Phylogeographic analysis of Goodman's mouse lemur reveals historical interconnectivity of Madagascar's Central Highlands and eastern rainforests

George P. Tiley[1,†], Marina B. Blanco[1,2], José M. Ralison[3], Rodin M. Rasoloarison[3,4], Amanda R. Stahlke[5], Paul A. Hohenlohe[5], Anne D. Yoder[1]

[1]Department of Biology, Duke University, Durham, NC 27708, USA

[2]Duke Lemur Center, Duke University, Durham, NC 27705, USA

[3]Département de Biologie Animale, Université d'Antananarivo, BP 906, Antananarivo 101, Madagascar

[4]Behavioral Ecology and Sociobiology Unit, German Primate Centre, 37077 Göttingen, Germany

[5]Institute for Bioinformatics and Evolutionary Studies, Department of Biological Sciences, University of Idaho, Moscow, ID 83844, USA

[†]Author for correspondence: george.tiley@duke.edu

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Abstract

The Central Highland Plateau of Madagascar is largely composed of savannah, interspersed with patches of closed-canopy forests. The origins of this landscape are now accepted as long preceding the arrival of humans in Madagascar, but whether savannah vegetation has remained static or expanded over time remains a topic of debate. By analyzing multiple populations of Goodman's mouse lemur (*Microcebus lehilahytsara*), a small-bodied nocturnal primate, we reconstruct its phylogeographic and demographic history to serve as a proximal estimate of the time at which the Central Highland and eastern forests became discontinuous. We applied coalescent methods to RADseq data to infer phylogeographic relationships, population structure, and migration corridors among sampling sites. Analyses revealed that forest fragmentation occurred rapidly and affected both the Central Highlands and eastern forests during a period of decreased precipitation near the last glacial maximum. There was clear structure between Central Highland populations versus those of the eastern rainforests, but there has also been historical migration across this presumed gene flow barrier. Findings support origins of the Central Highland savannah that predate human arrival and demonstrate that this natural landscape has long impacted the population dynamics of Goodman's mouse lemur.

Introduction

Madagascar is considered to be a biodiversity hotspot with high levels of species diversity and endemism [1]. Much of this diversity is at risk due to deforestation and forest fragmentation [2-4], which is due at least in part to human activity and agricultural practices [5,6]. But the timing of and degree to which humans have transformed these closely-canopy forests and have affected their connectivity has been a point of contention. Much of central Madagascar is covered with savannah, which was originally thought to be the product of fires from slash-and-burn agriculture and cattle farming [7,8]. The best available archeological evidence places humans in Madagascar between 2 thousand years ago (KYA) and 10 KYA [9-11]. This timeline does coincide with an increased frequency of fire based on charcoal records from sediment cores during the late to mid Holocene [12-14]. Pollen abundances from these same sediment cores imply the Central Highland vegetation transitioned from woody taxa to grasses at this time as well, although the transition likely began following Pleistocene glaciations [13,15] and similar shifts in plant composition were not limited to the Central Highlands [16,17].

Juxtaposed to the hypothesis of rapid human-mediated deforestation, ecological and phylogenetic studies of Madagascar's grasses suggest the Central Highland Savannah (CHS) originated well before the Holocene. Levels of endemism observed in the CHS are comparable to the putatively natural grasslands of Africa [18,19]. Although few C_4 lineages such as *Aristida* and *Loudetia* dominate the CHS [20-22], diversity among endemic C_4 grass clades are more consistent with global diversification of C_4 grasses during the Miocene [e.g 23] rather than many independent migration events [24]. Thus, we are left with a complex natural history where the CHS has ancient origins as a savannah, but has likely experienced deforestation and fragmentation associated with Pleistocene climate change and early human activity.

Elucidating the natural history of Madagascar's CHS is necessary for understanding patterns and the processes of speciation in many of Madagascar's endemic lineages. For example, biogeographic barriers to gene flow formed during historical climate change may explain recent radiations in many of Madagascar's endemic clades [25]. Barriers can be rivers, or open grasslands in the case of taxa restricted to forested environments, such as mouse lemurs [26]. Demographic studies of mouse lemurs may help develop a more accurate reconstruction of pre-human conditions in Madagascar and inform conservation research goals. A range of forest fragmentation hypotheses have been used to evaluate population declines in sifaka populations throughout the Holocene [27,28], but recognizing a more ancient fragmentation scenario into the Pleistocene would warrant consideration of additional historic demographic models and the need to explore data and methods more appropriate for older timescales [29]. Although losses in genetic diversity of endangered species putatively due to human action are observable [30-33], a better reconstruction of pre-human conditions can reveal if some populations were already vulnerable, especially given that some populations have been robust to recent habitat loss and fragmentation [34].

To evaluate hypotheses regarding scale of fragmentation for the Central Highlands, we leverage the unique distribution of a mouse lemur, *Microcebus lehilahytsara*. *M. lehilahytsara* is found in eastern montane forests but is considered a highland specialist that occurs in isolated forest fragments in the CHS [35,36]. It is the only mouse lemur species known to inhabit forest fragments in the CHS. The extended range of *M. lehilahytsara* may be due to the ability to enter a state of prolonged torpor [36]; capable of reducing their metabolism during extreme low temperatures in the CHS during the winter season. While prolonged torpor or hibernation is obligatory in *Cheirogaleus*, another member of the family Cheirogaleidae [37,38], mouse lemurs vary in their expression of daily torpor or hibernation, with some species reported to display daily torpor (less than 24 hours) exclusively and unable to deposit fat stores to sustain

88 prolonged torpor [38,39]. Recently, species-level analyses of molecular data that included *M.*
89 *lehilahytsara* from the CHS diverged from its sister species *M. mittermeieri* in the eastern
90 rainforests near 50 KYA [40]; the species status of *M. mittermeieri* has recently been called into
91 question [41]. Here, we sampled multiple populations of *M. lehilahytsara* from sites in the CHS
92 and eastern forests to further investigate the natural history of Madagascar's CHS with RADseq
93 data.
94
95 **Methods**
96 *Study sites and sampling*
97 *Microcebus lehilahytsara* individuals were captured at five field sites. Three of the locations,
98 from north to south, Riamalandy, Ambatovy, and Tsinjoarivo are mid-elevation rainforests,
99 situated along the eastern escarpment. Forests are fragmented along the corridor; although, the
100 distance between patches has been exacerbated by deforestation since the 1950s [4]. Annual
101 rainfall is ~1400 mm and daily temperatures vary between ~14 and 23 °C at Riamalandy, while
102 Tsinjoarivo and Ambatovy, receive 1700-2000 mm of rainfall with temperatures between 15 and
103 23 °C [42]. The other two locations, Ambohitantely and Ankafobe, are located within the Central
104 Highlands. Annual rainfall is around 1460 mm, and daily temperatures are between 12.5 and 23
105 °C [42]. Samples were collected between 2004 and 2016. Mouse lemurs were captured using
106 live Sherman traps and released back to the forest later the same day [see 36]. Detailed
107 descriptions of sampling sites are given in the Supplementary Methods (Supplementary
108 Material).
109
110 *RADseq library preparation and sequencing*
111 *Microcebus lehilahytsara* samples were gathered from existing collections and a combination of
112 genomic DNA from available tissue and previous whole-genome amplifications were used in
113 order to maximize sampling of these rare and vulnerable species. Library preparations for RAD

114 sequencing followed Ali et al. [43], using a single-digest protocol with the restriction enzyme
115 SbfI. A median insert size of 380 bp was selected for paired-end sequencing at the Duke
116 Sequencing Core on an Illumina Hi-Seq 4000 with an anticipated 15x coverage per individual.
117
118 *Assembly and orthology evaluation*
119 Data were assembled using STACKS v2.0b [44,45] using *de novo* methods. Final loci output by
120 STACKS were filtered such that at least three of the 5 sample geographic locations were
121 represented with at least half of the taxa present. RAD loci were supported by a minimum depth
122 of three reads; all other assembly and orthology details are the Supplementary Methods
123 (Supplementary Material).
124
125 *Phylogenetic analyses*
126 We estimated phylogenies of all *M. lehilahytsara* individuals or species trees of sampling sites
127 using four approaches: 1) concatenated ML analysis with RAxML v8.2.11 [46], 2) topological
128 invariants of quartets approximated with SVDquartets [47] implemented through PAUP* v4.0a
129 build 165 [48], 3) an implementation of the multispecies coalescent (MSC) model for SNP data,
130 SNAPP v.1.3.0 [49], through BEAST2 v2.4.8 [50], and 4) the MSC with joint gene tree
131 estimation using BPP v4.0 [51,52]. Phylogenetic hypothesis testing with RAxML and SNAPP
132 were performed with an approximately unbiased (AU) test [53] and Bayes factors respectively.
133 Complete details for phylogenetic analyses are in the Supplementary Methods (Supplementary
134 Material).
135
136 *Delimiting population structure and testing migration hypotheses*
137 To elucidate migration patterns between *M. lehilahytsara* populations in the CHS and eastern
138 forests, we developed competing migration hypotheses and tested models with Bayes factors.
139 We analyzed biallelic SNPs with STRUCTURE v. 2.3.4 [54], using the admixture model and

140 default priors (Supplementay Material). Pairwise fixation index (F_{ST}) were calculated between
141 populations in ARLEQUIN v. 3.5.2.2 [55] and an AMOVA analysis [56] was performed for
142 Central Highland versus eastern populations. Isolation-by-distance was investigated with a
143 Mantel test. Results were used to develop testable hypotheses that were evaluated with Bayes
144 factors for isolation-with-migration models in MIGRATE v4.2.14 [57].
145
146 Notably, we did not use the default uniform prior for nucleotide diversity (θ) estimates. Instead,
147 we used independent effective population size (N_e) estimates from three mouse lemur species
148 [30] to develop a prior distribution on θ (Supplementary Material). θ was rescaled N_e to using a
149 mutation rate of 1.64×10^{-8}, which is based on a direct germline estimate for mouse lemurs [58].
150 Two sets of analyses were conducted on phased RAD loci. First, we used all individuals binned
151 into either a CHS or eastern population. We were limited in the number of loci computationally
152 feasible for obtaining marginal likelihood estimates, and thus analyzed 100 jackknifed data sets
153 of 100 RAD loci. The second analysis attempted to differentiate between east-to-west migration
154 and west-to-east migration among Ambatovy, Ambohitantely, and Ankafobe, and did not allow
155 for missing data. The three-population analyses used four diploid individuals per population.
156
157 *Estimating divergence time of eastern and Central Highland mouse lemurs*
158 We used the MSC model for divergence time estimation among *M. lehilahytsara* lineages with
159 BPP v4.0 [51,52]. We fixed the topology recovered in the majority of analyses here and used
160 several lines of evidence to support rooting with Riamalandy (Supplementary Material),
161 including a mitochondrial phylogeny of *M. lehilahytsara* individuals and outgroups (Table S2). A
162 partitioned ML analysis and bootstrapping was conducted with PAUP* v4.0a build 163 [48].
163 Phased haploid sequences were used and at least two individuals per population were required
164 for each locus. We ran eight chains of 2500 samples and evaluated convergence. Final MSC
165 parameter estimates were based on combined chains. Coalescent branch lengths were

166 rescaled to absolute time using a germline mutation rate estimate [60] and generation time
167 estimates from wild [59] and captive [60] populations. Robustness of divergence times to prior
168 choice was also evaluated (Supplementary Material).
169
170 *Characterizing population size changes during Pleistocene glaciations*
171 We estimated change in population size over time with extended Bayesian skyline plots [61] in
172 BEAST2 [50]. For all sampling locations with four or more individuals, we jackknifed 100 RAD
173 loci eight times, with no missing data. Sequences were treated as phased and haploid. Each
174 jackknife replicate was run twice to check convergence. Large posteriors were required for
175 agreement on the number of change points and population size estimates between replicates;
176 each chain ran for 500 million generations after a 100 million generation burnin while sampling
177 every 10000 generations. Operators for increasing the sampling of change points followed
178 recommendations by Trucchi et al. [62]. We used linear piecewise reconstructions of population
179 size changes.
180
181 **Results**
182 *Circumscribing orthologous RAD loci*
183 All individuals included in our analyses had an average depth of coverage greater than 10x per
184 locus. In total, 92889 loci were recovered across all taxa with an average of 49201 loci per
185 individual (Table S1). On average, 31% of loci were shared between pairs of taxa (Figure S1).
186 The number of loci shared between individuals are largely not due to phylogenetic signal, as
187 there was only a slight negative correlation between evolutionary distance and the proportion of
188 shared loci (pearson's r = -0.19; p = 0.001). Rather, this pattern was driven by the number of
189 reads for each individual (pearson's r = 0.86; p = 3.54e-08). Only 54018 loci had at least one
190 biallelic SNP.
191

192 *Support for Central Highland and eastern Microcebus lehilahytsara*

193 All phylogenetic analyses recovered a well-supported split between *M. lehilahytsara* from
194 Central Highland localities (Ambohitantely and Ankafobe) and eastern forests (Riamalandy,
195 Ambatovy, and Tsinjoarivo) (Figure 1). But the relationships among eastern sampling locations
196 was contentious across analyses. The ML, SNAPP, and BPP analyses recovered Ambatovy as
197 sister to Tsinjoarivo (H_{TA}; Figures S2- S5); although, the posterior probability from SNAPP was
198 weak. SVDquartets strongly conflicted the H_{TA} topology, favouring Riamalandy sister to
199 Tsinjoarivo (H_{RT}) when analyzing all variable sites (Figure S6) or complete RAD loci (Figure S7).
200 However, the H_{RT} topology was sensitive to the underlying data. Analyses of biallelic SNPs
201 alone with SVDquartets recovered a third well-supported topology (H_{RA}; Figure S8). Although in
202 one out of ten resampled datasets, when treating all individuals as independent lineages,
203 Ambatovy was not recovered as a monophyletic clade. Topological hypothesis testing
204 differentiated among the three recovered phylogenetic hypotheses: H_{TA}, H_{RT}, and H_{RA}.
205
206 AU tests rejected H_{RT}, but could not reject H_{RA} despite H_{TA} being present in the majority of ML
207 bootstrap trees (Figure S9). Bayes factors from SNAPP provided insights into the prevalence of
208 both H_{RA} and H_{TA} hypotheses across analyses. SNAPP supported lumping Riamalandy and
209 Ambatovy together as opposed to lumping Ambatovy and Tsinjoarivo (Table 1). This result is
210 surprising, considering H_{TA} was the maximum clade credibility tree from the posterior sample in
211 the unconstrained analyses (Figures S3 and S4). All phylogenetic evidence suggested a shared
212 common ancestor for Ambatovy and Tsinjoarivo, but Tsinjoarivo may be more evolutionarily
213 distant than expected. Phylogenetic analyses showed strong support for both CHS and eastern
214 clades, and at least no very recent gene flow as evident by monophyly of our five sampling
215 locations. However, such analyses do not rule out historical gene flow between the CHS and
216 eastern forests.
217

Migration between eastern and Central Highland forests

There was admixture between eastern and Central Highland *M. lehilahytsara* as well as population structure within the eastern forests that mirrored SNAPP Bayes factors (Figure 1). STRUCTURE analyses recovered an optimal k = 3 in 19 out of 20 jackknifed datasets (Figures S10-S12). The remaining replicate recovered an optimal k = 2 (Figure S11). All analyses supported a clustering of Central Highland and eastern *M. lehilahytsara*, with some degree of admixture between the two. The Ambohitantely site largely clusters with Ankafobe, but individuals may share between 10% and 40% of their alleles with Ambatovy and Riamalandy. Tsinjoarivo often forms its own unique cluster, except in the one jackknife replicate where k = 2.

Admixture between Central Highland and eastern *M. lehilahytsara* was also supported by analyses of genetic variation with F_{ST} statistics. Pairwise F_{ST} revealed similar levels of genetic divergence between Ambatovy and Ambohitantely to that of Ambatovy and Tsinjoarivo, despite the more contiguous forest among eastern sampling locations (Table S3). The geographic structure between Central Highland and eastern *M. lehilahytsara* explains a small fraction of genetic variation, with most variation explained within populations (Table S4). A Mantel test revealed little evidence for isolation-by-distance (pearson's r = 0.09; p = 0.39), suggesting barriers to gene flow aside distance alone (Figure S13).

Bayes factors were computed for different isolation-with-migration models to further investigate the patterns of admixture observed between the Ambatovy, Ambohitantely, and Ankafobe genetic clusters. The *n-islands* model was unambiguously the best model for both analyses that included all individuals modeled as two populations (Figure S14; Table S5) or three populations (Figure 2). There were no large discrepancies in the estimated migration rates; however, the selected model suggested a lower N_e in Ankafobe and Ambohitantely compared to Ambatovy. Assuming a mutation rate of 1.64×10^{-8}, MIGRATE estimated contemporary population sizes to

244 be 12652 for Ankafobe and Ambohitantely, and 17226 for Ambatovy. Their N_e estimates,
245 although influenced by the prior, are not driven by the prior alone (Figures S15-S17).
246 Additionally, a higher N_e in Ambatovy is supported across all models regardless of the migration
247 and divergence scenarios (Figure 2). Because of Ambatovy's higher effective population size,
248 the number of migrants leaving towards Ambohitantely or Ankafobe per generation is
249 approximately 1.2, compared to 0.8 effective migrants per generation leaving Ambohitantely or
250 Ankafobe towards Ambatovy.
251
252 *Dating Population Divergences*
253 Using the H_{TA} topology supported by Bayes factor analyses, divergence times were estimated
254 using the MSC model. The topology was rooted with Riamalandy based on the analysis of
255 mitochondrial data from *Microcebus lehilahytsara* and outgroup taxa (Figure S18) and inference
256 of clock-like trees from BPP (Figure 3). Most MSC parameters converged across eight chains
257 with potential scale reduction factors approaching 1 (Table S6). A few parameters still retained
258 high variation between chains for both θ and τ, but this discrepancy was largely due to one or
259 two chains with inefficient mixing for different prior combinations (Figure S19-S26). Divergence
260 times were not strongly influenced by the priors (Figure 3) and implied that divergence between
261 the CHS and eastern forest, as well as the forest patches within them, occurred almost
262 simultaneously. Absolute estimates inferred that divergence among our sampled lineages
263 occurred near 31 KYA (Table 2).
264
265 *Population declines in the CHS*
266 Population sizes experienced some slight contractions near 25 KYA (Figure 4) that roughly
267 coincide with the divergence of sampling locations (Table 2). Analyses also suggested an uptick
268 in population size through the Holocene until the present. Ambatovy had consistently higher N_e
269 estimates across jackknife replicates. Ankafobe had the lowest N_e estimates. Intermediate N_e

270 estimates were observed for individuals at Ambohitantely and Tsinjoarivo, despite that
271 Ambohitantely is a more fragmented habitat in the CHS. There is high uncertainty in N_e based
272 on comparisons across jackknife replicates (Figure S27), but individual analyses tended to
273 converge (Figures S28-S31).
274
275 **Discussion**
276 *Population structure of M. lehilahytsara predates human colonization of Madagascar*
277 Our results provide additional evidence in favour of the now well-accepted view that
278 Madagascar's Central Highlands are an ancient mosaic of grassland and wooded savannah
279 [18,19], while elucidating the evolution of a uniquely distributed mouse lemur. Phylogenetic
280 analyses revealed an unambiguous split between CHS and eastern forest sampling locations
281 (Figure 1; Figures S2-S8), which was also supported by analyses of genetic structure (Figure 1;
282 Figure S12). Three competing topological hypotheses were uncovered concerning the
283 relationships among our eastern rainforest sampling locations, but the northern-most sampling
284 location, Riamalandy, is likely sister to more southern sites. Phylogenetic evidence suggested
285 that *M. lehilahytsara* dispersed as a ring around the higher elevation regions of the CHS, and
286 that the CHS acted to some extent as a barrier to gene flow during a period of vegetational
287 change and aridification through the LGM [16,63]. The northern end of this barrier may have
288 remained permeable since watersheds are hypothesized to have buffered the region from
289 aridification more evident in the southern extent of the CHS [25,41,63,64].
290
291 Tsinjoarivo is a population isolated by biogeographic barriers from Ambatovy and Riamalandy.
292 *M. lehilahytsara* from Tsinjoarivo was previously recognized as genetically distinct based on a
293 study of few mitochondrial and nuclear loci, although Bayes factors did not indicate Tsinjoarivo
294 to be an independent lineage under the MSC model compared to other *M. lehilahytsara* [65].
295 The findings of Hotaling et al. [65] were recapitulated here, with little to no admixture between

296 Tsinjoarivo and Ambatovy or Riamalandy (Figure 1; Figure S12). The isolation of Tsinjoarivo
297 from other eastern sampling locations is plausible, because it is separated by the Mangoro
298 River. Our BPP analyses date the separation of Tsinjoarivo from Ambatovy near 27 KYA, which
299 again coincides with a time of cool and dry conditions during the LGM [66,67]. Interpreting
300 absolute dates is tenuous at best, especially given these ages contradict the hypothesis that
301 riverine barriers formed during times of increased precipitation [25], but Madagascar is
302 ecologically complex and there is likely no single causal factor that shaped the distribution of *M.*
303 *lehilahytsara*. Our divergence time estimates may be indicative of more widespread forest
304 fragmentation and vegetative turnover linked to paleoclimatic oscillations.
305
306 *The mosaic of grassland and wooded savannah was not impermeable by mouse lemurs*
307 Three lines of evidence support that *M. lehilahytsara* had a large ancestral distribution that
308 covered both the CHS and eastern rainforest escarpment, and that the two were
309 interconnected. This large ancestral distribution underwent some contraction and developed
310 population substructure during a period coincident with the LGM. One is the successive
311 divergence times of the CHS and eastern forest populations (Figure 3 and Table 2). The
312 divergences between mouse lemurs from Ankafobe and Ambohitantely, Ambatovy and
313 Tsinjoarivo, and their common ancestors occurred near-simultaneously, and this result is not an
314 artifact of prior choice (Figures S19-S25). The pattern of divergence and population structure
315 formed by reduced gene flow is reflected in θ estimates from BPP. MSC estimates suggested a
316 large population size proceeding divergence events (Table 2), which likely explains why a larger
317 prior on θ resulted in a higher posterior probability and reduced uncertainty in the divergence
318 time for the MRCA of CHS and eastern *M. lehilahytsara*.
319

320 The strong preference for an *n-islands* model from MIGRATE (Figure 2; Figure S13; Table S5)
321 also supported the scenario of populations that diverged simultaneously from a single larger
322 distribution. There was little discernable direction in gene flow, which may explain why models
323 that incorporated divergence did not perform as well as the *n-islands* model. The migration rates
324 inferred for our data are arguably high, between 0.8 and 1.2 migrants exchanged per
325 generation. Such migration rates could explain the admixture in Ambohitantely (Figure 1; Figure
326 S12) and low F_{ST}, despite the many generations that must have passed (Tables S3 and S4).
327 Although, parameter identifiability is a concern for our parameter-rich models [57], especially
328 given that our short RAD loci individually contain very few informative coalescent events
329 (Figures S15-S17). That caveat in mind, the *n-islands* model agreed with the MSC results in that
330 eastern *M. lehilahytsara* has a higher N_e than *M. lehilahytsara* in the CHS. Analyses of genetic
331 variation with F_{ST} also suggested that the mosaic of grassland and wooded savannah in the
332 Central Highlands at least does not pose a more significant barrier to gene flow than the
333 Mangoro river (Table S3). Even with its close proximity to Ambohitantely and historical gene
334 flow across the Central Highlands, Ankafobe is genetically distant compared to eastern forest
335 populations (Figure S13). The increased genetic distance is possibly explained by the small
336 amount of suitable habitat (less than 1 Km^2) and inbreeding.
337
338 The third piece of evidence is that analyses of population size change over time share some
339 patterns with the MSC and isolation-with-migration model analyses (Figure 4; Figures S27-S31).
340 We found similar demographic trends among the four populations analyzed with EBSP
341 methods, and all displayed a slight population decrease before the LGM followed by a recovery.
342 These patterns roughly reflect N_e estimates from MSC analyses and the similar demographic
343 histories among populations would also agree with our hypothesis that *M. lehilahystara* had a
344 single widespread population that experienced fragmentation. Although, there are large

345 disparities in N_e estimates between EBSP and other methods, which raises concern for
346 interpreting absolute population size values from molecular data.
347
348 *Limitations of RADseq and demographic interpretations*
349 Scrutiny is needed in reconciling our results from various coalescent analyses, as we are
350 analyzing short RAD loci that individually carry little information about demographic history
351 (Figures S14-S17). And notably, no migration is assumed after divergence in the MSC model
352 implemented here; however, we expect estimation errors from unaccounted migration to make
353 divergence times older [40,68,69]. Therefore, the processes that shaped existing *M.*
354 *lehilahytsara* population structure must have occurred before human arrival and mid-Holocene
355 droughts. Although charcoal records [12,15], stratigraphic pollen occurrence [13,70], and
356 megafauna subfossils [11] all point to human-mediated large-scale ecological changes in the
357 Central Highlands [14], our analyses implied that *M. lehilahytsara* population divergences
358 coincide with Pleistocene climate change near the LGM. A similar narrative has been recovered
359 in other analyses of lemur populations [27,28] and an endemic olive tree [71]. Therefore, these
360 analyses represent first steps in revealing the demographic history of *M. lehilahytsara*, while
361 also contributing the evolving natural history of Madagascar's Central Highlands [18,19,22,42].
362
363 *Making conservation biology predictions from models of molecular evolution*
364 It is difficult to evaluate our N_e estimates in the context of conservation biology since comparable
365 numbers to other mouse lemur species are not available, and here we provided a range of N_e
366 estimates that are sensitive to model (i.e. isolation-with-migration, MSC, Extended Bayesian
367 Skyline) choice. The most recent IUCN evaluation of *M. lehilahytsara* considers the species to
368 be vulnerable [72] while geographic neighbors in the eastern rainforest are considered
369 endangered [31-33]. If *M. lehilahytsara* has adaptive traits that allowed the species to expand its
370 range into the CHS while related species were restricted to lowland forests, *M. lehilahytsara*

may have been more robust to Pleistocene climate change and even more recent anthropogenic disturbances. We hypothesize that torpor in *M. lehilahytsara* [36] has allowed its increased range, compared to other mouse lemurs. Although, *M. lehilahytsara* populations in the CHS have maintained smaller population sizes compared to those in the eastern forests (Table 2; Figure 4; Figure S27). These differences in population size are expected, given that the connectivity and area of available habitat is reduced. For example, the Ankafobe forest patch is less than 1 Km^2. CHS areas are also subjected to frequent wildfires, which easily spread across secondary grasslands and destroy isolated forest fragments [42].

M. lehilahytsara in the CHS is not simply a sink population from an eastern forest source. CHS populations have been evolutionarily independent from the eastern escarpment for approximately 5000 generations considering the lower end of the divergence time 95% HPD intervals for nodes A and B (Table 2). After divergence, CHS populations still exchanged migrants (Figure 2) with the eastern forests despite the historic mosaic of grassland and wooded savannah [22]. The geographic isolation with occasional migration to and from the east may have been important for sustaining large N_e despite the relatively small habitable areas. Our best migration model estimated an N_e of 13000 for both Ankafobe and Ambohitantely and MSC estimates are 19000 and 31000 respectively. There are discrepancies in the absolute N_e, but our results suggest that N_e has remained sufficiently large such that the strength of selection was strong enough to overcome fixation of deleterious alleles by drift alone [73]. The sustained lower N_e in the CHS compared to the eastern forests does imply a negative consequence of habitat loss and fragmentation, and that a loss of CHS populations could put *M. lehilahytsara* at risk, similar to closely related species in the eastern rainforest [40].

Mouse lemurs are iconic of Madagascar's endemism and diversity, but they can also be a valuable system for reconstructing the island's complex natural history. Molecular data from *M.*

lehilahytsara has bracketed population divergences, which we interpret as at least some forest loss and fragmentation in the CHS and eastern rainforest escarpment, to late Pleistocene. We interpret the phylogenetic hypothesis, demographic inference, and dating as rapid fragmentation during the LGM. Additional comparative work among populations of multiple species may further refine our reconstruction of the CHS biogeography while also informing the demographic history of mouse lemurs and their conservation.

Ethics

Research was conducted under permits N 0233/07/MINENV.EF/SG/DGEF/DPSAP/SSE, N 229/14/MEEF/SG/DGF/DCB.SAP/SCB, and N225/15/MEEMF/SG/DGF/DAPT/SCBT issued by the Ministry of Environment, Water and Forests of the Malagasy Government.

Data Accessibility

New sequence data is available through NCBI BioProject PRJNA560399. Individual SRA identifiers are in Table S7. Alignments and configuration files are available from the Dryad Digital Repository: XXXX

Author Contributions

GPT and ADY conceived the study. MBB, JMR, and RMR performed the fieldwork. ARS and PAH constructed RADseq libraries. GPT performed analyses. GPT, MBB, and ADY wrote the manuscript.

Competing Interests

The authors declare no competing interests.

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700 **Tables**

Table 1- Marginal likelihood estimates and model selection with SNAPP

Steps	Topological Hypothesis	Marginal Likelihood	Bayes Factor†	Model Probability
20	RA	-41399.18	0.00	1
	TA	-41847.70	-897.05	1.61×10^{-195}
	RT	-41689.27	-580.19	1.03×10^{-126}
40	RA	-41531.26	0.00	1
	TA	-41848.94	-635.35	1.08×10^{-138}
	RT	-42060.55	-1058.58	1.36×10^{-230}
60	RA	-41328.81	0.00	1
	TA	-41618.87	-580.12	1.07×10^{-126}
	RT	-42057.39	-1457.16	0

†Bayes factors computed by marginal log-likelihoods with respect to the best model as $2 * (\ln L_i - \ln L_{Max})$

701

Table 2 - Absolute divergence times and population sizes estimated† from BPP.

Node	N_e (x 10^3)	95% HPD	Divergence time (kya)	95% HPD
Ankafobe	19.2	17.3, 25.7	-	-
Ambohitantely	31.2	27.1, 40.9	-	-
Tsinjoarivo	23.1	14.0, 26.1	-	-
Ambatovy	55	33.5, 63.0	-	-
Riamalandy	204.5	193.4, 216.0	-	-
A	60.2	10.6, 201.3	26.7	15.9,30.4
B	22	15.1, 34.2	19	16.9, 25.2
C	875.1	776.9, 955.0	31	25.4, 42.7
D	108.3	106.6, 110.1	194.1	189.0, 198.8

†Estimates are based on combined posterior for priors $\theta = 0$ and $\tau = 1$.

702

703 **Figure Legends**

704 **Figure 1 – Well-supported phylogenetic lineages of *Microcebus lehilahytsara* reflect**
705 **genetic structure.** The maximum clade credibility tree from SNAPP. Node support represents
706 ML bootstrap, SVDquartets bootstrap, and posterior probabilities from SNAPP respectively. An
707 asterisk represents 100% support. Branch lengths are median coalescent units with 95%
708 highest posterior densities from SNAPP. STRUCTURE results are shown for an optimal k = 3.
709 GPS coordinates for each sampling site are shown on the map.

710

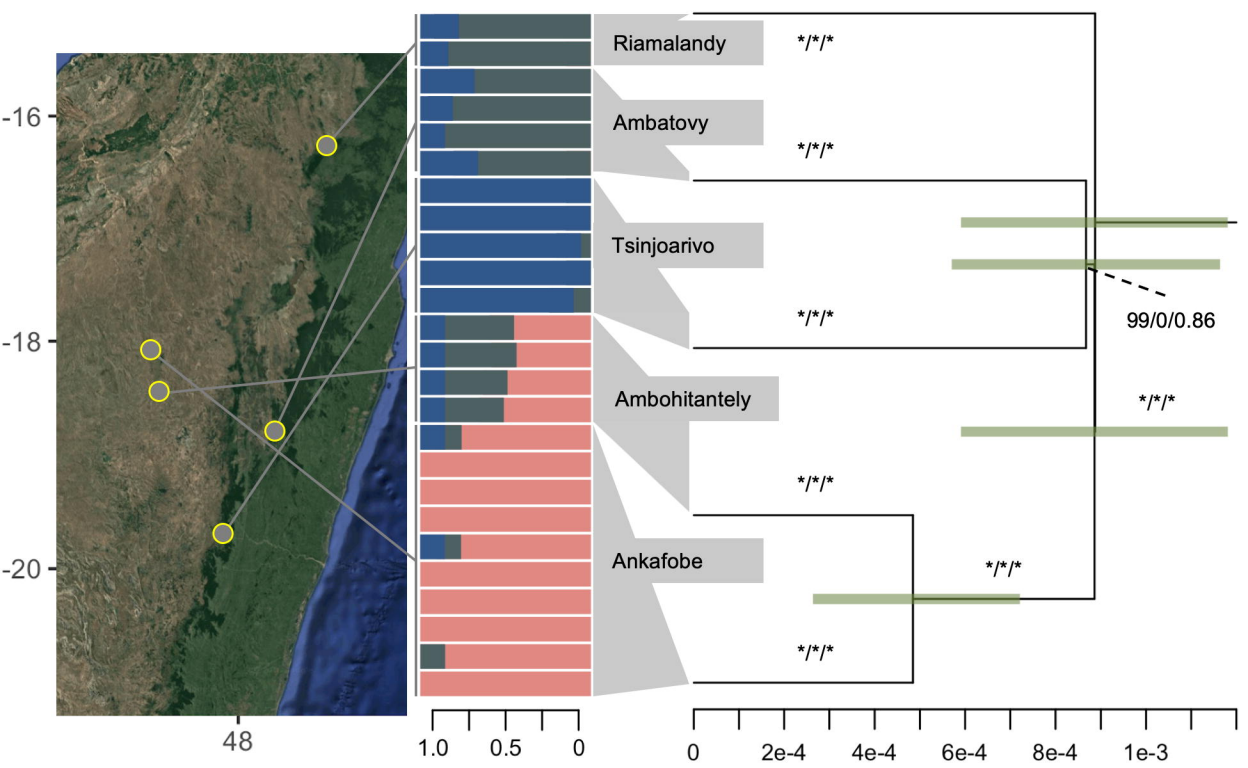
711 **Figure 2 – Migration models reveal gene flow between the Central Highlands and eastern**
712 **forests.** Bayes factors were used to test migration hypotheses between the eastern rainforest,
713 Ambatovy (grey), and two fragmented forest patches in the Central Highlands, Ambohitantely
714 (pink and grey) and Ankafobe (pink). These three locations were selected because of admixture
715 detected in Ambatovy based on STRUCTURE analyses. θ parameters are scaled as $4N_e\mu$ and
716 mutation-scaled migration parameters are shown near arrows. Divergence times for relevant
717 models are in coalescent units.

718

719 **Figure 3 – Divergence time estimates with BPP.** Posterior distributions of MSC parameters
720 for the combined posterior of prior choice 01. Branch lengths are in coalescent units and error
721 bars represent 95% highest posterior density intervals around the median node height. Violin
722 plots are the posterior distributions of node heights for prior choices 1 though 4 to show that
723 while divergence time estimates are sensitive to prior choice, our biological conclusions are not.

724

725 **Figure 4 – Population size change over time at sampling locations.** Posterior distributions
726 of θ at discretized bins of τ determined by the number of change points. Only Jackknife replicate
727 one is shown for each population. The blue box shows the duration of the LGM.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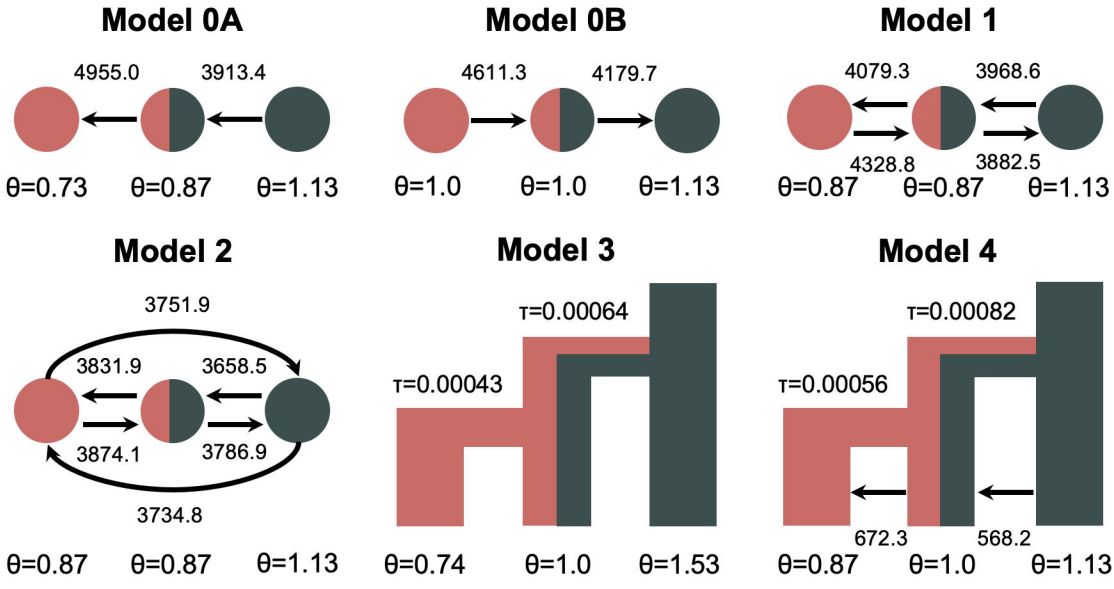
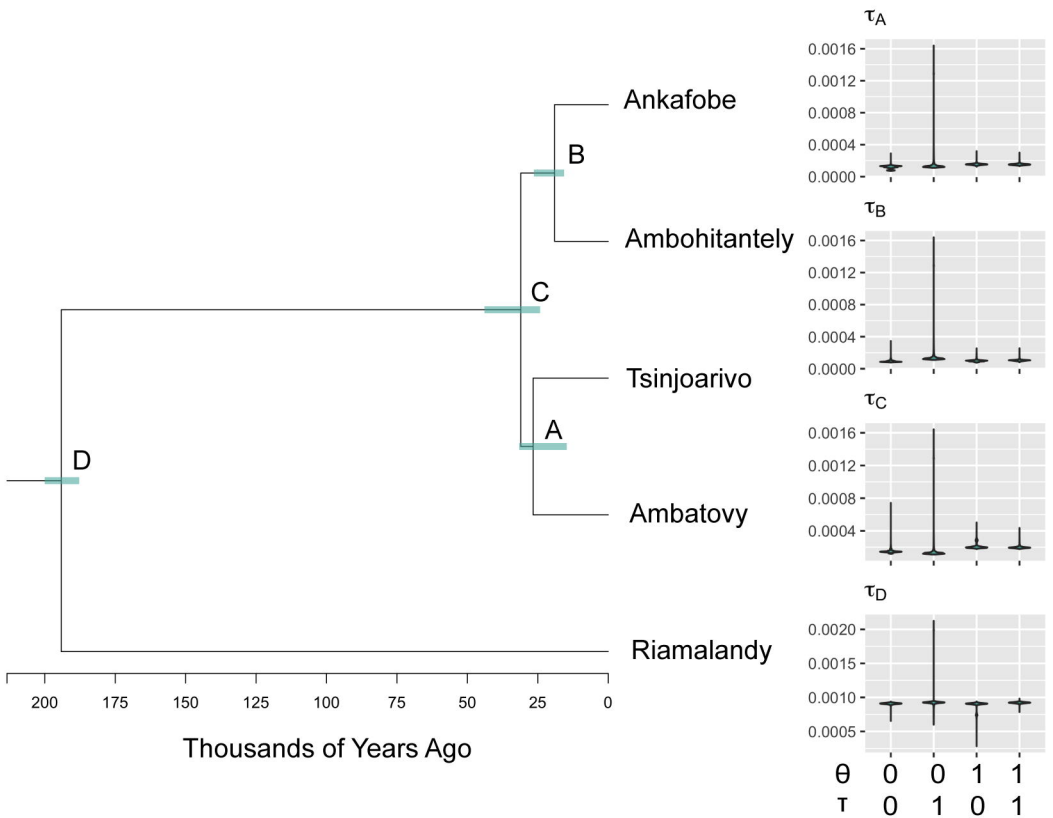


Model	Marginal Likelihood	Bayes Factor*	Model Probability
0A – East to west	-408766.23	NA	0
0B – West to east	-408169.12	1194.22	0
1 – Stepping stone	-404252.47	9027.52	0
2 – N-island	-402558.14	12416.18	1
3 – Divergence and isolation	-415036.4	-12540.34	0
4 – Divergence and migration	-407777.86	1976.74	0

*Bayes factors computed with natural logarithms of marginal likelihoods with respect to model 0A as 2 * ($\ln L_i - \ln L_{0A}$)
†θ values are presented as 10^{-3}


Ankafobe
Ambohitantely
Tsinjoarivo
Ambatovy
Riamalandy
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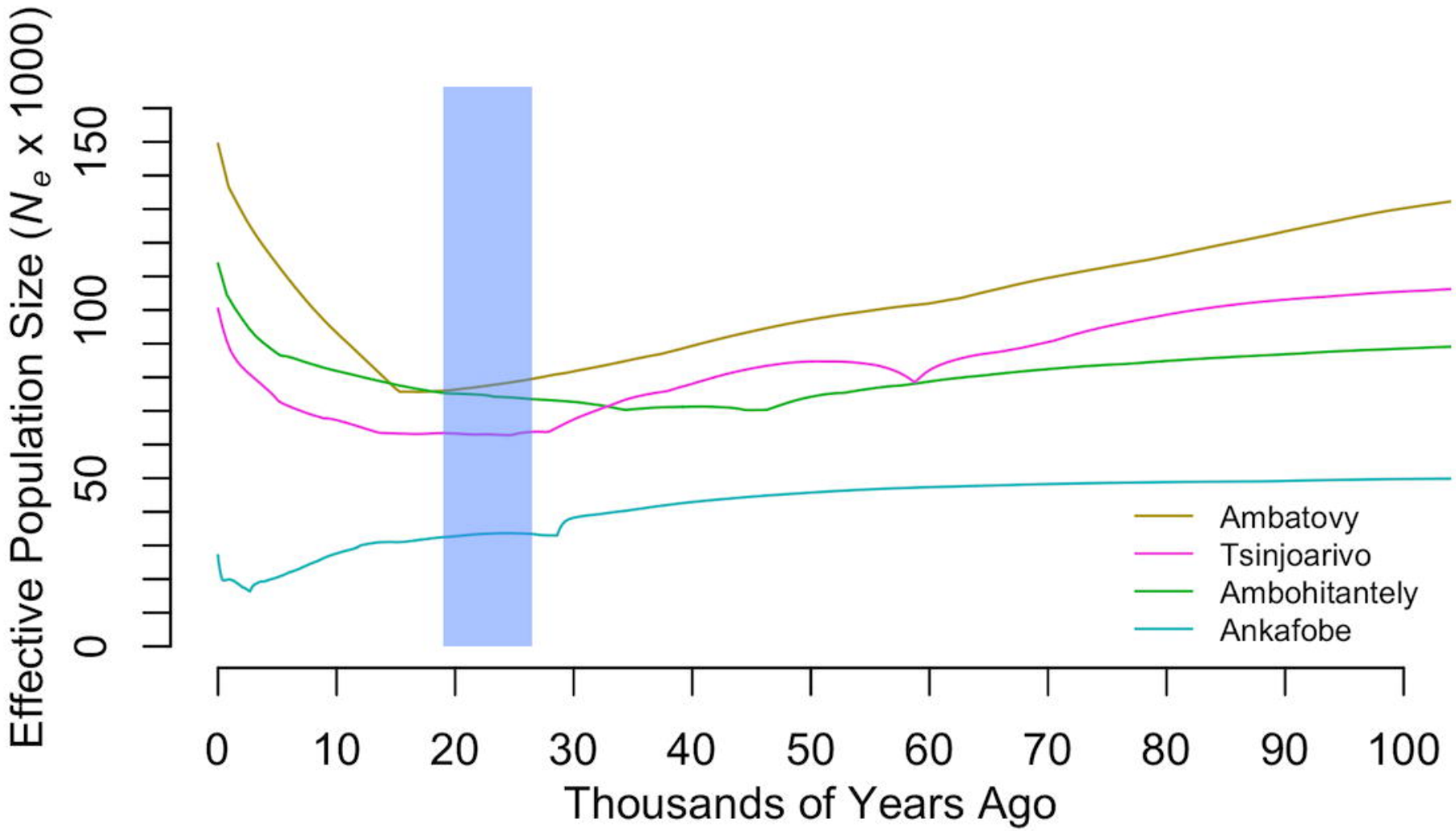
Effective Population Size (N_e x 1000)
0
50
100
150
0
10
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30
40
50
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70
80
90
100
Thousands of Years Ago
Ambatovy
Tsinjoarivo
Ambohitantely
Ankafobe