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Latitude drives diversification in Madagascar's endemic dry forest rodent *Eliurus myoxinus* (subfamily Nesomyinae)

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Numerous hypotheses have been proposed for the historical processes governing the rich endemism of Madagascar's biodiversity. The 'watershed model' suggests that drier climates in the recent geological past have resulted in the contraction of forests around major watersheds, thereby defining areas of endemism. We test whether this hypothesis explains phylogeographical patterns in a dry forest-dependent rodent, *Eliurus myoxinus*, an endemic species widely distributed through western Madagascar. We sequenced the mitochondrial cytochrome *b* locus and nuclear introns of the β-fibrinogen and the growth hormone receptor genes for *E. myoxinus*. Using a parametric bootstrapping approach, we tested whether the mitochondrial gene tree data fit expectations of local differentiation given the watershed model. We additionally estimated population differentiation and historical demographic parameters, and reconstructed the spatial history of *E. myoxinus* to highlight spatial and temporal patterns of differentiation. The data do not support the watershed model as a clear explanation for the genetic patterns of diversity within extant *E. myoxinus* populations. We find striking patterns of latitudinal genetic structure within western Madagascar, and indicate possible roles for environmental and ecological gradients along this axis in generating phylogeographical diversity. *Biological Journal of the Linnean Society*, 2013, **110**, 500–517.

ADDITIONAL KEYWORDS: endemism – phylogeography – watersheds.

INTRODUCTION

Madagascar is exceptional for its high levels of species richness and endemism across clades (Goodman & Benstead, 2005). Recent studies of diversity and endemism in Madagascar aim to identify both areas of rich biodiversity and the evolutionary processes generating these patterns (Kremen *et al*., 2008; Vences *et al*., 2009). Given Madagascar's long geological isolation from mainland Africa (approximately 160 Mya) and India (approximately 90 Mya) (de Wit, 2003), diversification on the island can be studied in endemic and demonstrably monophyletic groups that have long been established without the confounding influences of colonization and migration (Yoder & Nowak, 2006).

The biomes of Madagascar are notably heterogeneous (Du Puy & Moat, 1996) and the effects of climate, topography, and geology on diversification

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across taxa have been intensely researched in recent years (Pastorini, Thalmann & Martin, 2003; Wilmé, Goodman & Ganzhorn, 2006; Raselimanana *et al*., 2008; Pearson & Raxworthy, 2009; Townsend *et al*., 2009; Vences *et al*., 2009; Weyeneth, Goodman & Ruedi, 2011). The 'watershed model' (Wilmé *et al*., 2006) proposes an explicitly historical, biogeographical model for species diversification across the island. This model suggests that during dry periods in recent geological time, mesic forests and their associated biotic communities were constrained to river drainages. Rivers with low elevation headwaters define isolated centres of endemism, having served as historical refugia. Rivers with high elevation sources delimit 'retreat–dispersion zones' as a result of historical connections to other drainages via montane forest corridors.

Although the watershed model has received considerable attention (Pearson & Raxworthy, 2009; Townsend *et al*., 2009; Vences *et al*., 2009; Weyeneth *et al*., 2011), there are few historically explicit tests of differentiation, particularly for forest-dependent organisms. Testing the model necessitates very broad sampling across its centres of endemism and an explicitly phylogenetic approach (Köhler & Glaubrecht, 2010). To date, studies of the model generally assess present-day geographical distributions of species in a purely spatial context (Wilmé *et al*., 2006; Olivieri *et al*., 2007; Pearson & Raxworthy, 2009; but see also Townsend *et al*., 2009; Chan *et al*., 2012). Explicitly incorporating historical divergence is necessary to test the mechanisms proposed by the watershed model and to understand broadscale biogeographical processes on Madagascar. Wide-ranging and forest-dependent taxa, in particular, are appropriate for evaluating the watershed model and incorporating population genetic and coalescent-based approaches with respect to understanding the historical nature of differentiation.

Tuft-tailed rodents (genus *Eliurus*) are widely distributed across the diverse terrestrial biomes of Madagascar. *Eliurus* is the most speciose genus in the endemic subfamily Nesomyinae, with 12 recognized species (although certain species delineations remain partially unresolved; Carleton, 2003; Carleton & Goodman, 2007; Soarimalala & Goodman, 2011). Members of this genus inhabit both dry and humid forests, and are most easily recognized by their eponymous tufted tails. Despite the broad distribution of the genus, only one species, *E. myoxinus*, occurs across the dry deciduous forests and xerophytic scrub bush of western Madagascar, making it the most widespread member of the genus in these habitats. This species is also known from lowland humid forests in the north-east of the island, which have a pronounced annual dry season (Soarimalala & Goodman, 2003). Previous morphological research on *E. myoxinus* has found a tendency for greater body size towards the north (Carleton & Goodman, 2007; Rakotomalala, 2010); whether genetic patterns are also latitudinally structured is not known. Aspects of *E. myoxinus* population dynamics and natural history are poorly understood (Carleton, 2003; Randrianjafy, Ramilijaona & Rakotondravony, 2007; Soarimalala & Goodman, 2011), although it is strictly forest-dependent and occurs in dry and humid forests up to 900 m and 1240 m in elevation, respectively (Soarimalala & Goodman, 2011). Thus, it is an appropriate study organism for testing the watershed model because populations may historically have been restricted to forests along watersheds.

The present study tests the applicability of the watershed hypothesis to *E. myoxinus* across its known distribution. We also characterize the geographical patterns of genetic diversity and differentiation and explore potential alternative mechanisms of divergence in this species. We highlight possible underlying ecological and geographical factors maintaining local endemism in western Madagascar, and estimate the historical demography of the species as the first step in elucidating the underlying historical and ecological forces governing its spatial diversification.

MATERIAL AND METHODS

Sampling

Specimens of 123 *E. myoxinus* individuals from northern and western Madagascar (Fig. 1), as well as seven purported *E. myoxinus* (six from locality 2, one from locality 8) and six congeneric individuals from throughout Madagascar, were collected and deposited in the Field Museum of Natural History (FMNH) and the Université d'Antananarivo, Département de Biologie Animale (UADBA) (see Supporting information, Table S1). Animals were handled in accordance with the guidelines of The American Society of Mammalogists (Sikes, Gannon & the Animal Care and Use Committee of the American Society of Mammalogists, 2011). Muscle tissue was sampled from each individual and preserved in 0.5% ethylenediaminetetraacetic acid buffer. Localities were assigned to geographical regions loosely *sensu* Muldoon & Goodman (2010) (Table 1).

Sequence data collection

Whole genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen). We targeted three loci: the mitochondrial cytochrome *b* gene (cyt *b*), intron 7 of the nuclear β-fibrinogen gene (*β-fib*) and intron 9 of the nuclear growth hormone receptor gene (*GHR*).

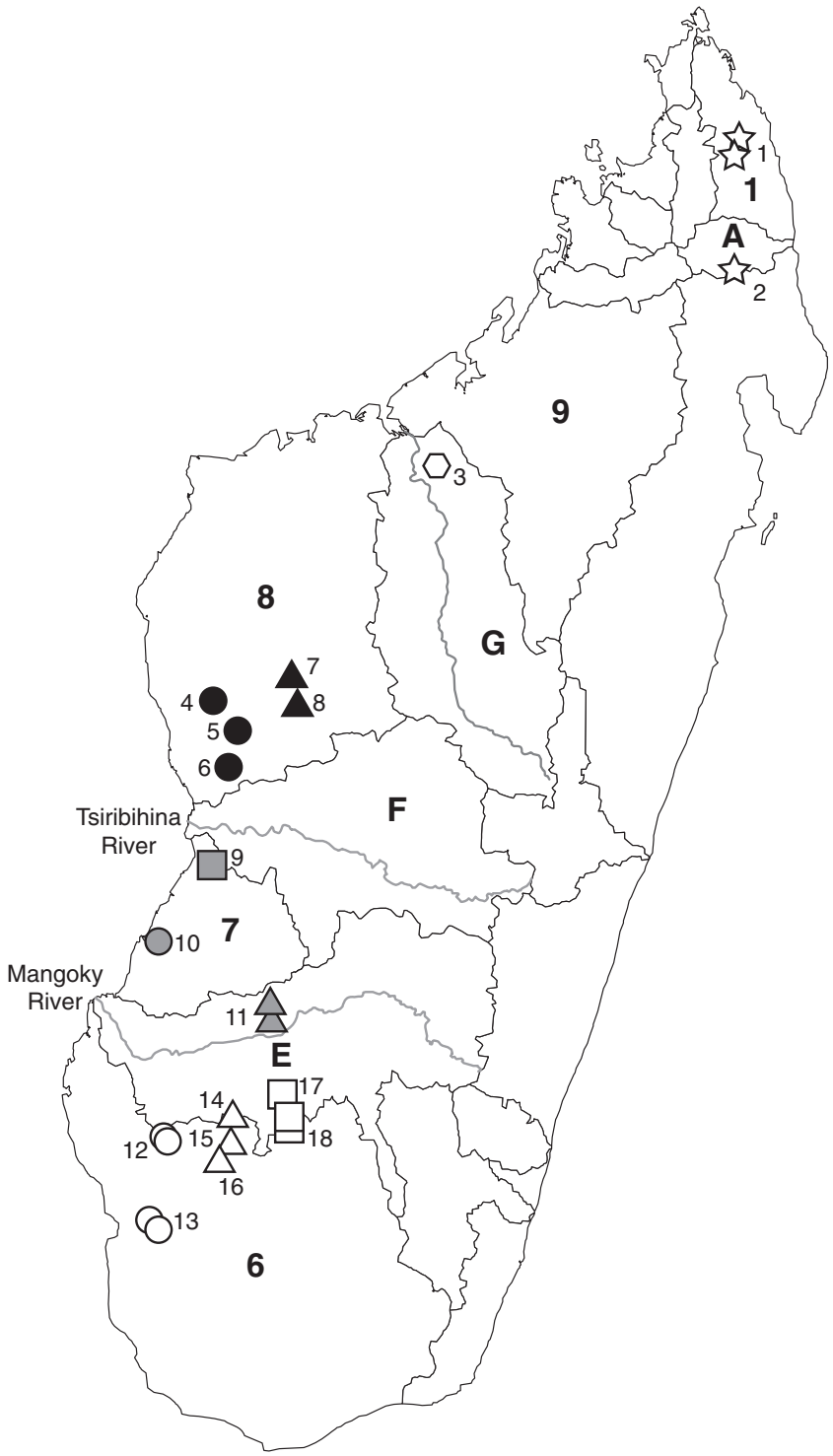


Figure 1. Sampling sites for *Eliurus myoxinus* and retreat–dispersion (letters) and centres of endemism (bold numbers) predicted by the watershed model of Wilmé *et al*. (2006). Localities for groups of proximal sites are indicated by a number. Symbols denote regional groupings of localities.

Table 1. Locality numbers and names for *Eliurus myoxinus* along with and sample sizes and summary statistics (mean nucleotide diversity π, mean number of segregating sites θ_S) for each locus

			cyt *b*			*β-fib*			*GHR*		
Number	Locality	Region	*N*	π	θ_S	2*N*	π	θ_S	2*N*	π	θ_S
1	Forêt de Binara	North	25	6.973	5.826	50	2.829	2.456	26	1.108	1.310
2	PN de Marojejy	North	19	4.632	3.147	38	1.562	1.428	38	0.925	0.476
3	PN d'Ankarafantsika	West	1	0	–	2	0	0	0	–	–
4	Forêt de Mamakibetro	West	1	0	–	0	–	–	2	9	5
5	Forêt de Bendrao	West	1	0	–	2	5	5	2	0	0
6	PN de Bemaraha	West	8	5.929	6.171	16	5.442	6.027	14	4.121	3.773
7	RS d'Ambohijanahary	West	3	2	2	6	2.933	3.066	4	5.667	2.727
8	Ambohijanahary Mountain	West	4	11.500	9.818	8	3	3.471	8	6.071	4.242
9	Forêt de Kirindy	West	1	0	–	4	6.5	6.545	2	0	0
10	PN de Kirindy-Mite	West	3	19.333	19.333	4	6	6	4	5.500	3.818
11	Forêt de Makay	South-west	4	4.500	4.909	8	2.893	3.471	4	1.167	1.091
12	Forêt d'Analavelona	South-west	23	12.008	14.902	46	4.626	3.868	46	3.049	2.503
13	Sept Lacs	South-west	1	0	–	4	2.667	2.727	4	0.500	0.545
14	Forêt de Vohibasia	South-west	1	0	–	2	1	1	2	1	1
15	Forêt de Vohimena	South-west	2	0	0	2	2	2	4	6.833	4.364
16	Forêt de Zombitsy	South-west	2	34	34	0	–	–	0	–	–
17	PN de l'Isalo	South-west	2	5	5	4	3.500	3.273	4	1.167	1.091
18	PN de l'Isalo, Canyon des Rats	South-west	20	5.311	5.074	40	3.432	3.762	40	4.821	2.821
	Mean across localities			25.807	26.635		4.534	8.611		3.521	5.939

PN, Parc National; RS, Réserve Spéciale. cyt *b*, cytochrome *b* gene; *β-fib*, intron 7 of the nuclear β-fibrinogen gene; *GHR*, intron 9 of the nuclear growth hormone receptor gene.

Congeneric taxa were included for cyt *b*, whereas only *E. myoxinus* were included in the *β-fib* and *GHR* datasets (see Supporting information, Table S1). Cyt *b* was amplified using the primers UMMZ04 (Jansa, Goodman & Tucker, 1999) and MVZ05 (Irwin, Kocher & Wilson, 1991; Jansa *et al*., 1999). *β-fib* polymerase chain reaction (PCR) amplification used the primers bfib-mammU and b17-mammL (Matocq, Shurtliff & Feldman, 2007). For the *GHR* locus, we used PRIMER3 (Rozen & Skaletsky, 2000) to design primers for the consensus sequence from a *Rattus rattus* and *Mus musculus GHR* intron alignment (GenBank accession numbers: JF412704.1 and J04811; Benson *et al*., 2004) (GHRI9F 5′-TAC CCC CAG TAC CAG TTC CA-3′; GHRI9-5R 5′-CCT TTG CTC CAA GGA TAC CA-3′).

PCRs were conducted in 25-μL reactions with 1 × buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM of each primer, 0.5 U (0.625 U for *GHR*) *Taq* polymerase and 1 μL of template DNA. Initial denaturation was at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 50 °C (cyt *b*) or 56 °C (*β-fib*, *GHR*) for 30 s (cyt *b*, *β-fib*) or 1 min (*GHR*), and 72 °C for 75 s, and a final extension at 72 °C for 8 min.

Samples were cleaned prior to DNA sequencing by incubating 5 μL of PCR product with 1.6 μL of sterile H_2O and 0.4 μL of ExoSAP-IT (USB Products) at 37 °C for 15 min followed by 80 °C for 15 min. Each locus was sequenced in complementary directions using the same primers as for the PCR; reactions included 0.75 μL of purified PCR product, 0.75 μL of 5 × buffer, 0.5 μL of primer and 0.2 μL of BigDye, version 3 in a total volume of 5 μL and were electrophoresed on an ABI 3730xl capillary sequencer.

Sequences were checked by eye, trimmed and assembled into contigs with SEQUENCHER, version 4.8 (GeneCodes). Alleles of heterozygous *β-fib* and *GHR* sequences were resolved through TA cloning and computational analysis using SEQPHASE (Flot, 2010) and PHASE (Stephens, Smith & Donnelly, 2001; Stephens & Scheet, 2005). We recovered 76 heterozygous individuals at *β-fib* and 71 at *GHR*; the alleles of 14 and 22 samples, respectively, were resolved directly via TA cloning and sequencing of at least six clones each. All DNA sequence data are deposited on GenBank (see Supporting information, Table S1).

We aligned sequences in MACCLADE, version 4.08 (Maddison & Maddison, 2008) and SE-AL, version 2.0 (Rambaut, 2007). Samples were pooled into 18 unique localities (Table 1), with no individuals within these groups being found at pairwise distances greater than 11 km. We used ARLEQUIN, version 3.5 (Excoffier & Lischer, 2010) to calculate the mean nucleotide diversity (π) (Tajima, 1983, 1993) and number of segregating sites (θ_S) (Watterson, 1975) for each locality.

Phylogenetic analysis

We constructed haplotype networks for *E. myoxinus* individuals at each locus using TCS (Clement, Posada & Crandall, 2000) with a connection limit of 90%. Seven individuals from localities 2 and 8 were distantly related to all other *E. myoxinus* at all loci (and in subsequent phylogenetic analysis of cyt *b*), indicating either misidentification or representatives of previously unrecognized cryptic species. Morphological and taxonomic investigation of these specimens is beyond the scope of the present study, although their phylogenetic placement may provide useful data for future studies. Thus, our phylogeographical study focuses only on verified samples from *E. myoxinus*, and we refer to the other individuals as *Eliurus* sp. A and B.

We estimated best-fit models of nucleotide sequence substitution for each cyt *b* codon position using DT-MODSEL (Minin *et al.*, 2003). Identical haplotypes were identified using COLLAPSE, version 1.2 (Posada, 2006), after trimming ends to the first variable sites. We estimated the phylogenetic relationships among unique haplotypes under both Bayesian and maximum likelihood (ML) frameworks. Analyses were partitioned by codon position, applying the best-fit nucleotide substitution models from DT-MODSEL (TrNef+Γ, HKY, and TrN+Γ for first, second, and third positions, respectively). Bayesian phylogenetic analyses were performed in MrBayes, version 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). We conducted two separate runs, each with a single chain sampled every 1000 generations for 100 million generations. We confirmed adequate mixing and convergence between runs in TRACER, version 1.5 (Rambaut & Drummond, 2009). The posterior distribution of trees was summarized on an unrooted majority rules consensus tree after discarding the first 25% of trees as burn-in. ML phylogenetic analyses were conducted in GARLI, version 2.0 (Zwickl, 2006); each of the 100 bootstraps consisted of five search replicates with termination thresholds of 20 000 for topology and 0.05 for tree score. Bootstrap scores were summarized on the consensus phylogeny from Bayesian analyses using the SumTrees function of DENDROPY (Sukumaran & Holder, 2010).

Testing the watershed model

We tested the congruence of cyt *b* data to expectations of population monophyly under the watershed model using a parametric bootstrapping approach. First, we specified a constraint tree under the watershed model: all individuals from the same centre of endemism were constrained to a monophyletic group, with no specified relationships among clades or among individuals within clades. Individuals from retreat–dispersion zones were unconstrained and specified as a basal polytomy (Fig. 2). We estimated the general model of sequence evolution for empirical data under the constraint tree using DT-MODSEL (Minin *et al.*, 2003). This model was then used to parameterize ML estimations of the phylogeny and associated parameters in GARLI, version 2.0 (Zwickl, 2006) for the empirical data, both under the constraint and unconstrained trees. We conducted five search replicates for each run, with search termination thresholds of 10 000 for topology and 0.05 for tree score. The substitution model parameters estimated under the constrained ML search were used to parameterize 1000 simulated DNA sequence datasets under the constraint tree in MESQUITE, version 2.75 (Maddison & Maddison, 2011). For each simulated dataset, we estimated the best constrained and unconstrained trees in GARLI, version 2.0. The differences between the log-likelihood (lnL) scores of the best constrained and unconstrained trees from each simulated dataset defined the null distribution expected under the watershed model. We compared the difference in lnL scores for the empirical data with this null distribution to determine the probability that our data fit expectations of population monophyly under the watershed model.

Population genetic analysis

Among localities with sample sizes greater than two, we used pairwise sequence divergence to estimate F_{ST} values for cyt *b* in ARLEQUIN. We tested for genetic signatures of population expansion within these same localities; locality-specific Tajima's *D* (Tajima, 1989) and Fu's F_S (Fu, 1997) were calculated for each locus, with 1000 simulated coalescent samples to determine significance.

We also estimated historical and current demographic parameters under a multilocus, two-population isolation-with-migration model in IMA (Hey & Nielsen, 2007) for four alternative divergence scenarios between western (Group I) and southwestern (Group II) populations (Fig. 3). We explored

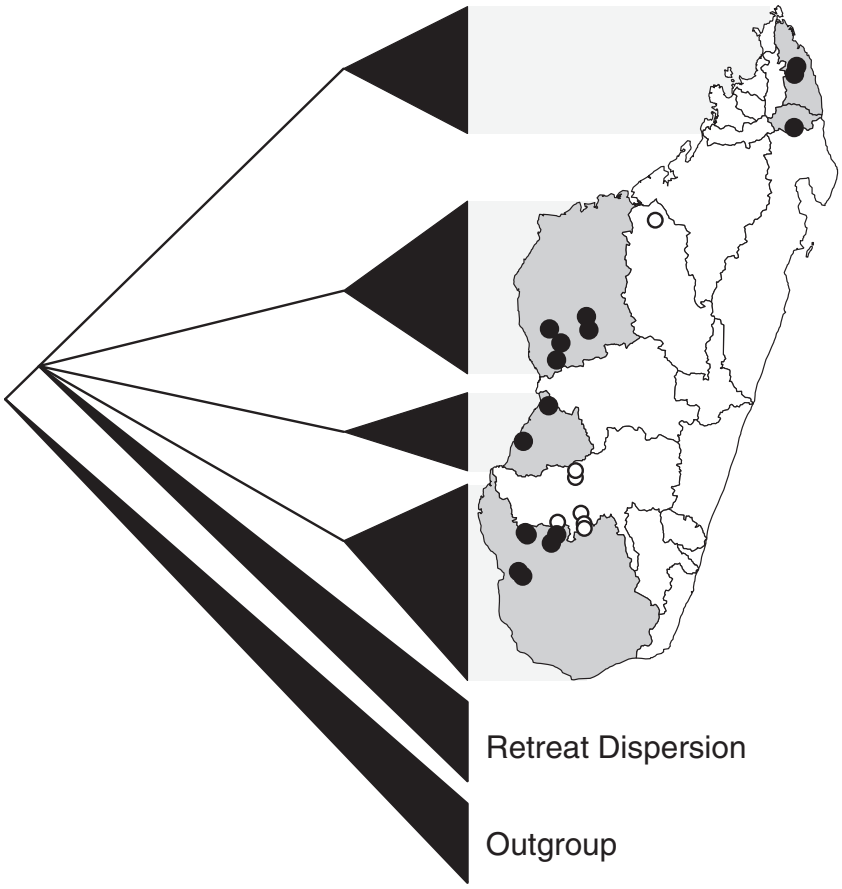


Figure 2. Visual representation of the constraint tree used in parametric bootstrap analyses testing the watershed model.

divergence across each of two major rivers: the Tsiribihina and the Mangoky. Additionally, we explored divergence scenarios including Kirindy-Mite individuals (locality 10) with western localities 4–9 (Kirindy-Mite Western) and, alternatively, with the south-western localities 11–18 (Kirindy-Mite South-western) (Fig. 3); in phylogenetic analysis of mitochondrial DNA individuals from this locality fell out with individuals from both regions (Fig. 4). We excluded four sequences from the cyt *b* alignment with missing data and for each model, conducted preliminary runs in IMA to determine appropriate parameter settings. Final runs consisted of at least three million steps with 500 000 steps discarded as burn-in. We used the 'load-trees' mode of IMA to calculate the log-likelihood ratio (LLR) of nested demographic models under each alternative divergence scenario.

We also explored spatial patterns of colonization and divergence under a continuous diffusion model (CDM) in BEAST, version 1.7.5 (Lemey *et al*., 2010; Drummond *et al*., 2012). We partitioned cyt *b* data by codon position and assumed a Gaussian Markov random field Bayesian Skyride model of population size change (Minin, Bloomquist & Suchard, 2008) and a relaxed-random walk model of spatial diffusion. We specified a Cauchy prior distribution on diffusion rate variance across tree branches (Lemey *et al*., 2010). For each run, we assumed a normal prior distribution of cyt *b* substitution rates with a mean of 2×10^{-8} substitutions per site per year (SD: 5×10^{-9} substitutions per site per year), which includes a range of reported substitution rates from other studies across murid rodents (Steppan, Zawadzki & Heaney, 2003; Suzuki *et al*., 2003; Luo *et al*., 2004; Abdel Rahman *et al*., 2008). The final analysis consisted of 100 million generations sampled every 10 000 steps. The posterior distribution of trees was summarized on a maximum clade credibility topology in TREEANNOTATOR (Drummond *et al*., 2012), after discarding the first 1001 trees as burn-in. We used SPREAD, version 1.0.5 (Bielejec *et al*., 2011) to visualize ancestral locality reconstructions on the consensus tree through time in Google Earth.

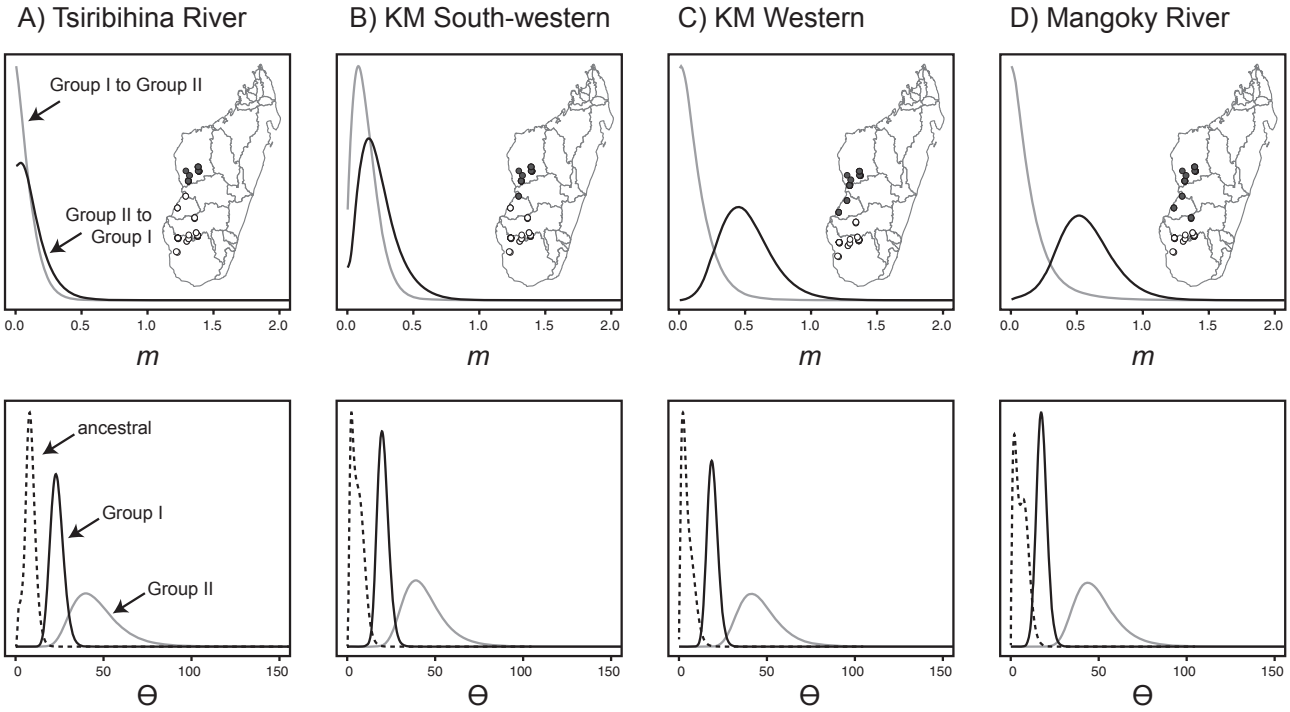


Figure 3. Probability densities for estimates of population sizes (θ) and migration rates (*m*) of *Eliurus myoxinus* from IMA analyses of four alternative two-population models: Tsiribihina River as barrier (A), Kirindy-Mite (KM) South-western (B), KM Western (C), and Mangoky River as barrier (D). Inset maps show partitioning of sampling localities between Group I (grey circles) and Group II (white circles) regions under each model.

Figure 4. Majority-rules consensus phylogeny from Bayesian phylogenetic analysis of unique cyt *b* haplotypes in *Eliurus myoxinus* and outgroups. Tips representing *E. myoxinus* haplotypes are labelled with sampling locality numbers and regional symbols (Fig. 1, Table 1). Nodes are annotated with Bayesian posterior probabilities (PP) and maximum likelihood (ML) bootstrap support for all nodes with PP ≥ 0.80. Outgroup tip labels include: FMNH, Field Museum of Natural History; UADBA, Université d'Antananarivo, Département de Biologie Animale; MR, Martin Raheriarisena.

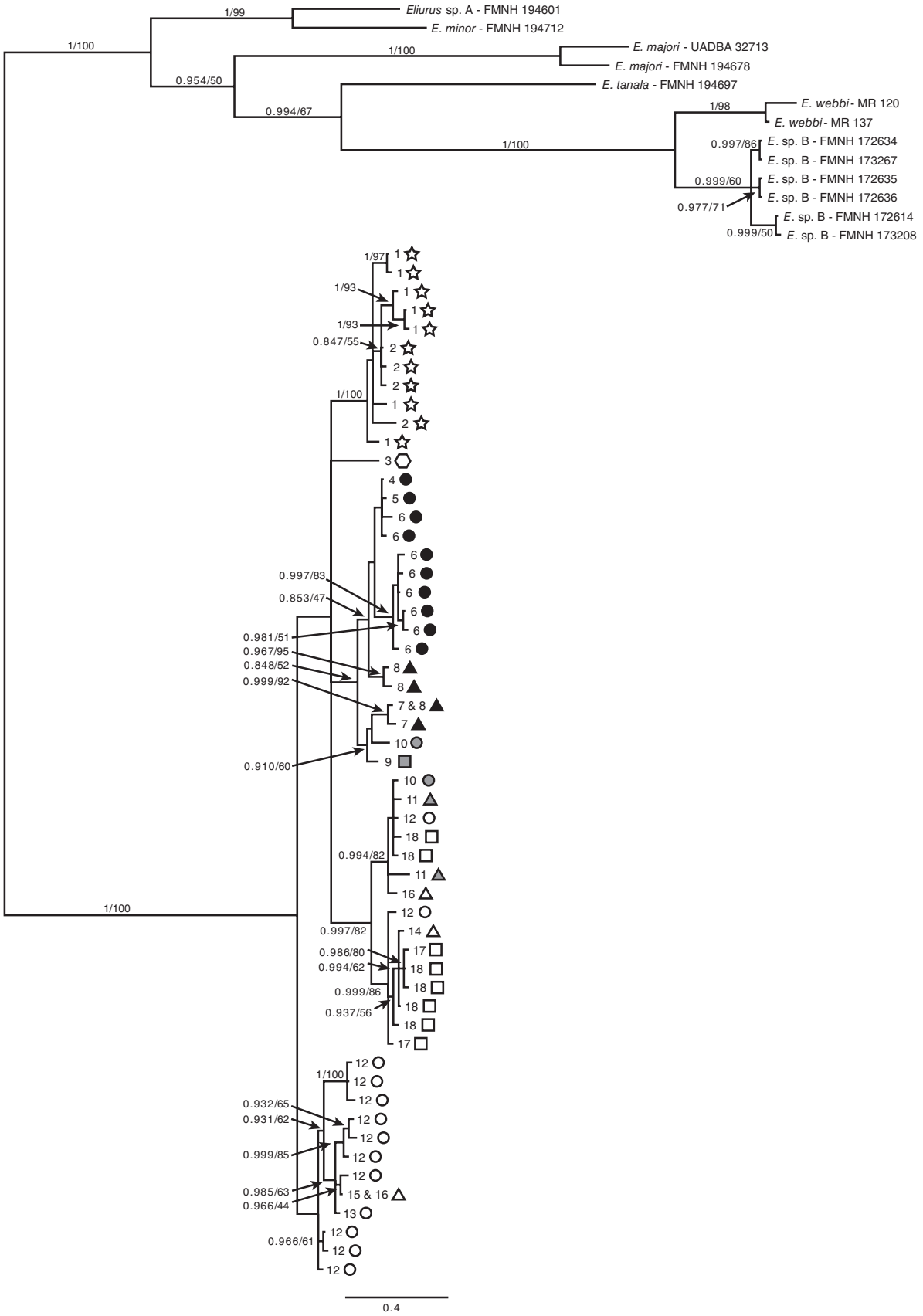
RESULTS

The final alignment of cyt *b* sequences was 1089 bp in length for 121 individuals of *E. myoxinus* and 14 congeneric individuals. For the full dataset, there were 272 parsimony informative sites; within *E. myoxinus*, there were 108 parsimony informative sites and 55 unique haplotypes. We recovered diploid genotypes for 118 *E. myoxinus* at *β-fib* and 102 individuals at *GHR*. Sequence alignments for *β-fib* comprised 673 bp, with one insertion/deletion (indel), 34 parsimony informative sites, and 62 unique alleles. *GHR* alignments included 392 bp with two indels of 4 bp each, 21 parsimony informative sites, and 45 unique alleles.

Mean nucleotide diversity (π) within localities ranged from 0–34 at cyt *b*, 0–6.5 at *β-fib*, and 0–6.833 at *GHR* (Table 1). Overall π values for these loci were 25.807, 4.534, and 3.521, respectively. The mean number of segregating sites (θ_S) within localities ranges from 0–34 for cyt *b*, 0–6.545 for *β-fib*, and 0–5 for *GHR*. Respectively, overall θ_S values for these loci were 26.635, 8.611, and 5.939.

Haplotype networks and phylogenetic analysis

At the 90% connection limit, we inferred four separate cyt *b* haplotype networks for *E. myoxinus* that correspond largely with four geographical areas (Fig. 5). For *β-fib*, all haplotypes except one (from locality 6, with a 15-bp indel) formed a single network. The most common haplotype occurred predominately in northern Madagascar. Among the remaining localities, weakly divergent haplotypes were sometimes found in similar geographical regions. We recovered a single network for *GHR*, and found very little geographical structure among haplotypes. The most common


Eliurus sp. A - FMNH 194601
E. minor - FMNH 194712
E. majori - UADBA 32713
E. majori - FMNH 194678
E. tanala - FMNH 194697
E. webbi - MR 120
E. webbi - MR 137
E. sp. B - FMNH 172634
E. sp. B - FMNH 173267
E. sp. B - FMNH 172635
E. sp. B - FMNH 172636
E. sp. B - FMNH 172614
E. sp. B - FMNH 173208
1/99
1/100
1/100
0.954/50
0.994/67
1/98
1/100
0.997/86
0.999/60
0.977/71
0.999/50
1/97
1/93
1/93
0.847/55
1/100
0.997/83
0.853/47
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0.967/95
0.848/52
0.999/92
0.910/60
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0.986/80
0.994/62
0.999/86
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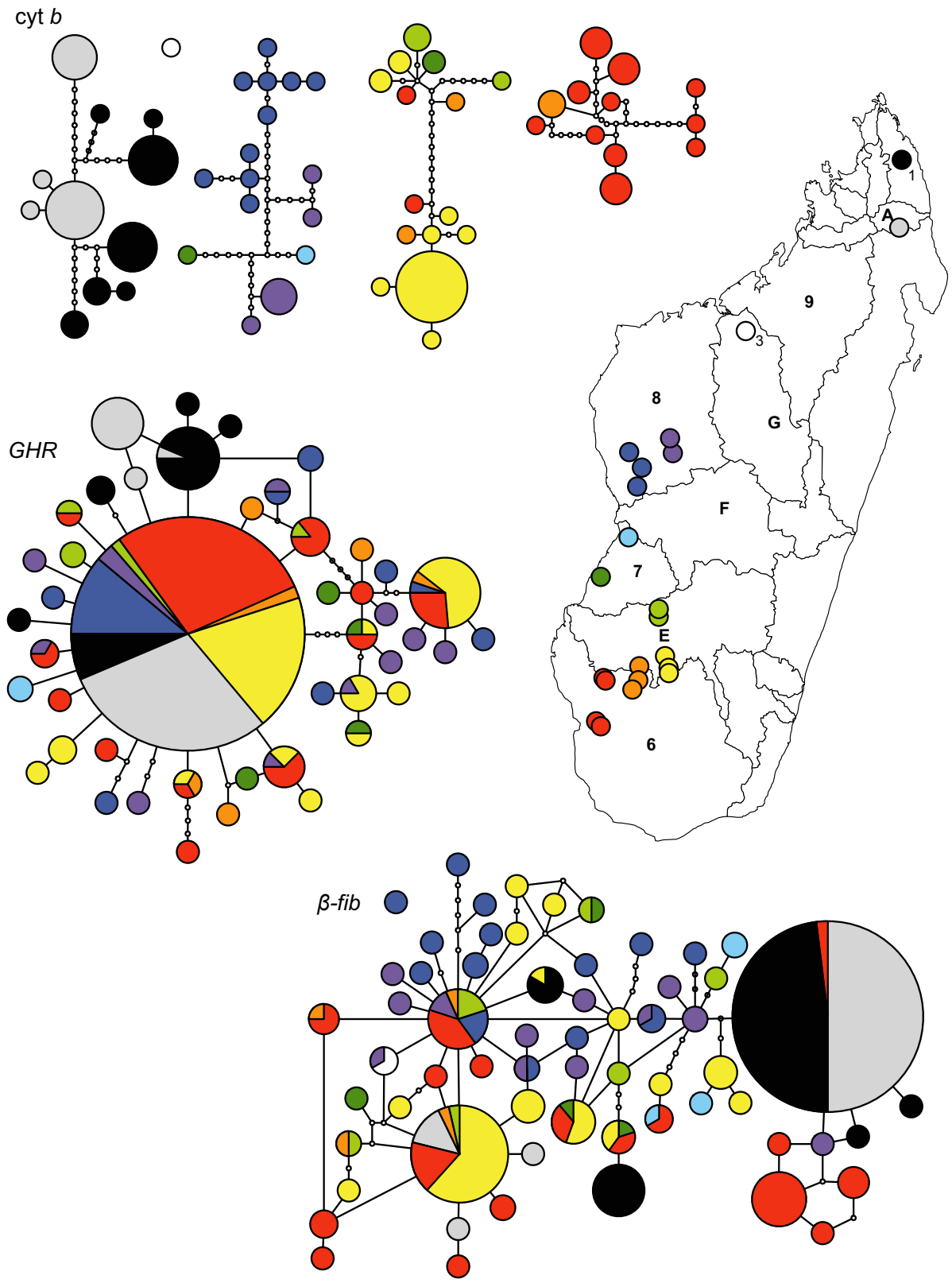
cyt *b*
GHR
β-fib
1
2
A
9
3
8
G
F
7
E
6

Figure 5. Haplotype networks for three loci: cytochrome *b* gene (cyt *b*), intron 7 of the nuclear β-fibrinogen gene (*β-fib*), and intron 9 of the nuclear growth hormone receptor gene (*GHR*) for *Eliurus myoxinus*. Circles represent sampled haplotypes, lines denote one mutational step, and small circles are inferred haplotypes. Sizes of the circles reflect the relative frequency of that haplotype and colours reflect the sampling localities (see inset map).

haplotype was found throughout the range of our samples, and most other haplotypes of high frequency were shared among disparate localities. The major exceptions to this were several common haplotypes unique to northern localities 1 and 2. The differences in the overall structure of the haplotype networks for these three loci reflect the smaller effective population size and higher substitution rate of the mitondrial locus, cyt *b*, relative to the two nuclear loci.

Bayesian and ML phylogenetic analyses of cyt *b* revealed largely congruent patterns of support across the tree. We recovered strong support for monophyly of *E. myoxinus* [posterior probability, PP = 1; ML bootstrap support (BS) = 100] (Fig. 4), excluding seven individuals identified in the field as *E. myoxinus*. In general, relationships at the base of the *E. myoxinus* clade were not well-resolved, although we do find strong support for a northern clade (localities 1 and 2; PP = 1, BS = 100) (Fig. 4). All individuals from western localities 4 through 9 and one individual from Kirindy-Mite (locality 10) clustered together with low support (PP = 0.848, BS = 52). The other two individuals from Kirindy-Mite formed a well-supported clade with some south-western individuals (PP = 0.997, BS = 82). The remaining south-western samples clustered together with low support (PP = 0.755; BS = 72).

Testing the watershed model

For our test of the watershed model, unconstrained ML phylogenetic analysis of cyt *b* data resulted in an lnL score of –4623.895. The lnL score for the constrained search was –4807.613. This difference (183.718 lnL units) was greater than expected by simulations under the watershed model and, thus, we rejected the watershed model for *E. myoxinus* ($P < 0.001$).

Population genetic analysis

Pairwise F_{ST} values for cyt *b* among localities with sample sizes greater than three were largely significant after Bonferroni correction (Table 2), indicating pronounced genetic structure. Pairwise F_{ST} values among localities 7, 8, 10, and 11 were not significantly different from zero, although this may be a result of small sample sizes.

Tajima's *D* and Fu's F_S were insignificant at all three loci for most population estimates, suggesting demographic stability. Tajima's *D* for *β-fib* at Bemaraha (locality 6) and Fu's F_S for both *β-fib* and *GHR* at Ambohijanahary Mountain (locality 8) were the only significant test statistics (Table 3). This pattern of significance is likely a result of the small sample sizes obtained from these two localities.

Multilocus isolation-with-migration analyses consistently support a pattern of population expansion from a small ancestral population, regardless of the grouping of localities (Fig. 3). In all four divergence scenarios, LLR tests reject demographic models with an ancestral population size ($\theta_{Ancestral}$) equal to the current population sizes ($\theta_{Group\ I}$, $\theta_{Group\ II}$) (Table 4). For the Mangoky River and Kirindy-Mite Western scenarios (Fig. 3), we reject all models except a single model with no immigration into the south-west ($m_{Group\ II} = 0$). When Kirindy-Mite is instead included with the south-western localities, there are three models that we are unable to reject, all of which have unequal population sizes ($\theta_{Group\ I} \neq \theta_{Group\ II} \neq \theta_{Ancestral}$). Under the Tsiribihina River scenario, we reject all models in which either or both of the current population sizes ($\theta_{Group\ I}$, $\theta_{Group\ II}$) are equal to the ancestral population size ($\theta_{Ancestral}$). Although accepted demographic models for the Tsiribihina River scenario included both symmetrical and asymmetrical migration ($m_{Group\ I} = m_{Group\ II}$, and $m_{Group\ I} \neq m_{Group\ II}$, respectively) (Table 4), migration estimates were close to zero (Fig. 3). For the other three scenarios, migration rates suggest greater immigration into the western populations from the south-western populations ($m_{Group\ I} > m_{Group\ II}$) (Fig. 3).

CDM analyses estimated that the ancestral population of *E. myoxinus* was located near the Mangoky River (see Supporting information, File S1), although the 80% highest posterior density for location was not continuous at all nodes. Assuming a mean substitution rate of 2% per Myr, initial divergence between the western and south-western populations occurred approximately 280 000 years ago, followed by narrow-ranged expansion within those two regions. According to our CDM, the northern localities were colonized later, approximately 215 000 years ago. Population expansion occurred in the western regions before expansion in the south-western and northern regions, approximately 150 000 years ago. Under this model, it appears that migration between the western and south-western regions has occurred recently, within the past 50 000 years.

Table 2. Pairwise F_{ST} values at cyt *b* (below diagonal) and pairwise geographical distances (km) between sampling localities of *Eliurus myoxinus* (above diagonal)

Locality	1 (N = 25)	2 (N = 19)	6 (N = 8)	7 (N = 3)	8 (N = 4)	10 (N = 3)	11 (N = 4)	12 (N = 23)	18 (N = 20)
1	–	130.5	831.2	717.7	736.4	1020.8	1020	1193.1	1123.6
2	**0.31212**	–	731.2	620	635.6	914.9	904.9	1079.2	1004.3
6	**0.78019**	**0.83997**	–	114.4	95.5	197	254	395.8	379.7
7	**0.78721**	**0.85213**	**0.72566**	–	30	310.1	348.4	503.4	472.1
8	**0.73244**	**0.78992**	**0.47199**	0.18012	–	286.9	319.7	477	442.9
10	**0.70724**	**0.76068**	0.63759*	0.49738	0.35035	–	128.7	206.2	230.2
11	**0.81196**	**0.86303**	**0.84369**	0.8835*	0.74603*	0.18754	–	175.1	125.7
12	**0.71402**	**0.73346**	**0.70941**	**0.69386**	**0.64814**	0.5602*	**0.67778**	–	125.1
18	**0.80636**	**0.84067**	**0.84904**	**0.8392**	**0.79618**	**0.55758**	0.62071*	**0.72025**	–

F_{ST} values significantly different from zero at $\alpha = 0.05$ after Bonferroni correction ($P < 0.003$) are indicated in bold; values with asterisks are significant without correction ($P < 0.05$).

DISCUSSION

THE WATERSHED MODEL

The watershed model of Wilmé *et al*. (2006) is an attractive hypothesis because it recognizes the dynamic nature of historical ecosystems as a potentially significant influence on diversification. In other nonvolant mammals, extant spatial patterns of diversity and local endemism conform to the predictions of this hypothesis, although these patterns were instrumental in the formulation of the model (e.g. lemurs of the genera *Microcebus* and *Varecia*; Wilmé *et al*., 2006). There is evidence that the hypothesis applies to some reptiles, although perhaps in conjunction with extant climatic regimes (Pearson & Raxworthy, 2009). A widespread forest-dependent mammal, such as *Eliurus myoxinus*, should be an ideal candidate for fitting the expectations of this model. However, the phylogeographical structure and historical diversification of *E. myoxinus* across the dry forests of Madagascar do not support this hypothesis and suggest that its differentiation has been shaped by alternative ecological factors.

ECOLOGICAL GRADIENTS

Importantly, latitudinal gradients in climate and ecosystem composition appear to influence the overall phylogeographical structuring of *E. myoxinus*. This pattern has been suggested for other mammalian clades across regions in Madagascar (Pastorini *et al*., 2003; Yoder *et al*., 2005; Heckman *et al*., 2007; Weisrock *et al*., 2009). At the broadest geographical scale within the monophyletic clade of *E. myoxinus*, we recover genetic clusters generally corresponding to four geographical regions. The two northern-most localities are divergent from all other samples, although limited sampling from intermediate areas prevents determination of whether this results from a true biogeographical break or whether there is merely an underlying pattern of isolation-by-distance (Irwin, 2002). These northern environments are unlike the dry, deciduous forest and xerophytic bush habitats typical of *E. myoxinus* (Carleton, 2003) in the more southerly localities, which partially explains the striking latitudinal gradient of diversity. Locality 2 is in a region consisting of extensive humid forest vegetation with a pronounced annual dry season in the lowlands; locality 1 is characterized by a mosaic of humid and transitional dry deciduous forests (Goodman, 2000; Du Puy & Moat, 2003; Goodman & Wilmé, 2006). *Eliurus myoxinus* often occupies transitional habitats, particularly within humid forest zones (Carleton, Goodman & Rakotondravony, 2001). Such habitat differences may help distinguish this northern clade of localized endemism and genetic

Table 3. Tajima's *D* and Fu's F_S tests of neutral evolution for *Eliurus myoxinus* at each of three loci for localities with sample sizes of three or greater

	cyt *b*		*β-fib*		*GHR*	
Locality	Tajima's *D*	Fu's F_S	Tajima's *D*	Fu's F_S	Tajima's *D*	Fu's F_S
1	0.71808	3.9487	0.44296	3.64459	−0.43816	−1.5412
2	1.69593	4.99642	0.25298	1.92765	1.80416	0.42742
6	−0.20171	−3.4302	**−1.64221**	−3.8026	−1.16423	−0.9771
7	0	1.60944	−0.25127	0.19432	–	
8	1.75122	2.86031	−0.6648	**−5.6187**	−0.36946	**−3.3658**
10	0	5.37476	–		–	
11	−0.82943	3.77706	−0.816	−1.6949	–	
12	−0.7626	1.25713	0.62036	−1.9089	−1.14386	−1.8049
18	0.17565	2.945	−0.28175	−0.9427	−0.05671	1.08076

Bold values are significant at $P < 0.05$ for Tajima's *D* test, and $P < 0.02$ for Fu's F_S test (Fu, 1997). Dashes indicate localities where fewer than three individuals were sampled for a given locus.

differentiation within *E. myoxinus*, which is also markedly distinct with respect to morphological measurements (Carleton & Goodman, 2007).

Ecological and climatic differences may also explain the phylogeographical divergence and latitudinal structure among the remaining localities. Populations in western Madagascar occur in seasonally dry forest, whereas the south-western populations occur in regions dominated by xerophytic bush (Du Puy & Moat, 2003). These two regions also correspond loosely to two different areas of endemism outlined by the 'current climate hypothesis' (Pearson & Raxworthy, 2009), which suggests that climate gradients structure extant diversity on the island. Although the current climate hypothesis does not propose historical mechanisms for population divergence, the concordance between genetic groups and predicted areas of endemism suggests that ecological differences in response to climatic variation, at the very least, may maintain distinct lineages in extant populations.

We observe additional population genetic differentiation both among populations in western localities of Madagascar and among those in the south-west (Table 2). These may indicate barriers to dispersal within broad geographical regions limiting, or perhaps even preventing, gene flow. Small mammal communities in Madagascar have been profoundly shaped by climate and habitat variation (Muldoon & Goodman, 2010). This variation is most pronounced along the island's east–west gradient; thus, it is not surprising to identify notable east–west differentiation among western localities and among south-western localities. These patterns of differentiation can be associated with climatic gradients. For example, significant genetic differences are found between geographically proximal populations at Analavelona (locality 12) and Isalo (locality 18) in the south-west, along an east–west axis. Specimens of *E. myoxinus* were collected from the mesic canyons of Isalo and the upper portion of the Analavelona Massif, which have distinctly more humid vegetation than surrounding low-lying areas. Taken together, these sampling zones represent Quaternary relics of wetter climatic conditions across southern Madagascar (Raxworthy & Nussbaum, 1997; Carleton *et al*., 2001). Notable shifts occurred in vegetation zonation along altitudinal gradients in association with broad patterns of climate change during the Late Pleistocene and Holocene (Straka, 1996; Burney *et al*., 2004). These repeated shifts resulted in cycles of population connectivity and disjunction for a variety of different organisms, including rodents (Rakotoarisoa, Raheriarisena & Goodman, 2013). In conjunction with the elevational gradient, these shifts may explain the differentiation between Analavelona and the localities of Isalo. Hence, although Quaternary fluctuations in climate may not have resulted in the broadscale patterns of differentiation expected by the watershedmodel, they have apparently influenced the population genetic structure at finer spatial scales.

Historical demography

Such fluctuations in climate played a similar role in the initial divergence among populations of *E. myoxinus*. Although we cannot determine the exact phylogeographical processes accompanying divergence in this species, we are able to explore the demographic processes that may have accompanied divergence under alternative scenarios. Under the continuous diffusion model, we find rapid colonization northward from the south-west, followed by

Table 4. Log probability scores for nested population models in IMA and log-likelihood ratio values (–2LLR) for each model relative to the full model for each of four divergence scenarios (Fig. 3)

	Tsiribihina River		Mangoky River		Kirindy-Mite South-western		Kirindy-Mite Western	
Model	logP	–2LLR	logP	–2LLR	logP	–2LLR	logP	–2LLR
ABCDE (full model)	–3.8247		–4.0364		–4.0764		–3.9327	
ABCDD	–3.9603	0.2713	–6.9279	**5.7829**	–4.1126	0.0724	–5.9965	**4.1277**
ABCD0	–3.8278	0.0063	–4.0347	–0.0034	–5.7153	3.2778	–3.9325	–0.0002
ABC0D	–4.0491	0.4489	–8.5723	**9.0717**	–4.1566	0.1603	–13.6261	**19.387**
ABC00	–4.1452	0.6411	–102.5782	**197.0836**	–22.8567	**37.5606**	–90.9101	**173.9548**
AACDE	–5.0305	2.4117	–7.2695	**6.4663**	–6.4947	**4.8365**	–6.8045	**5.7437**
AAADE	–12.7725	**17.8957**	–18.3907	**28.7086**	–13.1682	**18.1835**	–15.2655	**22.6657**
AACDD	–5.9089	4.1684	–15.8988	**23.7249**	–7.179	**6.2052**	–12.4635	**17.0616**
AAC00	–6.6398	5.6302	–129.1342	**250.1956**	–28.5183	**48.8838**	–108.5998	**209.3344**
AAADD	–12.8166	**17.9839**	–25.4615	**42.8502**	–18.2231	**28.2934**	–20.8503	**33.8353**
AAA00	–17.9392	**28.229**	–156.9577	**305.8425**	–42.4051	**76.6573**	–115.626	**223.3867**
ABADE	–12.6018	**17.5543**	–14.5899	**21.107**	–13.1202	**18.0876**	–14.1799	**20.4945**
ABADD	–12.651	**17.6526**	–22.004	**35.9352**	–17.5013	**26.8498**	–20.8482	**33.8311**
ABA00	–16.8848	**26.1202**	–146.4062	**284.7395**	–42.3214	**76.4899**	–110.4208	**212.9763**
ABBDE	–9.0029	**10.3565**	–8.9335	**9.7942**	–8.8743	**9.5958**	–9.5063	**11.1473**
ABBDD	–9.7189	**11.7883**	–9.1548	**10.2367**	–10.014	**11.8752**	–10.4182	**12.9712**
ABB00	–12.2222	**16.7951**	–104.8201	**201.5674**	–29.1197	**50.0866**	–93.732	**179.5987**

–2LLR for rejected models are indicated in bold. The five characters of the model name correspond to population parameters in the order: $\theta_{\text{Group I}}$, $\theta_{\text{Group II}}$, $\theta_{\text{Ancestral}}$, $m_{\text{Group I}}$, and $m_{\text{Group II}}$, where θ and m are mutation scaled effective population sizes and migration rates, respectively. Parameters specified by the same uppercase letter were held equal for that model; parameters specified as zero were held at zero for that model. For example, in model ABCDD, $\theta_{\text{Group I}} \neq \theta_{\text{Group II}} \neq \theta_{\text{Ancestral}}$, $m_{\text{Group I}} = m_{\text{Group II}}$

simultaneous spatial expansion in the north, west, and south-west. We estimate subsequent secondary contact between the western and south-western populations at Kirindy-Mite. The four scenarios explored in IMA also estimate a small ancestral population size and much larger sizes for western and south-western regions, indicating demographic expansion. The estimated ages of these events must be interpreted cautiously because they are based on the assumed mean substitution rate. However, these results do suggest relatively recent population expansion during the Quaternary, indicating a potential role for recent climatic fluctuations in structuring historical demography.

It is also possible that rivers have had consequences for patterns of diversification in *E. myoxinus*. Major rivers such as the Tsiribihina and Mangoky, which flow from the east to western drainage basins, have been proposed as barriers to dispersal in taxa such as lemurs and small mammals (Martin, 1972; Pastorini *et al*., 2003; Craul *et al*., 2008; Rakotomalala & Goodman, 2010). The Tsiribihina River may be a significant and long-standing barrier to dispersal for *E. myoxinus*, based on estimates of no or very low migration in IMA analyses (Fig. 3). By contrast, we recover asymmetrical population connectivity with migration from the south-west to the west under analyses that assume initial divergence across the Mangoky River (Fig. 3), indicating that this river may not be a hard barrier for this species.

Although we are unable to disentangle the specific processes responsible for patterns of phylogeographical divergence in *E. myoxinus*, it is clear that it has had a dynamic history associated with environmental changes in the recent past. Further studies with greater sampling, particularly in the areas between the Tsiribihina and Mangoky Rivers, and between the western and south-western localities, will help determine the exact mechanisms that underlie this population divergence.

Conclusions and implications

Using phylogenetic analyses, the present study statistically evaluates the watershed model of Wilmé *et al*. (2006) for a broadly distributed, forest-dependent rodent, *E. myoxinus*. We reject the watershed model as an explanatory mechanism of diversification in the history of this species. Although an alternative model explaining the processes of differentiation of this species cannot be identified at this time, there is preliminary evidence for the influence of environmental gradients and ecological zones, which are factors that have previously been correlated with community evolution in nonvolant Malagasy mammals (Muldoon & Goodman, 2010).

Ecological models for Madagascar have tended to emphasize the importance of east–west constraints to dispersal (Goodman & Ganzhorn, 2004; Yoder & Heckman, 2006; Vences *et al*., 2009). In small mammals, this cline, which shows dramatic changes in abiotic and biotic factors, is directly correlated with β-diversity and dispersal barriers (Muldoon & Goodman, 2010). For *E. myoxinus*, a species that is broadly distributed in the lowland dry forests across western Madagascar, patterns of genetic differentiation and local endemism lie along a north–south axis, characterized by more subtle climatic and vegetational clines than the east–west axis. This pattern has been found in other land vertebrates (Yoder *et al*., 2005), including reptiles (Boumans *et al*., 2007; Raselimanana *et al*., 2008; Chan *et al*., 2012) and lemurs (Pastorini, Forstner & Martin, 2001; Pastorini *et al*., 2003; Heckman *et al*., 2007). It is unclear what exact biogeographical processes underlie the patterns we find in the present study. Further studies in other taxa and localities of this region will be particularly important with respect to achieving a complete model of the evolutionary mechanisms acting within western Madagascar.

The processes driving patterns of diversification and endemism in Madagascar's dry forests still remain to be fully elucidated (Ganzhorn *et al*., 2001; Carleton & Goodman, 2007; Olson *et al*., 2009). Historical variation in habitats, driven by the complexity of the geology, watersheds, and ecosystems in western Madagascar, has potentially led to speciation and diversification in this species, as with other taxa (Vences *et al*., 2009). The forests of Madagascar have been subjected to heavy anthropogenic deforestation and fragmentation (Harper *et al*., 2007), with impacts on endemic species diversity (Irwin *et al*., 2010). Limited funding and support necessitate the specific targeting of threatened, concentrated centres of micro-endemism (Myers *et al*., 2000; Craul *et al*., 2007; Kremen *et al*., 2008). If we are to preserve the Malagasy biota, including the structured diversity found within *E. myoxinus*, we must strive to understand the complex forces governing speciation and differentiation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Table of *Eliurus* samples used in the present study, including specimen catalogue numbers, locality information, and GenBank accession numbers for sequenced loci.

File S1. Visualization of results from phylogeographical analysis of *Eliurus myoxinus* under a continuous diffusion model (Google Earth; KML file). Lines depict movement and polygons represent the 80% highest posterior density for ancestral locality. Line colours reflect the node heights from the consensus tree (from red to black; oldest to most recent) and polygon colours reflect the timing of dispersal (from light blue to dark blue; oldest to most recent).