A Phylogeographic Study of the Endemic Rodent *Eliurus carletoni* (Rodentia: Nesomyinae) in an Ecological Transition Zone of Northern Madagascar

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**Abstract**

We conducted a mitochondrial phylogeographic study of the endemic dry forest rodent *Eliurus carletoni* (Rodentia: Nesomyinae) in an ecological transition zone of northern Madagascar (Loky-Manambato) and 2 surrounding regions (Ankarana and Analamerana). The main goal was to assess the evolutionary consequences on this taxon of the complex landscape features and Quaternary ecological vicissitudes. Three haplogroups were identified from the 215 specimens obtained from 15 populations. High levels of genetic diversity and significant genetic differentiation among populations were observed. The different geographical subdivisions of the study area by regions, by river catchment zones, and the physical distance between populations are not correlated with genetic patterns. In contrast, population structure is mostly explained by the geographic distribution of the samples among existing forest blocks. *E. carletoni* experienced a genetic bottleneck between 18,750 and 7,500 years BP, which correlates with periods when moister climates existed on the island. Overall, our data suggest that the complex genetic patterns of *E. carletoni* can be explained by Quaternary climatic vicissitudes that resulted in habitat fluctuations between dry and humid forests, as well as subsequent human-induced fragmentation of forest habitat.

**Key words:** Madagascar, mammals, mitochondrial DNA, Rodentia, phylogeography

Madagascar is known for its unique biota and patterns of endemism at an island-wide scale to levels of microendemism at a local scale (e.g., Wilmé et al. 2006; Kremen et al. 2008). The island is also geographically complex, with varied topography, notable landscape heterogeneity, and numerous watershed systems (Chaperon et al. 1993). Several hypotheses, based on large-scale contemporary and historical factors, have been proposed and tested to explain these patterns of endemism, such as riverine barriers, ecogeographic constraints, and watershed retreat and dispersion (reviewed in Vences et al. 2009). Given the varied physical geography of Madagascar, further taxon specific investigations conducted at a regional scale are needed to provide additional insights into the evolution of endemic taxa and their biogeographic patterns.

The fields of phylogeography and population genetics provide powerful frameworks for conducting such investigations by examining the distribution of genetic variation and the history of populations and lineages (e.g., population structure, phylogeographic groups, and historical demography). The associations of genetic patterns with landscape features and landscape-level environmental changes through time form the basis for understanding different biogeographic patterns, and can also be informative about the spatial and temporal dynamics of historical factors, particularly when fossil evidence is missing (e.g., Beheregaray 2008; Hickerson et al. 2010; Chan et al. 2011).

Our main study area, the Loky-Manambato region, is located in the northeastern portion of Madagascar (Figure 1).
This geographically complex region delimits a notable biogeographical transition zone between dry deciduous and humid forests (Goodman and Wilmé 2006a; Nusbaumer 2011) and contains a distinct biota, including microendemic taxa (Goodman and Wilmé 2006a). The region is demarcated by 2 permanently flowing rivers, the Loky to the north and the Manambato to the south; the first has its source at around 1000 m elevation and the second at 1400 m (Dormois 1954). The central portion of the region is subdivided by a third river, the relatively shallow and seasonal Manankolana, with its source at 500 m (Dormois 1954). These rivers partition the Loky-Manambato region and adjacent areas into 4 distinct river zones (I–IV, Figure 1). The topography of the region includes elevation ranges from sea level to 1170 m with a greater elevational gradient in the western portion (Table 1, Figure 1). The remaining forests of Loky-Manambato have been reduced and fragmented by human activities over the past few centuries and consist of a 44 000 ha collage of dry forests, humid forests, and transitional forest blocks ranging in size from 150 to 6000 ha (Goodman and Wilmé 2006b) (Figure 1). The distribution of these 3 natural forest types is closely associated with local climatic variables (Nusbaumer 2011). The habitat matrix between forest blocks is largely anthropogenic grassland and agricultural land. Finally, the Loky-Manambato region is bordered by 2 regions of similar vegetation types, the Réserve Spéciale d’Analamerana, about 20 km to the north and the Parc National d’Ankarana, about 60 km to the northwest (Figure 1A, B).

Fossil bone and pollen deposits from the Quaternary from different portions of Madagascar have revealed that humid forests were generally more widespread than today (see Figure 1B) and are associated with episodes of wetter climatic conditions (Matsumoto and Burney 1994; Goodman and Rakotozafy 1997; Gasse and van Campo 1998; Ray and Adams 2001; Burney et al. 2004). No fossil deposits are known from the Loky-Manambato region, but the same patterns of vegetation shifts as documented elsewhere on the island most likely occurred. Further, the

Figure 1. Map of Madagascar showing Eliurus carletoni collecting localities and mtDNA distributions. (A, B) Location of the 3 study regions including Loky-Manambato (LM), the Parc National d’Ankarana (ANK), and the Réserve Spéciale d’Analamerana (RSA), as well as habitat distribution in the north. For figures A and B, rectangles demarcate the region presented in the subsequent map. (C) In the LM region and neighboring areas, specimens were collected from forest blocks (designated as A–N) scattered across the different river zones (I–IV) (see Table 1). Pie diagrams represent proportions of mtDNA clades: A (black), B (grey), and C (white) across populations (n = 215; total bp = 1975); for the ANK population (not shown), all sampled individuals belong to clade A.
expansion of humid forests, associated with the contraction of dry forests, may have been directly associated with the fragmentation of populations of organisms restricted to dry forests.

The recently named rodent, *Eliurus carletoni*, belonging to the endemic Malagasy subfamily Nesomyinae, is restricted to northern (Ankarana, type locality) and northeastern (Analamana and Loky-Manambato) dry forests (Goodman et al. 2009) (Figure 1A). The species is the most common endemic rodent in Loky-Manambato and occurs in dry forests and occasionally in the bordering transitional forests, generally below 600 m, and after intensive trapping efforts it is undetected in the regional humid forests (Raheriarisena and Analamerana a few years earlier (n = 10). These samples include those employed by Rakotoarisoa et al. (2010) and an additional 104 specimens. Animals were trapped at 25 collection sites in 15 forest blocks across the 3 regions, all from elevations below 600 m. This species is only known from forest habitat, within which it can readily disperse, and is presumed not to traverse nonforested zones. Given the close proximity of collection sites within the same forest block, we combined them for each forest block into a single population. Geographic coverage, geographic terms, sample sizes, and other characteristics of sampled populations are presented in Figure 1 and Table 1. Voucher specimens are housed in the Field Museum of Natural History, Chicago,

<table>
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<tr>
<th>Regions</th>
<th>River zones</th>
<th>Forest blocksa (number of collection sites)</th>
<th>Sizes (ha)</th>
<th>n</th>
<th>Diversity indices</th>
<th>Neutrality tests</th>
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<tr>
<td></td>
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<td>π (SD)</td>
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</table>

α = sample size; N_h = number of haplotypes; H = haplotype diversity; π = nucleotide diversity under Tamura and Nei (1993) gamma distances; D = Tajima neutrality statistic (Tajima 1989); F_s = Fu’s neutrality statistic (Fu 1997); R_s = Ramos-Onsins and Rozas’ neutrality statistic (Ramos-Onsins and Rozas 2002); NA = sample size is too small to compute test statistics. Asterisks and the values in bold are statistically significant (p < 0.05).

**Table 1** Different geographical levels within the study area and indices of diversity of populations of *Eliurus carletoni* across forest blocks (a single study population in each forest block) along with the results of neutrality tests on the combined cyt b + CR

### Materials and Methods

#### Biological Samples

We used 215 tissue samples (muscle or liver) in this study from voucher specimens of *E. carletoni* obtained between 2003 and 2005 at collection sites across the Loky-Manambato region (n = 205), as well as from Ankarana and Analamerana a few years earlier (n = 10). These samples include those employed by Rakotoarisoa et al. (2010) and an additional 104 specimens. Animals were trapped at 25 collection sites in 15 forest blocks across the 3 regions, all from elevations below 600 m. This species is only known from forest habitat, within which it can readily disperse, and is presumed not to traverse nonforested zones. Given the close proximity of collection sites within the same forest block, we combined them for each forest block into a single population. Geographic coverage, geographic terms, sample sizes, and other characteristics of sampled populations are presented in Figure 1 and Table 1. Voucher specimens are housed in the Field Museum of Natural History, Chicago,
and the Département de Biologie Animale, Université d’Antananarivo, Antananarivo.

DNA Isolation, PCR Amplification and Mitochondrial Variation

The same protocols described in Rakotoarisoa et al. (2010) were used in this current study to extract DNA, amplify, and sequence the entire cytochrome \( b \) (cyt \( b \), 1143bp) and the control region (CR, ~900bp) of the additional 104 specimens (Genbank accession numbers JQ866514–JQ866617, JQ866410–JQ866513). SEQUENCHER 4.2.2 (Gene Codes Corp, Ann Arbor, MI) was used to edit and generate consensus sequences. We avoided the inclusion of nuclear paralogs in the datasets by checking for the presence of double peaks in the electropherograms and by verifying the absence of stop codons, deletions, and insertions in the corresponding amino acid sequences after translations were carried out using published \( E\). \( \text{linnus} \) DNA sequences as templates (e.g., Jansa et al. 1999).

We aligned cyt \( b \) sequences using MUSCLE version 3.6 (Edgar 2004) followed by translation of sequences into amino acid to verify the absence of stop codons. For CR, we employed Clustal W2 (Larkin et al. 2007; available at: http://www.ebi.ac.uk/Tools/clustalw2/index.html) with a gap opening penalty of 10, a gap extension penalty of 0.05, and 5 iterations. The alignments were verified by visual inspection.

Average pairwise sequence divergence of each mtDNA region was computed using MEGA (Tamura et al. 2007) based on the uncorrected genetic distance “\( p \)”. Because both DNA regions are linked and nonrecombinant, we combined them in subsequent analyses. Haplotype and nucleotide diversity statistics (Nei 1987) were summarized across populations using Arlequin version 3.0 (Excoffier et al. 2005) based on the Tamura and Nei (1993) gamma distances (gamma = 0.5). Average uncorrected genetic distances “\( p \)” within and among populations were computed using DnaSP version 5.0 (Librado and Rozas 2009).

Phylogenetic and Network Analyses

To control for potential artifacts associated with underlying phylogeographic patterns in our molecular datasets, we first performed phylogenetic analyses to determine the exact clade membership of the 104 specimens of \( E\). \( \text{carletoni} \) not examined by Rakotoarisoa et al. (2010), prior to population genetic and demographic analyses. The phylogenetic dataset consisted of 225 trimmed sequences of cyt \( b \) + CR (1975 bp). Ten sequences from 5 other \( E\). \( \text{linnus} \) spp. and \( \text{Gymnuraconyx roberti} \), another nesomyine rodent, were included as outgroups.

We used MrBayes 3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) to conduct Bayesian Markov Chain Monte Carlo analyses of the dataset with the GTR+I+G model identified by Modeltest 3.06 (Posada and Crandall 1998) and the Akaike Information Criterion (AIC; Akaike 1974). The analyses were run for 10 million generations with 10 independent and incrementally heated MCMC chains and using default prior settings. The majority rule consensus tree and the posterior probability support for each node were generated using trees obtained after the stabilization of the −ln likelihood and for which a value of the average standard deviations of split frequencies was ≤0.01 (Ronquist et al. 2005). In addition, we used RAxML (Stamatakis et al. 2008) to determine the best Maximum Likelihood tree from the dataset under the GTR+G model and to generate bootstrap support values for each node based on 1000 replicates.

We employed TCS (Clement et al. 2000), which implements a statistical parsimony method (Templeton et al. 1992), to further examine clades identified by the phylogenetic analyses. A haplotype network was reconstructed from the aligned cyt \( b \) + CR sequences using a 95% parsimony connection limit. Ambiguous loops that likely resulted from homoplasy (e.g., recurrent mutation or sequencing errors) were broken using predictions from coalescent theory (Pennington and Posada 2002). The geographic distributions of these haplotypes were incorporated in the network to detect possible correlations between patterns of haplotype clustering and geographic overlays.

Genetic Structuring and Landscape Factors

To assess the degree to which landscape characteristics contributed to the genetic structuring in \( E\). \( \text{carletoni} \), we computed the Analysis of Molecular Variance (AMOVA) statistic \( \Phi_{ST} \) using Arlequin version 3.0 (Excoffier et al. 1992, 2005) and explicitly tested hypotheses associated with different characteristics of the study area. Specifically, we examined whether genetic variation was associated with (1) the grouping of populations by study regions (Analamerana, Loky-Manambato, and Ankarana), (2) subdivisions by river zones (I–IV within and bordering the Loky-Manambato region, Figure 1C), or (3) the occurrence of each population in forest blocks (A–N in the Loky-Manambato region, Figure 1C). For each AMOVA design, nonparametric permutation procedures (Excoffier et al. 1992) were performed based on 10 000 runs to verify if corresponding indices were significantly different from 0 and to assess the statistical significance of the partitioning of different variance components into hierarchical levels. To control for potential artifacts associated with clade membership, we repeated all AMOVA analyses for each major clade(s).

Alternatively, to assess whether geographical distance alone explains patterns of genetic variation, we analyzed Isolation-by-Distance (IBD) by regressing values of \( \Phi_{ST} / (1−\Phi_{ST}) \) obtained from pairwise population comparisons against the natural log-transformations of geographic distances (see Rouset 1997). In addition, a partial Mantel test as implemented in the IBD program (http://ibdws.sdstate.edu/) (Jensen et al. 2005) was used to statistically test the significance of correlations between genetic and geographic distances among populations, while controlling for the effects of the zones delineated by rivers. To examine the effects of distance across different geographic scales, the analyses were performed first for all 3 regions (“regional scale”) and then only for those within the Loky-Manambato region (“local scale”). Potentially confounding effects of sample size
and clade membership were verified by repeating the same analyses and designs, but only on populations with \( n \geq 10 \) samples, and finally on members of major clade(s).

We also computed the nearest-neighbor statistic \( S_{nn} \) (Hudson 2000) to characterize further the genetic differentiation among populations. \( S_{nn} \) uses a symmetric island model and assumes an infinite-sites model of mutation and it is specifically appropriate when samples sizes are low and haplotypic diversity is high. Because \( S_{nn} \) measures how often haplotypes and their most closely related sequences (i.e., nearest neighbors in sequence space) are found in the same locality (Hudson 2000), this statistic provides insight into the patterns of differentiation. \( S_{nn} \) is close to 0.5 for panmictic populations and 1 for highly differentiated populations. These analyses were first performed on all populations, and then repeated on populations with \( n \geq 10 \). Computation of \( S_{nn} \) and its statistical significance was conducted using permutation tests (\( n = 1000 \)) in DnaSP version 5.0 (Librado and Rozas 2009).

**Patterns of Historical Gene Flow**

We used MIGRATE 3.0.3 (Beerli 2006, 2008) to explore patterns of matrilineal gene flow among populations (i.e., forest blocks) in the Loky-Manambaro region, but, because of limited samples from Ankaranana and Analamerana, individuals from these latter 2 regions were not included. In addition, direct line distances between the 3 regions are >3 times the average geographic distances between Loky-Manambaro sites, implying that local migration is likely to be more important than between the 3 regions. To overcome computation limitations of the MIGRATE program associated with the number of forest blocks studied in the Loky-Manambaro region (\( n = 13 \)), we used previously computed pairwise genetic and geographic distances (see IBD analyses) to identify the nearest neighboring forest blocks with genetically similar populations that could be combined. As forest blocks within close geographic proximity are likely to have been connected until recent times, the combination of these populations should provide a reasonable approximation of the patterns of gene flow. Employing a user-defined nearest-neighbor stepping stone model, we then estimated average migration rates based on the parameter \( \theta \) using a Bayesian search strategy with 3 long chains, a sampling increment of 100, 4 heated chains with a heating scheme of 1, 1.5, 3, and 10, and 100 000 trees sampled. The runs were repeated 4 times to verify consistency.

**Demographic History**

We performed neutrality tests on each population comprising 2 class I tests, Tajima’s (1989) \( D \) and Ramos-Ornins and Rozas’ (2002) \( R_2 \), as well as a class II test, Fu’s (1997) \( F_s \) test. Class I and II tests used information about frequency of mutation spectrum and haplotype distribution, respectively. Overall, the relative performance of each neutrality test depends on the age and extent of the demographic event in question, and the characteristics of the sequence data (e.g., Ramos-Ornins and Rozas 2002; Depaulis et al. 2003; Ramírez-Soriano et al. 2008). Thus, a combination of tests should detect and characterize the nature of any major event (e.g., sudden population growth or contraction). We restricted neutrality tests to populations with \( n \geq 10 \) to minimize the potential artifact of low sample sizes. All tests were performed with Arlequin version 3.0 (Excoffier et al. 2005) and DnaSP version 5.0 (Librado and Rozas 2009).

Although neutrality tests are informative, they only use part of the sequence data (e.g., mutation frequency spectra and haplotype distribution). We complemented these tests with another coalescent-based approach, the Bayesian Skyline Plot as implemented in Beast (Drummond et al. 2005). This method does not require a priori assumptions associated with the model of demographic history; it can also capture nonmonotonic variation of \( N_e \) over time and provide a credibility interval of \( N_e \) estimates to account for phylogenetic errors and uncertainty associated with the gene genealogies (Drummond et al. 2005). The variability of the marker(s) has been shown to affect the extent of phylogenetic errors associated with gene genealogies and eventually the \( N_e \) estimates (Heled and Drummond 2008). Thus, we performed the Bayesian Skyline Plot analyses on the noncoding mtDNA region under the GTR+I+G model and with a strict molecular clock. After 70–100 million iterations and 10% burn-in, Tracer version 1.4.1 (Rambaut and Drummond 2007, available at \( \text{http://tree.bio.ed.ac.uk/software/tracer} \)) was used to verify that the runs were successful (e.g., likelihood profile, effective sample size ESS > 200) and to create plots of the number of effective females (\( N_e \)) over time. These analyses were conducted 4 times to verify their consistency. To assess support for the demographic scenario identified by these analyses, we conducted a Bayes Factors Test (Kass and Raftery 1995) as implemented in Tracer version 1.4.1 and compared the Bayesian skyline model against a constant population size model (Suchard et al. 2001).

Because CR substitution rates are not yet available for any member of the subfamily Nesomyinae, we used published rodent rates. Given possible variation among different taxonomic groups of rodents and the recently proposed time dependency of molecular rates (e.g., Ho et al. 2007; Howell et al. 2008), we selected 2 very different rates for an approximation of the dates of demographic events and a broad comparison with the fossil record to test our prediction (see Discussion). Substitution rates of 4% (Geraldes et al. 2008) and 10% per site per myr (e.g., Prager et al. 1993; Gündüz et al. 2005) were used. Unlike other published rodent rates, which were obtained from the examination of a portion of CR (usually the hypervariable regions), these 2 rates were originally inferred for the whole DNA region, which make them suitable for the current study.

**Results**

**Mitochondrial Variation and Population Diversity**

For 215 individuals of \( E. \ carletoni \), 52 and 59 haplotypes were identified, respectively, for \( \text{cyt b} \) (1104 bp) and CR
Average pairwise uncorrected genetic distances “p” were 1.0% for cyt b and 1.2% for CR. The concatenated *E. carletoni* sequences have an average “p” of 1.3% and 197 segregating sites. Table 1 summarizes mitochondrial variation across forest blocks, each containing a single study population. Sample size ranged from 3 to 41 specimens across the 15 forest blocks and the number of haplotypes varied between 2 and 197 segregating sites.
All but one population had locally restricted haplotypes. Proportions of haplotypes that were unique to each population (mean 70%, range 50–91%) are relatively high (Table 1). Uncorrected average sequence divergence within populations ranged from 0.5% to 1.5%, whereas up to 2% nucleotide divergence was observed between populations. Most populations exhibit high haplotype diversity ($H$) (mean 0.81, range 0.60–1.00), but notable differences were observed for nucleotide diversity ($\pi$) (mean 0.0088, range 0.0010–0.0160). Both patterns of genetic diversity ($\pi$ and $H$) do not appear to be an artifact of sample sizes ($n$) (Pearson’s product–moment correlation tests between $n$, $\pi$, and $H$ were not statistically significant; $P > 0.05$).

**Phylogenetic and Network Analyses**

The phylogenetic dataset consisted of 225 sequences for *E. carletoni* and outgroups, which comprised 86 unique haplotypes with 578 variable sites, of which 383 were parsimoniously informative. The Bayesian and Maximum Likelihood analyses generated identical trees based on well-supported branches with 3 main clades (phylogroups A, B, and C; Figure 2), similar to the clades recovered by Rakotoarisoa et al. (2010). The topological patterns were characterized by short internodes between the clades and their unresolved relationships. Genetic distances between clades are ~2% based on mean uncorrected “$p$” values of cyt $b$. With respect to the affinity of the additional 104 specimens, all belonged to clade A, which comprised 84% of the animals incorporated in this study (Figure 2). Out of the 15 populations (forest blocks) sampled, 1 had individuals from all 3 clades, whereas 4 populations had individuals in 2 clades (either A and C or A and B) (Figure 1C).

The TCS analysis of the combined *E. carletoni* cyt $b$ and CR sequences also recovered the same 3 clades previously identified by phylogenetic analyses (Rakotoarisoa et al. 2010). The main haplotype group (clade A) exhibited 6 ambiguous loops, of which 5 were easily resolved. Overall, the network was characterized by the absence of a central and dominant haplotype, the existence of long interlinking branches, and many intermediate frequency haplotypes and singletons (Figure 3).

Two main relationships were observed between the network patterns and the different geographic datasets (Figure 3). First, although a few haplotypes were shared, most have localized distributions, either restricted to regions (Ankarana: 100%, $n = 7$; Loky-Manambato: 88%, $n = 70$; and Analamerana: 66%, $n = 3$) or to river zones (zone I = 78%, $n = 51$; zone II = 67%, $n = 31$; zone III = 63%, $n = 8$; and zone IV = 80%, $n = 10$). The same pattern was also observed...
Table 2 AMOVA analyses based on the different geographical levels within the study area

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<td>Total</td>
<td>179</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df = degree of freedom.
*Significant at P = 0.05.
*Based on the forest blocks in the Loky-Manambato region (each forest block contains a single study population).

The AMOVA analyses revealed statistically significant genetic structuring of populations of *E. carletoni* (Φ_ST = 0.32, P < 0.001). The same results were obtained when the analyses were restricted to clade A (Φ_ST = 0.30, P < 0.001); the analyses could not be performed separately on clades B and C because of low sample sizes (see Figure 2). In the Loky-Manambato region, approximately one-third of the overall genetic variation was among forest blocks (i.e., among populations) (Table 2). In contrast, neither the subdivision of the study area by regions nor by river zones contributed significantly to the observed patterns of genetic variation (% of total variance < 7%, P > 0.05; Table 2). These distributional patterns of genetic variation across the different geographical levels hold when the AMOVA analyses were restricted to clade A; the only exception was for the river zones which were associated with a small, but statistically significant, proportion of genetic variation (Φ_C = 0.08; 8.63% of variance component; P < 0.05). A significant and relatively high value of the Hudson (2000) statistic  \( S_{\text{hr}} \) (0.75–0.81, \( P < 0.0001 \)) was obtained for all populations analyzed together or those with \( n \geq 10 \).

The partial Mantel test did not detect any statistically significant correlation between genetic and geographic distances, thereby refuting the IBD models at both local (Loky-Manambato: distance = 4–42 km; slope = 2.25, \( P = 0.07 \)) and regional (Loky-Manambato, Analamerana, Ankaranana: distance = 4–83 km; slope = 2.15, \( P = 0.09 \)) scales. Similarly, very low coefficients of determination were obtained in the regression analyses after fitting linear trend lines on the scatter plots (\( R^2 \approx 0.03 \), see Supplementary Figure S1a,b online); this indicated the absence of linear relationships between genetic and geographic distances. The same results were obtained whether these analyses were restricted to members of clade A (slope = 0.05, \( P = 0.15 \); see Supplementary Figure S1c online) or to populations with \( n \geq 10 \) (result not shown).

For the MIGRATE analyses, we combined populations from a few adjacent forest blocks to overcome computation limitations (see Methods) and obtained an approximation of the patterns of migration. Based on the specified partial migration model, the geographic pattern and relative importance of migration rates are presented in Figure 4. Overall, migration patterns are very complex. Nevertheless, 2 weak relationships seem to be present: (1) lower migration rates are inferred among forest blocks separated by rivers (e.g., D vs. C; AB vs. C; H vs. EFG; Figure 4) and (2) higher migration rates are inferred between certain forest blocks in close geographical proximity (e.g., C vs. EFG; J vs. EFG).

Demographic History

Overall, the neutrality tests across populations generated different patterns of statistical significance and values (Table 1). The test of population expansion, Ramos-Onsins and Rozas’ (2002) \( D_s \), was nonsignificant across all populations. In contrast, Fu’s (1997) \( F_s \) tests were statistically significant with positive values for 6 out of the 9 populations tested (i.e., with \( n \geq 10 \)). Deviations from neutrality can result from different factors, such as the effects of selection, demographic events, and population admixture, which are often difficult to disentangle (e.g., Depaulis et al. 2003). However, under the assumption of neutral evolution, a positive and significant \( F_s \) value is often associated with a genetic population bottleneck or population subdivisions (Fu 1997). Finally, unlike \( F_s \), Tajima’s (1989) \( D \) was not significant across all populations. Generally, statistics based on haplotype frequency (e.g., \( F_s \)) are more powerful at detecting recent and moderate bottlenecks, whereas tests that rely on frequency spectrum of mutations (e.g., \( D \)) are best at detecting old and severe bottlenecks (Depaulis et al. 2003).

We carried out the Bayesian Skyline Plot analyses only on clade A (\( n = 190 \), Figure 2) to avoid potential artifacts associated with the inclusion of animals from different clades. The analyses revealed a decrease in the median \( N_e \) (Figure 5), Bayes Factors Test showed that the Bayesian...
skyline model inferring a decrease of $N_e$ was significantly better than a model of constant population size ($\log_{10}$ Bayes Factor $= 5.31$). The degree to which $N_e$ declined corresponds to a weak or moderate bottleneck event and the time of its onset ($t$) was inferred to be between 18 750 and 7500 years BP based on the rate of substitution of 4% and 10% per myr for CR.

Discussion

In this study, we predicted that genetic and demographic patterns of *E. carletoni* in the Loky-Manambato region correlate with landscape features and the presumed local late Quaternary ecological vicissitudes. Overall, we found that *E. carletoni* exhibits high haplotype diversity with localized distributions of haplotypes, complex patterns of gene flow, and a significant population structure. The 3 clades identified by a previous study were also recovered and a moderate population bottleneck was inferred between 18 750 and 7500 years BP. In the following sections, we discuss the geographic and temporal aspects associated with these patterns to assess our prediction.

**Genetic Patterns of *E. carletoni* and the Role of Landscape Features**

We tested the predicted correlation between genetic patterns of *E. carletoni* and landscape features in the Loky-Manambato region within the context of the genetic evolution of the species across its known geographical range, focusing on the following 4 aspects: (1) dispersion at a broad regional scale in northern Madagascar (*Figure 1A*), (2) rivers acting as barriers to gene flow; (3) effects of IBD at local and regional
scales, and (4) effects of isolation associated with forest cover fragmentation and the presence of unsuitable matrix between forest blocks in the Loky-Manambato region.

Our analyses at the regional level suggest that this species’ distribution did not play a central role in creating the identified genetic patterns, particularly given the absence of a clear association between the 3 distant study regions and patterns of the haplotype network, as well as the distribution of genetic variation. Although regional dispersion does not explain the principal patterns of genetic variation in *E. carletoni*, and as shown by the extensive clustering of haplotypes from Ankarana in the haplotype network its effects are not absent (Figure 3).

Not 1 of the 3 rivers in the Loky-Manambato region serve as a strict barrier to gene flow and no associated pattern of haplotype clustering was uncovered. Nevertheless, the level of dispersal across rivers is likely limited as suggested by the low relative migration rates and the small, but statistically significant, riverine barrier effects on genetic variation within clade A. Quéméré *et al.* (2010) found that the Manankolana River also affects the patterns of contemporary gene flow of a large diurnal lemur species currently restricted to the Loky-Manambato region.

Another landscape factor considered was geographic distance, which in certain cases can explain the distribution patterns of genetic variation due to its effects on gene flow. In this study, we did not detect any evidence of IBD, either at local or broad regional scales. This suggests that landscape feature(s) significantly disrupt gene flow and/or populations are not at equilibrium (e.g., due to changes in population size in the recent past) (Trizio *et al.* 2005).

Unlike the first 3 factors mentioned above, a large portion of mitochondrial variation is associated with the delineation of existing forest blocks (Table 2), which is consistent with our main prediction. At first glance, this suggests that habitat fragmentation may explain most of the observed population level patterns. However, given the rate of evolution of mtDNA, other aspects should be examined to assess the contributions of human perturbations on patterns of genetic variation. These aspects include the history of deforestation in the Loky-Manambato region, as well as a more precise assessment of geological history (Bermingham and Avise 1986; Cunningham and Moritz 1998).

**Figure 5.** Bayesian Skyline Plot of CR depicting changes in female effective population size of *Eliurus carletoni* over time. The thick line represents the median estimate and the 95% highest posterior density limits are above and below. *N* is the effective female population size, *mu* is the mutation rate per generation, and *t* is the onset of the moderate bottleneck. The *y*-axis is plotted on a log scale.
as late as 5000–3000 years BP (Matsumoto and Burney 1994; Goodman and Rakotozafy 1997; Burney et al. 2004).

Hence, despite the absence of a precise CR substitution rate for *E. carletoni*, the general agreement between its inferred historical demography and the presumed history of forest cover during recent geological time in Loky-Manambabo strongly suggests that dry forest contractions were associated with the observed decrease in $N_c$. Obviously, our date estimates depend on the substitution rates used. It should be noted that a rate >30% per myr (for the whole CR) would be needed for the initial decrease of $N_c$ to postdate the moister climate period in the north (~3000 years BP). Overall, these findings are consistent with our prediction and provide evidence for past landscape changes as an important possible factor influencing the genetic patterns of *E. carletoni*.

**On the Evolution of *E. carletoni***

We examined several aspects of the genetic and historical demographic patterns of the forest-restricted rodent *E. carletoni* and found varying degrees of support for the role of 3 landscape and historical factors influencing the genetic evolution of this species.

First, distributional patterns of genetic variation in this rodent indicate a significant role of anthropogenic forest fragmentation and loss. Deforestation is an important landscape factor in the Loky-Manambabo region given that the remaining forest cover now represents less than 20% of the local surface area. The exact period when the remaining forest blocks were isolated is unknown, but based on satellite imagery and aerial photos this took place at least 50 years ago (Jimenez and Vargas 2000, unpublished report), although estimates of a few hundred years have been suggested (Quémeré et al. 2010). In a broader context, the first evidence of people colonizing Madagascar is about 2300 years ago (Jimenez and Vargas 2000, unpublished report), although estimates of a few hundred years have been suggested (Quémeré et al. 2010). In a broader context, the first evidence of people colonizing Madagascar is about 2300 years ago. Human transformation of the landscape only reached a significant scale during the last millennium (2300 year BP). Obviously, our date estimates depend on the substitution rates used. It should be noted that a rate >30% per myr (for the whole CR) would be needed for the initial decrease of $N_c$ to postdate the moister climate period in the north (~3000 years BP). Overall, these findings are consistent with our prediction and provide evidence for past landscape changes as an important possible factor influencing the genetic patterns of *E. carletoni*.

Finally, historical demographic analyses suggest that current genetic patterns of *E. carletoni* may have been influenced by prehuman vegetational shifts associated with the climatic history of the Loky-Manambabo region and the extent of local dry forests (e.g., see Cunningham and Moritz 1998). Currently, disjunct humid forests can be found toward the upper reaches of local massifs (Raheriarisena and Goodman 2006; Nusbaumer 2011). Hence, the expansion of this habitat type in the Loky-Manambabo region during mesic periods of the last 25 000 years would have been in unison with contraction and fragmentation of local dry forests. Given the complex topography of the region, genetic impacts probably varied among *E. carletoni* populations due to localized range shifts of humid forest, causing a greater impact on certain populations (e.g., levels of genetic diversity). Regardless of the exact pattern, timing and duration of past vegetational shifts, the changes in the extent of regional dry forests would have likely resulted in a nonhomogenous distribution of genetic variation prior to human interventions. This, in turn, may have contributed to current patterns, including the localized haplotype distributions and the high tendency of most closely related haplotypes to occur in the same forest blocks as inferred from the $F_{st}$ value.

In conclusion, genetic patterns of the dry deciduous forest rodent *E. carletoni* are complex and additional studies are needed to elucidate certain aspects. The data and analyses presented herein provide general support for the hypothesis that the genetic evolution of this rodent was shaped by the geographic complexity of the Loky-Manambabo region and the history of forest cover during recent geological time.

For future studies, the examination of fast evolving and biparentally inherited markers will be important to test hypotheses generated by our mtDNA analyses, including the potential contribution of natal philopatry common in rodents (Solomon 2003), and to obtain further insights at a different evolutionary time scale. The inclusion of additional populations from certain river zones and regions will provide a more accurate assessment of the effects of large-scale geographic factors. The current findings provide testable hypotheses about vegetational shifts linked with paleoclimatic changes in the ecologically heterogeneous Loky-Manambabo region and associated phylogeographic and biogeographic correlates.

**Supplementary Material**

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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