Landscape genetics of an endangered lemur (*Propithecus tattersalli*) within its entire fragmented range

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Abstract

Habitat fragmentation may strongly reduce individuals' dispersal among resource patches and hence influence population distribution and persistence. We studied the impact of landscape heterogeneity on the dispersal of the golden-crowned sifaka (*Propithecus tattersalli*), an endangered social lemur species living in a restricted and highly fragmented landscape. We combined spatial analysis and population genetics methods to describe population units and identify the environmental factors which best predict the rates and patterns of genetic differentiation within and between populations. We used non-invasive methods to genotype 230 individuals at 13 microsatellites in all the main forest fragments of its entire distribution area. Our analyses suggest that the Manankolana River and geographical distance are the primary structuring factors, while a national road crossing the region does not seem to impede gene flow. Altogether, our results are in agreement with a limited influence of forest habitat connectivity on gene flow patterns (except for North of the species' range), suggesting that dispersal is still possible today among most forest patches for this species. Within forest patches, we find that dispersal is mainly among neighbouring social groups, hence confirming previous behavioural observations.

Keywords: causal modelling, dispersal, habitat fragmentation, lemur, primate, *Propithecus tattersalli*, spatial autocorrelation

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Introduction

Habitat loss and fragmentation are among the greatest threats to biodiversity worldwide (Myers *et al.* 2000; Ganzhorn *et al.* 2003), particularly so for tropical ecosystems (Sala *et al.* 2000; Foley *et al.* 2005; Laurance & Peres 2006). They reduce habitat area, quality and connectivity for many wild species with potentially dramatic consequences for population viability (Andrén 1994; Fahrig 2003; Walker *et al.* 2008). In particular, it can lead to a rapid decline of population size and genetic variation within habitat fragments (Crnokrak & Roff 1999; Frank-

ham *et al.* 2002; Frankham 2005; Olivieri *et al.* 2008; Craul *et al.* 2009). It is believed that, in the medium or long term, it can lead to an increase in the level of inbreeding, the erosion of species' evolutionary potential, and an increase in the risk of extinction (Saccheri *et al.* 1998; Keller & Waller 2002; Johansson *et al.* 2007). Local extinctions of small fragmented populations are relatively common (Fahrig & Merriam 1994; Kindlmann & Burel 2008). Consequently, persistence of species strongly depends on the ability of dispersing individuals to move across heterogeneous landscapes to re-colonize empty patches and maintain the functional connections among remaining populations. One aim of conservation is to identify the most suitable areas in the matrix (i.e. putative corridors) to restore and maintain habitat connectivity among populations (Taylor & Fahrig 2006; Cushman

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et al. 2009). Consideration of the matrix heterogeneity and the level of permeability of its components to dispersal movements are thus key issues for the management of endangered species with patchy distributions (Taylor *et al.* 2006; Lindenmayer *et al.* 2008).

The effects of environmental features on individual dispersal are difficult to assess using mark-recapture or radio-tracking approaches because a relatively large number of individuals have to be captured and monitored (Vignieri 2005; Cushman 2006; Broquet *et al.* 2006). Furthermore, these direct methods are time consuming, often invasive and thus not generally suitable for the study of threatened species. Genetic data, which can be obtained using non-invasive sampling methods, offer an attractive alternative approach to infer patterns of dispersal. Genetic methods can highlight the natural and anthropogenic factors shaping migration patterns between habitat patches (Holderegger & Wagner 2008; Balkenhol *et al.* 2009). The genetic study of functional habitat connectivity (i.e. the 'degree to which the landscape impedes or facilitates movements among resources patches', Taylor *et al.* 2006) has received growing interest principally because of the improvement of methodological and technical tools for assessing habitat heterogeneity (e.g. high-resolution satellite images, Geographic Information Systems). This has favoured the emergence of 'Landscape genetics' (Manel *et al.* 2003), a new interdisciplinary research area combining population genetics and landscape ecology for quantifying the effect of landscape composition, configuration and matrix quality on spatial patterns in neutral and adaptive genetic variation (Storfer *et al.* 2007; Holdegger & Wagner 2008). For instance, several recent landscape genetic studies used previously existing data with new statistical tools to uncover the spatial genetic discontinuities corresponding to anthropogenic barriers such as roads or canals (Epps *et al.* 2005; Coulon *et al.* 2006; Perez-Barberia *et al.* 2008; Radespiel, *et al.* 2008b). Others characterized environmental features that facilitate migration such as riparian areas in jumping mice (Vignieri 2005), high-slope areas in bighorn sheep (Epps *et al.* 2007) or eucalyptus forests in small marsupials (Banks *et al.* 2005).

In this study, we investigated the impact of forest fragmentation on patterns of genetic differentiation in the golden-crowned sifaka (*Propithecus tattersalli*). This relatively large, social species of lemur is found only in the Daraina region of north-eastern Madagascar (Fig. 1) and has one of the most limited ranges of any lemur (2450 km^2) (Mittermeier *et al.* 2006). The island of Madagascar is among the most important biodiversity hotspot and conservation priority areas in the world (Myers *et al.* 2000; Ganzhorn *et al.* 2003). However, deforestation and habitat fragmentation have increased precipitously during the 20th century, primarily due to human pressure

and particularly in the evergreen forests along the eastern coast and the deciduous forests of western Madagascar (Green & Sussman 1990; Smith 1997; Harper *et al.* 2007). Most previous studies have focused on the impact of habitat fragmentation on species richness (Ganzhorn *et al.* 2003; Scott *et al.* 2006; Dunham *et al.* 2008; Allnutt *et al.* 2008) or on the distribution of species among and within patches (Lehman *et al.* 2006; Irwin 2007; Quéméré *et al.* 2010). However, for Malagasy species, the genetic consequences of habitat fragmentation have been poorly studied (but see Olivieri *et al.* 2008; Radespiel *et al.* 2008a,b Craul *et al.* 2009 for recent exceptions). The Daraina region, where *P. tattersalli* lives, is expected to suffer from significant disturbances in the near future and was identified as a high-priority area for biodiversity conservation (Ganzhorn *et al.* 1999; ZICOMA, 1999). While we have shown that the population densities were higher than previously thought (Quéméré *et al.* 2010), recent political instability has led to a renewed level of hunting and poaching. Thus, to facilitate conservation in this species, studies focused on identifying the natural and anthropogenic factors influencing patterns of dispersal and levels of genetic diversity are needed.

To characterize the *P. tattersalli* spatial genetic structure, we first combined field observations and remote sensing data within a Geographic Information System (GIS) to provide an a priori definition of the landscape heterogeneity in Daraina area. Specifically, we assessed (i) the extent of forest habitat and structural connectivity and (ii) environmental features such as rivers, roads or riparian corridors that may impede or facilitate individual dispersal. To obtain the genetic data we used a non-invasive sampling strategy (Taberlet *et al.* 1997; Kohn *et al.* 1999) to genotype 230 individuals from the main forest patches of the golden-crowed sifaka range using 13 microsatellites. We first assessed the level of genetic diversity across all individuals sampled and then within each of the main inhabited forest patches. To build a species-specific model of habitat suitability (i.e. a posteriori definition of the landscape reflecting the golden-crowned sifaka behavioural response in terms of distribution and dispersal), we employed two individual-based approaches: (i) the commonly used Bayesian clustering method STRUCTURE (Pritchard *et al.* 2000; Falush *et al.* 2003), which identifies panmictic subpopulation units (i.e. clusters) delineated by discrete boundaries and (ii) a causal modelling approach based on resemblance matrices (Legendre 1993; Cushman *et al.* 2006) which evaluates the ability of alternative hypotheses to identify a landscape model (i.e. a combination of environmental factors) that predicts rates and patterns of gene flow. We compare the results from the two approaches and discuss their relevance for *P. tattersalli* conservation strategies.
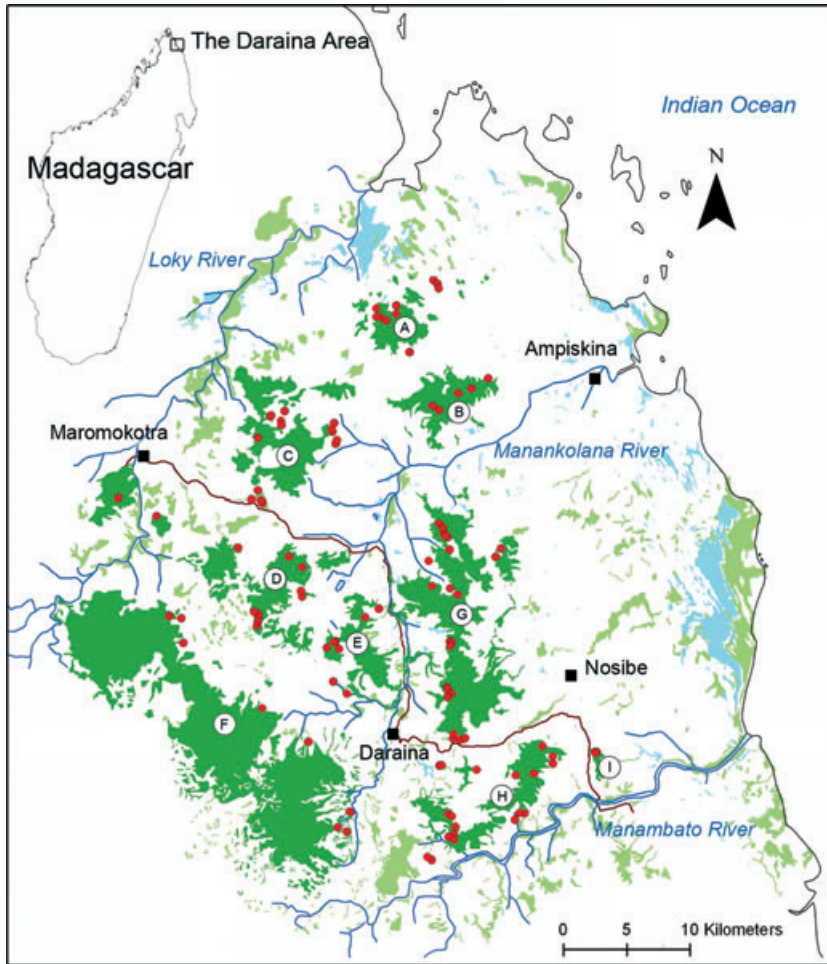
Fig. 1 Sampling distribution of golden-crowned sifakas. Distribution range of *Propithecus tattersalli* in the Daraina area. The *green* colour represents the forest fragments where we detected *P. tattersalli* individuals. *Red circles* correspond to the location of the sampled social groups. The commune chieftowns are represented by *black squares*. The national road is represented by a *brown line* and the main rivers by *blue lines*. The correspondence between *circled letters* and forest fragment complex name is indicated in Table 1.

Methods

Study area and landscape structure characterization

To capture the landscape heterogeneity in the Daraina region and differentiate forest and non-forest vegetation areas, we conducted a landscape classification using a LandSat Thematic Mapper imagery acquired in 2002 and the maximum likelihood supervised method, implemented in the software ENVI 4.1. The accuracy of the classification was checked in the field and using SPOT imagery. The forest habitat in the Daraina region is highly fragmented, covering about 17% of the total area (44,000 ha) and with only 6% of the patches larger than 1000 ha ($n = 9$) and 20% larger than 100 ha ($n = 28$) (Fig. 1). Forest patches (mainly lowland dry forests) are surrounded by a heterogeneous matrix of riparian gallery forests, agricultural areas, zebu cattle grazing pastures, human settlements and by large grasslands and dry scrubs. Some of the forest patches were included in 2005 in the Station Forestière à Usages Multiples—'Multiple Usage Forest Station' (SFUM) Loky-Manambato managed by the Malagasy NGO

Fanamby, in order to minimize direct anthropogenic pressures such as intensive wood and mining exploitation. In practice, it appears that golden-crowned sifaka populations are present both within and outside the SFUM (Vargas *et al.* 2002; Quéméré *et al.* 2010). Finally, it is important to note that the Daraina region, delineated by the Loky and the Manambato rivers, is crossed by a third large river (the Manankolana River) flowing south to northeast and by the unpaved national road RN5A running southeast to northwest (Fig. 1).

Sampling strategy and DNA analysis

We conducted a non-invasive hierarchical sampling design including social groups from across the species range. Faecal samples were collected in all the main forest patches within the golden-crowned sifaka's distribution (Fig. 1). Within the larger patches, we also collected samples from several valleys, and within these valleys several neighbouring social groups were sampled to capture genetic variation at multiple spatial scales. We collected faecal pellets for genetic analyses during two field missions in 2006 and 2008. The data

from 292 putative individuals belonging to 107 social groups are analysed here. All the samples were obtained immediately after defecation as described in Quéméré *et al.* (2009), and their geographical coordinates were assigned at the faeces collection point. DNA extraction was performed following the 2CTAB/PCI protocol adapted from Vallet *et al.* (2008). The extracted DNA was amplified using a set of 13 microsatellite loci isolated for *Propithecus tattersalli* (8 loci) and for the closely related species *P. coquereli* (5 loci) (see Quéméré *et al.* 2009 for details of protocols). In order to ensure the reliability of genotypes, we performed a sequential replicate-based approach as described in Frantz *et al.* (2003). This was found to be more efficient for *P. tattersalli* than the commonly used multi-tube approach (Taberlet *et al.* 1996) because the same reliable consensus genotypes were reached using a smaller mean number of replicates (Quéméré *et al.* 2009). To quantify the reliability of our genotyping, we calculated the mean quality index (QI) across samples and loci following the rules defined in Miquel *et al.* (2006). Using the software CERVUS 3.03 (Kalinowski *et al.* 2007), we computed the probability of identity (pID) (i.e. the overall probability that two individuals drawn at random from a given population share identical genotypes at all typed loci) and identity assuming sibling (pIDsib).

Genetic diversity and structure analysis

The level of genetic variation for the whole data set and within each of the nine studied forest patches was measured as the mean number of alleles per locus (MNA), the allelic richness (AR), the observed heterozygosity (H_o) and the expected heterozygosity (H_e) (Nei 1978). In order to investigate the level of genetic structure among forest patches, we estimated the pairwise F_{st} between all populations (Wright 1978) following Weir & Cockerham (1984). Departure from HWE was calculated with F_{is} (Wright 1978). We tested the significance of F_{is} and F_{st} values using permutations. We performed all analyses using the GENETIX software (Belkhir *et al.* 1996–2004), except for Allelic Richness (AR), which was computed using FSTAT (Goudet 2001).

Identification of population units and structuring environmental factors

STRUCTURE *analysis.* To detect discontinuities in genetic variation and define genetically distinct units, we first used the commonly used individual-based clustering Bayesian methods implemented in the STRUCTURE 2.2 (Pritchard *et al.* 2000; Falush *et al.* 2003). We performed 10 runs of 500,000 iterations with a burn-in period of 100,000 for each *K* value (with *K* ranging from 1 to 12

for the first level analysis) using the admixture model and assuming correlated allele frequencies (Pritchard *et al.* 2000; Falush *et al.* 2003). We used a uniform prior for alpha, the parameter representing the degree of admixture, with a maximum of 10.0 and set Alphapropsd (the standard deviation of the proposal distribution) to 0.05. Lambda, the parameter representing the amount of correlation in the parental allele frequencies, was set at 1.0 and the prior F_{st} was set to the default values with a mean of 0.01 and a standard deviation of 0.05. For each run, we checked the stabilization of the likelihood. To determine the optimal number of clusters, we compared the log-likelihood of the data given the number of clusters (Ln $P(X|K)$), evaluated the individual membership coefficient (*Q*) (Pritchard *et al.* 2007) and computed the standardized second order rate ΔK of change of Ln $P(X|K)$ (Evanno *et al.* 2005), for different values of *K*. The ΔK method is known to detect only the uppermost level of structure (Evanno *et al.* 2005). We thus repeated the process in a hierarchical way until no substructure could be uncovered and by assigning at each hierarchical level, the individuals to the group where more than 90% of their genome is assigned (i.e. membership proportion *Q* > 0.9). Since the golden-crowed sifaka individuals within social groups are expected to be closely related (Meyers 1993), the 'optimal' value of *K* might be reflecting family-induced structure rather than the overall geographical population structure as it has been recently noticed in gorillas (Bergl & Vigilant 2007) or in salmon and simulated data (Anderson & Dunham 2008). We, therefore, treated the clustering analyses as a data-exploration tool as was suggested by Chikhi and Bruford (2005) or Anderson and Dunham (2008). Since the assumption of correlated allele frequencies may also lead to an overestimation of *K* (Pritchard et al. 2007), we repeated the analyses using the independent allele frequency model with lambda set to 1.0. We report only the results from runs assuming correlated allele frequencies, since the outcomes of our analyses were not affected by this change in model choice. To discuss the relationship between genetic discontinuities and environmental features, we computed the mean membership coefficients of each social group for each cluster and plotted the results on the map of the Daraina region. These coefficients were assigned to the group sampling locations, and we used the inverse distance weighted (IDW) interpolation function implemented in ArcGis 9.2 to predict the overall pattern of cluster membership through the species range.

CAUSAL MODELLING *analysis.* To identify the environmental factors influencing *P. tattersalli* dispersal across landscape, we employed a causal modelling on resemblance approach (Legendre 1993; Cushman *et al.* 2006).

This factorial, multi-model approach aims to combine least cost path modelling and partial Mantel tests to identify the landscape organizational models (i.e. combination of environmental factors) which appear to drive the patterns of genetic relatedness among individuals (Cushman *et al.* 2006). Contrary to clustering methods, this approach needs to define a priori the environmental factors potentially driving genetic structure. We detail now the different steps of this approach.

First step—identification of potential drivers of genetic structure. By combining field observations, remote sensing data and GIS tools, we identified four potential drivers of *P. tattersalli* genetic structure in Daraina area. These are the geographical distance [Factor D], the national road (as a barrier) [Factor R], the Manankolana River (as a barrier) [Factor M], and the forest habitat structural connectivity (for simplicity we refer to it as 'canopy') [Factor C]. We did not test the effect of slope/altitude since *P. tattersalli* range mostly corresponds to lowland landscapes. Human density was also not considered since it is relatively low in the Daraina area and a strong *fady* (i.e. local taboo) limited the direct pressures on the golden-crowned sifaka (poaching and hunting mentioned above appear to have been caused by non-local people settling in the region during a 'gold rush' in the 1990s and during the recent political instability).

Second step—computation of genetic and resistance distance matrices. Genetic dissimilarity [G] among individuals was estimated by computing the matrix of Rousset (2000)'s genetic distance a_r between pairs of individuals using the program SPAGeDI (Hardy & Vekemans 2002). The isolation-by-distance hypothesis [D] was modelled as a single hypothesis with genetic dissimilarity predicted to increase linearly with Euclidian distances. A matrix of Euclidian distances was thus computed between pairs of individual locations. The two Isolation-by-barrier hypotheses (Isolation-by-road [R] or Manankolana River [M]) were represented as categorical model matrices (Legendre & Legendre, 1998) with 0s and 1s for pairs of individuals on the same or different side of the barrier, respectively. To model the Isolation-by-canopy hypothesis [C], we computed three resistance surfaces representing three level of selectivity for the forest connectivity. We first carried out a gradual forest numeric model (FNM) (Coulon *et al.* 2004) from the landscape classification (see above): the smoothing function implemented in the software ENVI 4.1 was used to assign a value between 1 and 10 to each pixel corresponding to $30 \times 30 \text{ m}^2$ squares indicating the amount of open area surrounding the pixel within a

radius of 200 m. (i.e. resolution of the friction maps). We used this value because (i) it corresponds to the estimated radius of sifaka social groups' territory (Meyers 1993) and (ii) it allows us to consider small elements like riparian forests that are likely to favour sifakas dispersal (Adriaensen *et al.* 2003; Broquet *et al.* 2006). The three isolation-by-canopy resistance surfaces [C1, C2 and C3] corresponded to a low/medium/high level of selectivity in which the most opened areas are two/five/ten times, respectively, more difficult to cross than the most forested areas (Fig. S1, Supporting Information). We then used these resistance surfaces to estimate cost distances between individuals and to produce three pairwise matrices using the PATHMATRIX tool (Ray 2005). Cost distances are the cumulative costs corresponding to the least-cost route between pairs of individual locations.

Third step—identification and test of organizational models. We considered 15 possible organizational models corresponding to all the patterns of causality among the four factors described above (Table 2c and Fig. S2, Supporting Information). To assess the support of each of these models, we followed the procedure detailed in Cushman *et al.* (2006). Each of the 15 models is related to a set of hypotheses corresponding to statistical relationships between genetic structure and putative structuring environmental factors (Table 2a and c). Support for a model can be simply computed as the number or the proportion of statistical predictions that are fulfilled. A model is fully supported only if the entire set of hypotheses is verified. To evaluate the relationship between the genetic structure and each of the potential factors, we used simple (Mantel 1967) and partial (Smouse *et al.* 1986) Mantel tests within a causal modelling framework (Legendre 1993). We first computed the Mantel correlation and associated Monte Carlo *P* value for each simple and partial Mantel correlation between genetic-distance matrix and the matrices corresponding to the different factors or set of factors (Table 2b). We then compared the significant and non-significant (NS) tests to the expectations under the 15 models (Table 2c) to identify the organizational model with the greatest support (i.e. most consistent with the observed pattern of genetic data). For each model, we computed a support rate corresponding to the ratio of the number of non-rejected hypotheses to the total number of tested hypotheses. The confidence intervals of the support rates were obtained using a resampling procedure (Manly 1997) in which we randomly selected 75% of the 230 individuals without replacement (100 iterations). We recomputed the support rates for each of the 15 models using the 100 data sets repeating the procedure detailed above.

Spatial genotypic structure at the patch scale

Isolation-by-Euclidian distance appears to be an important factor structuring genetic diversity in *P. tattersalli* populations (see the 'Results' section below). In order to investigate the spatial scale of variation of the dispersal process, we performed a spatial autocorrelation analysis in GENALEX 6.2 (Peakall & Smouse 2006) for individuals belonging to the same forest patch hence assuming the Euclidian distance and the rate of dispersal as the only factors influencing genetic structure. Data from all patches were combined using the 'Multiple Pops' option. The spatial autocorrelation analysis assesses the genetic similarity between pairs of individuals at different geographical distance classes. These classes were defined so as to (i) represent sensible biological classes (see below), (ii) be of similar length (in meters), and (iii) have a minimum number of within class comparisons. The first distance class was defined to only include comparisons among individuals from the same social group (i.e. the 'within groups' class). The following distance classes corresponded to increments of 500 m, up to 5000 m, and had between 58 and 264 pairwise comparisons. The autocorrelation patterns may result from processes other than dispersal, such as mutation, which is known to be affected by sampling scheme (Guillot *et al.* 2009). Since we collected the samples at random across the forest patches (Fig. 1), we assume that our sampling scheme does not reflect species density and is not informative about the process. Thus, the patterns of autocorrelation should reflect the sifaka dispersal and their social and reproductive structure. Since behavioural data indicated that *P. tattersalli* dispersal is biased toward neighbouring social groups (Meyers 1993), we should obtain a significant positive autocorrelation in the first distance classes and the correlogram should flatten out at the scale where dispersal is not connecting social groups (Aars *et al.* 2006). The significance of *r* values was tested by doing 1000 random permutations of the data to compute the 95% confidence interval about the null hypothesis of no spatial substructure ($r = 0$) compared with the bootstrap 95% confidence intervals around each estimate of *r* (Peakall *et al.* 2003).

Results

Genetic diversity and patterns of differentiation among patches

We obtained multi-locus genotypes from 250 out of the 292 extracted samples (~78%). Overall the faecal samples were of good quality, with a mean QI per sample of 0.81 (SD = 0.16) and the mean QI per locus of 0.82 (SD = 0.04). This relatively high quality is not necessar-

ily surprising given that all samples were immediately collected after defecation. All 13 loci used were polymorphic with an average of 7.7 alleles (SD = 1.98) per locus across all samples. The pID (Evett & Weir 1998) was 8.82×10^{-14} and the pIDsib (Evett & Weir 1998) was 7.98×10^{-6} for a 13-locus genotype, allowing ample power to discern individuals. Among the 250 samples, 20 were discarded as they corresponded to the same multi-locus genotype as another individual from the same social group at all 13 loci. We note here that we expected this to happen since defecation of the members of a sifaka group often occurs over a relatively short period of time and often under the same tree. Based on our 2006 experience, our sampling strategy during the 2008 fieldwork season was hence done so as to ensure that all individuals were sampled in a group, accepting the risk of sampling the same individual more than once. The remaining 230 individuals were thus considered for the analyses. Altogether this corresponded to a total of 2960 genotypes (i.e. 30 missing genotypes ~1%).

Genetic diversity of golden-crowned sifakas across all nine forest patches, as measured by H_e had a mean value of 0.66, ranging between 0.57 (Antsiasia) and 0.73 (Ampondrabe). The average AR was 3.83 ranging from 3.00 (Antsiasia) to 4.36 (Bekaraoka) (Table 1). Average H_o values were slightly higher than H_e in most of the sampling sites resulting in mostly negative F_{is} values ranging from −0.18 to 0.01 with a significant departure from HWE for three sites (Bobankora, Ampondrabe and Antsaharaingy). In these three cases, significant heterozygosity excess was observed for only one to four loci which appear to drive the overall result. We found that the genetic diversity levels within all forest patches were also relatively high and that this AR was slightly correlated with patch surface areas but not significantly (Pearson correlation, *P* value = 0.06). Levels of differentiation, as measured by pairwise F_{st}, were significant between most of the sites (values ranging between 0.03 and 0.30—Table S1, Supporting Information) except between the neighbouring Bemokoty and Tsarahitsaka forest patches ($F_{st} = 0.01$). The highest F_{st} values were found for Antsiasia (average pairwise $F_{st} = 0.2$) and Antsaharaingy (0.16) located in the far northern and south-eastern edges of the distribution area, respectively (Patches I and A—Fig. 1). By excluding these two isolated patches, which were responsible for the largest F_{st} values, we observed that the level of differentiation among patches located on the same side of the Manankolana River was much more limited (F_{st} values between 0.01 and 0.07) in comparison with the values obtained among patches located on different sides (F_{st} ranged between 0.09 and 0.16).

Table 1 Summary statistics for the nine forest patches populations

Forest massif [ID]	Sample size	MNA	AR	Ho	He n.b.	Fis
ANTSAHARAINGY [A]	22	4.62	3.29 (0.75)	0.69 (0.19)	0.58 (0.15)	−0.18***
AMPONDRABE [B]	16	4.77	4.19 (0.86)	0.79 (0.12)	0.73 (0.09)	−0.08*
AMPOETANY [C]	37	5.23	3.71 (0.51)	0.68 (0.10)	0.65 (0.09)	−0.05
BEMOKOTY [D]	25	5.08	3.99 (0.77)	0.69 (0.13)	0.67 (0.10)	−0.03
TSARAHITSAKA [E]	20	4.92	3.88 (0.84)	0.69 (0.10)	0.66 (0.10)	−0.04
BAA [F]	18	5.15	4.05 (0.87)	0.69 (0.15)	0.69 (0.09)	−0.01
BEKARAOKA [G]	49	6	4.36 (0.73)	0.72 (0.12)	0.72 (0.09)	0.01
BOBANKORA [H]	31	5.54	4.03 (0.87)	0.70 (0.13)	0.66 (0.12)	−0.07**
ANTSIASIA [I]	6	3	3.00 (0.71)	0.60 (0.22)	0.57 (0.19)	−0.06
Average	25	4.92	3.83 (0.77)	0.69 (0.14)	0.66 (0.11)	−0.03

ID, forest massif reference in Fig. 1; MNA, mean number of alleles; H_o, observed heterozygosity; H_e, n.b., unbiased expected heterozygosity; AR, allelic richness; Standard deviations are in brackets.
*$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.

Identification of population units and environmental factors

STRUCTURE *analysis.* The STRUCTURE analysis showed that the likelihood of the data increased quickly up to $K = 3$ and then reached an asymptote (data not shown). For $K > 3$, the results were not consistent among runs and the uncovered genetics units corresponded to related individuals belonging to the same or neighbouring social groups. It thus appeared that $K = 3$ captured the major genetic structure in our sample while producing a very high average value for Q (0.92 ± 0.11). The three clusters, KA, KB and KC, corresponded to three regions with limited overlap, and KA was mostly composed of individuals from east of the Manankolana River (Fig. 2). Using the method of Evanno *et al.* (2005), the results were at first slightly different as the highest ΔK value was obtained for $K = 2$ which corresponded, with very few exceptions, to individuals sampled on the Eastern (i.e. KA) or Western (i.e. KB and KC) sides of the Manankolana River (data not shown). When we repeated the ΔK analysis within these two main clusters, as suggested by Evanno *et al.* (2005), we found that the western river side group could be further divided into two subgroups with one genetically distinct group corresponding to most of the individuals from Antsaharaingy in the extreme north of the distribution area. We found no substructure on east of the Manankolana River (Fig. 2). When we interpolated the KA cluster membership coefficients obtained with STRUCTURE on the map of the Daraina region (Fig. 2), we observed that the main genetic discontinuity overlapped with the geographic location of the river, even though the analysis did not use any prior geographical information. In contrast, the genetic clusters did not coincide with the national road bisecting the area, although it is parallel to the river for about one third of its length. This

strongly suggests that one of the main factors structuring present-day genetic diversity is the Manankolana River and not the road.

Causal modelling analysis. The results of the simple Mantel tests for the seven independent factors in Fig. 3 show (i) that all correlations are significant, but also that (ii) all correlation values are relatively similar (between 0.25 and 0.33), with only one exception, namely the road which exhibits a much lower value ($r = 0.05$). The two highest values appear to be those corresponding to the Manakolana River [M] and Euclidean distance [D] hypotheses, followed by the different 'canopy' models. For each of the 15 hypothetical organizational models, Table 2c represents the expected results of the causal modelling analysis in terms of partial Mantel tests. For instance, model 1 [D + C + M] assumes that in *Propithecus tattersalli* gene flow is influenced by all factors except the road. If this model were correct, we would expect the first partial Mantel test corresponding to the correlation between road and pattern of gene flows controlling for distance (RG.D) to be NS because the road factor is absent of the model. We observed that this is indeed the case (Table 2b, $r = -0.06$, $P = 1.00$). In order to make this Table 2 easier to read, we highlighted the cases where the *P*-value of the partial Mantel tests matched with the expectation of the model (i.e. when the hypothesis is thus consistent and hence potentially supported). The global support rates of the models (i.e. the ratio of the number of non-rejected hypotheses to the total number of tested hypotheses) vary between 0.14 (±0.06) for model 15 (only one prediction was correct) and 0.83 (±0.04) for model 1 (10 predictions out of 12) (Table 2c). None of the 15 models is fully supported, but seven models (models 1–7) seem to provide relative good predictions in terms of partial Mantel
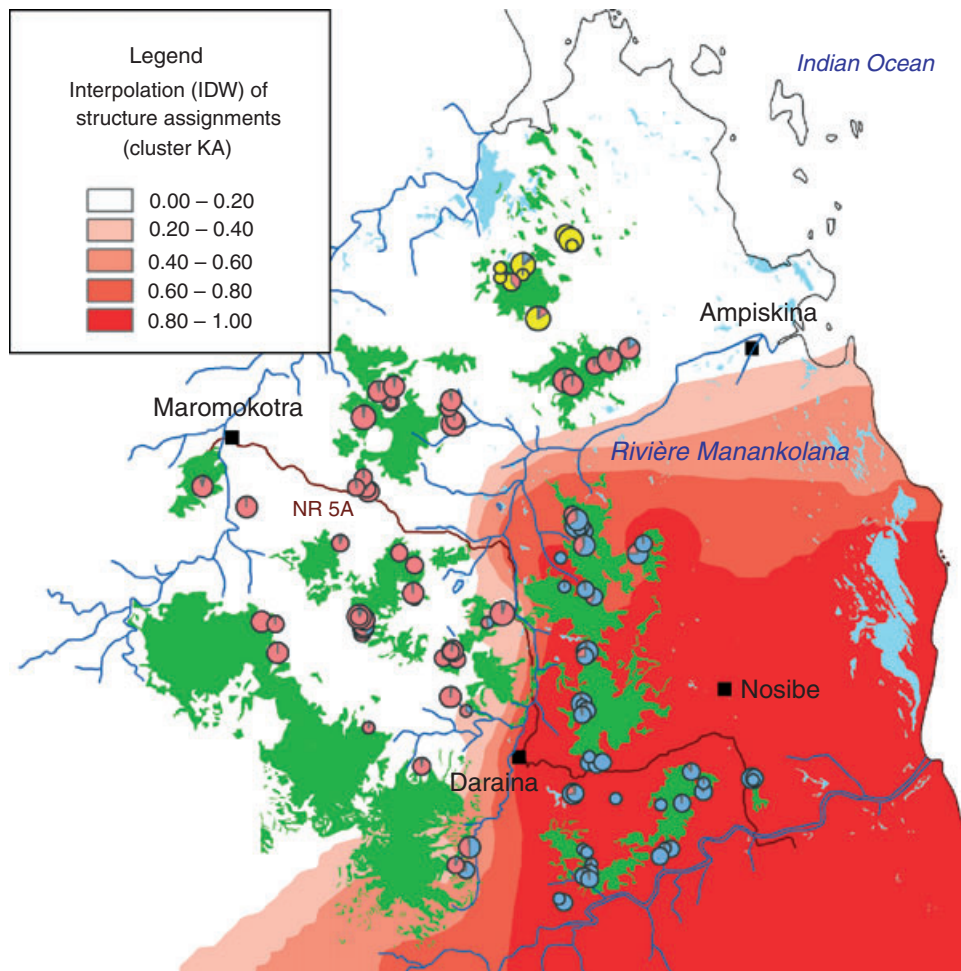
Fig. 2 Membership of social groups genetic clusters. The social groups are represented by pie charts at the location where they were sampled (*n* = 105). The size of the pie charts is proportional to the number of sampled individuals in the social groups (between one and five individuals). The colours indicate the average membership coefficients for each social group to each of the three clusters uncovered by STRUCTURE (*blue*, *red* and *yellow* colour for cluster KA, KB and KC, respectively). The red gradient illustrates the interpolated (IDW) membership coefficients of the cluster 1 (see text for details).

tests (support > 0.6), three of which have support values ≥0.8 (models 1–3). Among the six best-supported models, five appear to have the geographical distance factor [D], three the Manankolana [M], or canopy [C] factor.

Identification of the best organizational model. To identify the best model, we considered not only the global support of each model but also analysed in detail the output of associated predictions. Indeed, the different factors are not independent and a partial Mantel test may reveal a significant correlation not because of the direct effect of a factor but rather through a correlation with another factor. For instance, we can see a significant canopy effect partialling out the river (Hypothesis CG.M, *P* value < 0.01). However, we also observed that there is no effect of canopy controlling for distance (Hypothesis

CG.D, NS). This suggests that the effect of canopy in model 1 is caused by a correlation between canopy and geographic distance. In others words, model 1 should be
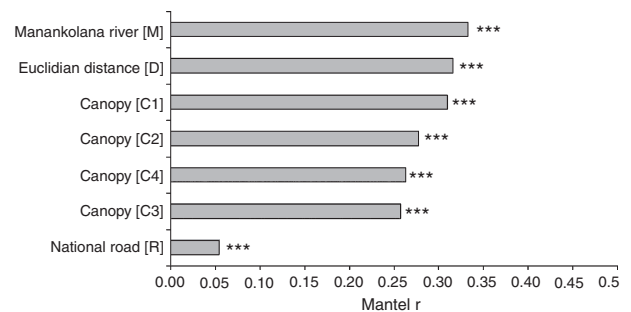

Fig. 3 Results of simple Mantel tests. *= $P < 0.05$, **$P < 0.001$, ***$P < 0.001$.

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Table 2 Evaluation of the 15 organizational models

(a) Resistance hypothesis (Partial Mantel test)	(b) R	P value	(c) 15 Organizational models 1 (D+C+M)	2 (D)	3 (D+M)	4 (C+M)	5 (D+C+M+R)	6 (D+C)	7 (D+M+R)	8 (D+R)	9 (C)	10 (C+M+R)	11 (M)	12 (D+R+C)	13 (R+M)	14 (C+R)	15 (R)
RG.D	−0.06	1	NS	NS			S	NS	S	S		S		S	S	S	S
RG.M	0.046	0.00	NS		NS	NS	S		S	S		S	NS	S	S	S	S
MG.D	0.26	<0.001	S	NS	S	S	S	NS	S	NS		S	S	NS	S		
MG.R	0.33	<0.001	S		S	S	S		S	NS		S	S	NS	S	NS	NS
DG.M	0.23	<0.001	S	S	S	NS	S	S	S	S		NS	NS	S	NS		
DG.R	0.32	<0.001	S	S			S	S	S	S		NS		S	NS	NS	NS
C1G.D	−0.04	0.98	S	NS	NS	NS	S	S	NS	NS	S	S		S		S	
C2G.D	−0.04	0.96															
C3G.D	−0.01	0.70															
MG.C1	0.29	<0.001	S		S	S	S	NS	S		NS	S	S	NS	S	NS	
MG.C2	0.28	<0.001															
MG.C3	0.26	<0.001															
DG.C1	0.19	<0.001	S	S	S	NS	S	S	S	S	NS	NS		S		NS	
DG.C2	0.16	<0.001															
DG.C3	0.07	<0.001															
C1G.M	0.19	<0.001	S		NS	S	S	S	NS		S	S	NS	S	NS	S	
C2G.M	0.20	<0.001															
C3G.M	0.23	<0.001															
RG.C1	-0.03	0.97	NS			NS	S	NS	S	S	NS	S		S	S	S	S
RG.C2	-0.04	1.00															
RG.C3	-0.06	1.00															
C1G.R	0.25	<0.001	S			S	S	S	NS	NS	S	S		S	NS	S	NS
C2G.R	0.28	<0.001															
C3G.R	0.31	<0.001															
Support rate	Mean		0.83	0.81	0.80	0 73	0 73	0.69	0.64	0.47	0.52	0.50	0 53	0.48	0.39	0.30	0.14
	SD		0.04	0.06	0.05	0.05	0.04	0.03	0.04	0.05	0.06	0.04	0.06	0.04	0.04	0.05	0.06

F = Forest; C = Canopy; M = Manankolana River; R = Road; D = Euclidian distance; G = Genetic distance.
In Table 2a, we represented the set of diagnostic resistance hypotheses (partial mantel tests) related to each model. A period separates the main matrices on the left from the covariate matrix on the right that are partialled out in the partial Mantel test. For example, RG.D is a partial Mantel test between the road (R) and the genetic distance (G) matrices with the geographical distance matrix (D) partialled out. For each model, the expectations for the Mantel tests were indicated in Table 2c. S and NS mean that the corresponding partial Mantel tests are expected to be significant or NS, respectively for this model. The *grey boxes* are a visual help to identify tests where the P value (Table 2b) matched the expected result of the test (see text for a simple example). Boxes where a particular test is meaningless for a specific model were represented in black. The support rates corresponding to the ratio of total hypotheses minus the rejected hypotheses to the total number of tested hypotheses, and their standard deviation values (SD) are indicated for each model.

modified to exclude canopy, which would reduce to model 3. Similarly, model 2 predicts that the pattern of gene flow is only a function of distance. However, we found a significant correlation between the river and the pattern of gene flow while controlling the effect of distance (Hypothesis MG.D, S). This suggests that the river should be added to this model, producing again model 3.

Model 3 (D + M) thus appears to explain best most significant and NS correlations while keeping the number of factors to a limited level (two factors instead of three for model 1). Additionally to the tests performed on the whole data set and to control for the Manankolana River effect, we also performed causal modelling analyses on each side of the river. For both sides, the Isola-

Table 3 Organizational models for the two sides of the river

Resistance hypothesis (Partial Mantel test)	R	P value	D	C+D	D+R	C4	R+D+C	R	C4 + R
Eastern side									
DG.R	0.212	<0.001	S	S	NS		S	NS	NS
C4G.R	0.115	0.002		S	NS	S	S	NS	NS
RG.D	−0.035	0.896	NS	NS	S		S	S	S
RG.C4	−0.055	0.977		NS	S	NS	S	S	S
C4G.D	−0.012	0.641	NS	S	NS	S	S		S
DG.C4	0.185	<0.001	S	S	S	NS	S		NS
Support rate	Mean		**0.99**	0.837	0.33	0.51	0.5	0.01	0.01
	SD		0.05	0.047	0.05	0.07	0.05	0.04	0.05

Resistance hypothesis (Partial Mantel test)	R	P value	C4 + D	D	R+D+C4	C4	C4 + R	D+R	R
Western side									
DG.R	0.2529	<0.001	S	S	S		NS	NS	NS
C4G.R	0.238	<0.001	S		S	S	NS	NS	NS
RG.D	−0.03208	0.958	NS	NS	S		S	S	S
RG.C4	0.02793	0.059	NS		S	NS	S	S	S
C4G.D	0.138	<0.001	S	NS	S	S	S	NS	
DG.C4	0.1625	<0.001	S	S	S	NS	S	S	
Support rate	Mean		**0.92**	0.75	0.75	0.63	0.41	0.25	0.12
	SD		0.08	0	0.08	0.13	0.08	0.08	0.13

See Table 2 for legend.

tion-By-Distance model was the only fully supported model, hence confirming our previous results (data not shown). We also modelled the Isolation-by-canopy hypothesis as a new categorical model matrix [C4] with the values of 1 and 0 corresponding to pairs of individuals located within the same patch, or in different patches, respectively. Interestingly, we observed, in this case, that the model D + C (Distance and Canopy) was the best supported model for individuals located on the west side of the river whereas the model D was still the best model for the east side (Table 3). When we excluded individuals from Antsaharaingy, the model D was again the best supported for the west side (data not shown).

Scale of isolation-by-distance

Significant and positive autocorrelation values were obtained in the first four distance classes (Fig. 4), corresponding to distances less than 1500 m. The highest value was observed in the first distance class corresponding to comparisons between individuals from the same social groups ($r = 0.244$, P value < 0.001). The autocorrelogram then flatten out and the autocorrelation coefficient stabilised around 0.08 in the following three distance classes before reaching values around zero between 1500 and 2000 m. Then, the values remained slightly negative but were not significant up to 5000 m, as expected when there is isolation by distance.
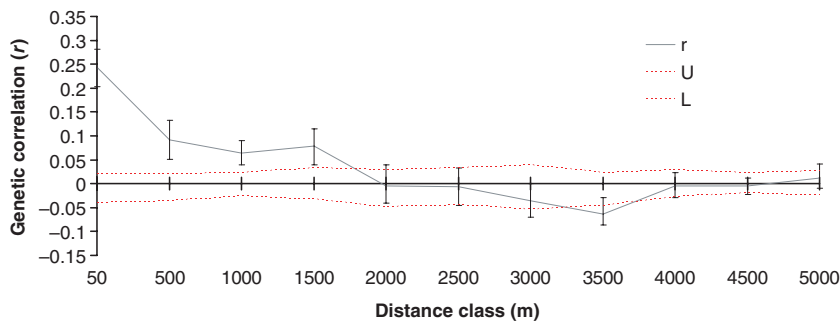

Fig. 4 Spatial autocorrelation analysis. Correlogram plots of the spatial genetic autocorrelation coefficient r as a function of distance for individuals within forest fragments. Upper (U) and lower (L) bounds for the 95% confidence interval for the null hypothesis of no spatial structure ($r = 0$) and the upper Ur and lower Lr 95% error bars about r as determined by bootstrap resampling are shown.

Discussion

A high-genetic diversity within a small distribution range?

The level of genetic diversity observed for the golden-crowned sifaka (MNA = 7.7 and H_e = 0.74 across loci and for all samples) appeared higher than we expected for a species living in one of the smallest range and most fragmented habitats of any lemur. Comparing this estimate with those obtained from closely related species remains problematic as population genetic studies on other sifakas are still limited and often carried out using a restricted number of individuals or locations due to the difficulty in capturing sifakas. Only one study conducted on a large number of *Propithecus verreauxi* (>200 individuals) from the Beza Mahafaly Special Reserve protected area (Lawler *et al.* 2001; Lawler *et al.* 2003) could be used as a comparison. Although this species is known to have a wider range and a less disturbed habitat than *Propithecus tattersalli*, the authors found similar values of genetic diversity (MNA = 9.29 and H_e = 0.74). Furthermore, the level of genetic diversity found in this study are higher than those observed for three *Microcebus* and one *Lepilemur* species (*L. edwardsi*), even though these species are expected to have larger effective population sizes (Olivieri *et al.* 2008; Craul *et al.* 2009). For *L edwardsi*, the low diversity observed (MNA ≤ 4 across 14 loci and H_e = 0.55) seems to be due to the fact that the species is currently heavily hunted and undergoing a significant reduction in population size (Craul *et al.* 2009). The comparisons with the *Microcebus* should be taken cautiously because of ascertainment bias introduced by the choice of the microsatellite loci. Loci used in Olivieri *et al.* 2008 were originally developed for *Microcebus murinus* and were thus expected to produce an underestimation of genetic diversity (Chikhi 2008). Altogether we would like to emphasize that the comparison of levels of genetic diversity across species remains a tricky issue, as it heavily depends on how loci were isolated and how the sampling was performed.

The role of the Manankolana River as a major gene flow barrier

One of the main results of our study was that we clearly identified the Manankolana River as a major geographical feature shaping genetic differentiation in *P. tattersalli* and as possibly the main barrier to dispersal in the Daraina region. This result was surprising at first given that the river is dry most of the year, and particularly during our fieldwork, and hence should not be more difficult to cross than many other open areas. One possible interpretation could be that this pattern is the result of significant human presence as many villages and rice fields are located near the river. Another plausible explanation to this pattern is related to the sifakas dispersal behaviour. Indeed, behavioural studies by Meyers (1993) have shown that most of the dispersal events between groups appear to take place during the mating season, which occurs in the middle of the rainy season when the river is full and therefore represents a significant barrier to dispersal. This is supported by the fact that rivers are increasingly recognised as barriers to gene flow in many primate species, both outside (Eriksson *et al.* 2004; Goossens *et al.* 2005) and in Madagascar (Mittermeier *et al.* 2008) and that this role has also been confirmed across other taxa in Madagascar (Wilmé *et al.* 2006; Yoder & Nowak, 2006). This is particularly clear in the *Microcebus* and *Lepilemur* genera where many new species have been shown to have very small ranges whose limits are rivers (Radespiel *et al.* 2008a, b; Louis *et al.* 2006; Mittermeier *et al.* 2008). Also, the geographical range of *P. tattersalli* is nearly entirely delimited by two rivers, the Manambato and the Loky. The only exception is a small population north-west of the Loky (Fig. 1), in the most upstream region of this river.

The role of isolation by distance

One of the main results provided by the causal modelling approach is that the geographical distance (together with the Manankolana River) appeared to be the main population-structuring factor in *P. tattersalli*. This suggests that *P. tattersalli* effective migration events are mostly between neighbouring groups, and was confirmed by the spatial autocorrelation analysis. This analysis showed that the highest autocorrelation values were found at the smallest spatial scale, within groups (Fig. 4), suggesting that most individuals within social groups are closely related. This result is probably due to the fact that sifaka social groups are likely including lineages of philopatric related females and young males born in the group who have not dispersed yet (Meyers 1993). We also observed a significant spatial autocorrelation among pairs of individuals in the first four distance classes (i.e. at distances <1500 m). These four classes included 87% of the distances between individuals and the nearest groups hence suggesting that (i) our sampling scheme was appropriate to assess fine-scale structure and (ii) most dispersal events within forest patches occur among neighbouring groups. This result is again in agreement with the behavioural studies on *P. tattersalli* (Meyers 1993) and also on *P. verreauxi* (Richard *et al.* 1993). These authors observed, in a 5-year census period, that all Verreaux's sifakas males migrated to groups no more than two home ranges

away from their natal group. As suggested by Richard (1985) and Richard *et al.* (1993), these patterns of adjacent transfers cause some neighbouring groups to have related males, thus resulting in a 'neighbourhood-like' social organization.

Impact of forest habitat fragmentation on P. tattersalli dispersal

The clustering and causal modelling approaches brought us complementary and interesting results about how the golden-crowned sifakas respond to current habitat fragmentation at both landscape and patch scales. At the landscape scale (i.e. entire species range), the results of the causal modelling approach suggest that canopy connectivity is not a major population structuring factor. This is in agreement with the clustering analysis since two of the three uncovered genetic units included several forest patches, suggesting that effective dispersal is actually occurring between at least some patches. A relatively large network of riparian gallery forests has been maintained and we suggest that they could have contributed to preserving dispersal between most of the forest patches. However, when we explored the results at the smaller, regional scale, the situation appeared to be more complex. Indeed, the STRUCTURE results showed that habitat fragmentation and hence connectivity had a strong impact on dispersal, since we found a significant genetic discontinuity in the very north of the Daraina area, identifying Antsaharaingy as a cluster (KC). This highlights one limitation of the causal modelling approach that can only identify general processes working at the entire sampling scale (i.e. the landscape scale). Here, habitat fragmentation appears to be important in only one region, but this effect is averaged out with other regions where fragments are still connected, leading to a NS effect when the whole region is considered. Interestingly, we observed that the model implementing Isolation-by-canopy hypothesis obtained the highest support when we restricted the causal modelling approach to individuals located on the west side of the Manankolana River and used a categorical model (C4) to represent the Isolation-by-canopy hypothesis.

Altogether (if we exclude the isolated Antsaharaingy forest), our results are in agreement with a weak influence of forest habitat structural connectivity on sifaka dispersal, not because habitat fragmentation is not a potential threat for this species but because in most regions, forest fragments are still close enough from each other or still connected by corridors. Two radically different historic scenarios of forest fragmentation may explain our results. First, if the forest fragmentation process in the Daraina area was recent then we would

expect to find a limited level of genetic differentiation between forest patches, even if migration had stopped at the present time. Indeed, the genetic structure can have a substantial time lag in its response to changes in gene flow (Wright 1978; Waples 1998). This possible time lag is difficult to assess since it may be influenced by many factors such as the nature of the genetic markers employed, the population size and the level of substructure before fragmentation, and mating patterns (Balkenhol *et al.* 2009). Although aerial and satellite images suggest that forest patch boundaries in the Daraina region have been relatively stable in the last 50 years (Jimenez & Vargas, 2000), we currently have no data before this date. We thus cannot determine whether the habitat was continuous before the 1950s. The current patterns of genetic differentiation could thus potentially be the result of very recent fragmentation, say during the first half of the 20th century. Under the second scenario, the forest fragmentation could be significantly more ancient, perhaps a few centuries or millennia old. In this scenario, the golden-crowned sifaka may have 'adapted' to the fragmented landscape, and genetic differentiation observed today would correspond to the actual migration events between present-day populations and fragments. This second scenario is supported by the fact that we observed sifakas moving on the ground on several occasions (EQ pers. obs.). Jimenez & Vargas (2000) also observed *P. tattersalli* individuals crossing more than 200 m of grasslands to reach the closest forest. They also showed that golden-crowned sifakas were able to survive in riparian forests and forest fragments smaller than 10 ha, which were sometimes located more than 2 km away from the closest large forest. Additionally, they noted that straight line distances better explained the presence of *P. tattersalli* in forest fragments than the presence or absence of corridors among forest patches.

On the use of causal and least-cost modelling

Here, we presented a practical case in which causal modelling was used to identify key features influencing patterns of genetic differentiation. As expected we found that the use of simple Mantel tests to identify structuring factors (see Epps 2007; Coulon 2004) could produce spurious correlations (Cushman *et al.* 2006). The reason for this is that different factors may be correlated among themselves, and a significant test may not be due to a particular factor but to another one to which it is correlated. The causal modelling approach, by explicitly stating different partial tests and comparing the support for competing hypotheses, appeared able to identify at least some of these spurious correlations. Used together with STRUCTURE, it allowed us to

identify important factors and importantly some limitations. First, the analysis is necessarily dependent on the factors identified a priori as potentially important. This means that some ecological and behavioural knowledge of the species may be required on habitat and corridor preferences to define least-cost paths. Second, potentially important factors in one sub-region may not be detected as such when the analysis is performed across the whole region, as we saw above. The joint use of clustering methods may thus be necessary, particularly as they do not require much prior information.

Another point to keep in mind in our case is that the configuration of the forest habitat in the Daraina region may have played a role in the results. Indeed, the surface area of several forest patches was relatively large compared to the distances between patches and to the total area of the distribution range. The distance between some individuals belonging to the same patch could thus be higher than the distance between individuals belonging to different patches. This could explain why it was difficult to demonstrate an effect of connectivity. Indeed, we found a high correlation between the geographical distance and the least-cost distances that were maximizing the amount of forest areas in a particular path. This was observed for all the levels of selectivity used for the canopy factor. Thus, we suspect that least-cost modelling analyses might work better when the geographical scale of the study is significantly greater than the dispersal ability of the species of interest (Vignieri 2005; Epps *et al.* 2007; Spear & Storfer 2008; Perez-Barberia *et al.* 2008; Clark *et al.* 2008).

Conclusions and implications for conservation

Altogether, our results suggest that the golden-crowned sifakas maintain dispersal among most of the forest fragments. However, a regular monitoring of this species is needed since important human disturbances are expected in the near future in the Daraina area. There is an impending installation of a major gold-mining company and the anticipated tarring of the national road. Furthermore, the recent political events in Madagascar have led to an increase in *Propithecus tattersalli* killing by poachers. The SFUM currently includes the following forest patches: Bekaraoka, Bobankora, BAA, Antsaharaingy and Ampondrabe. Unfortunately, these patches do not include Ampoetany, Tsarahitsaka and Bemokoty which harbour the highest population densities (Quéméré *et al.* 2010) among the populations belonging to the genetic clusters from the west side of the river. Clearly, the incorporation of these fragments to the SFUM seems to be a necessity from both the genetic and demographic point of view.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Models of habitat structural connectivity.

Fig. S2 Description of the 15 tested organizational models.

Table S1 Pairwise F_{ST} values.

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