that populations within this bioregion share a relatively recent history. Shared alleles (such as NH06) indicate some level of movement between populations while unique alleles (such as NH01 from Vryburg, NH02 from Tosca, NH03 from Schweizer-Reneke, NH04 from Severn and NH10 form Stella) indicate that a certain level of isolation exists. Added to which the fairly low to intermediate migration detected may be as a result of the marker used, which excludes male-biased dispersal.

The exchange of migrants from Tswalu (locality 6), Severn (locality 5), Vorstershoop (locality 2) and Tosca (locality 1) to Vryburg (locality 7) is considered as large (greater than one). This locality (Vryburg) was one of the only localities that exhibited such a large number of immigrants per generation and it also received migrants from almost half of the other populations (possibly a sink populations). The θ-estimates further suggested that this region has the largest effective female population size which may be attributable to more favourable environmental conditions. It is well documented that population sizes of rodents such as *M. namaquensis* occurring in semi-arid to arid environments such as the Eastern Kalahari Bushveld bioregion fluctuate with annual rainfall (White et al., 1997). Rain increases seed production of grasses, shrubs and trees which in turn results in peaks in population numbers, favouring dispersal. Dispersal, in the form of migration or individual movement, affects the genetic structure of a population (Clobert et al., 2001), although it can also be influenced by landscape heterogeneity, resource distribution and population densities.

Gene flow may either constrain evolution by preventing adaptation to local conditions or promote evolution by spreading new genes throughout a species' distributional range (Slatkin, 1987). Continuously distributed species may be genetically structured if gene flow is either restricted or if they are under local selection (Congdon et al., 2000; Hudson et al., 1992). In contrast, some species are restricted to small spatial distributions due to their association with particular habitat conditions. This could be the case with the

Namaqua rock mouse, a species that is strongly associated with rocky koppies, outcrops and hillsides (Chimimba and Bennett, 2005). Migration most likely occurs between neighbouring populations, probably approximating a stepping stone model (Hutchison and Templeton, 1999; Slatkin and Barton, 1989). In addition, there are indications of potential metapopulation dynamics within *M. namaquensis* (sink and source populations).

A signature of sudden population size and spatial expansion could not be rejected given the demographic scenario with the mismatch distribution analysis (Fig. 4.3). This phenomenon of a population expansion was also detected in the star-like allele network from the TCS analysis (Fig. 4.4). A demographic scenario of a sudden population expansion could have been expected due to the favourable environmental conditions (i.e., good rain triggers an increase in food which in turn would result in an increase in population numbers) that these animals are exposed to from time to time. A record of past climate changes has been retained within the landforms of the 2.5 million $km^2$ of the Kalahari sedimentary basin (Deacon and Lancaster, 1988). For example, the last glacial maximum (LGM; 18 000 YBP) was characterised by colder and more arid conditions than at present (Shi et al., 1998) with dune-formation in central southern Africa. Dune-formation over the past 18 000 years to present have being inhibited by wetter conditions and strong winds than in the LGM (Thomas and Shaw, 2002). Cold upwellings from the Benguela current caused cold air coming in contact with warm air off the land to condense forming fog which provides a permanent water source (Pickford and Senut, 1999). This may have been the main environmental parameter that permitted dispersal into, and subsequent radiation, in areas that may have been previously inhospitable. Added to which these changes in climate would have had an influence in the vegetation and as more areas became suitable, individuals would have occupied them. During the last 2000 years, increased farming, burning and overgrazing reflect intensified human activity in the region leading to significantly altered landscapes.

The signature of sudden population expansion may have influenced habitat selection and inter-specific competition that are important factors in the co-existence of species (Ricklefs and Schluter, 1993). A rodent community study on rehabilitating dunes in the KwaZulu-Natal Province, South Africa showed that rodent densities decreased with an increase in habitat regeneration age, indicative of unsuitable environmental conditions (Ferreira and van Aarde, 1996). In comparison, negative correlation between population sizes of

sympatric small mammal species provided evidence that intra-specific competition could have consequences on population size and habitat use (Grant, 1972; Gurnell, 1985). Species may use the same habitat, but some species might either segregate among strata within a habitat or they can also segregate temporarily and be active at different times (Ziv and Kotler, 2003; Ziv et al., 1993) in either the presence or absence of competitors (other small mammals possible competing for the same food sources and space for shelter). Therefore, the spatial segregation (i.e., movement between populations) of *M. namaquensis* individuals into different habitats allows for the species to co-exist.

The phylogeographic analysis in the present study revealed no statistically significant geographic structuring of mtDNA variation among the 10 *M. namaquensis* localities from the Eastern Kalahari Bushveld bioregion in South Africa. At the highest clade level, the NCPA inference was that of past fragmentation or isolation by distance. Some form of past fragmentation seemed the more likely factor responsible for the observed spatial distribution of genetic variation given the lack of isolation by distance (see Mantel test results) and the unequal individual movement detected between *M. namaquensis* populations. Female-mediated gene flow between the 10 populations of *M. namaquensis* could thus not be refuted. Templeton et al. (1995) and Durand et al. (1999) emphasised that the various evolutionary processes shaping geographic associations of alleles are not mutually exclusive. In the present study, a pattern of gene flow superimposed on a signal of possible past fragmentation can be explained by relatively recent vicariance and subsequent secondary contact between populations.

The allele network contained one star-like phylogeny within clade 2-1 (Fig. 4.4). The central haplotype (NH08) was shared between six localities (24 individuals) with a geographic range in excess of 500 km. Although this allele was not identified as the ancestral allele by the TCS analysis (NH06 being identified as an ancestral allele; see Fig. 4.4), it can still be argued that such a star-like pattern with a central common allele independently connected with numerous fairly rare alleles at the tips can be regarded as a signal of recent population expansion (Avise, 2000). This finding was further corroborated by the mismatch distribution analysis where the Eastern Kalahari Bushveld populations (corresponding to clade 3-1) presumably experienced a sudden population size expansion between 3 000 to 10 000 years ago.

For clade 2-1 (consisting of individuals from Stella (locality 3), Vryburg (locality 7), Severn (locality 5), Kuruman (locality 9), Vanzylsrus (locality 4) and Tosca (locality 1); see Fig. 4.4), when using the traditional nested clade phylogeographic analysis, the evolutionary inference was that of restricted gene flow/dispersal with some long-distance dispersal. These results were also confirmed by the MIGRATE analysis that showed movement of individuals between localities with large geographic distances of over 300 km. To some extent, this clade exhibited restricted gene flow because allele NH10 was shown to be locality-specific to the Stella population (locality 3).

Comparisons with published data showed that *M. namaquensis* falls within the continuous end (category IV) of the spectrum of categories proposed by Avise et al. (1987) to classify phylogeographic structuring. Category IV entails phylogenetic continuity, lack of spatial separation and gene flow in the species not sub-divided by long-term zoogeographic barriers. The Springhare, *Pedetes capensis* occurs in a uniform habitat and this is reflected in its phylogeographic pattern which shows a lack of genetic divergence among samples over a broad geographic range (Matthee et al., 1997). The sibling red veld rats, *Aethomys chrysophilus* De Winton, 1897, and the Tete veld rat, *A. ineptus* Thomas and Wroughton, 1908, from southern Africa also showed similar patterns to that seen in the Springhare, reflecting a lack of genetic divergence among samples over a broad geographic range (Russo et al., 2006). This phylogeographic pattern is also evident in the yellow mongoose, *Cynictis penicillata* (Jansen van Vuuren, 1995) from southern Africa, the old field mouse, *Peromyscus polionotus* (Avise et al., 1983) and the woodrat from the eastern United States of America (*Neotoma*; Hayes and Harrison, 1992). In contrast to this, a pattern of phylogenetic discontinuity (category I) has been reported in other taxa showing discontinuous intra-specific mtDNA phylogenetic networks, with a strong geographic orientation such as the pocket gopher (*Geomys pinetis*; Avise et al., 1979) and deer mouse (*Peromyscus maniculatus*; Lansman et al., 1983). Category I pattern of phylogenetic discontinuity has also been detected in southern African small mammals such as the rock hyrax, *Procavia capensis* (Prinsloo, 1993), red rock rabbits of the genus *Pronogalus* (Matthee and Robinson, 1996), the scrub hare, *Lepus saxatilis* (Kryger, 2002), and within the Grassland lineage of the Namaqua rock mouse, *M. namaquensis* (Russo, 2003).

## 5. Conclusion

The genetic analysis of samples of *M. namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa, in the present study suggests that the species has recently expanded its population size, and that geographic expansion must have followed habitat modification associated with recent environmental changes. It was also evident from the present study that there was gene flow between populations and more so between some populations than others (possible sink and source populations). The description of the genetic structure of *M. namaquensis* is fundamental to understanding the history and evolutionary potential of the species.

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## References

Apfelbaum, L. I., Massarini, A.I.,. Daleffe, L. E., Reig, O.A., 1991. Genetic variability in the subterranean rodents *Ctenomys australis* and *Ctenomys porteousi* (Rodentia: Octodontidae). Biochem. Syst. and Ecol. 19, 467–476

Avise, J.C., 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge.

Avise, J.C., Giblin-Davidson, C., Laerm, J., Patton, J.C., Lansman, R.A., 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. Proc. Natl. Acad. Sci. USA 76, 6694-6698.

Avise, J.C., Shapira, J.F., Daniel, S.W., Aquadro, C.F., Lansman, R.A., 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. Mol. Biol. Evol. 1, 38-56.

Avise, J.C., Arnold, R.M., Ball, E., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., Saunders, N.C., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18, 489-522.

Avise, J.C., Bowen, B.W., Lamb, T., 1989. DNA fingerprinting from hypervariable mitochondrial genotypes. Mol. Biol. Evol. 6, 258-269.

Beerli, P., Felsenstein, J., 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. USA 98, 4563-4568.

Butzer, K.W., 1973. Pleistocene ‘periglacial” phenomena in southern Africa. Boreas 2, 1-11.

Chimimba, C.T., Bennett, N., 2005. Order: Rodentia. In: Skinner, J.D., Chimimba, C.T. (Eds.), The mammals of the southern African. Cambridge Univ. Press, pp 156-163.

Cerling, T.E, Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., 1997. Global vegetation change through the Miocene/Pliocene boundary. Nature 389, 153-158.

Clement, M., Posada, D., Crandall, K., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657-1660.

Clobert, J., Danchin, E., Dhondt, A.A., Nichols, J.D., 2001. Dispersal. New York, Oxford University Press.

Congdon, B.C., Piatt, J.F., Martin, K., Friesen V.L., 2000. Mechanisms of population differentiation in marbled murrelets: historical vs contemporary evolutionary processes. Evolution 54, 974-986.

Deacon, J., Lancaster, N., 1988. Late Quaternary environments of southern Africa. Oxford University Press, Oxford.

Demboski, J.R., Sullivan, J., 2003. Extensive mtDNA variation within the Bellow-pine chipmunk, Tamias amoenus (Rodentia: Sciuridae), and phylogeographic inferences for northwest North America. Mol. Phylogenet. Evol. 26, 389-408.

Demastes, J.W., Spradling, T.A., Hafner, M.S., Hafner, D.J., Reed, D.L., 2002. Systematics and phylogeography of pocket gophers in the genera *Cratogeomys* and *Pappogeomys*. Mol. Phylogenet. Evol. 22, 144-154.

deMenocal, P.B., 2004. African climate change and faunal evolution during the Pliocene-Pleistocene. Earth Planet. Sci. Lett. 220, 3-24.

Dupanloup, I., Schneider, S., Excoffier, L., 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11, 2571-2581.

Durand, J.D, Templeton, A.R., Guinand, B., Imsiridou, A. Bouvet, Y., 1999. Nested clade and phylogeographic analysis of the chub, *Leucisrus cephalus* (Teleostei, cyprinidae), in Greece: Implications for Balkan Peninsula biogeographic. Mol. Phylogent. Evol. 13, 566-580.

Excoffier, L., Lavel, G., Schneider, S., 2005. Arlequin, Version 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. Online 1, 47-50.

Excoffier, L., Smouse, P., Quattro, J., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479-491.

Fedorov, V.B., Stenseth, N.C., 2001. Glacial survival of the Norwegian lemming (*Lemmus lemmus*) in Scandinavia: inference from mitochondrial DNA variation. Proc. R. Soc. Lond., Ser. B 268, 809-814.

Ferreira, S., van Aarde, R.J., 1996. Changes in community characteristics of small mammals in rehabilitating coastal dune forests in northern KwaZulu Natal. Afr. J. Ecol. 34, 113-130.

Fox, B.J., 1982. Fire and mammalian secondary succession in an Australian coastal heath. Ecology 65, 1332-1341.

Gaggiotti, O.E., 1996. Population genetic models of source-sink metapopulations. Theor. Pop. Biol. 50, 178-208.

Gaines, M.S., McClenaghan, L.R., 1980. Dispersal in small mammals. Annu. Rev. Ecol. Syst. 11, 163-196.

Grant, P., 1972. Convergent and divergent character displacement. Biol. J. Linn. Soc. 4, 39-68.

Grill, A., Amori, G., Aloise, G., Lisi, I., Tosi, G., Wauters, L.A., Randi, E. 2009. Molecular phylogeography of European *Sciurus vulgaris*: refuge within refugia? Mol. Ecol. 18, 2687-2699.

Griswold, C., Baker, A.J., 2002. Time to the most recent common ancestor and divergence times of populations of common chaffinches (*Fringilla coelebs*) in Europe and North Africa: insights into pleistocene refugia and current levels of migration. Evolution 56, 143-153.

Gurnell, J., 1985. Woodland rodent communities. Symp. Zool. Soc. Lond. 55, 377-411.

Harpending, H., 1994. Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Hum. Biol. 66, 591-600.

Hartl, D.L., 1980. Principles of population genetics. Sinauer Associates, Sunlerland, Massachusetts.

Hastings, W.K., 1970. Monte Carlo sampling methods using Markov chains and their applications. Biometrika 57, 97-109.

Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. Nature 405, 907-913.

Hayes, J.P., Harrison, R.G., 1992. Variation in mitochondrial DNA and the biogeographic history of woodrats (*Neotoma*) of the eastern United States. Syst. Biol. 41, 331-344.

Hey, J., Machado, C.A., 2003. The study of structured populations - new hope for a difficult and divided science. Nat. Rev. 4, 535-543.

Hilborn, R., Krebs, C.J., 1976. Fates of disappearing individuals in fluctuating populations of *Microtus townsendii*. Can. J. Zool. 54, 1507-1518.

Hudson, R.R., Boos, D.D., Kaplan, N.L., 1992. A statistical test for detecting geographic subdivision. Mol. Biol. Evol. 9, 138-151.

Hutchison D.W., Templeton, A.R., 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53, 1898-1914.

Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the cytochrome *b* gene of mammals. J. Mol. Evol. 32, 128-144.

Jansen van Vuuren, B., 1995. Mitochondrial variation and geographic population structure in the yellow mongoose (*Cynictis penicillata*). MSc thesis, University of Pretoria, Pretoria.

Kim, I., Phillips, C.J., Monjeau, J.A., Birney, E.C., Noack, K., Pumo, D.E., Sikes, R.S., Dole, J.A., 1998. Habitat islands, genetic diversity, and gene flow in a Patagonian rodent. Mol. Ecol. 7, 667-678.

Kotler, P.B., Brown, J.S., 1988. Environmental heterogeneity and the coexistence of desert rodents. Ann. Rev. Ecol. Syst. 19, 281-307.

Krebs, C.J., 1971. Genetic and behavioral studies on fluctuating vole populations. Proceedings of the Advanced Study Institute on the Dynamics of Numbers in Populations, Oosterbeek. 1970, 243-256.

Krebs, C.J., Wingate, I., LeDuc, J., Redfield, J., Taitt, M., Hilborn, R., 1976. *Microtus* population biology: dispersal in fluctuating populations of *M. townsendii*. Can. J. Zool. 54, 79-95.

Kryger, U., 2002. Genetic variation among South African hares (*Lepus* spec.) as inferred from mitochondrial DNA and microsatellites. PhD thesis, University of Pretoria, Pretoria.

Lansman, R.A., Avise, J.C., Aquadro, C.F., Shapira, J.F., Daniel, S.W., 1983. Extensive genetic variation in mitochondrial DNA's among geographic populations of the deer mouse, *Preomyscus maniculatus*. Evolution 37, 1-16.

Lidicker, W.Z., 1975. The role of dispersal in the demography of small mammals. In: Golley, F.B., Petrusewicz, K., Ryszkowski, L. (Eds.), Small mammals: their production and population dynamics. Cambridge University Press. London, United Kingdom, pp. 103-128.

Manni, F., Guerard, E., Heyer, E., 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Hum. Biol. 76, 173–190.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209-220.

Mares, M.A., Lacher, T.E., 1987. Current Mammalogy. In: Genoways, H.H. (Ed.), Ecological, morphological and behavioural convergence in rock-dwelling mammals. Plenum Press, New York, pp. 307-348.

Martin, Y., Gerlach, G., Schlötterer, C., Meyer, A., 2000. Molecular phylogeny of European muroid rodents based on complete cytochrome *b* sequences. Mol. Phylogenet. Evol. 16, 37-47.

Matthee, C.A., 1993. Mitochondrial DNA variability and geographic population structure in Pronolagus rupestris and P. randensis (Mammalia: Lagomorpha). MSc thesis, University of Pretoria, Pretoria.

Matthee, C.A., Robinson, T.J., 1996. Mitochondrial DNA differentiation among geographical populations of *Pronolagus rupestris*, Smith's red rock rabbit (Mammalia: Lagomorpha). Heredity 76, 514-523.

Matthee, C.A., Robinson, T.J., 1997. Mitochondrial DNA Phylogeography and comparative cytogenetics of the Springhare, *Pedetes capensis* (Mammalia: Rodentia). J. Mamm. Evol. 4, 53-73.

Matthee, C.A., Flemming, A.F., 2002. Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in southern Africa. Mol. Ecol. 11, 465-471.

Michaux, J.R., Libois, R., Paradis, E., Filippucci, M.-G., 2004. Phylogeographic history of the Bellow-necked fieldmouse (*Apodemus flavicollis*) in Europe and in the Near and Middle East. Mol. Phylogenet. Evol. 32, 788-798.

Monmonier, M.S., 1973. Maximum-difference barriers: an alternative numeritcal regionalisation method. Geogr. Anal. 5, 245–261.

Mucina, L., Rutherford, M.C. (Eds.). 2006. The vegetation of South Africa, Lesotho and Swaziland. Strelitzia19. South Afircan National Bioidiversity Institute, Pretoria.

Nabholz, B., Glémin, S., Galtier, N., 2008. Strong variation of mitochondrial mutation rate across mammals - the longevity hypothesis. Mol. Biol. Evol. 25, 120-130.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, USA.

Nei, M., Tajima, F., 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97, 145-163.

Neigel, J.E., Avise, J.C., 1993. Application of a random-walk model to geographic distributions of animal mitochondrial DNA variation. Genetics 135, 1209-1220.

Nicolas, V., Granjon, L., Duplantier, J-M., Cruaud, C., Dobigny, G., 2009. Phylogeography of spiny mice (genus Acomys, Rodentia: Muridae) from the south-western margin of the Sahara with taxonomic implications. Biol. J. Linn. Soc. 98, 29-46.

Nicolas, V., Bryja, J., Akpatou, B., Konecny, A., Lecompte, E., Colyn, M., Lalis, A., Couloux, A., Denys, C., Granjon, L., 2008. Comparative phylogeography of two sibling species of forest-dwelling rodent (*Praomys rostratus* and *P. tullbergi*) in West Africa: different reactions to past forest fragmentation. Mol. Ecol. 17, 5188-5134.

Nunney, L., Campbell, K.A., 1993. Assessing minimum viable population size: demography meets population genetics. TREE 8, 234-239.

Panchal, M., 2007. The automation of nested clade phylogeographic analysis. Bioinformatics 23, 509-510.

Patton, J.L., Da Silva, M.N.F., Malcolm, J.R., 1996. Hierarchical genetic structure and gene flow in three sympatric species of Amazonian rodents. Mol. Ecol. 5, 229-238.

Petit, E., Excoffier, L., Mayer, F., 1999. No evidence of bottleneck in the postglacial recolonisation of Europe by the Noctule bat (*Nyctalus Nactula*). Evolution 53, 1247-1258.

Pickford, M., Senut, B. 1999. Geology and palaeobiology of the central and southern Namib desert, southwestern Africa. Memoir 18, 1-155.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817-818.

Posada, D., Crandall, K.A., Templeton, A.R., 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol. Ecol. 9, 487-488.

Prinsloo, P., 1993. Molecular and chromosomal phylogeny of the Hyracoidea. PhD thesis, University of Pretoria, Pretoria.

Prinsloo, P., Robinson, T.J., 1992. Geographic mitochondrial DNA variation in the rock hyrax, *Procavia capensis*. Mol. Biol. Evol. 9, 447-456.

Rajabi-Maham, H., Orth, A. Bonhomme, F., 2008. Phylogeography and postglacial expansion of *Mus musculus domesticus* inferred from mitochondrial DNA coalescent, from Iran to Europe. Mol. Ecol. 17, 627-641.

Rautenbach, I.L., 1978. The mammals of the Transvaal. PhD thesis, University of Natal, Pietermaritzburg.

Ricklefs, R.E., Schluter, D., 1993. Species diversity in ecological communities: historical and geographical perspectives. The University of Chicago Press, Chicago and London.

Riddle, B.R., Hafner, D.J., Alexander, L.F., 2000. Phylogeography and systematics of the Peromyscus eremicus species group and the historical biogeography of North American warm regional deserts. Mol. Phylogenet. Evol. 17, 145-160.

Rogers, A.R., Harpending, H., 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9, 552-569.

Rozas, J., Sanchez-Delbarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism anlaysis by the coalescent and other methods. Bioinformatics 19, 2496-2497.

Russo, I.M., 2003. Molecular systematics of southern African *Aethomys* (Rodentia: Muridae). MSc thesis, University of Pretoria, Pretoria.

Russo, I. M., Chimimba, C. T., Bloomer, P., 2006. Mitochondrial DNA differentiation between two *Aethomys* species (Rodentia: Muridae) from southern Africa. J. Mammal. 87, 545-553.

Schneider, S., Excoffier, L., 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152, 1079-1089.

Shi, N. Dupont, L.M., Beug, H-J., Schneider, R., 1998. Vegetation and climate changes during the last 21,000 year in S.W. Africa based on a marine pollen record. Veg. Hist. Archaeobot. 7, 127-140.

Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. Science 236, 787-792.

Slatkin, M., Barton, N.H., 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43, 1349-1368.

Slatkin, M., Hudson, R.R., 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129, 555-562.

Smithers, R.H.N., 1971. The mammals of Botswana. Mem. Natl. Mus., Rhodesia 4, 1-340.

Smit, H.A., Robinson, T.J., Jansen van Vuuren, B., 2007. Coalescence methods reveal the impact of vicariance on the spatial genetic structure of *Elephantulus edwardii* (Afrotheria, Macroscelidea). Mol. Ecol. 16, 2680-2692.

Swofford, D.L., 2003. PAUP*: phylogenetic analysis using parsimony (* and other methods), Version 4. Sinauer Associates, Sunderland, Massachusetts.

Templeton, A.R., 2008. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7, 381-397.

Templeton, A.R., Boerwinkle, E., Singh, C.F., 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and analysis of alcohol dehydrogenase activity in Drosophila. Genetics 117, 343-351.

Templeton, A.R., Routman, E., Phillips, C.A., 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger salamander, *Ambystoma tigrinum*. Genetics 140, 767-782.

Templeton, A.R., Singh, C.F., 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. Genetics 134, 659-669.

Thomas, D.S.G., Shaw, P.A., 2002. Late Queternary environmental change in central southern Africa: new data, synthesis, isssues and prospects. Q. Sci. Rev. 21, 783-797.

Thompson, J.D., Gibson, T.J., Plewniak, T.F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876-4882.

van Rooyen, N., Bredenkamp, G., 1996. Shrubby Kalahari Dune Bushveld, Karroid Kalahari Bushveld, Kalahari Plains Thorn Bushveld. In: Low, A.B., Rebelo, A.G. (Eds.), Vegetation of South Africa, Lesotho and Swaziland. Department of Environmental Affairs and Tourism, Pretoria, pp. 33-35.

Weir, B.S., 1996. Genetic data analysis II: methods for discrete population genetic data. Sinauer Associates Inc., Sunderland, Massachusetts, USA.

White, R.M., Kerley, G.I.H., Bernard, R.T.F., 1997. Pattern and controls of reproduction of the southern African rodent *Gerbillurus paeba* in the semi-arid Karoo, South Africa. J. Arid Environ. 37, 529-549.

Withers, P.C., Louw, G.N., Henschel, J.R., 1980. Energetics and water relationships of Namib desert rodents. S. Afr. J. Zool. 15, 131-137.

Yang, Z., 1996. Among-site rate variation and its impact on phylogenetic analyses. TREE 144, 1941-1950.

Yang, Z., Goldman, N., Friday, A., 1994. Comparrison of models from nucleotide substitution used in maximum likelihood phylogenetic estimation. Mol. Biol. Evol. 11, 316-324.

Yu, F., Yu, F., McGuire, P.M., Kilpatrick, C.W., Pang, J., Wang, Y., Lu, S., Woods, C.A., 2004. Molecular phylogeny and biogeography of woolly flying squirrel (Rodentia: Sciuridae), inferred from mitochondrial cytochrome *b* gene sequences. Mol. Phyogenet. Evol. 33, 735-744.

Ziv, Y., Abramsky, A., Kotler, B.P., Subach, A., 1993. Interference competition, and temporal and habitat partitioning in two gerbil species. Oikos 66, 237-246.

Ziv, Y., Kotler, B.P., 2003. Giving up densities of foraging gerbils: the effect of interspecific competition on patch use. Evol. Ecol. 17, 333-347.

**Appendix 4.1** Geographic coordinates of all collecting localities of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion, South Africa that were analysed in the present study. Locality numbers 1-10 correspond to those in Fig. 4.1. Bioregion terminology follows that of Mucina and Rutherford (2006).

| LOCALITY | PROVINCE | GEOGRAPHIC COORDINATE |
|---|---|---|
| 1. Farm: Welbedeur, Tosca | North West | 25°42'53"S; 23°58'43"E |
| 2. Farm: Arizona, Vorstershoop | North West | 25°57'00"S; 23°13'55"E |
| 3. Farm: Rus en Vrede, Stella | North West | 26°10'23"S; 25°13'27"E |
| 4. Farm: Loversleap, Vanzylsrus | Northern Cape | 26°38'20"S; 22°01'04"E |
| 5. Farm: Jones, Severn | Northern Cape | 26°35'22"S; 22°41'46"E |
| 6. Tswalu Kalahari Reserve, Sonstraal | Northern Cape | 27°12'51"S; 22°'27'22"E |
| 7. Farm: Waterloo & Vlakfontein, Vryburg | North West | 27°03'34"S; 24°45'58"E |
| 8. Farm: Donkerpoort, Schweizer-Reneke | North West | 27°14'46"S; 25°06'01"E |
| 9. Farm: Strelley, Kuruman | Northern Cape | 27°39'48"S; 23°23'04"E |
| 10. Farm: Steenkampsput, Upington | Northern Cape | 28°06'13"S; 20°54'10"E |