Journal of Mammalogy, 94(1):218–230, 2013

Gene flow in mongooses endemic to Namibia's granite inselbergs despite past climatic fluctuations and isolating landscape features

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Past climatic fluctuations have had a significant impact on the patterns of genetic variation within taxa restricted to montane regions and forested biomes; however, little is known about whether equivalent processes have occurred in arid biomes. Northwestern Namibia's inselbergs provide a unique ecosystem in which to study the effects of major climatic events and geographical isolation on the genetic structuring of taxa in a subtropical arid biome. We investigated the phylogeographic structure of the black mongoose (*Galerella nigrata*), an inselberg habitat specialist endemic to this region, using mitochondrial DNA (mtDNA) haplotypes (cytochrome *b*, 1,089 base pairs) and nuclear microsatellite genotypes (15 loci) from 6 inselberg populations. Analyses of molecular variance and spatial analyses of molecular variance of the 14 mtDNA haplotypes identified 2 significant geographic barriers to the dispersal of black mongooses; these barriers occurred across the vast arid plains between Ruacana and Hobatere, and between Ohorongo and inselbergs to the south. The occurrence of mtDNA haplotypes that were restricted to specific populations also indicated some degree of isolation, likely resulting from limited gene flow and drift induced by the desertification of the landscape between inselbergs during the lead-up to the most recent glacial maxima. Despite this pattern of isolation, the widespread distribution of 1 mtDNA haplotype suggested that populations of black mongoose have, in general, been well connected in the past. Ongoing gene flow was further supported by Bayesian clustering of microsatellite genotypes, which showed 2 clusters, each spread across the mongoose's distribution, and a lack of significant differentiation between populations. In addition, a constant population size was indicated by a Bayesian skyline plot. These analyses suggested that inselbergs across the entire study area might have supported black mongoose populations during some or all of the most recent major climatic fluctuations, with periods of isolation during glacial maxima and reestablished connectivity during interglacial periods.

Key words: arid biome, *Galerella*, glacial maxima, mongoose, phylogeography, Pleistocene

DOI: 10.1644/11-MAMM-A-379.1

Climate change during the Quaternary strongly affected the distribution of lineages within taxa across the globe by causing repeated contraction, fragmentation, and expansion of biota. Effects on the biota of forest biomes have been well documented (e.g., Krosch et al. 2009; Macqueen et al. 2009; Michaux et al. 2005; Nicolas et al. 2008; Qiu et al. 2009; Resende et al. 2010; Spellman et al. 2007). From these studies it is clear that forested biomes have undergone repeated latitudinal changes in response to major climatic oscillations. Altitudinal changes in the current distributions and genetic structures of montane specialists also have been extensively studied (Carstens and Knowles 2007; DeChaine and Martin 2005; Shepard and Burbrink 2009; Taylor et al. 2009). For these montane specialists, major climatic fluctuations are known to cause shifts in the availability of favorable environments as these expand or contract along elevational gradients, with consequent effects on their genetic structure (DeChaine and Martin 2005; Hewitt 2000, 2004). Although we have an increasingly clear picture of the impacts of major climatic changes on forest and alpine biome assembly and maintenance, relatively little is known about the equivalent patterns and processes in arid-zone biomes (Byrne et al. 2008).

Arid regions have few alpine areas; however, inselbergs in these regions tend to have experienced similar isolation to mountain peaks, likely a result of Quaternary climatic fluctuations. Inselbergs are isolated rock hills, ridges, or

mountains that rise abruptly from much flatter surrounding plains. Processes of adaptation and speciation are facilitated on inselbergs because they tend to have microhabitat conditions quite unlike those of the surrounding lowlands (Porembski and Barthlott 2000). Phylogeographic studies focusing on these unique regions are important for effective conservation of current taxa, as well as understanding the historical processes that have led to current patterns of genetic differentiation. Studies on the floral species composition of inselbergs worldwide have been relatively common (e.g., Burke 2003a) and genetic studies have provided evidence of genetic isolation (e.g., Byrne and Hopper 2008; Fay et al. 2001; Sarthou et al. 2001; Zaghloul et al. 2006). However, very little is known about the species compositions and genetic structures of fauna in these areas.

In northwestern Namibia, the landscape is characterized by granite inselbergs, separated by lowland sandy plains (Fig. 1). These inselbergs are characteristically made of large granite domes and boulders of Cretaceous origin, formed more than 20 million years ago (Geological Survey 1997) and they tend to be extremely large and isolated. Some, such as Brandberg, which has a basal area of more than 500 km^2 and a height of over 2,000 m, dominate the landscape, whereas others, such as the Erongo Mountains, are more-weathered granite boulders forming large, interconnected rolling hills. Like other inselbergs, the Namibian inselbergs show unusually high levels of endemism in vertebrates and plants (Burke 2003b; Simmons et al. 1998). Although the genetic structures of taxa specific to this unique ecosystem in northwestern Namibia are unknown, it might be expected that species restricted to inselberg habitat would exhibit significant genetic differentiation among populations on different inselbergs. Indeed, studies on species occurring in isolated rocky habitats in southern Namibia and South Africa, such as the thick-toed gecko (*Pachydactylus rugosus*—Lamb and Bauer 2000) and the southern rock agama (*Agama atra*—Matthee and Flemming 2002), show significant genetic differences between populations on separate outcrops. The genetic structures of taxa restricted to Namibia's granite inselbergs could have been shaped both by geographic isolation and by dramatic changes in the landscape surrounding these inselbergs as a result of major climatic oscillations. During arid periods of the Pleistocene, it might have been difficult for dispersing organisms to successfully navigate the landscape between inselbergs, whereas the landscape may have become more hospitable and easier to traverse during past periods of lesser aridity.

The climatic history of Namibia has involved large fluctuations in the level of aridity across the landscape. Approximately 25,000 years ago southern Africa was much more arid than present conditions and there was extensive dune activity across the whole of Namibia coinciding with the last glacial maximum (Stokes et al. 1997). Partridge (1997) reported that widespread desert across southern Africa between 14,000 and 23,000 years ago received only a fraction of the rainfall experienced today. During these times the distributions of savannah and semidesert taxa may have retreated eastward away from Namibia with the savannah and semidesert habitats, because resources would have been scarce in areas of extreme desert. It appears that by 12,000 years ago the climate of Namibia, although unstable, supported trees and recolonizing

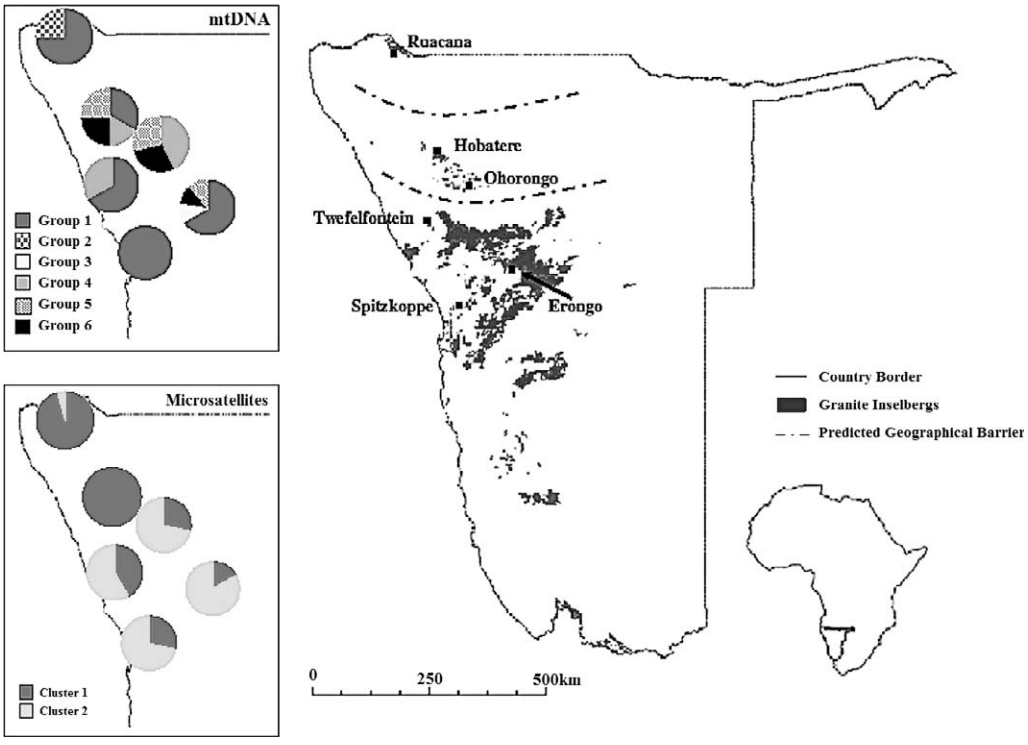

Fig. 1.—Map of Namibia, Africa, showing locations of the 6 study sites in relation to the distribution of granite inselbergs.

savannah habitats (Stokes et al. 1997). We can surmise that many taxa reexpanded their ranges back into Namibia from refugial areas around this time. After a relatively brief period when extreme desert conditions returned to the area (between 10,200 and 10,800 years ago), climatic conditions stabilized and the desert retreated again to a strip along the west coast, leaving a semiarid–savannah vegetation distribution across Namibia similar to that seen today (Stokes et al. 1997).

To gain a better understanding of the effects of landscape and past climatic fluctuations on ecosystems endemic to Namibian inselbergs, we investigated the phylogeographic structure of the black mongoose (*Galerella nigrata*), an inselberg specialist. Initially described by Thomas (1928), the black mongoose is a small, diurnal carnivore weighing under a kilogram that feeds on insects, small mammals, birds, and reptiles. A habitat specialist, the black mongoose lives on isolated granite inselbergs across northwestern Namibia. The entire known range of this species extends from Spitzkoppe and Erongo in central western Namibia to Ruacana in the north. It is possible that the range of the black mongoose extends north to inselbergs in southern Angola; however, there is no information available on the distribution of fauna in this region. It is currently unknown whether black mongooses disperse between these "terrestrial islands". However, there are 2 large potential geographic barriers within the mongoose's known species range that may be predicted to limit dispersal. These barriers consist of large expanses (up to 250 km) of semidesert with no granite outcrops (Fig. 1), where the environment is extremely harsh, with sparse vegetation, sporadic rainfall averaging between 50 and 300 mm annually, and temperatures that exceed 35°C for several months every year.

This is the 1st phylogeographic study of northwestern Namibia's arid zone biome and the 1st genetic assessment of any species in this region of inselbergs. The aim of our study was to test for genetic structuring of the black mongoose across northwestern Namibia, using both the cytochrome-*b* (*Cytb*) region of mitochondrial DNA (mtDNA) and nuclear microsatellites. We predicted that small population sizes and limitations to gene flow caused by unfavorable habitat between inselbergs would cause strong genetic structure across populations, reinforcing isolation caused by the Pleistocene climatic oscillations.

Materials and Methods

Study area.—A total of 46 black mongoose samples from 6 locations were used in this study. Forty-three of these genetic samples were obtained directly from trapping at 6 different areas within northwestern Namibia. A further 3 samples from Erongo Conservancy were supplied by G. Veron (Natural History Museum, Paris, France). The study areas (Fig. 1) were Ruacana (17°24′S, 14°12′E; $n = 6$), Hobatere Conservancy (19°19′S, 14°28′E; $n = 13$), Ohorongo Farm (19°51′S, 15°08′E; $n = 7$), Twefelfontein Conservancy (20°30′S, 14°25′E; $n = 3$), Erongo Conservancy (21°27′S, 15°52′E; $n = 10$), and Spitzkoppe Conservancy (21°50′S, 15°09′E; $n = 7$). These 6 locations span the entire known range of the black mongoose. Given the extremely low density of mongooses at each location, the area and intensity of trapping effort (detailed below), and a high rate of recapture (up to 80%) without evidence of trap bias, we are confident that we caught a substantial and representative portion of the mongooses present at each site, although of course we cannot know what proportion of individuals were trappable.

Trapping of black mongooses and processing of genetic samples.—All trapping and handling methods followed guidelines of the American Society of Mammalogists (Sikes et al. 2011) and were approved by the University of Queensland Animal Ethics Committee. Trapping took place over two 6-month field seasons (January–July) in 2007 and 2008. Live traps (257 × 250 × 700 mm) baited with local butchery remains were used to capture individuals. On each trapping day, 20 traps were set from dawn until dusk and checked at 2-h intervals. A total of 110 trapping days were spent at Hobatere, the main study site. A further 40 trapping days were spent at each of the following sites: Ruacana, Ohorongo, Erongo, and Spitzkoppe. Because of exceptionally low trapping success at Twefelfontein, trapping effort was increased to 60 days. The positions of traps were changed every 5 days and the cumulative trapping area at each site tended to cover either the entire inselberg or group of inselbergs (Ruacana, Twefelfontein, and Spitzkoppe) or at least 8 km^2 where there were many inselbergs in close proximity to one another (Hobatere, Ohorongo, and Erongo).

A small ear notch was taken from each animal when it was captured, for identification purposes and to provide a sample for genetic analysis. Ear tissue samples were stored in 100% ethanol for the duration of the field season and then transferred to 20% salt-saturated dimethylsulfoxide solution (Yoder et al. 2006) for transportation to, and storage in, the laboratory. The ammonium acetate salt extraction method outlined by Nicholls et al. (2000), with an extended digestion time (of 24 h), was used to extract total genomic DNA from tissue samples. Given that hybridization has been found between the black mongoose and its sister species, the slender mongoose (*G. sanguinea*—S. Rapson, pers. obs.), all mtDNA sequences and microsatellite genotypes were screened and compared to those of slender mongooses. Ten individuals suspected of being hybrids based on phenotypes or genetic data were removed from all analyses, and are not included in the 46 samples used in this paper.

Genetic analysis of mtDNA.—The *Cytb* (mtDNA) gene was sequenced in this study (1,089 base pairs). We used primers L14724 (5′-GATATGAAAAACCATCGTTG-3′) and H15915 (5′-TTCATCTCTCCGGTTTACAAGAC-3′ [modified from Irwin et al. 1991]) for initial amplification of the *Cytb* region and then employed 2 additional internal primers (InternalL [5′-AAGCCACCCTAACACGATTC-3′] and InternalH [5′-AGGGTTGTTAGATCCTGTTT-3′]) for sequencing reactions. We performed all polymerase chain reaction amplifications in a final volume of 10 µl using 1x AmpliTaq Gold buffer (Applied Biosystems, Melbourne, Australia), 0.5 units of AmpliTaq Gold

Taq polymerase, 0.2 mM of each deoxynucleoside triphosphate, 0.3 μM of each forward and reverse primer, 3 mM of $MgCl_2$, and 20 ng of DNA template. Reaction mixtures were denatured at 95°C for 15 min before undergoing 35 amplification cycles (94°C for 30 s, 50°C for 30 s, and 72°C for 1 min), followed by a final annealing temperature of 50°C and extension at 72°C for 10 min. Polymerase chain reaction products were purified with 2 μl of ExoSAP-IT enzyme (USB Corporation, Cleveland, Ohio). We sequenced fragments bidirectionally using standard Big Dye Terminator (Applied Biosystems) reactions and reaction products were separated on a capillary sequencer (Applied Biosystems/Hitachi 3130xl Genetic Analyzer). Sequences were assembled using CHROMAS PRO (Technelysium Ltd., Brisbane, Australia) and then aligned using Clustal W (Larkin et al. 2007) in MEGA4 (Tamura et al. 2007). General sequence statistics were calculated in MEGA4. All sequences have been deposited in GenBank (accession numbers JX402101–JX402114).

The jModelTest program (Felsenstein 2005; Guindon and Gascuel 2003; Posada 2008) selected the Tamura and Nei distance model of sequence evolution for phylogenetic analysis of the *Cytb* data set. An unrooted maximum-likelihood tree was constructed using RAxML 7.0.0 (Stamatakis 2006). Support for clades determined by RAxML 7.0.0 was tested by constructing a separate neighbor-joining tree in MEGA4 using 1,000 bootstrap replicates. A statistical parsimony network was assembled using TCS1.21 (Clement et al. 2000). For illustrative purposes, haplotypes were further assigned into groups defined by sets of haplotypes separated by more than 2 base changes and mapped to examine the possibility that closely related haplotypes were structured geographically.

For geographic analysis we conducted an analysis of molecular variance (AMOVA) implemented in Arlequin 3.1.1 (Excoffier et al. 2005). This program incorporates information on genetic divergence among haplotypes while investigating the significance of genetic partitioning across the landscape (Excoffier et al. 2005). Five alternative groupings of inselbergs were tested, exploring groups that were suggested by the geographic proximity of inselbergs and potential geographic barriers to dispersal (Fig. 1), as well as by the proximity of haplotypes in the statistical parsimony network. Significance levels were adjusted by a sequential Bonferroni correction. In addition, spatial analyses of molecular variance (SAMOVAs) using SAMOVA 1.0 (Dupanloup et al. 2002) were run repeatedly with several different possible group structures from $K = 2$ up to $K = 6$. Significance tests were performed with 1,000 permutations. SAMOVA allowed us to identify groups of populations that had the highest level of genetic differentiation from each other, taking into account the spatial distribution of haplotypes as well as their genetic distances and relative frequencies (Dupanloup et al. 2002).

Two Mantel tests in GENALEX (Beck et al. 2008; Smouse et al. 2008) were calculated to test for isolation by distance. Tests used a standard genetic distance matrix and either a standard geographic distance matrix or a least-cost path geographic distance matrix. To create a geographic distance matrix using the least-cost path, a black mongoose–specific landscape resistance map was created in ArcView (Esri, Redlands, California) using rock type and elevation as resistance measures. A map depicting rock type was modified from the Atlas of Namibia Project (2002) and a high-resolution elevation map was obtained from the United States Geological Survey (2007). It was assumed that the mongooses prefer to move on granite inselbergs with no preference for elevation, but that when crossing between granite inselbergs they would prefer to move at higher elevations, because these tend to be associated with other types of rocky outcrops. These assumptions were based on data from radiotracking of black mongooses (S. Rapson, University of Queensland, pers. comm.). Thus, a new synthetic map was created where each pixel was weighted according to these assumptions. We then computed a cost–distance layer using the "costdistance" function in ArcView (associated with the extension PATH-MATRIX—Ray 2005). The "costdistance" function computes the cumulative cost of moving across the resistance surface from a single source point to all possible destination points within the spatial extent of the resistance surface. We used the same programs to complete the least-cost path analysis using the "costpath" function. The "costpath" function maps the single path of least cumulative resistance from a specified source location to a single specified destination location. Using maps produced by the "costpath" function, the least-cost path distance between each pair of study sites was calculated and used to create a distance matrix.

Tajima's D (Tajima 1989), Fu and Li's D^* (Fu and Li 1993), and Fu's F_S (Fu 1997) statistics were calculated using DnaSP version 4.50.3 (Rozas et al. 2003). Each study site was tested separately; sites were amalgamated as northern, central, and southern groups (according to groups defined by SAMOVAs); and also all sequences from across the entire study area were tested as a single population. To test the significance of these statistics, random samples were generated using coalescent simulations and permuted 10,000 times. Demographically, a negative result for any of these statistics could reflect population expansion when a population would likely have an excess of recent mutations (or rare alleles).

Relative unidirectional migration rates among the 6 study sites were evaluated using the program Migrate (version 3.0.3—Beerli 2008; Beerli and Felsenstein 1999, 2001). Migrate is a maximum-likelihood estimator based on the coalescent theory. It estimates possible migration events using a Markov chain Monte Carlo approach. A transition : transversion ratio of 49.76, obtained using jModelTest, was incorporated into the analysis. Six independent runs were performed and used to generate a mean relative value of migration (Nm) and its associated standard deviation. Each run used 10 short Monte Carlo chains of 5,000 steps and 3 long chains of length 50,000, with a sampling increment of 100. The initial 10% of data was discarded to avoid dependence on starting conditions. In addition, a static heating scheme with 4 temperatures was used (set to 1.0°, 1.2°, 1.5°, and 3.0°—Geyer and Thompson 1995). Because insufficient data were available to accurately

estimate effective population sizes, which can then be used to calculate individual estimates of actual migration, we have simply compared relative migration estimates.

A Bayesian skyline plot (Drummond et al. 2005) was constructed based on the coalescence of the mtDNA sequences using BEAST version 1.7.1 (Drummond et al. 2012). Each codon position was partitioned separately, and a strict molecular clock model, an HKY substitution model, a 4-category gamma site model, and a random starting tree were used. ESS values for the posterior distribution and the skyline were >1,000, indicating convergence. The Markov chain Monte Carlo was run for 10,000,000 steps and the first 1,000,000 steps were discarded. Trees were then analyzed in Tracer version 1.5 (Rambaut and Drummond 2007) to generate a skyline plot.

Genetic analysis of nuclear DNA.—Fourteen polymorphic microsatellite primer sets previously developed for other mongoose species were used in this study: Hj15, Hj35, Hj45, and Hj56 (Thulin et al. 2002); Ss7.1, Ss10.1, Mm18-2, Mm18-3, Mm19, and MmAAAC5 (Waldick et al. 2003); and Hic2.52, Hic3.22, Hic4.30, and Hic4.59 (Rodrigues et al. 2009). Forward primers were modified to include an M13 label. A QIAGEN Multiplex PCR kit (QIAGEN, Melbourne, Australia) was used to amplify loci in 5 different multiplexes (A: Hj15, Hj45, Hj56, and MmAAAC5; B: Ss7, Ss10, Mm18, and Mm19; C: Hj35 alone; D: Hic2.52, Hic3.22, and Hic4.59; and E: Hic4.30 and Mm18-2). All reactions were carried out in 10-μl reaction volumes according to the manufacturer's instructions with 1× QIAGEN Multiplex Mix, 0.125 μM each of the forward primer and the corresponding fluorescently labelled M13 primer, 0.25 μM of the reverse primer, and 20 ng of the template DNA. Polymerase chain reaction conditions were as outlined above but with an annealing temperature of 55°C. Fragments were separated by capillary electrophoresis on an Applied Biosystems/Hitachi 3130xl Genetic Analyzer. Microsatellites were scored using Genemapper version 3.7 (Applied Biosystems) and MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) was used to check for potential scoring errors and the presence of null alleles. Deviations from Hardy–Weinberg equilibrium and general diversity statistics for microsatellite loci were calculated using GenAlEx 6.1 (Peakall and Smouse 2006). Linkage disequilibrium among loci was tested using 10,000 iterations of the Markov chain Monte Carlo simulation implemented by GENEPOP version 3.4 (Raymond and Rousset 1995). Significance levels were adjusted using a sequential Bonferroni correction.

We employed the Bayesian clustering method implemented in STRUCTURE 2.3.X (Hubisz et al. 2009; Pritchard et al. 2000) to infer genetic structure within the overall population of black mongooses. The results generated were based on simulations of 1–20 inferred populations (k) using 10^5 iterations after discarding the inital 10^5 iterations, using an admixture model and correlated allele frequencies with default settings. The inferred number of populations ($k = 2$) was deduced using the delta log-likelihood statistic (Evanno et al. 2005) and both of the identified clusters were subsequently reanalyzed to test for further within-cluster structure. For geographic analyses, we performed AMOVAs and SAMOVAs, employing the same methods outlined for mtDNA analyses. GenAlEx was used to perform Mantel tests looking for evidence of genetic isolation by distance using both standard geographic distance and the least-cost path method (outlined above). To test for evidence of sex-biased dispersal, we performed Mantel tests on male and female genotypes separately. Φ_{ST}-values were calculated in Arlequin 3.1.1 using 10^4 permutations. F_{ST}-values were calculated using GENETIX 4.05 (Belkhir et al. 2004) to assess population differentiation. Significance of Φ_{ST}- and F_{ST}-values was tested by permutation with a sequential Bonferroni correction.

A genetic assignment method, implemented in BayesAss+ version 1.3 (Wilson and Rannala 2003), was used to estimate recent rates of gene flow (Berry et al. 2004; Pearse and Crandall 2004). This program employs Markov chain Monte Carlo resampling to estimate asymmetrical rates of gene flow between predefined populations; in this case populations were defined by sampling location. We averaged the results from 6 independent runs, each with 3×10^6 iterations, sampling the chain every 2,000 generations and using a delta value of 0.06 for inbreeding (calculated using GenAlEx 6.1), discarding the first 100,000 iterations.

Results

Genetic analysis of mtDNA.—A total of 41 samples were sequenced for *Cytb* and 14 haplotypes were identified (Table 1). The remaining 5 samples were discarded due to incomplete sequencing. There was no evidence for insertions–deletions, ambiguities, or stop codons at any site in the 41 sequences used. Nine 1st-codon sites were variable, 5 sites were variable in the 2nd codon position, and 14 were variable in the 3rd position. Ten of these substitutions were nonsynonymous. The distances between haplotypes ranged from 0.00094 to 0.01146 and the overall mean distance was 0.00452 ± 0.00113. Nine of the 14 haplotypes were population specific (Table 1). Spitzkoppe had the lowest number of haplotypes, with only 1 haplotype, whereas Hobatere had the highest, with 6 haplotypes, although Hobatere also had the highest number of samples. Ruacana had the highest proportion of private haplotypes (Table 1).

The topologies of both the maximum-likelihood tree and the neighbor-joining tree were identical and, although they showed well-resolved genetic structure, this structure was not organized by geographic region (Fig. 2). The most common haplotype (A) was present in all study areas except Ohorongo (Table 1) and Spitzkoppe contained only this most common haplotype. To simplify analysis, we grouped haplotypes separated by 2 or fewer base changes (Fig. 3). Haplotype group 1 spanned all study areas except Ohorongo. Groups 2 and 3 contained only a single haplotype each and were each restricted to a single location: group 2 was only found at Ruacana and group 3 was restricted to Erongo (Table 1; Fig. 1).

Table 1.—Frequency of mitochondrial DNA cytochrome-*b* haplotypes in black mongooses (*Galerella nigrata*) by population. Haplotype group number refers to those groups depicted in Fig. 3.

Haplotype	Group	Ruacana	Hobatere	Ohorongo	Twefelfontein	Erongo	Spitzkoppe	Total[a]
A	1	0.25	0.17	—	0.33	0.56	1.00	15
B	1	0.25	—	—	—	—	—	1
C	1	0.25	—	—	—	—	—	1
D	1	—	—	—	—	0.11	—	1
E	2	0.25	—	—	—	—	—	1
F	1	—	0.17	—	—	—	—	2
G	1	—	—	—	0.33	—	—	1
H	3	—	—	—	—	0.11	—	1
I	4	—	0.08	0.43	—	—	—	4
J	4	—	0.08	—	0.34	—	—	2
K	5	—	0.25	0.14	—	0.11	—	5
L	5	—	—	0.14	—	—	—	1
M	6	—	0.25	—	—	0.11	—	4
N	6	—	—	0.29	—	—	—	2
Total no. individuals		4	12	7	3	9	6	41

[a] Total number of individuals with each haplotype.

The remaining 3 groups were confined to the 4 central populations (Table 1; Fig. 1).

The AMOVAs on mtDNA data revealed significant variation among all 6 locations and among populations when grouped as "northern" (Ruacana), "central" (Hobatere and Ohorongo), and "southern" (Twefelfontein, Erongo, and Spitzkoppe) regions. Other tested groupings were nonsignificant after sequential Bonferroni correction (Table 2). Similarly, SAMOVA results yielded an optimum of 3 groups: group 1 at Ruacana; group 2 spanning Hobatere and Ohorongo; and group 3 including Twefelfontein, Erongo, and Spitzkoppe ($F_{CT} = 0.218$, $P = 0.0195$). Results of both Mantel tests (using standard geographic distance and least-cost path methods) showed no evidence for isolation by distance ($R^2 = 0.0286$, $R\text{xy} = 0.169$, $P = 0.08$; $R^2 = 0.0973$, $R\text{xy} = 0.312$, $P = 0.14$, respectively).

We obtained nonsignificant ($P > 0.1$) but negative values for Tajima's D, Fu and Li's D^*, and Fu's F_S statistics when analyzing: Erongo ($D = -1.02$; $D^* = -1.15$; $F_S = -1.05$), southern populations (Twefelfontein, Erongo, and Spitzkoppe) together ($D = -1.32$; $D^* = -1.58$; $F_S = -1.43$), and the entire data set as 1 population ($D = -0.87$; $D^* = -1.03$; $F_S = -0.87$). The Bayesian skyline plot (Fig. 4) clearly indicates a constant overall population size, with no evidence of either population expansion or bottlenecks. Migration analyses suggested that black mongoose populations at Hobatere and Spitzkoppe had both contributed larger proportions of emigrants to other populations within the study area than had the other studied populations, yet had received comparatively smaller proportions of immigrants (Table 3), whereas the opposite was observed for Ohorongo, Twefelfontein, and Erongo.

Genetic analysis of nuclear DNA.—All 14 microsatellite loci were polymorphic across the 46 individuals sampled. The number of alleles per locus varied from 3 to 24 with a mean of 9 ± 1.5 alleles per locus. Only locus Ss10.1 in the Erongo population deviated significantly from Hardy–Weinberg equilibrium ($P = 0.001$) due to homozygote excess (observed heterozygosity = 0.6, expected heterozygosity = 0.64). No evidence for null alleles was found and there was no linkage disequilibrium between loci after sequential Bonferroni correction. Both Ruacana and Hobatere had large numbers of private alleles (14 at Ruacana and 15 at Hobatere). Table 4 summarizes allelic variation for each population.

STRUCTURE found 2 microsatellite clusters (Fig. 5, mapped on Fig. 1). The 2 clusters occurred across the species' distribution, although the 2 most northerly populations, Ruacana and Hobatere, were dominated by microsatellite cluster 2 (Fig. 1). No subclusters were found when these 2 clusters were reanalyzed. AMOVAs of microsatellite data revealed significant levels of variation among locations as well as significant levels of genetic differentiation among northern, central, and southern regions for all tested groups of populations after sequential Bonferroni correction (Table 2). SAMOVAs found no optimum number of groups ($P > 0.1$ for all values of K). Results of the Mantel test using standard geographic distance revealed no evidence for isolation by distance ($R^2 = 0.0017$, $R\text{xy} = -0.042$, $P = 0.19$). In contrast, the Mantel test using the least-cost path method showed strong evidence for isolation by distance for all individuals ($R^2 = 0.12$, $R\text{xy} = 0.35$, $P = 0.01$) and for males ($R^2 = 0.08$, $R\text{xy} = 0.28$, $P = 0.01$) but not for females ($R^2 = 0.0024$, $R\text{xy} = 0.049$, $P = 0.40$). Population differentiation assessed by Φ_{ST} and F_{ST} was significant for 5 pairwise comparisons after sequential Bonferroni correction (Table 5). Although geographically separated from all other populations, the population at Ruacana was only significantly differentiated from Erongo and Spitzkoppe. In contrast, the neighboring populations of Hobatere and Ohorongo were significantly differentiated from each other. Spitzkoppe was significantly different from the 2 most northern populations, Ruacana and Hobatere (Table 5). Migration analyses in BayesAss+ suggested significant levels of unidirectional migration from Spitzkoppe to Erongo, Twefelfontein, and Ohorongo (0.25, 0.13, and 0.21, respectively). All other estimates of migration were found to be nonsignificant at $P < 0.1$. Standard deviations for all distributions were <0.05.

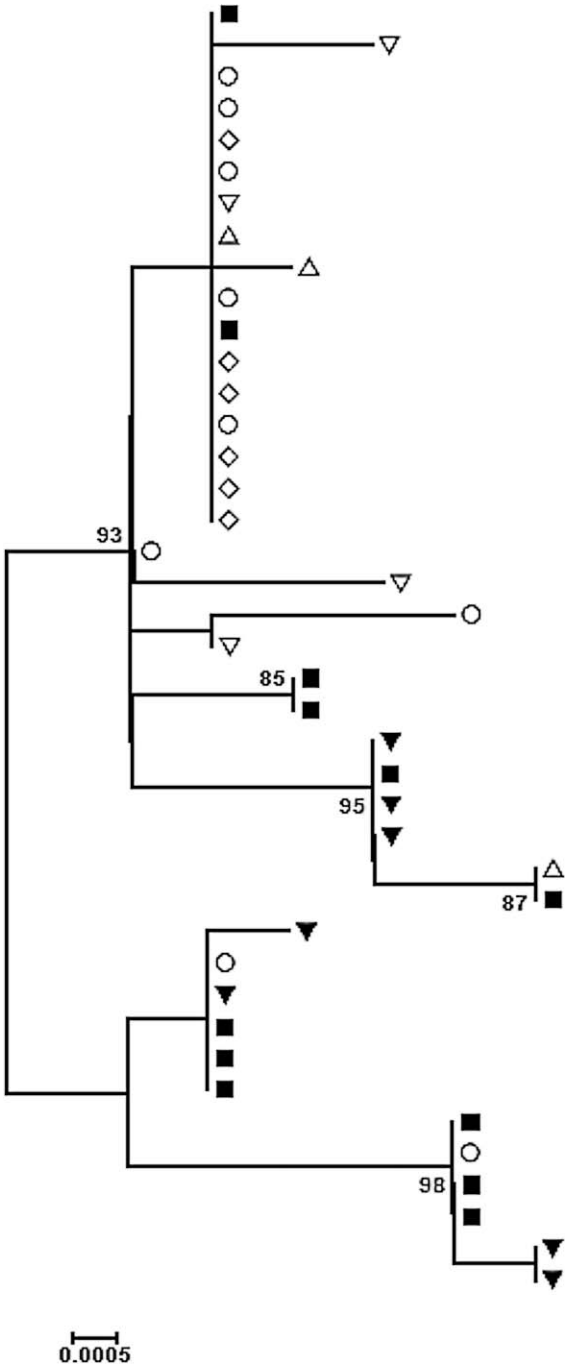

Fig. 2.—Unrooted neighbor-joining tree obtained from analysis of the cytochrome-*b* data set in MEGA4. Bootstrap values are reported for each node unless inferior to 70. ▽ = Ruacana; ■ = Hobatere; ▼= Ohorongo; △ = Twefelfontein; ○ = Erongo; ◇ = Spitzkoppe.

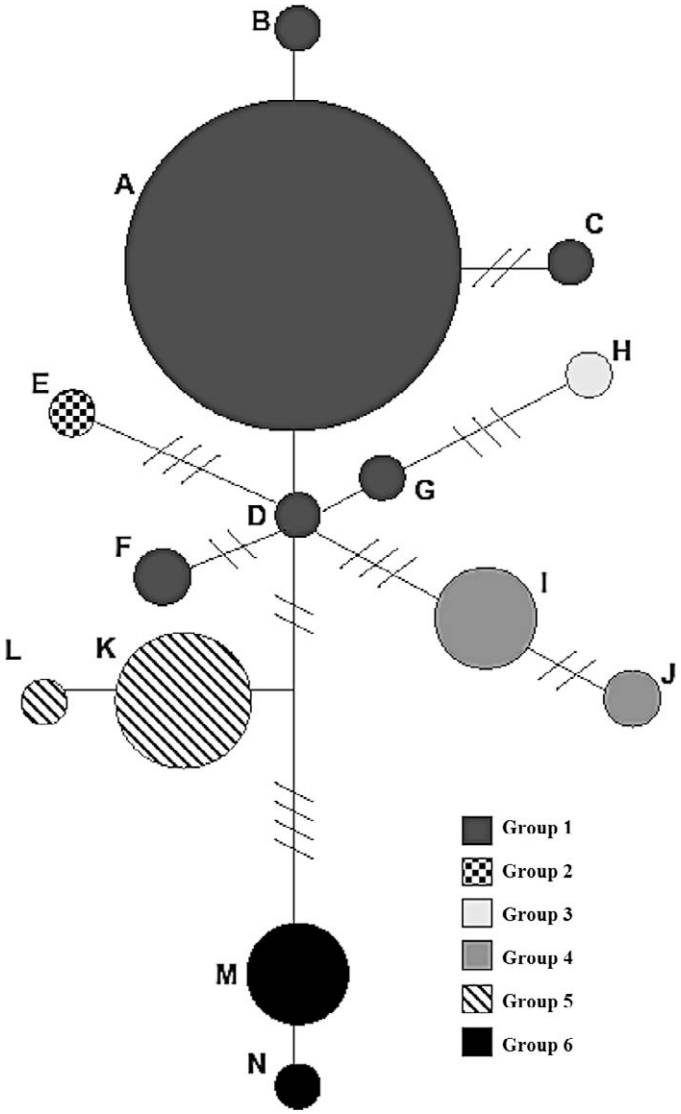

Fig. 3.—Statistical parsimony network showing 14 haplotypes of cytochrome *b*. Each haplotype is represented by a circle scaled by the relative number of individuals that shared that particular haplotype (Table 1). Cross lines indicate the number of mutations where the number of mutations is greater than 1. Haplotypes have been further arranged into 9 groups as indicated by shading. Haplotype group membership was defined as haplotypes separated by more than 2 base changes.

Discussion

The aim of our paper was to test for evidence of population structure that would indicate restricted movement between populations. Both AMOVAs and SAMOVAs showed evidence of restricted mtDNA gene flow when populations were grouped as northern, central, and southern populations, as was predicted geographically (Fig. 1). Similar studies on several southern African rupicolous taxa such as the thick-toed gecko (Lamb and Bauer 2000), the southern rock agama (Matthee and Flemming 2002), the rock hyrax (*Procavia capensis*—Prinsloo and Robinson 1992), Smith's red rock rabbit (*Pronolagus rupestris*—Matthee and Robinson 1996), and the Cape rock elephant shrew (*Elephantulus edwardii*—Smit et al. 2007) also have found genetic differences between geographically separated populations.

The pattern of genetic movement across a landscape can be influenced by both biotic factors such as strong territoriality, social organization, inter- and intraspecific interactions (Dias 1996; Matthee and Robinson 1996; Nathan 2001) and abiotic

TABLE 2.—Analysis of molecular variance results for mitochondrial DNA (mtDNA) and microsatellite analyses of black mongooses (*Galerella nigrata*).

Test	Structure	Groupings[a]			mtDNA			Microsatellites		
		Northern	Central	Southern	% variation	Φ_{ST}-statistics	*P*-value	% variation	Φ_{ST}-statistics	*P*-value
1	All geographic locations as separate populations				12 among locations, 88 within locations	0.11916	0.01466	6 among locations, 94 within locations	0.06009	<<0.001***
2	All geographic locations placed into 3 groups: northern, central, and southern	Ruacana	Hobs and Ohorongo	Twefel, Erongo, and Spitz	17 among groups, 83 within groups	0.17493	0.00293***	5 among groups, 95 within groups	0.04972	<<0.001***
3		Ruacana	Hobs, Ohorongo, Twefel, and Erongo	Spitz	12 among groups, 88 within groups	0.12040	0.03324	4 among groups, 96 within groups	0.04386	<<0.001***
4	All geographic locations placed into 2 groups: northern and southern	Ruacana		Hobs, Ohorongo, Twefel, Erongo, and Spitz	5 among groups, 95 within groups	0.05107	0.21994	5 among groups, 95 within groups	0.05391	0.00098***
5	All geographic locations placed into 2 groups: northern and southern	Ruacana and Hobs		Ohorongo, Twefel, Erongo, and Spitz	0 among groups, 100 within groups	−0.00651	0.45357	6 among groups, 94 within groups	0.05496	<<0.001***

[a] Hobs =Hobatere; Twefel = Twefelfontein; Spitz = Spitzkoppe.
*** Significance after sequential Bonferroni correction.

factors in the form of geographic barriers (e.g., Dalen et al. 2005; Miller et al. 2006; Rueness et al. 2003). We know that the black mongoose is a habitat specialist living on isolated granite inselbergs and is rarely observed in the surrounding plains (Shortridge 1934; S. Rapson, University of Queensland, pers. comm.); however, there is no information on its dispersal capabilities. Recent studies have shown that individuals tend to be solitary with considerable home-range overlap (Rathbun and Cowley 2008; S. Rapson, University of Queensland, pers. comm.). Thus, it appears likely that it is the geographic barriers between granite inselbergs that hinder the movement of black mongooses across the landscape, rather than biotic factors such as strong territoriality or a complex social organization. The barriers between these regions can be up to 250 km wide and are semidesert environments where there is little sustenance for animals such as mongooses and no shelter from temperatures that regularly exceed 35°C. Significant levels of genetic differentiation have been documented for species worldwide when populations are separated by unsuitable habitat (e.g., arctic fox [*Vulpes lagopus*—Geffen et al. 2007], red tree vole [*Phenacomys longicaudus*—Miller et al. 2006], and Canadian lynx [*Lynx canadensis*—Rueness et al. 2003]).

Although there is evidence for restricted gene flow in black mongooses, the distribution of mtDNA haplotypes across multiple populations suggests that the isolation of black mongoose populations has not been complete. This differs from the pronounced genetic discontinuities seen in the rock hyrax (Prinsloo and Robinson 1992), Smith's red rock rabbit (Matthee and Robinson 1996), and the Cape rock elephant shrew (Smit et al. 2007) and suggests that the black mongoose may have a greater dispersal capability across inhospitable habitat than these small mammals. During the less-arid phases of the Quaternary climatic oscillations (between glacial maxima) the savannah habitat expanded across the landscape between Namibia's granite inselbergs as the desert retracted back toward the west coast (Stokes et al. 1997). Black mongooses may have successfully navigated the large distances between granite inselbergs during these times. Although examination of the mtDNA data indicates historical connectivity among the 6 study populations, some of the rare haplotypes at the tips of the network branches are restricted to specific populations (Fig. 3). This would suggest that these populations have experienced some degree of isolation in their more recent history.

As global temperatures increased following the last glacial maximum and the desert retreated toward the west coast of Namibia, we would expect that the landscape between inselbergs became increasingly hospitable and easier to traverse. Indeed, a general lack of significant genetic differentiation at microsatellite loci between neighboring populations (with the exception of Hobatere and Ohorongo) does suggest an increase in the recent movement of black mongooses across the landscape. In STRUCTURE analyses, although there were 2 clusters, both were spread across the whole region, indicating there were no substantial geographic barriers to gene flow. In addition, both AMOVAs and

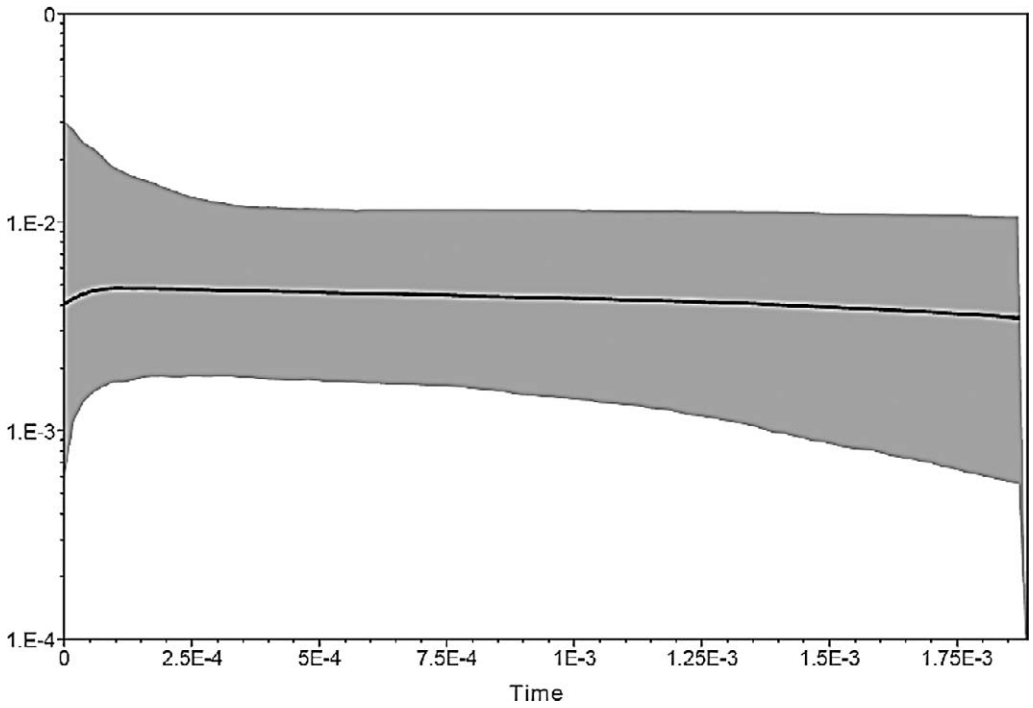

Fig. 4.—Bayesian skyline plot showing the effective population size (y-axis) over time. The x-axis shows time in units of mutations per site from current day. The median effective population size is shown with 95% highest posterior density intervals.

SAMOVAs of microsatellite data were unable to define any clear spatial structure in the data. The unusually high numbers of private alleles in populations at Ruacana and Hobatere likely reflect older divergences, larger population sizes reducing the loss of alleles through genetic drift, or reduced gene flow from these more geographically isolated populations.

There was strong support for a geographic component to explain the genetic variation at microsatellite loci for male black mongooses across the landscape—as the geographic distance of the least-cost path for dispersal increased between populations, so too did the genetic distance for all individuals and for males. It is likely, therefore, that male black mongooses have followed a stepping-stone model for dispersal with low genetic distances between close populations due to gene flow. Mongooses have occasionally been observed moving along riverbeds and small canyons during times of drought and these could provide corridors for dispersal between inselbergs that are a relatively short distance apart. Given the tendency of mammals to show male-biased dispersal (reviewed by Greenwood 1980), philopatry is a likely explanation for the lack of evidence for female isolation by distance from microsatellite data in black mongooses, and for the discrepancy between population structuring in AMOVAs and SAMOVAs for mtDNA but not for microsatellite data.

Estimates of migration based on microsatellite genotypes suggested that a substantially greater number of individuals have been successfully moving from Spitzkoppe to neighboring populations in the north, when compared to emigration estimates from other populations (Table 3). Spitzkoppe is an extremely isolated single large granite rock formation with a relatively small area of suitable habitat available for black mongooses. This may encourage a disproportionately large number of dispersal attempts away from Spitzkoppe, compared to other study populations, and hence result in a larger number of successful migrants.

Table 3.—Migration estimates among each of the study populations of black mongooses (*Galerella nigrata*) using coalescent analyses in MIGRATE based on mitochondrial DNA haplotypes. Estimates of migration from columns (source) to rows (recipient) are expressed as a proportion of the total estimated number of migration events between all study populations.

	Ruacana	Hobatere	Ohorongo	Twefelfontein	Erongo	Spitzkoppe	Total immigrants
Ruacana	—	0.0028	0.0000	0.0000	0.0195	0.1620	0.18
Hobatere	0.0000	—	0.0000	0.0551	0.0404	0.0000	0.10
Ohorongo	0.0000	0.1964	—	0.0000	0.0000	0.0000	0.20
Twefelfontein	0.0000	0.0000	0.0379	—	0.0000	0.0985	0.14
Erongo	0.0000	0.1118	0.0000	0.0000	—	0.1416	0.25
Spitzkoppe	0.1341	0.0000	0.0000	0.0000	0.0000	—	0.13
Total emigrants	0.13	0.31	0.04	0.06	0.06	0.40	1.00

Table 4.—Summary of microsatellite loci variation between inselberg populations of black mongooses (*Galerella nigrata*). The mean and standard error over 14 loci are shown for each of 6 populations. N = number of individuals; Np = number of private alleles; Ar = allelic richness corrected for minimum population size; Na = number of different alleles/locus; Ne = number of effective alleles = $1/(\Sigma\pi^2)$; H_O = observed heterozygosity = number of heterozygotes/N; H_E = expected heterozygosity = $1 - \Sigma\pi^2$ F = fixation index = $(H_E - H_O)/H_E = 1 - (H_O/H_E)$.

Location	N	Np	Ar	Na	Ne	H_O	H_E	F
Ruacana								
$\bar{X}$	6	14	3.603	5.214	3.811	0.688	0.651	−0.087
SE			0.308	0.547	0.526	0.052	0.052	0.052
Hobatere								
$\bar{X}$	13	15	3.126	5.357	3.518	0.648	0.609	−0.029
SE			0.283	0.768	0.549	0.075	0.060	0.059
Ohorongo								
$\bar{X}$	7	2	2.954	4.214	3.124	0.510	0.547	0.051
SE			0.339	0.656	0.477	0.084	0.074	0.097
Twefelfontein								
$\bar{X}$	3	4	3.143	3.143	2.666	0.595	0.496	−0.218
SE			0.390	0.390	0.377	0.094	0.076	0.064
Erongo								
$\bar{X}$	10	3	2.733	4.214	2.743	0.554	0.508	−0.037
SE			0.290	0.576	0.422	0.089	0.072	0.090
Spitzkoppe								
$\bar{X}$	7	3	2.757	3.929	2.893	0.500	0.496	−0.031
SE			0.338	0.642	0.574	0.079	0.077	0.062

In the Northern Hemisphere where the effects of glacial cycles were more severe, fauna tended to survive in specific refugial areas, responding to postglacial warming with rapid population and range expansion (Kerdelhue et al. 2009 and references therein). However, where the effects of climatic change on the effective population sizes were much less dramatic, such as in the Southern Hemisphere, populations were less likely to show evidence of rapid demographic expansion (Kerdelhue et al. 2009). Indeed, Tajima's *D*, Fu and Li's *D**, and Fu's F_S statistics all were found to be nonsignificant in this study. In addition, the Bayesian skyline plot shows no evidence of historic population expansion or bottlenecks in the overall population of black mongooses across our study area (Fig. 4). The apparent persistence of relatively stable populations over an extended period (encompassing major climatic variation) suggests that, although there may have been episodic periods of isolation and connectivity, no particular set of inselbergs provided consistent habitat refugia. Rather, inselbergs across the entire study area may have supported populations of black mongooses at different times during the various glacial cycles, with a number of different recolonization pathways following each period of isolation. In this scenario, inselbergs such as Hobatere, Ohorongo, and Erongo may have provided comparatively larger habitat refuges than others or may have supported populations in habitat refugia on a more regular basis, or both, thus maintaining a relatively larger mtDNA genetic diversity when compared to other study locations (Table 1; Fig. 1; Avise 2004). Indeed, the fact that these populations show a range of divergent mtDNA haplotypes (Table 1; Fig. 3) suggests that these inselbergs might have been refugial regions over several glacial cycles. Similarly, mountainous regions in the Australian arid zone also have been suggested as important past refugial areas during extreme climatic oscillations (for example, the Pilbara [Western Australia], Kimberley [northern coastal], and Arunta [central Australia] ranges [Byrne et al. 2008; Pepper et al. 2011]).

The mtDNA migration analyses show that Hobatere may have provided a large proportion of emigrants while receiving a comparatively low proportion of immigrants (Table 3); however, this pattern was not observed for the other populations with high diversity, Ohorongo and Erongo. It is therefore possible that Hobatere provided a refugial area for black mongooses during the most recent glacial maximum, with subsequent recolonization of Ohorongo and Erongo during less-extreme climatic conditions. However, larger sample sizes as well as comparative studies across multiple species are needed before any firm conclusions about the positions of consistent refugial regions can be made. Two divergent haplotypes identified in Ruacana (haplotypes C and E) and 1 in Erongo (haplotype H) are absent from all other populations. Although this may be attributed to not sampling all animals or all populations within the study area, it also could indicate additional colonization from refugia beyond our sampling area (Table 1).

Shepard and Burbrink (2009) found that responses to historic climatic events can vary substantially even among closely related taxa with a similar distribution. This emphasizes the importance of a comparative phylogeographic approach using multiple taxa. Thus, further studies on multiple species endemic to Namibia's granite inselbergs are recommended because they would enhance our understanding of both

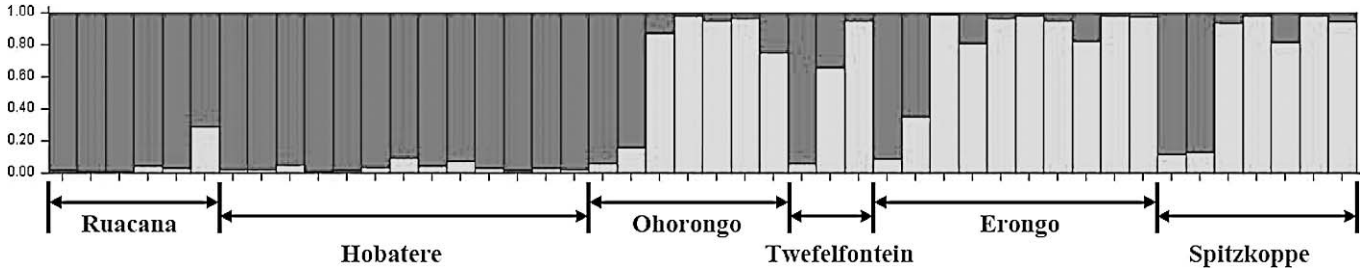

Fig. 5.—Population admixture bar plots of estimated genetic clusters for black mongooses (*Galerella nigrata*) according to Bayesian clustering results from STRUCTURE. The colors indicate assignment to each of $K = 2$ clusters.

Table 5.—Summary of pairwise population differentiation of black mongooses (*Galerella nigrata*) for microsatellite data. Φ_{ST}-values are presented above the diagonal and F_{ST}-values are presented below the diagonal.

	Ruacana	Hobatere	Ohorongo	Twefelfontein	Erongo	Spitzkoppe
Ruacana	—	0.04257	0.05971	0.03472	0.09102**	0.10883**
Hobatere	0.04064	—	0.05162**	0.01251	0.09321**	0.07726**
Ohorongo	0.05048	0.04996**	—	0.00320	0.05274	0.02710
Twefelfontein	0.02870	0.01466	0.01058	—	0.01003	0.03752
Erongo	0.09226**	0.09472**	0.04963	0.01484	—	0.04873
Spitzkoppe	0.10339**	0.07699**	0.01894	0.03162	0.04710	—

** Significance at $P < 0.05$ after Bonferroni correction.

historical and current evolutionary processes acting on this particular ecosystem as a whole.

Acknowledgments

Many thanks to a great number of people involved in this project, including the team at Etosha Ecological Institute and field volunteers A. Carter, B. Barth, and T. Mitchell. G. Veron (National Museum of Natural History, Paris, France) provided us with additional genetic samples, for which we are most grateful. The process of sequencing and genotyping was made so much easier by the assistance of S. Corley, and F. Hibert provided invaluable assistance when conducting the least-cost path analyses. We also are indebted to the owners, managers, and staff at Hobatere Lodge, Ohorongo Farm, Erongo Conservancy, Erongo Wilderness Lodge, Uukolonkadhi Censervancy, Hippo Pools Community Campsite, Spitzkoppe Conservancy, and Spitzkoppe Community Campsite. Funding for this project was provided by Go Green (Nedbank), Wilderness Wildlife Trust, and the Namibian Environment and Wildlife Society.

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Submitted 6 November 2011. Accepted 9 August 2012.

Associate Editor was Samantha M. Wisely.