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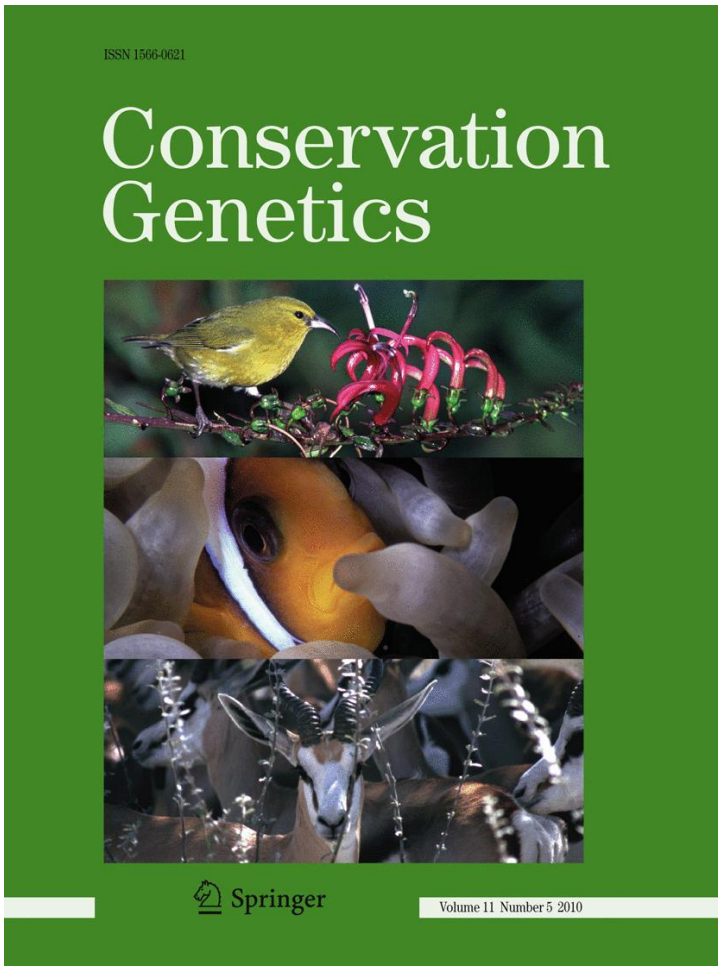

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RESEARCH ARTICLE

Endangered species in small habitat patches can possess high genetic diversity: the case of the Tana River red colobus and mangabey

David N. M. Mbora · Mark A. McPeek

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Abstract We used mtDNA sequence data from the Tana River red colobus and mangabey to determine how their population genetic structure was influenced by dispersal and habitat fragmentation. The colobus and mangabey are critically endangered primates endemic to gallery forests in eastern Kenya. The forests are a Pliocene–Pleistocene refugium that has recently undergone significant habitat loss and fragmentation due to human activities. We expected both primates to exhibit low levels of genetic diversity due to elevated genetic drift in their small populations, and to show a strong correspondence between genetic and geographic distance due to disruption of gene flow between forests by habitat fragmentation. Additionally, because mangabey females are philopatric, we expected their mtDNA variation to be homogeneous within forest patches but to be heterogeneous between patches. In contrast, colobus have a female-biased dispersal and so we expected their mtDNA variation to be homogeneous within and between forest patches. We found high levels of haplotype and nucleotide diversity as well as high levels of sequence divergence between haplotype groups in both species. The red colobus had significantly higher genetic variation than the mangabey did. Most of the genetic variation in both primates was found within forest fragments. Although both species showed strong inter-forest patch genetic structure we found no correspondence between genetic and geographic distances for the two primates. We attributed the high genetic diversity to recent high effective population size, and high sequence divergence and strong genetic structures to long-term habitat changes in the landscape.

Keywords MtDNA · Climate change · Africa · Genetic diversity · Conservation

D. N. M. Mbora · M. A. McPeek
Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA
e-mail: Mark.McPeek@Dartmouth.edu

D. N. M. Mbora
Tana River Primate National Reserve, P O Box 4, Hola, Kenya

D. N. M. Mbora (✉)
Department of Biology, Whittier College, PO Box 634, Whittier, CA 90608, USA
e-mail: David.Mbora@Dartmouth.edu; dmbora@yahoo.com; dmbora@Whittier.edu

Introduction

The current genetic diversity of any species has been influenced by many factors in the past. Past changes in climate are known to be a major influence on the distribution of populations and therefore their genetic structure (Hewitt 2000). In Africa, climate change caused major shifts in faunal assemblages during a time interval lasting 5.3 million years during the Pliocene–Pleistocene epochs (deMenocal 2004; Bobe and Behrensmeyer 2004). The genetic imprint of these habitat changes is evident in several mammals in East Africa; buffalo (Heller et al. 2008), elephant (Okello et al. 2008), hippopotamus (Okello et al. 2005) and baboons (Storz et al. 2002a, b). During that interval, lower temperatures and increased aridity in East Africa reduced and fragmented tropical forests and left them as isolated patches along major rivers and on high elevation (Bobe and Behrensmeyer 2004). These forest refugia have provided important habitat for forest dependent non-human primates (Fleagle 1999). Thus, the current population genetic structure of primates endemic to these forests should reflect their histories in these refugia.

Furthermore, primates endemic to these forests are now vulnerable to further loss of genetic diversity because of additional forest reduction and fragmentation caused by human activities (Mace and Balmford 2000).

The Tana River forests of southeastern Kenya are an example of forest fragments whose origin dates back to the increasing aridity of the Pliocene–Pleistocene interval in East Africa (Bobe and Behrensmeyer 2004). Botanical surveys of the Tana River forests suggest that evergreen forests were once more continuous in Africa (Hamilton 1981). In particular, 12% of tree species in the Tana are from the Guinea-Congolian region indicating an earlier period of continuous rain forest across the African continent (Medley 1992). The forests occupy the lower floodplain of the Tana River and are of great conservation importance. They are part of the east African coastal forests global biodiversity hotspot (Myers et al. 2000) and support a high diversity of rare plant and animal species (Andrews et al. 1975). In particular, they provide the only known habitat of two endemic primates: the Tana River red colobus (*Procolobus rufomitratus*) and the Tana mangabey (*Cercocebus galeritus*). Both species are critically endangered (Hilton-Taylor 2000) and ranked among the IUCN's top 25 most endangered primates (Grubb et al. 2003; Mittermeier et al. 2007). It is estimated that the population of the colobus is less than 1000 individuals and that of the mangabey does not exceed 2000 individuals (Butynski and Mwangi 1994).

In addition to the natural forest fragmentation caused by the meandering of the river in its old stage, recent human activities have further reduced and fragmented the forests causing precipitous declines in the primate populations, and extinctions in several of the fragments (Mbora and Meikle 2004). Thus, these forests offer a natural setting to study the effects of forest loss and fragmentation on population genetic structure of endemic, endangered forest primates.

The population structure seen in mitochondrial DNA can be particularly useful in understanding the effects of forest loss and fragmentation on population genetic structure of forest primates. Mitochondrial DNA is maternally inherited (Gyllensten et al. 1985), lacks recombination (Hayashi et al. 1985) and exhibits rapid sequence evolution (Brown et al. 1979). Consequently, any mtDNA lineages that diverge in populations (e.g. in forest fragments) are independent clones that rapidly accumulate divergent sets of mutations through time. Thus, in species where females are philopatric, there should exist little or no variation within, and much variation between populations, e.g. in many macaque species (*Macaca* spp.; Melnick and Hoelzer 1992) and vervet monkeys (*Cercopithecus aethiops aethiops*; Shimada 2000). In contrast, female dispersal should lead to much differentiation within and less differentiation between populations; e.g. in the hamadryas baboons (*Papio hamadryas hamadryas*; Hapke et al. 2001).

We analyzed the population structures of mtDNA variation (NADH dehydrogenase subunit 4, ND4, gene) of the Tana River red colobus and mangabey to determine how they are influenced by the pattern of dispersal and habitat fragmentation. We expected both primates to exhibit low levels of genetic diversity due to genetic drift in their relatively small populations, and for populations that were geographically close to one another to be more genetically similar because of greater gene flow (Wright 1978; Kimura and Weiss 1964). Mangabey females are largely philopatric (Kinnaird 1992), while red colobus females disperse on attaining sexual maturity (Marsh 1979). Thus, we expected the mtDNA variation in the mangabey to be relatively homogeneous within forest patches but to be heterogeneous between forest patches. Conversely, we expected the mtDNA variation of the red colobus to be relatively homogeneous within and between forest patches due to female dispersal in this species (Marsh 1979).

Materials and methods

Study area and species

The study area comprises approximately 26 km^2 of gallery forest occurring in scattered patches of various sizes on both sides of the Tana River in eastern Kenya (Fig. 1; Mbora and Meikle 2004). This area encompasses the entire distribution range of the Tana River red colobus and mangabey. These forests exist in an arid environment with an annual total rainfall of less than 400 mm. Forest is created and maintained by groundwater, and by periodic flooding of the river (Hughes 1990). The depth of the water table drops off rapidly from the edge of the river and limits the lateral extent of the forests to about 1 km on either side (Hughes 1990). The intervening matrix is mainly cultivated land, riparian grassland and dry shrubs.

We mapped the gallery forest using aerial photographs taken in 1994 and 1996, and selected 12 forest patches as study sites. We chose forest patches so that approximately equal forest area was sampled east and west of the Tana River, and inside and outside the Tana River Primate National Reserve (TRPNR) to capture the range of habitat conditions within the floodplain (Fig. 1). We surveyed each study forest to determine the number of resident groups of colobus and mangabeys, and identified a subset of groups within each forest for detailed studies of group size, age and sex composition over time. We systematically selected social groups that were easy to locate and to identify using "marker" animals. Since 2001, we have periodically surveyed the forests and monitored all these study groups (Mbora and Meikle 2004; Mbora, unpublished data).

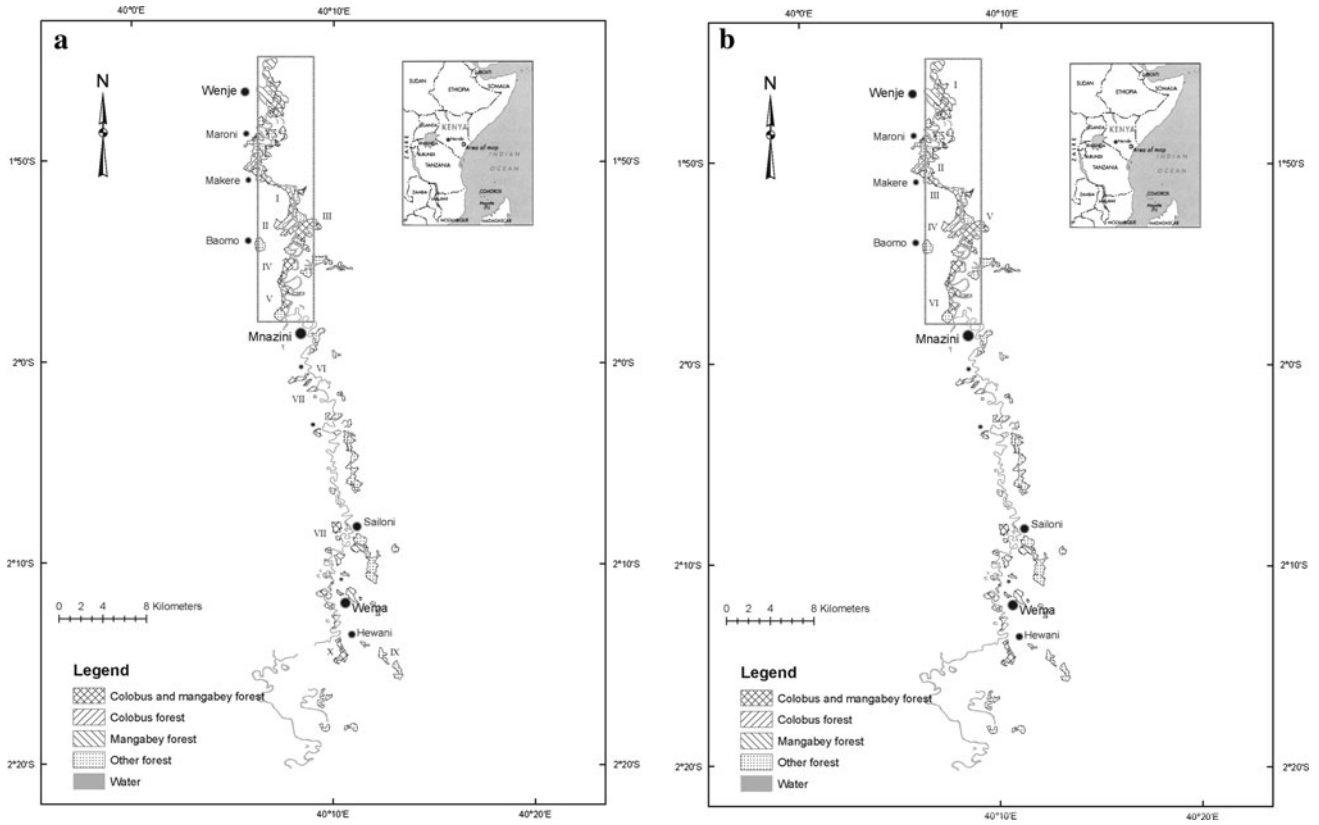

Fig. 1 Study area indicating the location and distribution of study forests in southeastern Kenya for the colobus (**a**) and mangabey (**b**). The frame on the top half of the map shows the approximate location of the Tana River Primate National Reserve. The Roman numeral codes identify study forests adjacent to them, and the major villages are named

The Tana colobus and mangabey are of similar body size but their behavioral ecologies and life history strategies are quite different (Kinnaird 1992; Marsh 1979). The red colobus is a specialist frugivore with limited vagility. It is almost exclusively arboreal and lives in relatively small social groups that exhibit high site fidelity (Marsh 1981). A canopy dweller, the colobus depends on a diet of mainly leaves obtained from a limited number of canopy tree species (Marsh 1981; Mbora and Meikle 2004). Thus, it was relatively easy to locate and observe colobus groups, to maintain contact with them and to determine their group composition while they were in the canopy. In contrast, the mangabey is a dietary generalist that is mostly terrestrial and highly vagile. It lives in much larger social groups and its diet comprises seeds and ripe fruit from a variety of tree species, and substantial amounts of animal prey (Kinnaird 1992). Mangabeys are quite skittish, and it was necessary to get groups well habituated to human presence in order to determine their size and composition and to obtain fecal samples for mtDNA analysis. Consequently, detailed observation of mangabeys focused on fewer social groups than in the colobus.

Collection of fecal samples and DNA extraction

In 2004 and 2005, from July to September, we collected fecal samples from study groups of colobus and mangabeys by following them, on separate days, from 0600 h to 1130 h, and then from 1500 h until nightfall. Upon observing an animal defecating, we collected a sample of the feces using a sterile collecting stick while wearing latex gloves. We aimed to take only a single sample from any particular animal, but to sample as many individuals from each social group and forest fragment as possible. Colobus feces are usually deposited in distinct pellets so we just collected 1–3 pellets depending on size. The mangabey does not produce its feces in distinct pellets so we extracted a sample of the feces from the outermost part of the dung bolus. In either case, the sample was placed into a tube containing 30 ml of 100% ethanol and labeled with a permanent marker to indicate the date, species identity, and coded to identify the troop and forest. The ethanol and sample were then mixed by inversion without shaking. The goal was to maintain the bolus form of the sample in order to avoid losing target cells along with the ethanol

supernatant in the next step. After 36 h, we carefully poured off the ethanol with the tube loosely capped, and transferred the remaining solid material into a new-labeled tube containing silica for drying and storage (Nsubuga et al. 2004). The second tube was also labeled with a permanent marker as above. The samples were stored at a cool temperature in a tent in the field, and at −80°C after arrival in the laboratory.

Approximately ≅200 mg of the fecal sample was extracted using the QIAamp DNA Stool kit (Qiagen) according to the manufacturer's instructions with minor modifications (Nsubuga et al. 2004). The dried samples were vortexed in 1.6 ml of ASL buffer and left overnight (12–16 h) in an agitator at 25°C. The intermediate steps followed the manufacturer's protocol, but we included an incubation step of 20 min followed by centrifugation for 2 min (Nsubuga et al. 2004) in the final step of the procedure where buffer AE elutes the DNA.

DNA samples used for positive controls in PCR reactions and numts diagnostics were extracted from tissue following standard phenol extraction methods (Dowling et al. 1990). Colobus DNA was extracted from two individuals of black and white colobus (*Colobus guereza*) liver tissue donated by Dr. Cathi Lehn (American Zoo and Aquarium Association's Biomaterials Banking Advisory Group), a tissue sample of *Procolobus badius* donated by Dr. Nelson Ting (University of Iowa). Mangabey DNA was extracted from muscle tissues preserved in ethanol. The muscle was acquired in Tana River following a fatal attack on a mangabey by an unidentified bird of prey in one of the forests in August 2005. In addition, total genomic DNA of two Uganda red colobus (*Procolobus tephrosceles*) was donated by Dr. TL Goldeberg (University of Illinois, Urbana-Champaign).

We used several measures to avoid cross contamination of samples, and contamination of our samples with concentrated DNA sources. In particular, all the laboratory procedures reported here were performed in a section of the laboratory dedicated to the analyses of DNA from the two primates; this laboratory does not work with any other species of primates or other vertebrates. We worked on the samples from the two study species sequentially rather than concurrently; we first worked on the colobus and then mangabey samples.

Genotyping and sequencing

We successfully amplified and sequenced DNA from 53 colobus individuals (53 stool samples) from 10 forests, and 36 mangabey individuals (35 stool samples and 1 tissue sample) from 6 forests (Fig. 1; Table 1). The following primer pair was used at a concentration of 5 mM; STRETCHM (5′-RCTTGCGTTGAGGCGTTCTG, H11 196) and ND4#1 (5′-CTTCTAACACTRACCGCCTGACT, L10952). The primers amplify the NADH dehydrogenase subunit 4 (ND4) of *Procolobus badius* corresponding to site 10203-11580 of the mitochondrial genome (accession no. DQ355301). We used 1 µl of the eluate from the extraction procedure as template in a 50 µl polymerase chain reaction (PCR) containing HotStarTaq DNA polymerase, PCR buffer with 3 mM $MgCl_2$ and 400 µM each dNTP (Qiagen). We performed a hot start PCR cycle in an MJ Research PTC-200 Peltier thermal cycler under the following conditions: an activation step at 95°C for 15 min; followed by 45 cycles at 95°C for 30 s, 57.9°C for 30 s and 72°C for 1 min, and a final extension step at 72°C for 10 min. Each reaction was replicated at least three times and included positive and negative controls, and the

Table 1 Attributes of the study populations of Tana River red colobus and mangabey

Forest name	Colobus			Mangabey		
	Population ID	Individuals genotyped	Haplotypes[a] found	Population ID	Individuals genotyped	Haplotypes[a] found
Wenje East				I	6	A
Makere East				II	7	A, B
Guru-Mchelelo	I	5	A, B, D, E, F	III	4	A
Congolani	II	8	A, C, E, F	IV	8	A
Sifa East	III	8	A, C, D, E	V	7	A, B
Lalafitu	IV	9	A, B, C, D, F			
Mnazini	V	2	D, F	VI	4	A, B
Kinyadu	VI	7	A, D, E, G			
Bubesa	VII	3	C, F			
Sailoni West	VIII	3	C			
Hewani East	IX	2	A, D			
Home forest	X	5	A, F			

[a] Haplotype groups as identified in Fig. 2

success of the PCR was assessed by electrophoresis of 5 µl of the product on a 1.5% agarose gel.

We purified the PCR products using the QIAquick PCR purification kit (Qiagen) following the manufacture's protocol, and cloned them using pGEM®-T easy Vector Systems (Promega Corporation). We prepared overnight cultures of cells containing pGEM® easy Vector by picking individual ampicillin-resistant colonies from fresh plates, inoculating 2 ml of LB broth containing 100 µg/ml ampicillin and shaking samples overnight at 37°C. We harvested the bacterial cells by centrifugation and purified single stranded DNA by extraction and precipitation using GenElute™ Plasmid Miniprep Kit (Sigma) following the manufacturer's protocol.

The bacterial clones from the above procedures were sequenced with an ABI Prism™ 3100 genetic analyzer (Applied Biosystems) using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequences were then aligned using SEQUENCHER™ (version 4.5) and verified for accuracy. In addition, we translated all the nucleotide sequences into protein sequences to determine if they contained any missense mutations or internal stop codons using DnaSP v. 4.10 (Rozas et al. 2003) and MEGA version 3.1 (Kumar et al. 2004). On translation of the nucleotide sequences into protein sequences, we did not find any missense mutations or internal stop codons (GenBank accession numbers FJ881863-FJ882004). Thus, all our sequences appear to be valid functional mtDNA.

Data analyses

We treated all sequences from fecal samples collected from the same forest patch as comprising a population. To compute the mtDNA sequence variation of the two species, we calculated the haplotype diversity, nucleotide diversity (π), and the proportion of nucleotide polymorphisms (θ) for each species (Nei 1987) using DnaSP v. 4.10 (Rozas et al. 2003). To examine the relationships between haplotypes detected in the each species in the landscape, we computed a minimum spanning network between haplotypes using ARLEQUIN 2.0 (Excoffier et al. 2005), and then used the connection lengths between samples (Operational Taxonomic Units) to draw a diagram of the minimum spanning network of haplotypes. We then mapped the distribution of haplotypes in the forests (Fig. 1; Table 1).

We constructed a neighbor joining phylogenetic trees using MEGA version 3.1 (Kumar et al. 2004) using MODELTEST (Posada and Crandall 1998) to determine the appropriate nucleotide substitution model for the data set. To investigate the possibility of a past bottleneck in both species, we conducted an analysis of pairwise sequence mismatch distributions (Rogers 1995) using ARLEQUIN 2.0 (Excoffier et al. 2005). The sequence mismatch distributions in a population that has experienced a population bottleneck should be smooth and have a peak, whose position identifies the time of the bottleneck (Harpending 1994).

We conducted two analyses to elucidate the role of current habitat fragmentation in shaping the population structure of the mtDNA variation among populations (forest patches) for each of the two species. First, we conducted an analysis of molecular variance (AMOVA) to determine how mtDNA variation was partitioned among and within populations (Excoffier et al. 1992). Second, we calculated the genetic distance between populations as pairwise F_{st} values (Weir and Cockerham 1984) and measured geographic distances between populations as linear centroid-to-centroid distances between forests using ArcMap GIS. We then tested for the correspondence between geographic and genetic distance using a mantel test (Mantel 1967) and linear regression analyses in the R-package (Casgrain et al. 2005).

Results

We found high levels of genetic diversity in both monkeys, but the colobus had significantly greater levels of genetic variability than the mangabey. We identified 34 haplotypes among the 53 red colobus sequences, and 18 haplotypes among the 36-mangabey sequences (Table 2; Fig. 2). In addition, when we compared metrics that account for differences in numbers of sequences for each species, we also found that red colobus had significantly greater haplotype and nucleotide diversity than mangabeys (Table 2). Comparison of the minimum spanning networks among haplotypes highlights the major difference that underlies these differences in genetic diversity. The red colobus haplotypes form seven distinct groups that are each separated from the next closest group in the network by 17–24 nucleotide substitutions (the average distance between adjacent groups was 20.5 mutational steps (Fig. 2a). The mangabey network contained only two such haplotype groups separated by 30 mutational steps from one another (Fig. 2b).

Although they differed in the overall levels of genetic diversity, the two species showed simpler patterns of spatial population structure. Five of the seven-haplotype groups identified in the red colobus were represented in four or more populations; two groups were found in six populations (Table 1; Fig. 1a). Similarly, the mangabey network had one diverse haplotype group that was widely distributed among the various populations, and a second haplotype group, that was found in only three populations (Table 1; Fig. 1b).

The sequence mismatch distributions exhibited a smooth distribution with a peak (Table 3). However, the smooth

Table 2 Genetic variability of mtDNA in Tana River red colobus and crested mangabey

mtDNA attribute	Colobus	Mangabey
Number of sites	203	196
Number of sequences	53	36
Number of segregating sites (S)	83	52
Number of mutations (Eta)	88	58
Number of haplotypes	34	18
Haplotype diversity (Hd)		
Mean	0.96	0.80
Variance	0.00	0.01
Lower 95% CI	0.96	0.78
Upper 95% CI	0.963	0.83
Nucleotide diversity (Pi)		
Mean	0.12	0.05
Variance	0.00	0.00
Lower 95% CI	0.12	0.04
Upper 95% CI	0.12	0.05
Theta (per site) from Eta	0.10	0.07
Theta (per site) from S		
Mean	0.09	0.06
Var (no recomb)	0.00	0.00
Theta (per site) from Pi	0.14	0.05
Average number of nucleotide differences		
Mean	24.33	9.27
Stochastic variance K (no recombination)	113.49	17.95
Observed variance	4.39	1.06
Raggedness	0.01	0.02
Fu's FS	−2.40	−1.48
P	0.04	0.08

and peaked mismatch distribution pattern was much more clearly defined in the colobus than in mangabey (Fig. 3).

The analysis of molecular variance (AMOVA) suggested that both species exhibit significant among population genetic differentiation (Colobus $F_{st} = 0.095$; Mangabey $F_{st} = 0.236$; Table 4a; Wright 1978). However, we did not find any significant association between genetic distance (population pair wise F_{st}) and geographic distance for the colobus (Mantel' $r = 0.13$, $P > 0.05$; Fig. 4a) or the mangabey (Mantel's $r = 0.28$, $P > 0.05$; Fig. 4b).

Discussion

Our data revealed unexpected high levels of genetic diversity and surprising similarities in the population genetic structures of the colobus and mangabey. Contrary to our expectation of low genetic diversity due to small populations, both primates had relatively high levels of haplotype and nucleotide diversity with high levels of sequence divergence between haplotype groups (Fig. 2; Table 2). Additionally, populations of both primates showed strong among population genetic differentiation, but little correspondence between genetic and geographic distances as we had predicted. We believe that the high genetic diversity, high sequence divergence, and strong genetic structures are a consequence of three complementary processes.

First, we have reason to believe that even though these primates currently have very low census populations, they have had much larger census populations in the recent past. The type specimens for the Tana colobus and mangabey were shot at Ozi forest at the mouth of the Tana River by Carl Peters in 1879 (Cited in Andrews et al. 1975). The Ozi area is located 100 km downstream of the limit of the current distribution range of the two species. However, the Ozi area and the area between the downstream limit of the distribution of the two species is currently not forested because all forest has been converted to farmland. This suggests progressive extinction of populations upstream over the last 100–150 years or so. Thus, our results further underscore the importance of considering historical demography when evaluating the genetic effects of current threats to wild populations (Dinerstein and McCracken 1990). In this case, it would seem that not enough generations have passed since the declines of these populations to the current levels to allow us to detect a reduction in genetic variation due to recent habitat losses. Therefore, loss of genetic diversity can be expected in the near future for these primates unless concerted management efforts are made to avoid such losses.

Second, the effects of long-term habitat changes in east Africa on the effective population sizes of the two species are strongly implicated. High levels of sequence divergences between haplotype groups, coupled with low levels of sequence divergences within haplotype groups, are characteristic of populations that have experienced population bottlenecks in the past (Avise et al. 1987). We believe that the history of habitat change in East Africa could explain the high diversity of mtDNA haplotypes as well as the high levels of sequence divergences among haplotype groups. Our analyses of pairwise sequence mismatch distributions suggested that the populations of the two primates experienced a major population bottleneck in the past (Rogers 1995; Fig. 3; Table 3). Allowing for a large error associated with the estimation of divergence time and based on the molecular clock rate for primate mtDNA of 11.5–17.3% per million years (Vigilant et al. 1991), the observed level of sequence divergence indicates that the haplotype groups observed in these primates have been diverging over the past 0.6–1 million years. Thus, these mtDNA polymorphisms seem to date back to the Pliocene–Pleistocene interval when major shifts occurred in the fauna of East African due to increased

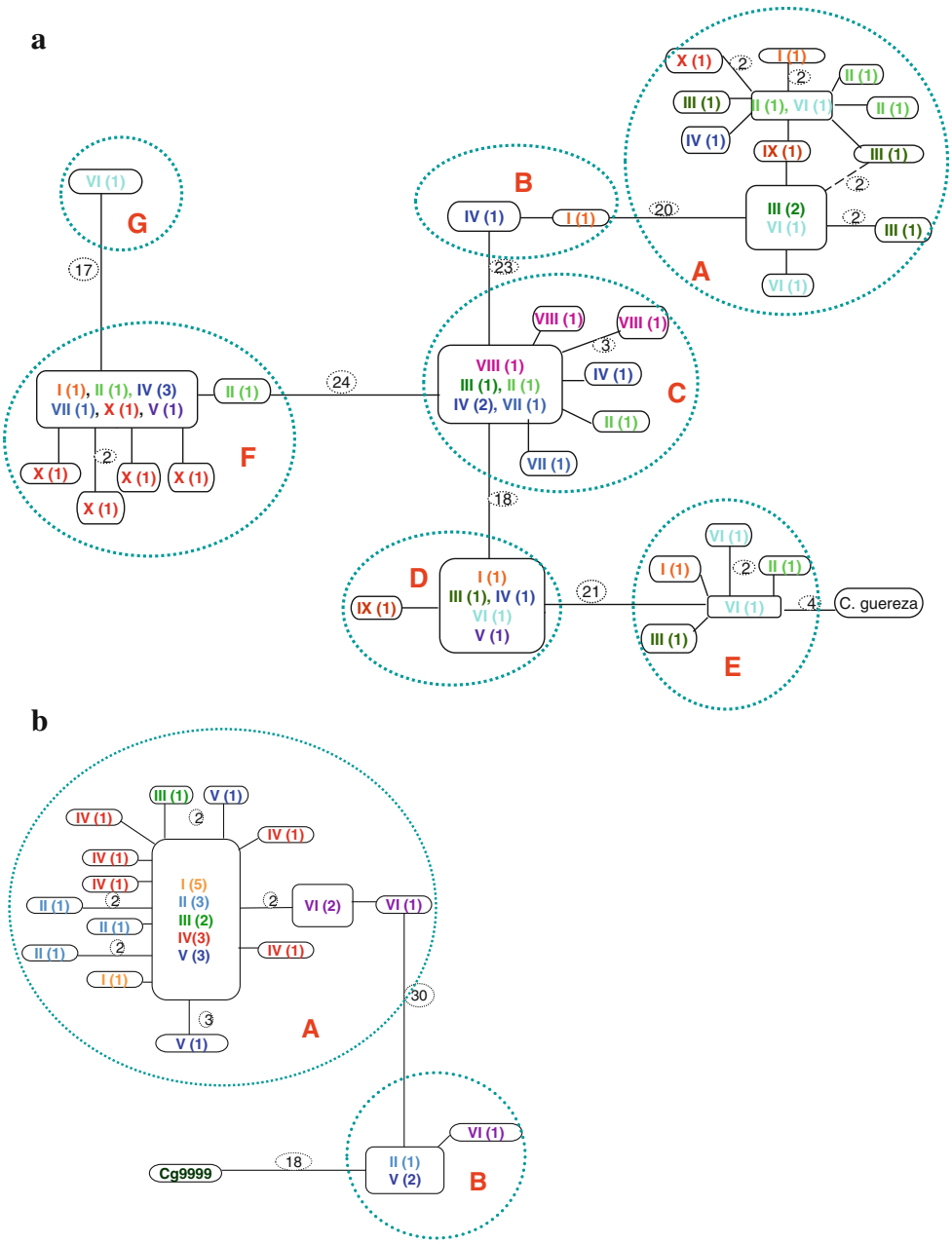

Fig. 2 Haplotype networks of the Tana River red colobus (**a**) and mangabey (**b**). Each polygon is a unique sequence, Roman numeral codes identify the study populations as in Fig. 1, and numbers within polygons are individuals (sequences). The large *dotted circles* surrounding groups of sequences identify the different haplotype groups

Table 3 Mismatch distribution analyses of the Tana River red colobus and mangabey mtDNA sequences

Species	τ	Observed mean	θ_0	θ_1	Raggedness
Colobus	27.41 (21.14–39.14)	24.57 (18.69–31.38)	3.62 (0.0–9.07)	81.99 (55.38–450.12)	0.013
Mangabey	1.20 (0.0–7.854)	9.94 (0.74–5.61)	1.59 (0.0–4.97)	5.12 (1.47–5289.50)	0.021

Values in brackets are 95%CI

aridity (Bobe and Behrensmeyer 2004; Heller et al. 2008; Okello et al. 2008; Okello et al. 2005; Storz et al. 2002a, b). Subsequently, the mtDNA polymorphisms may have been maintained by habitat heterogeneity in the Tana River landscape as populations in different habitat fragments were isolated from each other.

Mitochondrial DNA lineages with large sequence divergences in the same populations can result from secondary contact between previously isolated populations (Avise et al. 1987; Taberlet et al. 1992). Riverine forest along the Tana is an isolated remnant of a continuous rain forest belt that extended between the Congo basin and the

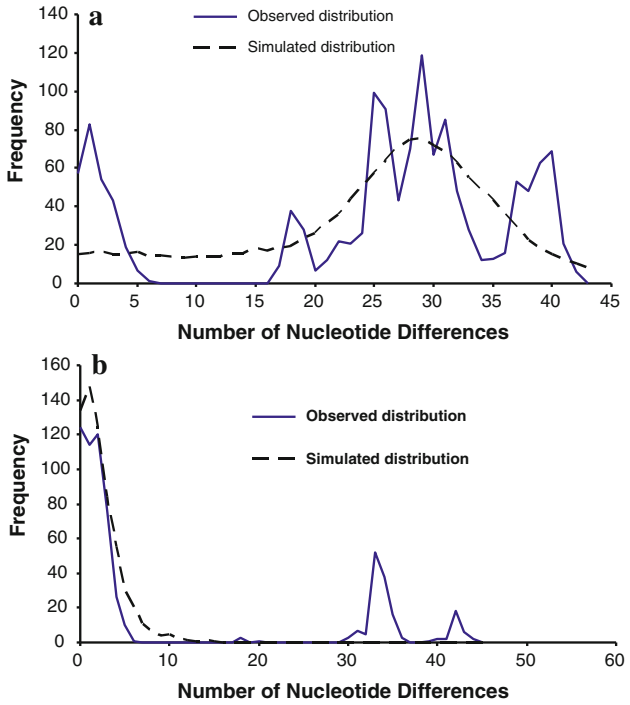

Fig. 3 Sequence mismatch distributions for the Tana River red colobus (**a**) and mangabey (**b**)

coast during moister periods of the Pleistocene (Livingstone 1975). Severe climatic drying isolated East African evergreen forests in the highlands and riverine localities such as the Tana (Hamilton 1981, Livingstone 1975). In the study area, the Tana River is in its old stage. Thus it meanders widely and occasionally changes course within the floodplain causing repeated fragmentation, isolation and reconnection of forest fragments over time (Hughes 1990; Mbora, personal observations). Consequently, primate populations in different forest patches must experience repeated extinctions and recolonizations through time, and therefore secondary contact between previously isolated mtDNA lineages can be assumed to occur (Avise et al. 1987; Taberlet et al. 1992).

The high level of mtDNA haplotype diversity found in these two primates is atypical but not unique among primates. For example, Wimmer et al. (2002) found 13 mtDNA haplotypes in a population of mouse lemurs that lived in a mere 9 ha of forests. Similarly, Liu et al. (2007) found 34 mtDNA haplotypes among the critically endangered snub-nosed monkey living in 11 remnant habitat patches. Both the colobus and mangabey showed sequence divergences between haplotype groups of up to 10 and 11% respectively. In the gray mouse lemur the divergence was up to 7.4% (Wimmer et al. 2002) and in the Yunnan snub-nosed monkey was up to 11% (Liu et al. 2007). Even though the mouse lemur and the snub-nose monkey studies focused on the control region of mtDNA, it seems that high levels of sequence divergences between haplotype groups, coupled with low levels of sequence divergence within groups are common in primate populations found in fragmented habitats.

Third, in addition to habitat changes, natal dispersal patterns and dynamics of social group formation over time may contribute to the pattern of haplotype sharing we observed in these primates. The Tana River red colobus is one of a handful of primate species in which females transfer between social groups (Marsh 1979). However, this primate is also an arboreal habitat specialist in which little dispersal is assumed to occur between forest fragments. The high frequency of haplotypes shared among forests (Table 1) suggests that dispersal does indeed occur between forest fragments.

In contrast to the colobus, female mangabeys are philopatric (Kinnaird 1992). Thus, we expected the genetic variation in this species to be homogeneous within forests but to be heterogeneous among forests. However, our analyses showed that mangabey groups in many forests shared the same haplotypes (Table 1). Since female philopatry in the Tana Mangabey is well established (Kinnaird 1992), the high level of haplotype sharing among forests is probably the result of shared common ancestry of the founding groups in those forests and the limited spread of new social groups in this primate (Melnick and Hoelzer 1996).

Among cercopithecine monkeys, new groups typically form by fissioning of existing groups along matrilines under conditions of environmental stress (e.g. food shortage in *Macaca sinica*: Dittus 1988). Following group

Table 4 AMOVA of the population structure of the (a) Tana River red colobus and (b) Tana River crested mangabey

Source of variation	Sum of squares (d)	Variance component	*P*	F_{st}	Percentage variation
(a) Tana River red colobus					
Among populations	169.81 (10)	Va = 1.19	0.035	0.095	9.52
Within populations	485.54 (43)	Vb = 11.32			90.48
(b) Tana River crested mangabey					
Among populations	59.87 (6)	Va = 1.218	0.024	0.24	24.64
Within populations	114 (29)	Vb = 3.93			75.36

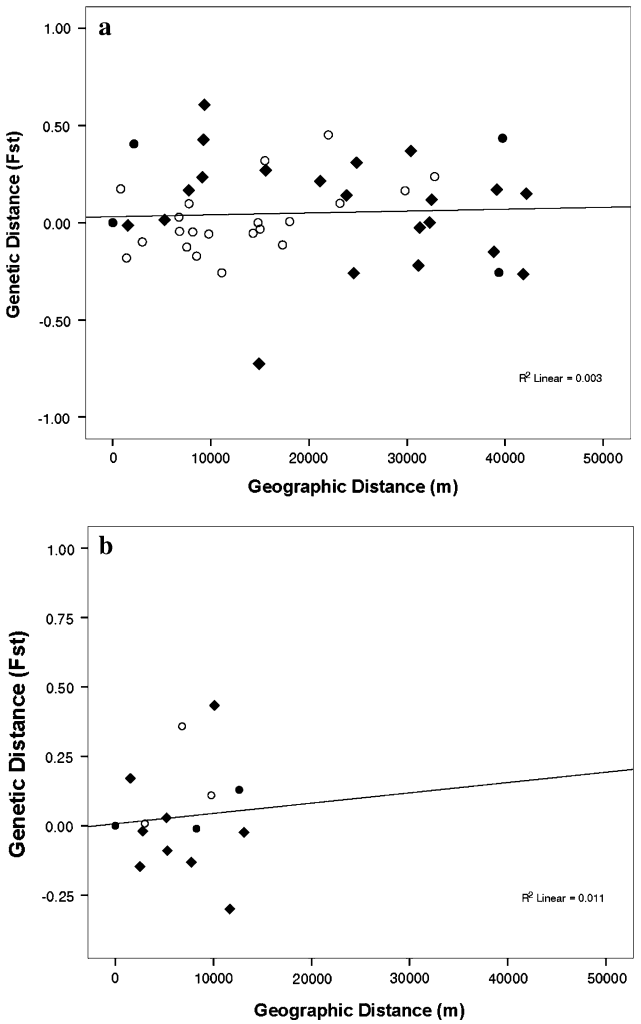

Fig. 4 Analysis of isolation by distance; genetic distance (pairwise F_{st}) vs. geographic (m) distance between populations of Tana River red colobus (**a**) and mangabey (**b**). *Filled circles* are pairs of forests east of the river, *empty circles* pairs of forests west of the river and *filled diamonds* are pairs of forests from the two sides of the river

fissioning, daughter groups are usually characterized by a higher average level of within-group relatedness than the parent group (Melnick and Kidd 1983; Whitlock and McCauley 1990). Because cercopithecine monkey social groups are generally characterized by low within group mtDNA diversity, group fissioning followed by colonization of new areas should lead to homogeneity of mitochondrial haplotypes between forests (Melnick and Hoelzer 1996).

Given the unexpected nature of our findings, we were very concerned that our results were being influenced by mixing numts and mitochondrial loci (Collura and Stewart 1995). Therefore, we conducted both whole mitochondrial genome and long-range PCR amplications on tissues samples of the mangable and closely related colobine taxa and found that our primer set did not amplify numts (Thalmann et al. 2004). What's more, our data in all cases satisfied the criteria listed by Zhang and Hewitt (1996) for diagnosing numts; (1) the data were collected as the result of a single PCR band (2) there were no sequence ambiguities upon alignment of sequences, (3) there were no nucleotide sequences that were unexpected, (4) there were no gaps or indels in the sequences, and, (5) the phylogenetic analysis yielded a plausible tree. Finally, on translation of the nucleotide sequences into protein sequences, all our sequences appeared to be valid functional mtDNA.

Our results have important implications for the conservation of these two critically endangered primates. First, our finding that both species have relatively high levels of mtDNA diversity emphasizes the need to enhance protection and conservation measures for them. This is important because some conservationists have argued recently that because the populations of the two primates are very small; their genetic diversity may be already compromised and therefore are not be worth conserving (World Bank 1996, p. 23). Indeed, our results show that the populations with the highest diversity of haplotype groups are located in the area of floodplain forest congruent with the location of the Tana River Primate National Reserve, which was established to protect these primates (Fig. 1; Table 1). Thus, we recommend that high priority be placed upon the protection and conservation of the primate populations found within the reserve area. More generally, this study shows that endemic endangered species with small fragmented populations can possess high levels of genetic diversity. Thus, such species should not be discounted from conservation action because of their small populations. We are cognizant, of course, that organisms comprise many genes. Therefore, it would be important to generate data from several independent loci in order to characterize rigorously the amount and spatial patterns of genetic variation of the populations over evolutionary time and geographic space. However, the view from mtDNA from these primates is very promising.

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