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Extending ecological niche models to the past 120 000 years corroborates the lack of strong phylogeographic structure in the Crested Drongo (*Dicrurus forficatus forficatus*) on Madagascar

JÉRÔME FUCHS[1,2]*, JUAN L. PARRA[3], STEVEN M. GOODMAN[4,5], MARIE JEANNE RAHERILALAO[5,6], JEREMY VANDERWAL[7] and RAURI C. K. BOWIE[1]

[1]*Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, 3101 Valley Life Science Building, Berkeley, CA 94720-3160, USA*
[2]*Percy FitzPatrick Institute, DST/NRF Centre of Excellence, University of Cape Town, Rondebosch 7701, South Africa*
[3]*Instituto de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Medellín, Colombia*
[4]*Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605, USA*
[5]*Association Vahatra, BP 3972, Antananarivo (101), Madagascar*
[6]*Département de Biologie Animale, Université d'Antananarivo, BP 906, Antananarivo (101), Madagascar*
[7]*Centre for Tropical Biodiversity & Climate Change, School of Marine and Tropical Biology, James Cook University, Townsville, QLD 4811, Australia*

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We conduct a phylogeographic study of the Crested Drongo (*Dicrurus forficatus forficatus*), a broadly distributed bird species on Madagascar. We first determined the demographic and spatial pattern inferred from mitochondrial and nuclear data, and then compared these results with predictions from a present to 0.120-Myr-old reconstruction of the spatial dynamics of the range of *D. f. forficatus* on Madagascar, enabling putative areas of stability (lineage persistence) to be detected. Weak genetic structure along an east–west pattern and comparatively low genetic diversity were recovered, with strong evidence of population expansion found at all ten loci sampled. The palaeoclimatic distribution models over the past 0.120 Myr suggest the presence of extensive areas of suitable climate in the east and west for the species since its colonization of Madagascar, a result in strong concordance with the spatial and genetic signal derived from our multilocus data set. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **108**, 658–676.

ADDITIONAL KEYWORDS: colonization – ecological niche modelling – genetic data.

INTRODUCTION

Two contrasting processes can explain the origin of the unique fauna of Madagascar: vicariance associated with its separation from greater Gondwana approximately 165 Mya and from India approximately 88 Mya (Coffin & Rabinowitz, 1992; Storey *et al*., 1995), or subsequent over-water dispersal from Africa or Eurasia. A solid picture of the timing and mode of colonization of Madagascar and the nearby Comoro Islands for several groups of land vertebrates is emerging (e.g. Nágy *et al*., 2003; Vences *et al*., 2003; Yoder *et al*., 2003; Marks & Willard, 2005; Weyeneth

*Corresponding author. Current address: UMR7205 Case Postale 51, Département Systématique et Evolution, Muséum National d'Histoire Naturelle, 75231 Paris, France. Email: fuchs@mnhn.fr

et al., 2008; Samonds *et al*., 2012). For birds, most lineages colonized Madagascar from either the Indo-Malaya or Afrotropic ecozones within the past five million years (Warren *et al*., 2003, 2005, 2006, 2010; Pasquet *et al*., 2007; Fuchs *et al*., 2008), although earlier colonization events (from the Eocene to the Miocene) have also been described (Cibois, Schulenberg & Pasquet, 2001; Kirchman *et al*., 2001; Beresford *et al*., 2005; Fuchs, Fjeldså & Pasquet, 2006).

In contrast, the phylogeographic patterns of the terrestrial land vertebrates of Madagascar are less well understood. Most consistent for terrestrial vertebrates is the occurrence of north–south (reptiles, Boumans *et al*., 2007; amphibians, Vieites *et al*., 2006; mammals, Yoder *et al*., 2000; birds, Cruaud *et al*., 2011) or west–east (amphibians, Vences & Glaw, 2002; birds, Fuchs *et al*., 2007; mammals, Pastorini, Thalmann & Martin, 2003) genetic breaks. Overlain on these patterns is the importance of other potential barriers to gene flow, such as rivers or altitudinal segregation (Pastorini *et al*., 2003; Goodman & Ganzhorn, 2004; Olson, Goodman & Yoder, 2004).

Several studies have addressed aspects of genetic structure within Malagasy bird species (e.g. Goodman, Tello & Langrand, 2001; Goodman & Weigt, 2002; Fuchs *et al*., 2007; Cruaud *et al*., 2011; Goodman, Raherilalao & Block, 2011). All of these studies have relied exclusively on mitochondrial DNA (mtDNA), which may differ at the level of species or population history because of several non-exclusive factors such as hybridization, introgression, and selection (Ballard & Whitlock, 2004; Bazin, Glemin & Galtier, 2006; Irwin, 2012). The Crested Drongo (*Dicrurus forficatus* Linnaeus, 1766) is a medium-sized (47 g) insectivorous passerine bird, endemic to several natural forest types, as well as wooded anthropogenic savannah on Madagascar (*Dicrurus forficatus forficatus*), and on Anjouan Island (*Dicrurus forficatus potior*) in the nearby (about 300–400 km) Comoros Archipelago (Sinclair & Langrand, 2003). In Madagascar, the species is absent above the altitudinal limit of the forest, at approximately 1950 m a.s.l., and is widely distributed in both dry and humid forest. The species is considered to be a generalist and occupies different ecological conditions (Rocamora & Yeatman-Berthelot, 2009).

Dicrurus forficatus constitutes a monophyletic lineage with respect to all other species of this genus (Pasquet *et al*., 2007; this study). Two subspecies are usually recognized, *D. f. forficatus* on Madagascar and *D. f. potior* on Anjouan (Rocamora & Yeatman-Berthelot, 2009). Its closest relative is the Aldabra Drongo (*Dicrurus aldabranus*), from which it probably diverged not later than 120 000 years ago, when a major inundation of the Aldabra Atoll is likely to have eliminated all the locally occurring terrestrial biota (Thomson & Walton, 1972). The recent release of palaeoclimatic layers going back *c.* 120 000 years (Singarayer & Valdes, 2010), together with the use of newly developed algorithms that project the spatial extent of lineage persistence while allowing for dispersal through time (i.e. shifting refugia; Graham *et al*., 2010), provides an ideal framework to cross-validate results from analyses of multilocus DNA sequences.

Here, we make use of genetic data gathered from ten loci for individuals collected across the range of *D. f. forficatus* on Madagascar to test whether recently proposed molecular clock rates and historical demographic models are in accordance with the timing of the major inundation of the Aldabra Atoll. Based on results from previous phylogeographic studies of terrestrial vertebrates on Madagascar we test the hypotheses of putative genetic structure in the Malagasy Drongo along two primary axes: (1) an east–west cline, along a humidity gradient; or (2) a north–south break recovered for several primarily forest-associated taxa. We further relate our phylogeographic results to the existence of areas where climate putatively remained suitable through time after the colonization of the species from the Comoro Islands, using recently published palaeoclimatic layers that extent back to the past 120 000 years.

MATERIAL AND METHODS

Geographical sampling

We sampled 35 individuals of *D. f. forficatus* from across its range on Madagascar, including samples from different elevational zones and biomes (i.e. humid forest, dry spiny forest; Fig. 1, Table S1 for details of sampling). Our sampling encompassed all putative areas where the species is hypothesized to have been continuously present for the past 120 000 years (see Results). Sequences from one individual of *D. f. potior* from Anjouan Island were available for the two mitochondrial loci (Pasquet *et al*., 2007; this study).

Laboratory procedures

DNA was extracted from blood and tissue (muscle, liver and kidney) using the Qiagen extraction kit (Valencia, CA, USA), following the manufacturer's protocol. We amplified and sequenced ten loci: two mitochondrial loci (ND2 and ATP6), seven autosomal loci (myoglobin, MB intron 2; β-fibrinogen, FGB intron 5; glyceraldehyde-3-phosphate dehydrogenase, GAPDH intron 11; calbindin, CAL intron 9; tropomyosin, TPM1 intron 6; transforming growth factor β2,

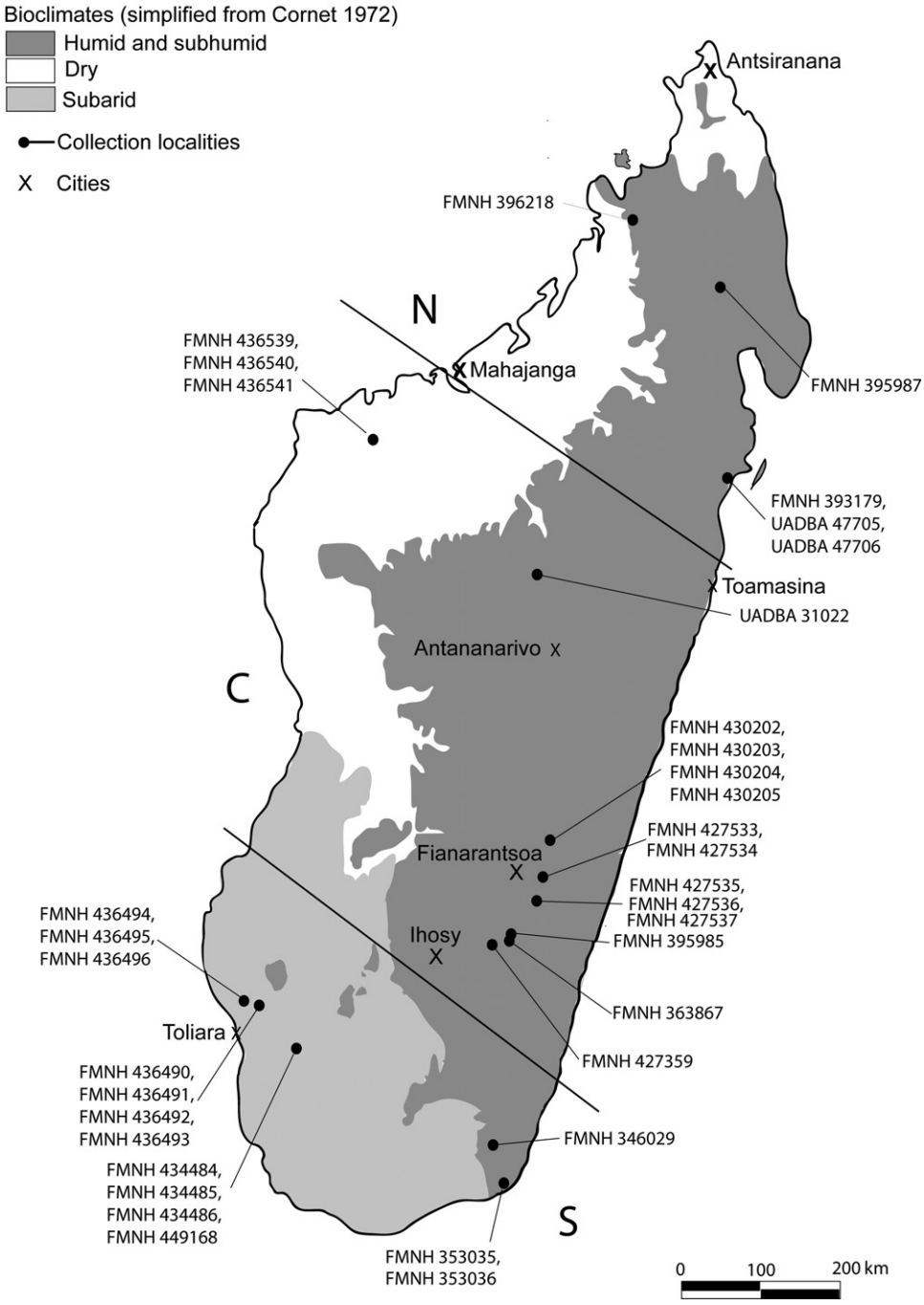

Figure 1. Map of Madagascar, indicating sampling localities, with an overlay of Cornet's (1974) bioclimatic zones (modified from Fuchs *et al*., 2007). The east–west divergence hypothesis corresponds to the comparison between the dry/subarid and the humid/subhumid bioclimates. The lines correspond to the north–south population structure hypothesis tested (N, northern; C, central; S, southern), based on the results of Boumans *et al*. (2007) and Cruaud *et al*. (2011).

TGFβ2 intron 5; and ornithine decarboxylase, ODC gene region introns 6–8), and one *Z*-linked locus (brahma protein, BRM intron 15). Locations on the chicken genome and primer sequences used for PCR-amplification and DNA sequencing are detailed in Table S2. Polymerase chain reactions (PCRs) were performed using standard protocols, with only the annealing temperature (54–60 °C) being varied. Heterozygous sites in nuclear loci (double peaks) were coded using the appropriate International Union of

Pure and Applied Chemistry (IUPAC) code. Newly generated sequences have been deposited in Genbank (www.ncbi.nlm.nih.gov, accession numbers JX624342–JX624713) and alignments have been deposited in the Dryad repository (Fuchs *et al.*, 2013). Molecular sexing was performed using the primer pair 2550F and 2718R under standard PCR-amplification conditions (Fridolfsson & Ellegren, 1999). We did not detect any conflict between molecular and anatomical sexing (when gonad information was available).

Resolving the allelic phase of the nuclear loci

We used PHASE 2.1.1 (Stephens, Smith & Donnelly, 2001; Stephens & Donnelly, 2003) to infer the all eles for each nuclear locus. Several runs, using different seed values, were performed. We used the recombination model (–MR option) and ran the iterations of the final run ten times longer than the other runs (–X10 option). We also made use of the Invitrogen cloning kit (Invitrogen, Carlsbad, USA), following the manufacturer's protocol, to confidently determine the gametic phase of alleles from heterozygous individuals that could not be statistically resolved at a probability level > 0.6 (see Harrigan, Mazza & Sorenson, 2008). Between six and eight clones were PCR-amplified and cycle-sequenced for each individual and locus. In a few instances, we were not successful in cloning some of the individuals where phase probabilities were below 0.63. In such instances, we removed the single nucleotide polymorphisms (SNPs) that could not be phased from the data set for analyses that need full phase information; these few SNPs primarily consisted of autapomorphic mutations, which are present at very low frequency (< 3%).

Selection and recombination

Prior to phylogeographic and population genetic analyses, we established whether our data were in agreement with the expectations of neutral evolution, and whether recombination was present in the nuclear loci. Violation of either of these assumptions is likely to alter the inferred phylogenetic topology and demographic parameter estimates. We used the McDonald–Kreitman test (MK test; McDonald & Kreitman, 1991), as implemented in DNASP 5.0 (Librado & Rozas, 2009), to test whether selection was acting on the mitochondrial protein-coding genes. We employed Fisher's exact test at a threshold of $\alpha = 0.05$ to assess sequence neutrality. Non-coding sequences (including the stop codon for both ND2 and ATP6) were excluded from the analyses, leaving 1719 bp (1038 bp for ND2 and 681 bp for ATP6) for 36 individuals (35 *D. f. forficatus* and one *D. aldabranus*, the closest species to *D. forficatus*; Pasquet *et al.*, 2007).

We tested for selection acting on the nuclear loci by using the Hudson–Kreitman–Aguadé test (HKA test; Hudson, Kreitman & Aguadé, 1987), as implemented in the software HKA (http://genfaculty.rutgers.edu/hey/home). Sequences from one *D. aldabranus* individual were used as the out-group. We could not include the TPM1 locus in the HKA test as no PCR-amplification product was obtained for this locus from the outgroup. We tested for intralocus recombination using the genetic algorithm for recombination detection (GARD) and single break point (SBP) algorithms, as implemented in HYPHY (Kosakovsky Pond, Frost & Muse, 2005; Kosakovsky Pond *et al.*, 2006).

Genetic diversity and demographic parameters

The number of segregating sites (S), nucleotide diversity (π), and Watterson's theta (θ) were estimated with DNASP 5.0. For analyses that require the use of fully phased data, and therefore ignore indels, we recoded these potentially informative characters by replacing the indel with a nucleotide that would induce a mutation: for example, a deletion at a site where only an 'A' was present was modified to a 'G'. When the insertion–deletion event involved more than a single nucleotide, we considered it as a single event; for example, an 'AA' deletion was modified in the data set by using 'AG'.

We used Fu's *Fs* test (1000 replicates) and Ramos-Onsins and Rozas R_2 statistic (Ramos-Onsins & Rozas, 2002), as implemented in DNASP 5.0, to detect signatures of demographic change. The significance of the R_2 statistic was assessed using 1000 coalescent simulations. We also used LAMARC 2.1.6 (Kuhner, 2006) to estimate the population growth parameter g in a coalescent multilocus framework. We specified the best-fit model for each locus according to the Bayesian information criterion, estimated using TOPALI 2.5 (Milne *et al.*, 2009), and simultaneously ran two searches of ten chains under a heating parameter for 300 000 generations (burn-in = 25 000 generations). We used uniform priors for θ (10^{-5} to 10) and g (–500 to 15 000), and repeated the analysis three times to ensure that the parameters were reliably estimated.

Time to most recent common ancestor of mtDNA haplotypes

We employed BEAST 1.6.0 (Drummond *et al.*, 2006; Drummond & Rambaut, 2007) to estimate the time to most recent common ancestor (TMRCA) for all mitochondrial haplotypes. We applied several differ-

ent molecular clock rates: (1) a ND2 molecular clock rate of 4.3% Myr^{-1} (Arbogast *et al*., 2006); (2) a ND2 molecular clock rate of 12.3% Myr^{-1} (Arbogast *et al*., 2006); (3) a ND2 molecular rate of 5.8% Myr^{-1} (Lerner *et al*., 2011); (4) an ATP6 rate of 5.2% Myr^{-1} (Lerner *et al*., 2011); and (5) a combination of the ATP6 and ND2 rates from Lerner *et al*. (2011). In all cases, we specified the substitution rate as a normal distribution (that is not a fixed value) and set the 95% credibility interval to fit the originally published range. For all analyses, we used a F81 model for each of the two loci, the best-fit model according to the Bayesian information criterion, and assumed a strict molecular clock model for each of the two mtDNA loci, as this clock model could not be rejected using a Bayes factor comparison over the lognormal clock model [e.g. ND2, 0.0615 substitutions per site per Myr (ssMyr); natural log Bayes factor lognormal clock model over clock model, 1.075, non-decisive].

We also applied two recently estimated neutral mutation rates from substitutions acting on fourfold degenerated sites, and their associated uncertainty [Bayesian method mean rate, 0.073 ssMyr; 95% highest posterior density (HPD) rate, 0.025–0.123 ssMyr; maximum likelihood method rate, 0.062 ssMyr, with a 95% CI of 0.3–0.9 ssMyr; Subramanian *et al*., 2009], to our mitochondrial data set (332 sites).

For each data set, we ran the Markov Chain Monte Carlo (MCMC) algorithm for 10^7 steps, with parameter values sampled every 10^3 steps. For each molecular rate, we conducted analyses using three diversification modes: a Yule tree prior, equivalent to a PureBirth model; a coalescent constant population size model; and a coalescent population expansion model. In summary, we ran 21 different analyses using different combinations of substitution rates and tree priors.

Two independent BEAST runs were performed for each data set. We made use of TRACER 1.5 (Rambaut & Drummond, 2007) to ascertain that our sampling of the posterior distribution had reached a sufficient effective sample size for meaningful parameter estimation.

Geographic structure

Network

We used TCS 1.21 (Clement, Posada & Crandall, 2000) to reconstruct a 95% statistical parsimony network for each locus. Insertion and deletion (indel) events were considered as informative mutational events. We used POFAD 1.03 (Joly & Bruneau, 2006) and SPLITSTREE 4.0 (Huson & Bryant, 2006) to build a multilocus network using the same set of individuals as employed in the STRUCTURE analyses (see below). We used uncorrected p-distances as input for POFAD, and made use of the standardized matrix for network reconstruction.

AMOVA

We tested for genetic differentiation between: (1) western ($N = 14$ individuals) and eastern populations ($N = 21$ individuals), following the partitioning scheme of Fuchs *et al*. (2007); and (2) a partitioning scheme comprising northern ($N = 5$ individuals), central ($N = 16$ individuals), and southern populations ($N = 14$ individuals), based on the phylogeographic patterns recovered by Boumans *et al*. (2007) and Cruaud *et al*. (2011). Attribution of each locality to a particular population was straightforward, as localities broadly overlap across studies. Two localities (Namoroka, FMNH 436539–436541, and Tampolo, FMNH 393179 and UADBA 47705–47706) were assigned to the central and northern populations, respectively, based on phylogeographic patterns recovered in some of the focal taxa in previous studies. The AMOVA was implemented in ARLEQUIN 3.5 (Excoffier & Lischer, 2010). For each locus, we used uncorrected pairwise distance and a gamma value for the rate heterogeneity parameter of 50 (decreasing or increasing this value had no impact on the result). The *P* value was assessed through 1023 random permutations of each data set.

STRUCTURE

We used STRUCTURE 2.3 (Pritchard, Stephens & Donnelly, 2000; Falush, Stephens & Pritchard, 2003) with the LocPrior option implemented (Hubisz *et al*., 2009) to infer how many populations could be distinguished based on the haplotypes of the eight nuclear loci. We only included individuals for which we obtained sequences for all loci ($N = 30$), and only the SNPs that could be satisfactorily phased. We compared the optimal number of populations estimated by STRUCTURE and the probability of each individual being assigned to a given population across analyses. We assumed an admixture model with correlated allele frequencies and let α vary among populations. We ran 5×10^6 iterations (burn-in: 5×10^5 iterations) with $1 \leq K \leq 5$. For each K, we ran ten iterations. The number of clusters (populations) was estimated using Evanno's ΔK (Evanno, Regnaut & Goudet, 2005), as calculated using STRUCTURE HARVESTER 0.6.8 (Earl & vonHoldt, 2012). The output file for the optimal K value, as determined from STRUCTURE HARVESTER, was analysed in CLUMPP 1.1.2 Jakobsson & Rosenberg, (2007) to summarize variance among runs, and thereby obtain

robust estimates of the membership coefficient. Plots were visualized in DISTRUCT 1.1 (Rosenberg, 2004).

Isolation by distance and resistance

To test for signatures of isolation by distance we correlated genetic distance (standardized matrix of genetic distances among individuals from POFAD 1.03) to both straight-line geographic distances as well as 'resistance' distances (*sensu* McRae, 2006). Straight-line geographic distances were obtained in kilometres (km) using the great circle distance method (Vincenty, 1975). Resistance distances are calculated based on all possible paths between two individuals, given a friction surface that describes the likelihood of movement through pixels (McRae *et al*., 2008). Resistance distances incorporate both the minimum distance and all alternative pathways between two points, and are based on the opposition that a resistor offers to the flow of electrical current between nodes (i.e. individuals). Resistance distances are in units of ohms. As a friction surface, we used the dynamic stability surface (i.e. the persistence of a suitable climate, see below) as being indicative of conductance through the landscape. Resistance distances were calculated using CIRCUIT ESCAPE 3.5 (Shah & McRae, 2008). To assess the significance of these relationships we performed 9999 randomizations of the distance matrices using the *ade4* library in R (Thioulouse *et al*., 1997).

Ecological niche modelling

To investigate the putative range dynamics of *D. f. forficatus* as a consequence of climatic fluctuations during the late Quaternary, we compiled a total of 156 unique localities for *D. f. forficatus* based on published and unpublished field data (S. M. Goodman and M. J. Raherilalao). Using this locality information, we used a maximum entropy algorithm (MAXENT 3.2.19) to estimate the current ecological niche (ENM) of *D. f. forficatus* and predict its potential geographic distribution (Phillips, Anderson & Schapire, 2006; Phillips & Dudik, 2008). MAXENT estimates the distribution of a species as the distribution of maximum entropy (i.e. the distribution closest to uniform) under the constraint that the environmental predictors or functions thereof are close to their empirical average (Phillips *et al*., 2006). MAXENT models were developed using standard default settings (automatic selection of response functions; regularization threshold, 0.001; number of background points, ~3500; background, Madagascar). We used the jack-knife feature (assuming no interaction between environmental variables) to estimate the contribution of each variable in predicting suitability (Phillips *et al*., 2006). After considering the performance of the model we projected it into the past, predicting potential distributions during the late Quaternary (see below). *Dicrurus f. forficatus* can be considered a generalist because it occupies both open anthropogenic habitat and forest on Madagascar (Hawkins, 1999).

In order to include all potential habitat accessible by this bird (VanDerWal *et al*., 2009a), we chose Madagascar as the background study area for modelling and started with eight bioclimatic variables expected to jointly represent the major modern climatic classes on the island: annual mean temperature; temperature seasonality; mean temperature of the warmest quarter (quarter defined as three consecutive months); mean temperature of the coldest quarter; annual precipitation; precipitation seasonality; precipitation of the wettest quarter; and precipitation of the driest quarter. As three temperature-related variables (annual mean temperature, and mean temperatures of the warmest and coldest quarters) and three precipitation-related variables (annual mean precipitation, and precipitation of the wettest and driest quarters) were strongly correlated, we only included the mean temperature of the coldest quarter and the precipitation of the driest quarter, thus reducing our variables to four; this further avoids the potential bias of over-fitting our model to current conditions. Variable correlations were assessed through Pearson's correlations, excluding variables that were related to each other with coefficients that are higher or equal to 0.75.

In order to evaluate the performance of the model on the current distribution of the species, we used a five-fold cross-validation procedure (Elith *et al*., 2011). We acknowledge that this evaluation procedure has potential disadvantages, such as non-independence between training and testing occurrence localities, and the retention of sampling biases in the testing localities (Peterson *et al*., 2011). We use all data points for the final model projected onto previous climate surfaces, as the best model is arguably the model inclusive of all possible data. To quantify model performance we used the testing coordinates to estimate the area under the receiver operating curve (AUC) that relates sensitivity (true presence) to the proportion of the study area predicted as present by the model (as it is based on presence-only data). AUC is a standard threshold-independent measure of model performance (Fielding & Bell, 1997, but see Lobo, Jimenez-Valverde & Real, 2008). We use the modified approach to calculate the AUC proposed by Peterson, Papes & Soberón (2008), which only considers the area under the curve within the domain of prediction of the algorithm and the predictive ability given by the user-defined omission error (Pepe, 2000).

We assumed a 10% admissible error for true positives given the level of uncertainty for some georeferenced localities. We assessed the significance of the adjusted AUC through the *Z*-statistic by comparing the mean adjusted AUC against the expected value under a random model within the domain of prediction (Peterson *et al*., 2008).

The models were projected onto 62 time slices representing 1000-year intervals from the present back to the last glacial maximum (22 000 years ago), continuing with 2000-year intervals until 80 000 years ago, and then every 4000 years back to 120 000 years ago. We paid particular attention to three time slices: the present (1950–2008); the Last Glacial Maximum (LGM; 21 000 years ago); and the Last Interglacial (LIG; *c.* 135 000 years ago). Nonetheless, stability is calculated using all projections (see below). Details on how the climate was downscaled are provided in the Supporting information.

Climate suitability through time

In order to estimate the suitability of climate through time for *D. f. forficatus* in Madagascar, we quantified both static (*sensu* Hugall *et al*., 2002; Graham, Moritz & Williams, 2006; VanDerWal, Shoo & Williams, 2009b; Bell *et al*., 2010) and dynamic (*sensu* Graham *et al*., 2010) versions of stability. Climate stability indicates long-term perseverance of climate, and it is hypothesized that climate stability allows the persistence of populations (Hugall *et al*., 2002; Carnaval *et al*., 2009). The difference between static and dynamic stability is that the former assumes that organisms cannot disperse (i.e. a pixel is only stable if the climate remains suitable at that pixel through time), and the latter considers a defined dispersal distance (i.e. a pixel is stable as long as there is suitable climate at or below the dispersal distance in adjacent time steps). In the case of members of the genus *Dicrurus*, it is expected that individuals were able to move relatively long distances to track their most suitable environmental conditions. Thus, as long as patches with suitable climate remained within dispersal distances, these areas could be considered stable dynamic refugia (Graham *et al*., 2010). For the static stability estimate, we summed the negative log of the suitability through time for each pixel. For the dynamic stability estimate, we followed the same guidelines specified in Graham *et al*. (2010). We used modified R scripts provided by S. Phillips (pers. comm.) to calculate stability surfaces.

RESULTS

In total, we collected 6171 bp of DNA sequence data (1752 bp of mtDNA sequence data and 4419 bp of nuclear DNA sequence data) for 35 *D. f. forficatus* individuals. We recovered 180 variable sites within our data set (79 mtDNA and 101 nuclear DNA). Twelve variable positions could not be phased above the 0.63 threshold or cloned for the nuclear introns; these sites were removed for all analyses needing full phase information (population assignment and network analyses).

The MK test performed with the mtDNA data did not detect any departure from neutrality (Fisher's exact test, $P = 0.70$). The HKA test indicated that none of the loci deviated from the expectation of neutrality (sum of deviations = 7.4944, d.f. = 12, $P = 0.82$). No recombination was detected for any of the nuclear loci.

Genetic diversity and demographic parameters

Mitochondrial locus

Our alignment of the ATP6 gene region consisted of 711 bp (684 from ATPase 6, 6 bp from a non-coding spacing region, and 21 bp from the flanking CO3). Twenty-four variable sites, delineating 19 haplotypes, were recovered. Our ND2 alignment consisted of 1041 bp. Fifty-six variable sites were detected among the 36 sequences, which included *D. f. potior* from Anjouan. We analysed the mtDNA data set (1752 bp) both with and without *D. f. potior*. The haplotype from the single individual of *D. f. potior* included in our analyses is nested within *D. f. forficatus*. Excluding *D. f. potior*, we recovered 33 haplotypes among the 35 individuals, most of which were differentiated from each other by only a single mutation (Fig. 2). The most distant haplotypes differed by 1% (between FMNH 430205, Ranomafana, and FMNH 436493–436496, Forêt des Mikea). The haplotypes from individuals sampled from Ranomafana (FMNH 430202–430205; Fig. 1) differ by up to 16 substitutions (0.9%), whereas individuals sampled from Tsimanampetsotsa (FMNH 434484–434486, FMNH 449168) only differ by 0.45%. Summary statistics for the mtDNA data set are detailed in Table 1; values recovered for both Fu's *Fs* ($Fs = -28.779$, $P < 0.001$), and Ramos-Onsins and Rozas' R_2 ($R_2 = 0.0307$, $P < 0.001$) are consistent with recent population expansion. The test for population differentiation between the western and the eastern sampled localities on Madagascar was significant (AMOVA $\Phi_{st} = 0.10919$, $P < 0.001$), as was the north–central–south spatial clustering of localities (AMOVA, $\Phi_{st} = 0.07093$, $P < 0.001$). Yet, only the pairwise comparison between the central and southern populations was significant ($\Phi_{st} = 0.096$, $P = 0.002$).

Nuclear loci

The number of variable sites varied considerably among the eight nuclear loci, with the lowest recov-

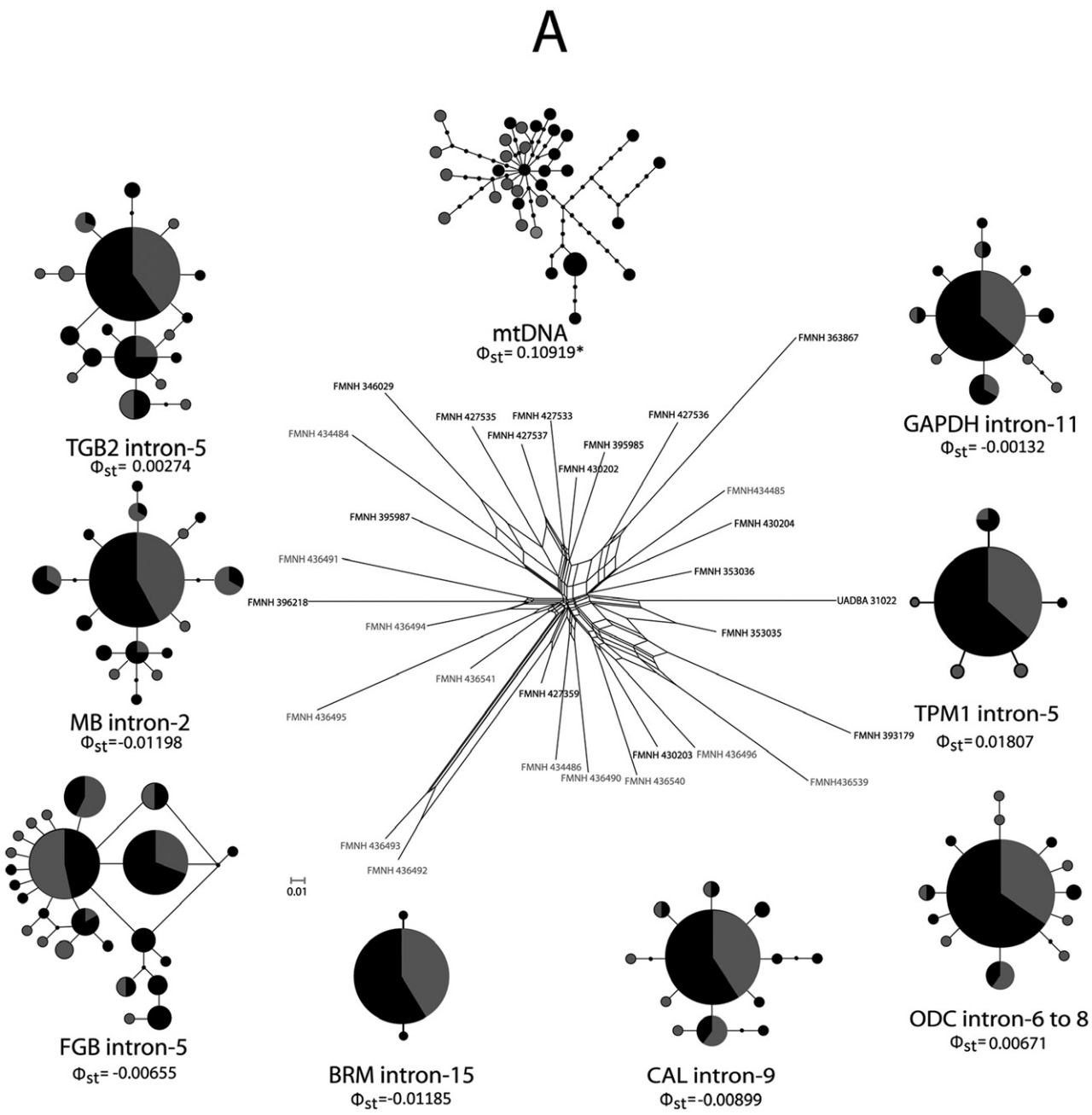

Figure 2. The 95% statistical parsimony network obtained for each locus for (A) east–west and (B) south–north comparisons. Pairwise Φ_{st} values between (A) western (grey) and eastern (black) populations and between (B) south (grey), central (black), and north (red in online version, light grey in printed version) populations were obtained using ARLEQUIN with uncorrected p distances (*population comparisons that were significantly different, $P < 0.05$). Black dots represent unsampled or extinct haplotypes. Circle sizes are proportional to haplotype frequencies. The multilocus network was obtained using SPLITSTREE and uncorrected p distances. For the multilocus network, we only included individuals for which we obtained sequences from all ten loci ($N = 30$).

ered for the *Z*-linked locus BRM intron 15, and the highest recovered for FGB intron 5. Strong evidence of population expansion for all loci was obtained for both Fu's *Fs* and Ramos-Onzins and Rozas' R_2 (Table 1). Haplotype networks from each of the nuclear loci exhibited a star-like pattern (Fig. 2), with most alleles differing from each other by a single mutation and a central allele with high frequency.

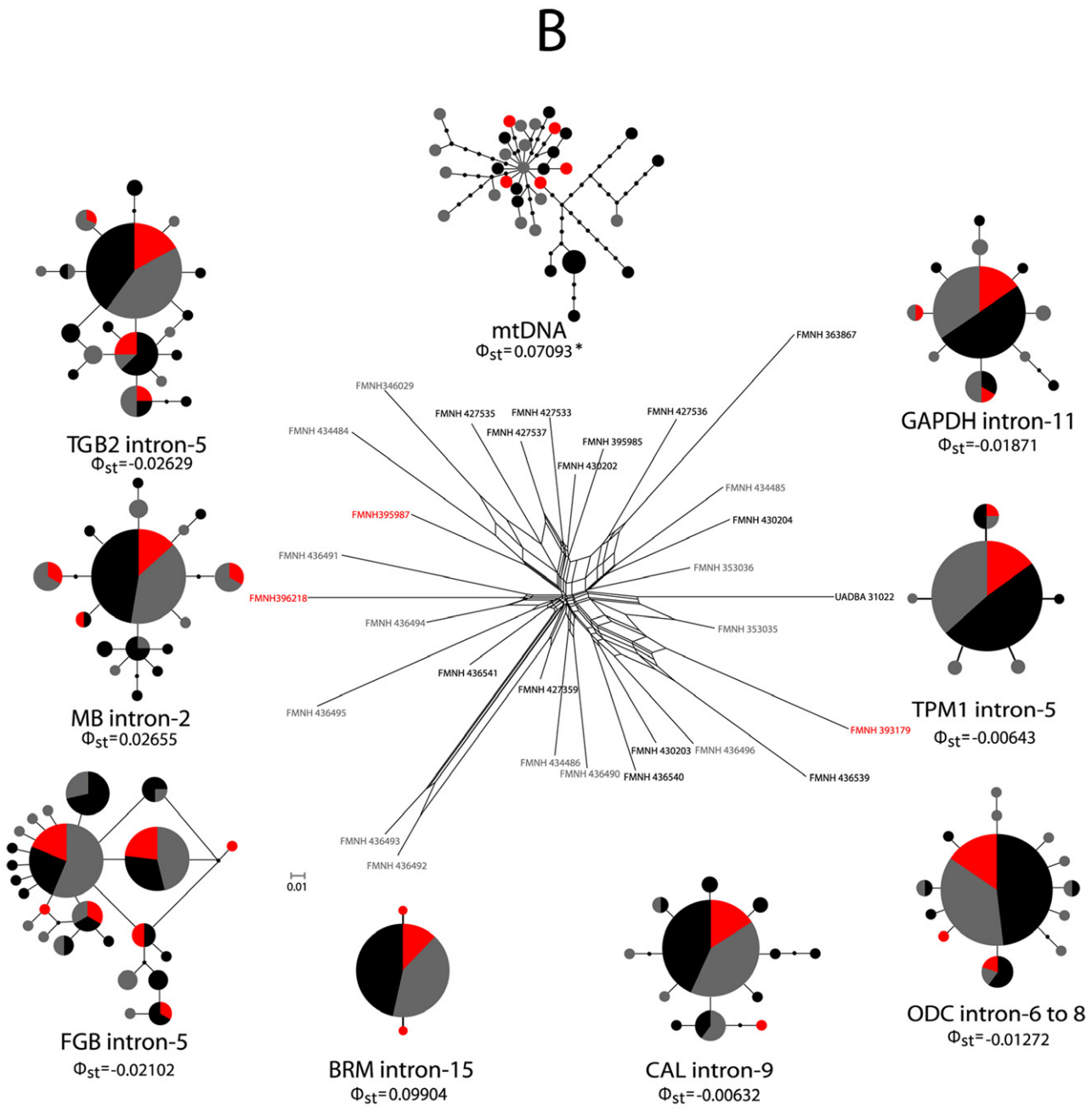

Figure 2. *Continued*.

This pattern was most pronounced for BRM, with only three alleles and an allele frequency of 97% for the most common allele. There was no genetic differentiation between the western and eastern populations according to Φ_{st} (Φ_{st} ranged from –0.01198 to 0.0671 among autosomal loci, $P > 0.20$), and neither for the south-to-north gradient (Φ_{st} ranged from –0.02629 to 0.09904 among autosomal loci, $P > 0.20$). For the *Z*-linked locus, we found a significant differentiation in the south-to-north gradient ($\Phi_{st} = 0.09904$, $P = 0.02$), but not in the western versus eastern comparison ($\Phi_{st} = –0.01185$, $P = 0.99$).

All data combined

The multilocus coalescent analyses performed with LAMARC suggest population growth, as a zero value for *g* was not included in the 95% confidence interval (95% CI) for any of the individual loci or for the combined data set. The maximum posterior estimate

Table 1. Number of single nucleotide polymorphisms (SNPs) per locus within *Dicrurus forficatus forficatus*

Locus	$N_{chromosomes}$	L	$N_{mutations}$ (including indels)	Max_{dist}/Avg_{dist} (in%)	S	π	θ	Fu's Fs (P)/R_2 (P)	SNPs included in structure/network analyses
mtDNA	35	1752	79	1/0.4	78	0.00451	0.01081	–28.779 (0.001)*/ 0.0307 (0.001)*	79
MB	64	718	18	0.6/0.2	18	0.00169	0.00530	–12.196 (< 0.001)*/ 0.0338 (0.006)*	13 (position 237, 398, 429, 286, 568), 713 bp
GAPDH	70	349	11 (2-bp insertion)	0.9/0.2	11	0.00167	0.00654	–9.791 (< 0.001)*/ 0.0351 (0.03)*	11
CALB1	60	685	13 (one 11-bp deletion)	0.3/0.07	13	0.00112	0.00407	–9.819 (< 0.001)*/ 0.0351 (0.03)*	11 (position 37, 381), 673 bp
TGFβ2	70	569	17 (2-bp deletion and one C substitution by two As)	0.5/0.2	17	0.00235	0.00620	–14.111 (< 0.001)*/ 0.0386 (0.02)*	14 (position 117, 124, 226), 564 bp
ODC	70	685	13 (four 1-bp indels)	0.44/0.08	13	0.00082	0.00394	–14.081 (< 0.001)*/ 0.031 (0.03)*	13
FGB	70	560	22	1.3/0.4	22	0.00391	0.00815	–17.717 (< 0.001)*/ 0.0457 (0.04)*	20 (position 150, 171), 558 bp
TPM1	70	498	5 (2-bp deletion and 1-bp insertion)	0.4/0.05	5	0.00056	0.00208	–5.065 (< 0.01)*/ 0.04 (0.07)	5
BRM	60	355	2	0.3/0.02	2	0.00019	0.00121	–3.287 (0.033)*/ 0.0898 (0.37)	2

Indels of more than 1 bp in length were considered as a single mutational event. We included all SNPs, including the one that did not receive a phase probability greater than 0.63, in the genetic diversity measures (number of segregating sites, S; nucleotide diversity, π; Watterson's θ) and population expansions indexes. As the phase of the haplotype may have an impact on the θ, Fs and R_2 values, we estimated the genetic diversity values on every possible combination of the SNPs that were not phased above the 0.63 threshold. Values were always strictly identical. *Significant values ($P < 0.05$) in the Fs and R_2 tests.

Table 2. Time to most recent common ancestor (TMRCA) for the mitochondrial data set using several different combinations of molecular clock and tree priors

Tree model	Molecular rate, substitution per site per lineage per Myr	TMRCA (95% HPD)	Reference
Yule	Four-fold, 0.073	144 000 (57 000–264 000) years	Subramanian *et al*. (2009)
Yule	Four-fold, 0.062	170 000 (81 000–290 000) years	Subramanian *et al*. (2009)
Yule	ND2, 0.0615	69 000 (43 000–95 000) years	Arbogast *et al*. (2006)
Yule	ND2, 0.0215	197 000 (126 000–272 000) years	Arbogast *et al*. (2006)
Yule	ND2, 0.029	197 000 (135 000–266 000) years	Lerner *et al*. (2011)
Yule	ATP6, 0.026	105 000 (55 000–158 000) years	Lerner *et al*. (2011)
Yule	ND2 & ATP6, combined	136 000 (91 000–181 000) years	Lerner *et al*. (2011)
Constant	Four-fold, 0.073	248 000 (78 000–501 000) years	Subramanian *et al*. (2009)
Constant	Four-fold, 0.062	252 000 (113 000–429 000) years	Subramanian *et al*. (2009)
Constant	ND2, 0.062	84 000 (56 000–121 700) years	Arbogast *et al*. (2006)
Constant	ND2, 0.0215	245 000 (163 000–346 000) years	Arbogast *et al*. (2006)
Constant	ND2, 0.029	182 000 (113 000–255 000) years	Lerner *et al*. (2011)
Constant	ATP6, 0.026	135 000 (73 000–205 000) years	Lerner *et al*. (2011)
Constant	ND2 & ATP6, combined	168 000 (113 000–228 000) years	Lerner *et al*. (2011)
Expansion	Four-fold, 0.073	165 000 (10 000–377 400) years	Subramanian *et al*. (2009)
Expansion	Four-fold, 0.062	179 000 (77 000–307 000) years	Subramanian *et al*. (2009)
Expansion	ND2, 0.062	73 000 (48 000–102 000) years	Arbogast *et al*. (2006)
Expansion	ND2, 0.0215	200 000 (119 000–284 500) years	Arbogast *et al*. (2006)
Expansion	ND2, 0.029	147 000 (86 000–211 000) years	Lerner *et al*. (2011)
Expansion	ATP6, 0.026	112 000 (61 000–171 000) years	Lerner *et al*. (2011)
Expansion	ND2 & ATP6, combined	136 000 (88 000–193 000) years	Lerner *et al*. (2011)

for the exponential growth parameter g was 2792.536 (95% CI 2046.53–3607.68).

TMRCA estimate

The mean estimates for TMRCA for all mtDNA haplotypes varied between 69 000 years (95% HPD 43 000–95 000 years, with a Yule tree prior and ND2 clock rate of 0.0615 s/s/l/Myr, i.e. 12.3% sequence divergence per million years) and 252 000 years (95% HPD 113 000–228 000 years, with a coalescent model assuming constant population size and a four-fold site rate of 0.062 s/s/l/Myr) (Table 2). The mean estimate of TMRCA in some estimates pre-dated the inferred inundation of Aldabra 120 000 years ago. All three such instances involved the use of a non-optimal tree model, namely either a Yule tree prior or a model of constant population size (Table 2). Our estimates of the TMRCA are in agreement with earlier studies suggesting that *D. f. forficatus* colonized Madagascar during the last 120 000 years, when the ancestor of *D. aldabranus* was able to colonize Aldabra after the inundation of the atoll. Hence, phylogenetic patterns (sister-group relationship), the mitochondrial clock, and geological data all indicatethat *D. f. forficatus* likely originated within the past 120 000 years.

Structure analyses and multilocus network

The population assignment analyses performed with STRUCTURE indicated that the highest likelihood was obtained with a specified number of populations of $K = 2$ (Fig. 3). Yet, the percentage of the genome assigned to each of the two clusters was even across the individuals, with the assignment probability between two populations varying between 0.27 and 0.47 for each individual: that is, all individuals would still carry a larger component of the genome from population one than from population two.

Isolation by distance and resistance

Individual genetic distances were positively associated with both geographic ($r = 0.15$) and resistance ($r = 0.21$) distances, but were only statistically significant for the latter ($P = 0.03$, Fig. S1).

Ecological niche modelling

Test data used in the five-fold cross-validation procedure yielded a mean adjusted AUC ratio of 1.35, with a standard deviation of 0.12, which is significantly higher than expected under a random model ($Z = 6.37$, $P = 1.85 \times 10^{-10}$). This suggests that the climatic variables did better than selecting points at

Figure 3. Assignment of *Dicrurus forficatus forficatus* individuals to genetic clusters using the STRUCTURE algorithm for $K = 2$ (mean log-likelihood across ten runs, –ln = 636.2). The individual from Anjouan (*Dicrurus forficatus potior*) was not included in the STRUCTURE analyses.

random to delimit the bounds of the current distribution of *D. f. forficatus*. The variable that contributed the most to the model was precipitation of the driest quarter (40.4%). Based on the response curve of this variable, this taxon has a propensity to occur in areas with high-to-moderate precipitation, but tends to avoid areas characterized by low precipitation. Areas with high suitability ranged along the steep eastern slope of the highlands, lowland areas of the east, particularly the north-east, and had a patchy distribution in the western and north-western lowlands (Fig. 4). Areas with low suitability were distributed throughout the interior of the island, including a portion along the central western coast. Low-suitability areas represented mostly dry habitat zones. Projecting these variables back in time suggests that *D. f. forficatus* probably occurred in several loosely connected patches of putative suitable habitat on Madagascar during the past 120 000 years (LGM and LIG, Fig. 4). Patches occupied along the western coast are separated from those along the eastern half of Madagascar by an area of low suitability.

DISCUSSION

Genetic inferences

Analysing genetic variation among the ten loci sequenced for 35 individuals sampled throughout the distribution range of *D. f. forficatus* recovered relatively low levels of genetic differentiation. No evidence of geographical or ecological structure associated with genetic variation was identified in the nuclear data sets (Φ_{st} ranged from –0.01198 to 0.0671, all P values were non-significant). In contrast, the mtDNA data suggested the presence of weak but significant structure between eastern and western populations ($\Phi_{st} = 0.10919$, $P < 0.001$). Below, we discuss hypotheses that may explain this weak pattern of genetic structure, especially in light of the considerable levels of genetic structure that have been recovered in other vertebrate species on Madagascar (e.g. Vences & Glaw, 2002; Vieites *et al*., 2006). Genetic studies of Malagasy vertebrates have typically documented mtDNA divergences of 2.2–11.4% within amphibian and squamate reptile 'species' (e.g. Boumans *et al*., 2007; Vieites *et al*., 2009), and within mammals around 12% for lemurs (e.g. Groeneveld *et al*., 2010) and 14% for shrew tenrecs (Olson *et al*., 2004). These data, at a first glance, indicate ancient divergences and population structure within several Malagasy terrestrial vertebrates, in sharp contrast to our results for *D. f. forficatus*.

Fuchs *et al*. (2007) also documented little genetic differentiation (maximum mtDNA genetic distance: 0.6%) among populations of the Madagascar Scops-Owl *Otus rutilus* across Madagascar, although significant partitioning of the genetic diversity was also found along the east–west axis ($\Phi_{st} = 0.173$, a value comparable with what we recover for *D. f. forficatus*). Likewise, although with limited sampling, little genetic differentiation was found on Madagascar for the sunbird *Nectarinia souimanga* (0.2–0.6%, Warren

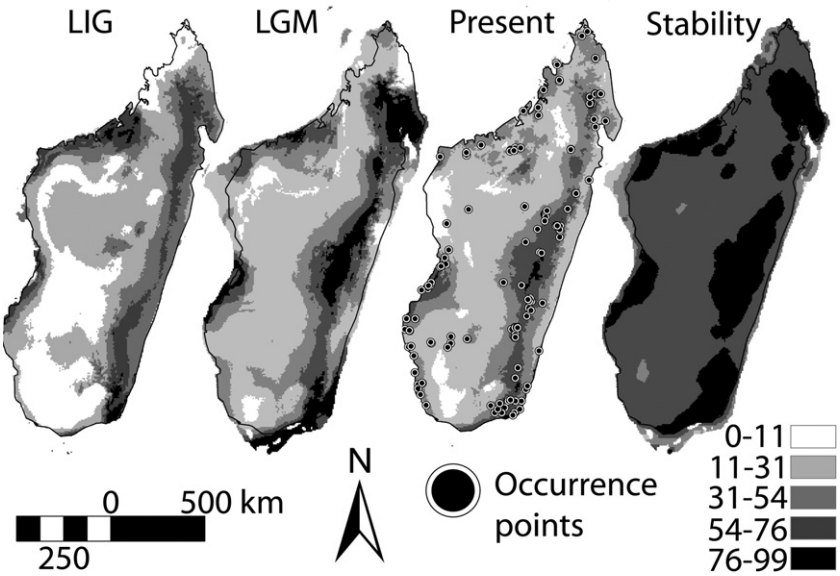

Figure 4. Stability and predicted geographic distributions for *Dicrurus forficatus forficatus* (suitability legend on lower right, 0–100%). The present niche model was developed using four environmental layers (see Material and methods) and 156 point localities (as shown on the present prediction). This model was projected onto several time slices, including the last glacial maximum (LGM) and the last interglacial (LIG). A stability map was produced considering all predictions through time and assuming a 20-m dispersal distance per year. Darker areas reflect higher suitability (LIG, LGM, and present maps), and hence putative refugia (stability map).

et al., 2003), the bulbul *Hypsipetes madagascariensis* (0–0.09%, Warren *et al*., 2005), the white-eye *Zosterops maderaspatana* (0–0.3%, Warren *et al*., 2006), and the kingfisher *Corythornis madagascariensis* (0–1.4%, Marks & Willard, 2005), although none of these species are strictly forest dependent, except for *C. madagascariensis*. The mitochondrial genetic differentiation we recovered for *D. f. forficatus* (mean, 0.5%; maximum, 1.0%) is similar to the values recovered for *O. rutilus* (mean, 0.24%; maximum, 0.6%) using a similar sampling scheme (34 individuals) and overlapping localities, suggesting that limited genetic differentiation among eastern and western populations may be common for non-forest-dependent Malagasy birds.

As expected given the limited mtDNA structure among populations, we recovered no spatial structure among sampling localities for any of the individual nuclear markers, or when they were combined in a large multilocus data set. Furthermore, for some nuclear loci, alleles were shared between *D. forficatus* and its sister species, *D. aldabranus*, a result that we attribute to the slower mutation rate and longer coalescent time inherent to the nuclear genome. The lack of clear structure among the nuclear loci is corroborated by the population assignment analyses from the nuclear data set, where only one biological population was inferred. One potential bias could be that we excluded the SNPs that could not be phased with a probability greater than 0.63. However, the number of SNPs we excluded from the data set is relatively low (12, nearly 12% of all variable nuclear sites), and almost exclusively involved sites that were autapomorphic and hence would not have provided any additional information with respect to population assignment (i.e. mutations only represented on one chromosome in the data set). In addition, individuals where the SNPs could not be phased appear to be randomly distributed, and there is no obvious association between localities and individuals where the full phase information could not be obtained.

Nucleotide diversity (Table 1) among all eight nuclear loci was low, suggesting that a selective sweep on mtDNA is very unlikely to be an explanation for the lack of observed deeply divergent mtDNA haplotypes. Finally, all loci analysed in our data set carried a strong signal of population expansion, as suggested by the significant negative values of Fu's *Fs* and low R_2 values (Table 1), and the significantly positive value of *g*. Hence, we conclude that the historical demography of *D. f. forficatus* encompasses a recent population expansion.

Ecological niche modelling

The geographical distributions predicted from ecological niche models recovered continuously favourable climate space for this species, and therefore putative

suitable habitat along the eastern slope of the eastern mountains and in two patches of the lowlands along the western coast of Madagascar for the past 120 000 years. The suitable areas in the eastern and western halves of Madagascar have remained relatively isolated since the LIG according to projections of the ENM on climate surfaces reconstructed for the past. Thus, following these results, we expect that if any signal of genetic structure were apparent in our molecular data, it would be between our eastern and western sampling localities. However, our ENM over the course of the past 120 000 years indicates that these eastern and western 'refugia' have been repeatedly connected through time, probably facilitating several episodes of gene flow across the eastern and western regions of the island. The changes in suitability through time show maximum suitability and connectivity around the LGM, with populations becoming relatively more isolated towards the LIG and the present. Thus, we might expect to see the signature of a past range expansion around the LGM.

Why limited genetic structure? Reconciling niche modelling and genetic data

An important factor that affects the genetic differentiation within a species is the elapsed time since its divergence from its closest relative. *Dicrurus forficatus* is a relatively young species that diverged from its closest relative, *D. aldabranus*, within the past 0.12–0.14 Myr (95% HPD 0.09–0.20 Myr, this study). The Aldabra Atoll could only have been colonized by the *D. aldabranus* lineage during the last 0.120 Myr, after the end of the higher sea levels that probably inundated all terrestrial biota (Thomson & Walton, 1972), an assumption used by Pasquet *et al*. (2007). All *D. f. forficatus* mtDNA haplotypes form a monophyletic group with respect to *D. aldabranus*, and the TMRCA for all *D. f. forficatus* mtDNA haplotypes. Given the short time frame involved for the evolution of *D. forficatus*, we would expect relatively little differentiation to have taken place during that period. This statement holds in particular for the nuclear loci we sequenced, which usually possess slower mutation rates than mtDNA loci (e.g. Lee & Edwards, 2008). Hence, the limited time since the divergence of *D. forficatus* from *D. aldabranus* may be one reason why stronger genetic structure was not detected, especially for the nuclear data, as mutations may not have had time to accumulate.

Drongos colonized several remote islands by overwater dispersal, including Madagascar and the Comoros, but also the Andaman Islands and several archipelagos in Asia and Australasia (Pasquet *et al*., 2007). Within the Comoros Archipelago, the nearest island to Madagascar is Mayotte, where a closely related species (*D. waldenii*) occurs (estimated colonization date 4.2 Mya; Pasquet *et al*., 2007), and the colonization of Anjouan by *D. f. potior* suggests that this species can reach distant locations even if a closer island is already occupied by a member of this genus. Thus, drongos exhibit a leapfrog colonization pattern among western Indian Ocean islands, a pattern shared with several other volant vertebrates (e.g. Weyeneth *et al*., 2008; O'Brien *et al.,* 2009; Chan *et al*., 2011). It is also critical to highlight that *D. f. forficatus* is not forest dependent, and occurs across a broad elevational gradient from sea level to about 2000 m a.s.l. (Hawkins, 1999). It is a habitat generalist that can be abundant in virtually all of the different terrestrial forested or partially wooded habitats of Madagascar (Wilmé, 1996; Raherilalao & Wilmé, 2008), including those that have drastically different climatic regimes and vegetational structure.

Different hypotheses have been proposed to explain the patterns of speciation in Malagasy forest-dependent taxa, such as riverine barriers, watersheds, or montane refugia (Wilmé, Goodman & Ganzhorn, 2006; Vences *et al*., 2009), which probably do not apply to *D. f. forficatus*, given its supposed strong dispersal capability and less restricted habitat requirements. Hence, current knowledge about the ecology and biology of the drongos, as well as the timing of divergence from its sister species, would point towards the general lack of population differentiation across the island.

Despite being weak, the genetic structure recovered across the east–west axis of Madagascar using the mitochondrial data was significant. Interestingly, our results indicate that genetic distance is better explained by ecological (climatic space) rather than linear geographic distances, suggesting that despite the generalist nature of this species, habitat is likely to act as an important dispersal filter, which may account for the east–west pattern of phylogeographic structure recovered. Indeed, suitable habitat for *D. f. forficatus*, as inferred in our palaeodistribution reconstructions, appears to have been present in both western and eastern Madagascar, and to have been stable through the portion of the Quaternary period when this species colonized the island (Fig 4). Our ENM suggests that the centre of the island was less climatically suitable through time, indicating that gene flow between the eastern and the western areas may have been repeatedly reduced over the past 120 000 years. As expected, areas where the climate and therefore putative habitat has most likely remained stable through time also hold some of the most divergent haplotypes. For example, haplotypes from individuals sampled from Ranomafana (FMNH 430202–430205, Fig. 1), one of the areas of

stability, differ by up to 16 substitutions (0.9%). In contrast, individuals sampled from areas with the lowest extent of climatic suitability through time have the smallest divergence among haplotypes (Tsimanampetsotsa, 0.45%; FMNH 434484–434486, FMNH 449168). Hence, it appears that sites characterized by climatic suitability through time harbour higher genetic diversity, whereas populations on the edge of the distribution range would only have a subset of the alleles in the source populations because of stochastic factors, as predicted by the abundant core theory (reviewed in Sexton *et al*., 2009).

In conclusion, we attribute the weak but significant geographic partitioning of genetic variation in *D. f. forficatus* to a combination of factors that include: (1) limited time for the accumulation of genetic differences between eastern and western populations, especially for nuclear data, with slower mutation rates and longer coalescent times; and (2) climatic oscillations leading to repeated contact between the eastern and western populations via population expansion/contraction over the past 0.120 Myr, resulting in brief periods of connectivity interrupted by longer periods of allopatry, during which mtDNA variation could accumulate and sort. This scenario of periodic connectivity and isolation might also explain why two populations were recognized in the STRUCTURE analyses of the nuclear data, even if all individuals belonged with less than 50% assignment probability to the second population. For *D. forficatus*, a west–east pattern of genetic structure (e.g. Fuchs *et al*., 2007) fits the data better than a south–north cline, as recovered for *Monticola* rock thrushes (Cruaud *et al*., 2011), a pattern that may be attributed to the denser distribution of areas of climatic suitability over time on the south–north cline than on the east–west cline.

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SUPPORTING INFORMATION

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

Figure S1. Correlation between genetic distances and geographic distances, and between genetic distance and the resistance matrix.

Table S1. List of samples used in this study. Voucher specimens have been deposited in the Field Museum of Natural History, Chicago (USA) and in the Département de Biologie Animale, Université d'Antananarivo (UADBA). Source of DNA consists of blood (B) or tissue (T). Information on habitat designation follows Schatz (2000) and Moat & Smith (2007). PN = Parc National, RNI = Réserve Naturelle Intégrale and RS = Réserve Spéciale.

Table S2. List of loci sequenced and primers used for PCR-amplification and sequencing.