UNIVERSITY OF CALIFORNIA

Los Angeles

Adaptive Diversification and Anthropogenic Impacts

on African Rainforest Biodiversity

A dissertation submitted in partial satisfaction of the

requirements for the degree of Doctor of Philosophy

in Biology

by

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PUBLICATIONS

- Freedman AF., Portier KM, Sunquist ME (2003). Life history analysis for black bears (*Ursus americanus*) in a changing demographic landscape. *Ecological Modelling*, **167**, 47–64.
- ---, Buermann W, Lebreton M, Chirio L, Smith TB (2009) Modeling the effects of anthropogenic habitat change on savanna snake invasions into African rainforest. *Conservation Biology*, **23**, 81–92.

- Funk DW, Noel LE, Freedman AH (2004) Environmental gradients, plant distribution and species richness in Arctic salt marsh near Prudhoe Bay, Alaska. *Wetlands Ecology and Management*, **12**, 215–233.
- Hamilton AM, Freedman AH, Franz R (2002) Influence of deer feeders, habitat, and sensory cues on predation rates on artificial turtle nests. *American Midland Naturalist*, **147**, 123–134.
- Pease KM, Freedman AH, Pollinger JP et al. (2009) Landscape genetic structure of California mule deer (*Odocoileus hemionus*): the role of contemporary ecological and historical factors. *Molecular Ecology*, **18**, 1848–1862.

ABSTRACT OF THE DISSERTATION

Adaptive Diversification and Anthropogenic Impacts

on African Rainforest Biodiversity

by

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Doctor of Philosophy in Biology University of California, Los Angeles, 2009 Professor Thomas Smith, Chair

The relative contributions of genetic drift and natural selection are central to the ongoing debate concerning mechanisms that generate rainforest biodiversity. While evidence has been presented supporting allopatric divergence via drift in rainforest refugia, more recent studies point to a greater role for divergent natural selection along ecological gradients. Thus, in chapter 1, I investigated the relative importance of rainforest refugia and ecological gradients in Cameroon to diversification in *Trachylepis affinis*, a rainforest skink. I performed genome scans to distinguish neutrally evolving loci from those under divergent selection across environments.

Generalized dissimilarity modeling was then used to build separate models of associations between either neutral or adaptive genetic variation and data on climate and vegetation from satellite remote sensing. In conjunction with niche models of species distribution change since the last glacial maximum, results from these analyses found little support for rainforest refugia as an important driver of diversification. A more significant role was found for divergent selection along gradients between rainforest and the rainforest-savanna ecotone, and between lowland and montane rainforest. In chapter 2, I used ecological niche models to investigate whether deforestation in Cameroon was facilitating the invasion of rainforest by savanna snake species. Models for three species demonstrated that rainforest habitats were normally unsuitable, but that deforestation created favorable microhabitats that were facilitating large-scale invasions of the rainforest zone. Because dramatic changes in land use since the early 20th century may be erasing the evolutionary potential of the rainforest-ecotone gradient, in chapter 3 I investigated the evolutionary implications of deforestation for the little greenbul (Andropadus virens), a common rainforest bird species. Using satellite-based estimates of forest cover, morphological data collected from museum specimens predating widespread deforestation, and recent morphological data, I showed that the gradient has become shallower in West Africa than in Central Africa, and that as a result, there has been a loss of morphological variation in West Africa in traits important to fitness. In contrast, I found no loss of morphological variation over time in Central Africa where there has been less deforestation and gradients have remained more stable.

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Genomic Signals of Diversification Along Ecological Gradients

in a Tropical Lizard

Introduction

Recent efforts to distinguish the relative roles of drift and natural selection in the evolution of populations suggest that selection may play the dominant role in diversification (Clegg *et al.* 2002; McKinnon *et al.* 2004; Coyne and Orr 2004; Smith *et al.* 2005a; Nosil *et al.* 2008). Although crucial to understanding which mechanisms are primarily responsible for observed patterns of biodiversity, simultaneous evaluation of these two processes is rare. Yet, such an approach would be useful in understanding speciation process in rainforests where debate over the relative roles of drift and selection has been particularly intense (Endler 1982; Mayr and O'Hara 1986; Smith *et al.* 1997; Moritz *et al.* 2000).

In equatorial Africa, persistence of rainforest refugia during cool, dry glacial periods are supported by paleoecological studies (Maley 1996; Dupont *et al.* 2000), and their importance to speciation has been inferred from patterns of species richness and endemism (Mayr and O'Hara 1986; Linder 2001; Tchouto *et al.* 2008; Wronski and Hausdorf 2008), as well as patterns of inter- and intraspecific genetic variation (Roy 1997; Quérouil *et al.* 2003; Bowie *et al.* 2006; Anthony *et al.* 2007). However, the mechanisms that generate morphological or reproductive divergence between refugia are seldom clear (Moritz *et al.* 2000); claims of vicariance generated by river barriers (Quérouil *et al.* 2003; Anthony *et al.* 2007) similarly lack precise mechanistic

explanations. In contrast, more recent studies in the tropical rainforests of Africa, South America, and Australia suggest divergent natural selection along ecological gradients frequently drives diversification even in the face of substantial gene flow (Smith *et al.* 1997, 2000, 2005a; Schneider *et al.* 1999; Moritz *et al.* 2000; Graham *et al.* 2004), i.e. "divergence-with-gene-flow" (Rice and Hostert 1993). While past molecular studies on tropical rainforest species have relied on neutral markers, a comparison of neutral variation with that under selection would permit a more direct assessment of the evolutionary processes that generate rainforest biodiversity.

A recent approach for evaluating the contribution of natural selection to diversification is to examine intraspecific heterogeneity in genomic divergence (Nosil *et al.* 2009). This typically begins with a genome scan from which a large number of anonymous genetic markers are generated. Samples are grouped by habitat or other ecologically relevant dimensions, and outlier loci whose genetic divergence among groups exceeds neutral expectations are identified as those likely to be under divergent selection. Comparisons between divergence patterns in neutral loci and those outliers purportedly under selection can then be made. The genome-wide effects of ecologymediated adaptive divergence can be identified by a signature of isolation-by-adaptation (IBA), where divergence at neutral loci is positively associated with adaptive divergence (Nosil *et al.* 2008, 2009). In this context, adaptive divergence is quantified in terms of phenotypes important to fitness, or environmental variables that are thought to be important with respect to local adaptation.

While genome scan studies can test for parallel divergent selection across habitats (e.g. Campbell and Bernatchez 2004; Bonin *et al.* 2006), loci purportedly under selection and IBA have rarely been evaluated concurrently (Nosil *et al.* 2009). Particularly lacking are studies that distinguish the evolutionary processes shaping neutral vs. "adaptive" outlier loci by contrasting their respective associations with environmental variables and geographic distance. Such analyses are now made more possible with the availability of globally available climate data, information on vegetation structure measured by satellite-borne instruments, and a growing array of landscape genetic techniques (Manel *et al.* 2003; Balkenhol *et al.* 2009). Because they focus on the association between genetic diversity and contemporary environment, inclusion of additional analyses that shed light on historical effects may help further resolve the causes of genetic variation. In particular, projections of paleodistributions using ecological niche models can reconstruct historic changes in geographic distribution that may have consequences for genetic diversity (Waltari *et al.* 2007; Carnaval *et al.* 2009; Pease *et al.* 2009)

Here we employ a combination of genome scans, outlier analyses, environmental modeling of gene-environment relationships, and ecological niche models in order to elucidate the evolutionary processes shaping diversification in *Trachylepis affinis* (Scincidae), an African rainforest lizard. Small ectothermic species such as *T. affinis* are ideal study organisms for two reasons. First, because they exhibit low overland dispersal rates (Hranitz and Baird; Sumner *et al.* 2001; Massot *et al.* 2003), the effects of environmental heterogeneity on genetic variation are likely detectable at relatively small spatial scales. Second, changing thermal regimes across environmental gradients have a

profound influence on ectotherm physiology, life history, locomotor performance, reproduction, and other components of fitness (Stevenson *et al.* 1985; Huey and Kingsolver 1989; Shine *et al.* 1997; Downes and Shine 1999).

We sampled 12 populations of T. affinis (Fig. 1A) in Cameroon throughout the range of habitats in which it is found, including rainforest (and hypothesized refugial areas), montane forest, and the gallery forests of the transitional ecotone between savanna and rainforest. We then performed an AFLP genome scan, partitioning AFLP markers into neutral loci, and outliers purportedly under divergent selection, in order to contrast their respective patterns of geographic variation and environmental associations. As a benchmark for neutral processes we also compared these to patterns of mitochondrial gene (mtDNA) sequence variation. MtDNA variation is frequently employed in studies of intraspecific phylogeography because it is thought to evolve neutrally under most circumstances (Avise 2000), although there are exceptions where selection may play a role (e.g. Cheviron and Brumfield 2009). We utilized variation in morphological traits thought to be important to fitness in lizards (Losos 1990; Losos et al. 1997; Vitt et al. 1997; Schneider et al. 1999) to support our argument that patterns of variation in many of the outlier loci are consistent with natural selection along the rainforest-ecotone gradient. To quantify and visualize gene-environment relationships, we employed generalized dissimilarity modeling (GDM; Ferrier et al. 2007), a recently developed nonlinear matrix regression technique that fits dissimilarity in a response variable (e.g. a genetic distance matrix), to dissimilarities in predictor variables (e.g. remotely sensed climate and



Figure 1. A. Location of sites in Cameroon where samples of *T. affinis* were collected overlaid onto percent tree cover derived from the MODIS satellite sensor (Hansen et al. 2002). Approximate locations of hypothesized rainforest refugia are taken from Maley (1996). Inset denotes major vegetation associations, adapted from White (1983). B. Predicted distribution for *T. affinis* based upon an Maxent ecological niche model (see text for details). Classification of areas of overprediction are based upon empirical observation of forest cover or known absence of *T. affinis* (particularly in southern Cameroon) based upon intensive field surveys conducted by AHF.

vegetation). We combined inferences from these relationships with traditional genetic structure analyses of neutral loci, an ecological niche model of distribution change since the Last Glacial Maximum (LGM), and historical demographic reconstruction. In integrating these diverse analytical approaches, our specific objectives were to: 1) quantify the proportion of the genome that is likely under divergent selection among environments, 2) identify the environmental gradients along which adaptive divergence may occur, and 3) simultaneously test rainforest refugia, riverine barrier, and ecological gradient speciation hypotheses.

Materials and Methods

Field sampling

Eleven of our 12 sites (Fig. 1A) were sampled during three consecutive field seasons (February – May 2004, March – August 2005, March – June 2006), while the twelfth site was sampled in 2001. Table S1 provides detailed information on localities, sample sizes, and habitats. Lizards were either captured by hand or in pitfall trap-drift fence arrays (Crosswhite *et al.* 1999). Immediately following capture, lizards were euthanized by placing them in a sealed jar with cotton dosed with approximately .5mL of 99% isoflurane. For each lizard, we determined sex in the field by the presence of hemipenes, and if unconfirmed, verified sex by presence of ovaries or testes in subsequent dissections. Individuals without detectable reproductive structures were classified as juveniles and excluded from morphological analyses. Eight measurements were taken on each lizard for traits related to body size, feeding and locomotion, and thus fitness (Losos

1990; Losos et al. 1997; Vitt et al. 1997; Schneider et al. 1999): snout-vent length (SVL), measured as the linear distance from tip of the snout to the cloacal opening; hind limb length; hind limb span; fore limb length; fore limb span; head length, from the posterior edge of the interparietal scale to the tip of the snout; head depth, at the deepest point of the head; and gape width, at the widest point of the head. Limb lengths were measured from the insertion point of the limb into the body wall to the distal tip of the claw on the fourth toe, and were taken from the left side of the body unless the limb was damaged. Hind (fore) limb spans were measured as the linear distance between the femoral-tibial (humero-ulnar) joints, while the femurs (humeri) were extended perpendicular to the body, and the tibia (ulnae) were oriented at right angles to the femurs (humeri). SVL, tail length, and limb lengths were measured with a metric ruler with 1-mm accuracy, and all other measurements were measured with dial calipers (.01 mm accuracy). All measurements were performed by AHF. Liver and/or tail tissue was collected and preserved in 95% ethanol. Lizards were then fixed with 10% formalin, and later transferred to 70% ethanol solution. Lizards collected by TBS in 2001-Palm d'Or, as well as a small number of individuals from Malimba—were preserved in 95% ethanol and then frozen. We applied correction factors to measurements that displayed preservation effects (SVL, gape width, and head depth), based upon re-measurement of 50 individuals by AHF from the 2004-2006 collection. All voucher specimens are to be deposited at the Museum of Vertebrate Zoology at University of California, Berkeley.

Morphological analyses

We performed principle components analysis (PCA) on morphological traits in order to characterize size (PC1) and shape (PC2) variation among habitats delineated with remote sensing (see below), and tested for sex, habitat and sex*habitat effects for the first two principle components with a general linear model (GLM), with sex and habitat fixed and random effects, respectively. Because sex effects were not significant, to evaluate morphological differentiation among habitats we pooled sexes and performed a one-way ANOVA on the PC scores, including Sidak post hoc tests adjusting for multiple comparisons between pairs of habitats. All tests were carried out with SPSS 16.0.

Laboratory methods

We extracted genomic DNA from tissue using QIAmp® DNA Mini Kit following the manufacturer's protocols. We performed amplified fragment length polymorphism (AFLP) genome scans on 18-24 individuals per sampling location for a total of 241 individuals. We followed the *MseI/Eco*R1 protocol of Bonin *et al.* (2005) modified from Vos *et al.* (1995), for restriction enzyme digests, ligation of adapters, pre-selective PCR amplification, and selective PCR amplification. Pre-selective primers were 5'-GACTGCGTACCAATTC-3' (*Eco*RI) and 5'-GATGAGTCCTGAGTAA-3' (*Mse*I). Selective primer sequences consist of pre-selective sequences with selective extensions of 3 nucleotides on the 3' end, of which the *Eco*RI primer was 6-FAM dye-labelled. We tested six selective primer combinations, four of which amplified. Sequences for these *Eco*RI /*Mse*I primer pairs were 1) –tag/ –cga, 2) –tag/ –cgt, 3) –tgc/ –cga, and 4) –tgc/ –

cgt. Genotyping PCR reactions were carried out on an ABI 3730XL sequencer using LIZ500 size standard. We analyzed genotypes using GeneMapper 3.7 (Applied Biosystems). To minimize genotyping error, all plates included at least 4 overlapping individuals, and 1-4 negative controls. Loci that scored inconsistently among plates, and for which bands were detected in any negative control were excluded. This produced a total of 191 repeatable loci, with 33 to 58 loci produced per selective primer combination.

For 175 individuals, we amplified a 693 base pair (bp) fragment of the mtDNA gene NADH dehydrogenase 4 (ND4), using primers ND4 [5'-CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC-3'] and Leu [5'-CAT TAC TTT TAC TTG GAT TTG CAC CA-3'] (Arevalo et al. 1994). A minimum of 10 individuals per sampling site were sequenced in order to provide sufficient information with which to generate inter-site genetic distance matrices (Table S1). We sampled a larger number of individuals from Kribi after initial detection of a deep phylogenetic break in ND4 within this site, in order to adequately estimate proportions of the two clades. Individuals from both clades were fixed at c-MOS and Beta Fibrinogen Intron 7 (AHF unpublished data) verifying that we did not confound species in our analysis. ND4 was amplified via PCR with a standard protocol (initial denaturation at 94 °C for 3 min; 45 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min). Amplification was performed in 25 µL reactions: 2.5 µL of 10x PCR buffer, 1.5 µL of MgCl₂ solution, 2.0 μL of dNTPs [2.5 mm each], 1.0 μL of each primer [10 μm], 0.1 μL Taq polymerase, 2.0 µL of DNA template [~50–100 ng double-stranded DNA], 1 uL 10x Bovine Serum Albumin [BSA] and 13.9 μ L of sterile water). To check for contamination, we ran

negative controls with each reaction, adding water instead of DNA. Sequencing was carried out on either a Beckman-Coulter CEQ 2000 or ABI 3730XL automated sequencer, with sequencing reactions performed using appropriate manufacturer protocols and dyedeoxy-terminator reagents. Sequences were manually aligned to each other in BioEdit version 7.0.9.0 (Hall 1999), and to a *T. affinis* Genbank accession (AF228553; Mausfeld *et al.* 2000). We verified that sequences were in proper reading frame and did not contain stop codons with DNaSP, version 4.50.3 (Rozas *et al.* 2003).

Environmental variables

We obtained information on climate, vegetation, and elevation at sampling sites, in order to group populations by habitat for tests of among-habitat divergent selection, for multivariate statistical modeling of relationships between genetic and environmental variation, and for building ecological niche models (see below). From WorldClim, version 1.4 (Hijmans *et al.* 2005), we obtained data on 19 bioclimatic variables at 1-km spatial resolution that include variation in annual means, extremes and seasonal variaiton in temperature and precipitation thought to be important in capturing the physiological limits of species (Nix 1986). These data are derived from monthly interpolated temperature and rainfall climatologies spanning the years 1950 to 2000. We included all of the available bioclimatic variables except isothermality, as it the context of our study its ecological significance is not easily interpretable (Table S2). In using ecological niche models to quantify range shifts associated with climate cycles, so as to better understand how they might influence patterns of genetic variation, we projected the present day

climate-genetic cluster relationships onto the LGM (21,000 BP) climate simulated with the Paleoclimate Modelling Intercomparison Project (PMIP; <u>http://www.pmip2.cnrs-</u> <u>gif.fr</u>) Community Climate System Model (CCSM3, http://www.ccsm.ucar.edu/, [Kiehl and Gent 2004]) downscaled to the resolution of contemporary environmental variables (Waltari *et al.* 2007). While comprehensive validation of environmental variables for the LGM generated by the CCSM3 model is not possible, predicted reductions in precipitation and temperature for tropical Africa produced by the PMIP models are broadly consistent with available terrestrial data (Jahns 1996, Maley 1996; Pinot *et al.* 1999; Dupont *et al.* 2000). Thus, the simulated LGM data are useful for exploring broad features of species distribution shifts associated with climate cycling.

To quantify spatial and temporal vegetation patterns, we used the monthly 1-km leaf area index (LAI) data derived from MODIS reflectance over a 5-year period (2000-2004; Myneni *et al.* 2002). To further reduce effects of persistent cloud cover and any natural interannual variability in the data, we created monthly climatologies by averaging the 5 years of data. We then used these composites to generate an annual maximum LAI (LAIMAX) layer. We also generated two normalized difference vegetation index (NDVI) data sets: maximum annual NDVI (NDMAX), and maximum NDVI during the "greening up" period (NDGR), when the flush of new vegetation is initiated from the onset of the rainy season. We calculated these indices from the same MODIS time series as the LAI metrics. As an additional fine-scale metric of land cover, we used the MODIS-derived vegetation continuous field (VCF) product as a measure of the percentage of tree canopy cover at 1-km resolution (Hansen *et al.* 2002). The VCF came from 2001 MODIS data

and separated open, fragmented, and deforested areas from those of intact old-growth forests. We also generated monthly composites of global Quick Scatterometer (QSCAT; Long *et al.* 2001) microwave measurements for 2001 and processed them to produce two variables: annual mean (QMEAN) and standard deviation of radar backscatter (QSD). The QSCAT data layers were reaggregated from their 2.25-km native resolution to 1-km to match the resolution of the other predictor variables. The QSCAT radar measurements are sensitive to surface roughness, surface moisture, leaf water content, and other seasonal attributes, such as deciduousness of vegetation. In addition, they have the added advantage of being unbiased by cloud cover. We also included elevation data at 1-km resolution from the Shuttle Radar Topography Mission (SRTM). Sources of environmental data and their interpretation are provided in Table S2.

Detecting outlier loci

We identified AFLP loci that deviate from neutral expectations (and that may be under divergent selection) with F_{ST} -based outlier tests that compare simulated to empirical levels of divergence among loci. Theoretical studies demonstrate that such tests have high power to detect divergent selection and are relatively robust to demographic history (Beaumont and Nichols 1996; Beaumont and Balding 2004; Beaumont 2005). They have been employed in a number of recent empirical applications (Campbell and Bernatchez 2004; Bonin *et al.* 2006; Nosil *et al.* 2008). We implemented outlier tests with the program Dfdist (Beaumont and Balding 2004). Dfdist first estimates empirical F_{ST} values among loci with a Bayesian approach (Zhivitovsky 1999). It then estimates the mean

empirical neutral F_{ST} value by removing the highest and lowest 30% of observed F_{ST} values from the data set, resulting in the "trimmed" mean. A distribution of F_{ST} values is then simulated so that they approximate the trimmed mean, with F_{ST} values conditional on heterozygosity estimated under an infinite island model (Wright 1943). Loci falling above the upper 95% confidence interval derived from the simulated data are considered outliers putatively under divergent selection. We used the recommended default settings for Dfdist, including the 30% threshold noted above, and a smoothing parameter of 0.04, excluding loci with a dominant allele frequency > 99%. Initial analyses indicated that results were insensitive to specified priors. A recent simulation study suggests variable proportions of outliers may be false positives when α is set to 0.05, and conversely, many may not be detected when selection coefficients are small (Caballero *et al.* 2008). To address this issue, we examined the influence of increasingly conservative α -values on our analysis.

We tested for outliers in two ways. First, we looked for outliers as a function of divergent selection among groups of populations defined by habitat and climate. We identified four discrete environments, by performing a Principle Components Analysis (PCA) on all environmental variables at our 12 sampling sites: rainforest, montane rainforest, lower ecotone, and upper ecotone (Fig. 2). Second, in order to identify population-specific effects, including local environmental features masked by PCA, we looked for outliers at the population level, coding each of our sampling areas as a population.



Figure 2. Habitats at sampling sites in Cameroon delineated with PCA on WORLDCLIM climate, MODIS (LAI maximum, % tree cover [VCF], NDVI maximum, and NDVI during greening up period), and radar (Quick Scatterometer mean and SD of backscatter) remote sensing. PC1 and PC2 explain 54% and 21% of habitat variation among sampling sites, respectively.

Although Dfdist intrinsically corrects for problem of multiple comparisons through the priors (Beaumont and Balding 2004), we also evaluated how controlling the false discovery rate (FDR) would influence our results, by setting an FDR threshold of \leq 5% for each of the outlier analyses. Specifically, we calculated q-values for each locus within each run, where q equals the proportion of false positives expected if the locus is considered significant (Storey and Tibshirani 2003), considering as confirmable outliers those loci with q \leq 0.05. Q-values were estimated using the program Q-value (Storey and Tibshirani 2003), with the proportion of truly null loci, π_0 , estimated with the bootstrap method.

Genetic distance and structure

Based upon our results from Dfdist, we partitioned our AFLP data into a neutral set, and one comprised of outlier loci purportedly under divergent selection. To minimize the influence of loci under selection that were not detected as outliers, we did not include in our neutral data set any loci that lost statistical significance at α -levels below 0.05. To summarize the overall pattern of neutral variation, we pooled neutral loci (Campbell and Bernatchez 2004; Bonin *et al.* 2006). For these, we constructed a matrix of Nei's genetic distances following the method of Lynch and Milligan (1994) using AFLP-Surv, version 1.0 (Vekemans *et al.* 2002). Because natural selection may operate along different environmental clines for different loci, and so as to avoid obscuring their separate spatial patterns and environmental associations, we built a separate distance matrix for each outlier AFLP locus, comprised of normalized, absolute differences in band frequency.

Genetic structure due to historical factors such as isolation in glacial refugia might lead to genetic variation explained neither by environment nor geographic distance, i.e. if refugia at varying distances from each other were effectively isolated to a similar degree. Such a scenario would be likely for species with limited dispersal ability such as skinks that rely of forested habitat, and for Cameroon in particular where hypothesized refugia are relatively close geographically. Thus, to look for evidence of population structure without the requirement that it be explained by environmental variables, for the AFLPneutral data set we identified genetic clusters in the AFLP-neutral dataset with the program Structure, version 2.1 (Pritchard *et al.* 2000). We analyzed runs for k = 1 - 10clusters, with a no admixture, allele frequencies correlated model (100,000 steps burn-in, 500,000 steps). We determined the most likely number of genetic clusters (k) with the method of Evanno et al. (2005), and by examining plots of likelihood scores, and the extent to which increasing k added clusters merely comprised of low proportional membership coefficients to individuals with high coefficients for other clusters. We expected that if historical isolation in rainforest refugia was responsible for neutral genetic divergence, genetic clusters would be detected that were congruent with the hypothesized refugial areas (Maley 1996).

Spatial modeling of gene-environment relationships

To quantify the extent to which variation in each of our genetic data sets was explained by environmental variables, and to develop spatial predictions of environmentally associated genetic variation, we employed Generalized Dissimilarity Modeling (GDM;

Ferrier *et al.* 2007). GDM is a matrix regression technique that fits a linear combination of I-spline basis functions (Ramsay 1988), permitting non-linear responses to environmental variation to be modeled. First, dissimilarities of a set of predictor variables are fitted to the genetic dissimilarities (the response variables). Then, predictor contributions to explaining the observed response variation are tested by Monte-Carlo permutation, and only those that are significant are retained in the final model. These procedures result in a function that best describes the relationship between environmental and genetic variation, and that can then predict spatial patterns of genetic variation across a study area of interest. To compare the AFLP-neutral to an independent neutral data set, we built an F_{ST} matrix (Weir and Cockerham 1984) from the ND4 sequences, using distances among haplotypes computed from a Trn+I model of sequence evolution selected with the Bayesian Information Criterion (BIC) using Modeltest 3.8 (Posada 2006).

To assess the relative contribution of environment versus distance to explaining the observed variation, we performed independent and combined analyses of environmental variables and distance. For each genetic data set, we built three classes of GDM: 1) environmental variables and distance, 2) only distance, and 3) only environmental variables. Subtracting 2) from 1) provides an approximate estimate of how much genetic variation is explained by environment, above and beyond strict geographic distance, excluding environmental variation correlated with geographic distance. Comparison of the results from these three models also provides an indication of the correlation between geographic distance and environmental difference effects. For neutral

markers, we also included as a predictor the least-cost path (LCP) distance between sampling localities, with cost inversely weighted by habitat suitability estimated from the ecological niche model. This allowed us to incorporate the ecological costs of dispersal per se, and to evaluate the Sanaga River (a large river which bisects our study area; see insert Fig. 1A) as a potential dispersal barrier. Specifically, we assigned a large cost to traversing the river, leading to an LCP which is forced to circumnavigate the river's headwaters. The LCP between sites used as a distance matrix input into GDM was generated with the program Pathmatrix (Ray 2005) implemented in ArcView version 3.2a (ESRI).

Although GDM can include geographic and other types of distances as predictor variables, in its present formulation it does not include covariates. GDM using only distance versus all variables can be compared to assess how much additional variation is explained by environment, but it does not, strictly speaking, measure the correlation between genetic and environmental variation when geographic distance is controlled for, or that between genetic and geographic distance when environmental variation is controlled for. To complement GDM analyses and better understand the proportions of genetic variance explained by distance versus environment , we conducted simple and partial Mantel tests (Manly 1997), with Isolation By Distance Web Service (IBDWS), version 3.15 (Bohonak 2002), with genetic distance matrices as response variables, and environmental and distance matrices as predictors. For each GDM that included both geographic distance and environmental variables as possible predictors to be included, we built a customized, weighted mean environmental distance matrix, with weights assigned

to environmental variables according to their respective coefficients in the GDM model. This weighting scheme is reasonable, because GDM splines are constrained to be monotonically increasing, such that model coefficients should provide reasonable estimates of an environmental variable's predictive power. Variables not included in the GDM were not part of the matrix, and for the few occasions when more than one I-spline was fitted to a variable, we used the sum of those coefficients in calculating the variable weight. Although partial Mantel tests are robust under most circumstances (Castellano and Balletto 2002), there may be some bias in significance testing (but not in correlation coefficients themselves) of partial Mantel tests, particularly when strong correlations exist between independent variables (Raufauste and Rousset 2001; Rousset 2002). The correlation between geographic distance and environmental distance was substantial in our study. Thus, in order to test the hypothesis that, across the genome, environment explains more variation than geographic distance, we also performed paired t-tests on the partial correlation coefficients from partial mantel tests on each genetic data set input into GDM.

Ecological niche modeling

We restricted our GDM analyses to areas within Cameroon where *T. affinis* is expected to occur using an ecological niche modeling approach. First, we supplemented our species localities with those obtained for west and central Africa from the scientific literature, museum records, and technical reports resulting from field surveys by expert herpetologists (Table S3). Using 52 localities from these sources (Fig. S1), we then

modeled the distribution of *T. affinis* with Maxent, version 3.2.1, a recently developed general-purpose algorithm for presence-only data (Phillips *et al.* 2006). In a recent, comprehensive, inter-model comparative study, Maxent ranked among the highest performers (Elith *et al.* 2006). Maxent offers a particular advantage in that it performs well with only a small number of point localities (Hernandez *et al.* 2006). Specifically, we used Maxent to construct the environmental envelope of *T. affinis* using all of our environmental variables, and project that envelope into geographic space in the form of probabilities of species occurrence per 1-km pixel. We defined areas above the minimum predicted probability of presence at an actual species locality as being part of the species distribution.

Because historical range expansions and contractions can create clines in genetic variation along the axis of expansion (Hewitt 1996; Wegmann *et al.* 2006), selectively neutral loci might have a significant environmental association if range expansion takes place along an environmental gradient. Providing an explanation for such patterns is important, so as not to confound them with the effects of loci under selection. In equatorial Africa, periodic climate cycles lead to rainforest expansion into previously more arid savanna areas during interglacials, and contraction out savannas during glacial periods (Maley 1996; Dupont *et al.* 2000). As a result, the contemporary rainforest-savanna might be able to explain neutral genetic variation because of its correlation with the axis of range dynamics. To evaluate this possibility, we examined changes in the distribution of *T. affinis* between the last glacial maximum (LGM) and the present by building an additional niche model comprised solely of contemporary climate variables,

then projected the environment-species relationship calculated by Maxent onto the LGM using the CCSM paleoclimate environmental variables. For this analysis, we excluded BIO 9, BIO14, BIO15, BIO17 and BIO19 (Table S2), because CCSM projections for the LGM contained visually detectable anomalies likely due to how algorithm split annual time series in order to delineate seasons. For the purposes of visualization, we restrict our presentation of predicted distributions to our study area in Cameroon.

Historical demography

To provide additional insight into the underlying historical demography that may have shaped patterns of genetic variation revealed with GDM, we perform additional analyses with the ND4 sequence data. We defined groups of populations based upon (1) sets of geographic populations that did not show signs of admixture between genetic clusters estimated from the AFLP-neutral data with the program Structure, and (2) sympatric ND4 clades exhibiting deep divergence that suggested secondary contact. For each group of populations, we calculated mismatch distributions (Rogers and Harpending 1992), and tested for signatures of both demographic and spatial expansion (Fu 1997; Schneider and Excoffier 1999; Ray *et al.* 2003; Excoffier 2004). To provide additional tests for population expansion, we performed Fu's tests (Fu 1997), for which large negative values are an indication of demographic expansion. These analyses along with standard molecular diversity indices were computed with the program Arlequin (version 3.1, Excoffier *et al.* 2006).

Results

Morphological variation among habitats

Consistent with previous research on African passerines (Smith *et al.* 1997, 2000, 2005a,b), we detected divergence in morphological traits likely important in fitness (Losos 1990; Losos *et al.* 1997; Vitt *et al.* 1997; Schneider *et al.* 1999) along the rainforest-ecotone gradient. The first two principle components explained 90% of morphological variation. Both PC1 and PC2 were significantly different among habitats (Fig. 3; ANOVA, N = 238, df = 3; PC1, F = 8.303, P < 0.001; PC2, F = 17.572, P < 0.001). All traits loaded heavily on PC1, indicating it describes overall body size, while increasing values for PC2 were correlated predominantly with increasing head depth, and to a much lesser extent smaller forelimb length and span (Table S4). Sidak post hoc tests indicated that rainforest was significantly different in PC 1 from lower ecotone (P = 0.045) and upper ecotone (P < 0.001), while upper ecotone was significantly differentiated from all other habitats along PC 2 (all $P \le 0.001$).

Genomic Heterogeneity and Structure

Of 191 repeatable AFLP loci, 116 had dominant allele frequencies \leq 99%, and were included in Dfdist analyses. Of these, outlier tests based upon either the 12 sampling localities or habitat types identified 15 loci (8% of repeatable loci) that deviate from neutral expectations (Fig. 4). Six of 7 of the outliers identified in between-habitat analyses were also recovered in the among-population analysis. The number of loci per



Figure 3. Means of principal components 1 and 2 on morphological traits in *Trachylepis affinis*, showing morphological divergence between rainforest and ecotone habitats. FOR = rainforest, MONT = montane forest, LOEC = lower ecotone, and UPEC = upper ecotone. Habitats labeled with different letters indicate significantly divergent morphology between them. Error bars represent \pm 1SE.Habitat types were determined from climate variables, remote sensing, and elevation (see Fig. 2 and Materials and Methods for details on variables).



Figure 4. Plots of empirical F_{ST} on heterozygosity showing neutral loci (hollow circles), outliers under selection at $P \le 0.05$ (hollow triangles), and at $P \le 0.01$ (filled circles). The solid line represents the upper 95% confidence of neutral F_{ST} values conditional on heterozygosity simulated in Dfdist (Beaumont and Balding 2004).
selective primer combination was small (range = 17–40), thus size homoplasy is unlikely to represent a confounding factor in our analyses (Caballero *et al.* 2008). Increasingly conservative α values had a small effect on the estimated proportion of the genome likely to be under selection, as even at an extremely conservative α =0.0001, that proportion only decreased to 3% (Fig. 5). For the remaining significant outliers, this pattern would not change with even more conservative α values, as their respective *P* values were effectively zero. For these loci, false discovery had no influence on results, as in at least one tests Q-values were zero; for an additional locus showing the "gap" GDM pattern (see below), false discovery was also improbably (Q=0.005).

For subsequent analyses, we then partitioned the AFLP loci into a set of 101 neutral loci, and a separate set of the 15 outliers. From the neutral AFLP data set, Structure provided the most support for three genetic clusters, with the vast majority of individuals being almost exclusively assigned membership to one cluster (Fig. 6). For each of three geographic areas, individuals from an area were almost exclusively assigned to one of the three genetic clusters: South of the Sanaga (Fig. 1A [sites 1 - 3], 3), Southwest Province (Fig. 1A [sites 6 - 8], 3), and ecotone (Fig. 1A [sites 9 - 12), 3). Within the South of Sanaga area, three individuals from Palm d'Or had closer affinity, based upon membership coefficients, with individuals from Southwest Province. Greater admixture between clusters occurred at Malimba just north of the Sanaga (Fig. 1A [site 4], 3), and to a lesser extent at Mapenja (Fig. 1A [site 5], 3). The South of Sanaga and Southwest Province clusters were generally consistent with Maley's (1996) hypothesized refugial areas (Fig. 1A, 6), while admixture at Palm d'Or and Malimba (proximate to



Figure 5. Number of outlier loci displaying a particular GDM spatial pattern, and the estimated percentage of the genome evolving in a non-neutral fashion, as a function of changing α levels for outlier tests. For a specific α , a locus is counted if its *P* values is $\leq \alpha$ for either one of the (habitat or population-level) outlier tests. Percentages are estimated as the number of outlier loci out of 191 (i.e., the total number of repeatable loci).



Figure 6. AFLP-neutral genetic structure estimated with Structure (Pritchard et al. 2000) for k=3, the best number of genetic clusters determined with the method of Evanno *et al.* (2005).

each other but separated by the Sanaga River) suggests the Sanaga is not a significant barrier to gene flow.

GDM: Neutral Loci

GDM of pooled neutral loci that included both geographic distance and environmental variables (i.e. the full model) as possible predictors explained 75% of genetic variation (Table 1). Environment was important in explaining neutral genetic differentiation. Genetic differentiation followed a cline from the coast inland into the ecotone, which was largely explained by geographic distance, mean annual precipitation, and precipitation of the driest month (Fig. 7A). Only 23% of variation was explained by a model based solely on geographic distance, with the full model including environment explaining 75% (Table 1). In other words, adding environmental variables increased the explanatory power of the model by 52%. A model incorporating environment but excluding distance explained only 3% less variation than the full model. Unlike the Structure analysis, no differentiation associated with hypothesized refugia was detected (Fig. 1A, 7A). In addition, in the full model the least-cost path around the Sanaga was not selected in model fitting, confirming findings from Structure demonstrating that the Sanaga River does not present a substantial barrier to gene flow.

GDM: Outlier Loci

Consistent with morphology as well as past research on African passerines (Smith *et al.* 1997, 2000, 2005a), seven loci were differentiated along the rainforest-ecotone gradient

	GDM model		
Locus	Distance only ^a	Environment only ^b	Distance + environment ^c
AFLP-neutral	23	72	75
ND4	31	_	63 ^d
1.39	70	91	94
1.114	28	66	67
1.136	71	92	93
2.5	65	90	90
2.38	67	85	87
2.125	64	89	89
2.141	0		41
3.98	7		72
4.98	12		69
4.102	3		69
4.105	0		70
4.129	0		67
4.142	3		56
4.154	0		40
4.165	64	82	64

Table 1. Percent of genetic variation explained by GDM models of neutral AFLPs, individual AFLP loci under selection, and mtDNA gene ND4 in *Trachylepis affinis*.

^a Model only includes geographic distance as a predictor variable.

^b Model allows for selection of any environmental variable, excluding geographic distance and the least cost path around the Sanaga River (LCP). Entries are blank because geographic distance was not included in the full model, and so environment-only and environment + distance models are identical.

^c Model allows for selection of environmental variables, geographic distance, and LCP, although the current implementation of GDM cannot estimate the percent of variation explained when LCP is selected.
 ^d LCP was included, but had a warm weak effect. Therefore, the percent variation explained

LCP was included, but had a very weak effect. Therefore, the percent variation explained is a close approximation of the total amount explained by all factors considered.



Figure 7. Spatial predictions of genetic turnover in *Trachylepis affinis* based upon GDM, and variables selected in each GDM model that allows for environmental variables and geographic distance as possible predictors. Colors between panels are not comparable, while within panels, areas with similar shading along the color scale are predicted to be

Figure 7 (continued)

genetically more similar. A. 101 neutral AFLP loci, B. outlier locus 2.38, under divergent selection between rainforest and ecotone habitats, C. outlier locus 3.98, under selection along a lowland forest \rightarrow ecotone \rightarrow montane gradient, and D. outlier locus 4.142, displaying convergent selection between areas proximate to hypothesized refugial areas, and which are divergent from an intervening "gap" area in the coastal forest as well as ecotone.

(Fig. 7B, S2). For these loci, geographic distance was always included as a predictor, and was the most important one for four loci (Fig. 7B, S2). Mean annual precipitation was the most important predictor for all but one of the remaining loci. In this instance, precipitation of the wettest quarter was more important. In general, either wettest quarter or wettest month precipitation helped describe patterns of genetic differentiation in these loci. The full model (including both environmental variables and geographic distance) explained 67 - 94% of genetic variation, approximately 20% more than models incorporating geographic distance alone. Models only using environmental variables (i.e. excluding geographic distance) were as powerful as the full model in explaining genetic variation for two loci, and nearly so for the remaining loci (Table 1). The lack of difference in explanatory power between the full and environment-only models may arise, in part, from large correlations between geographic distance among sampling sites and environmental difference in a number of bioclimatic variables (Table S5). This suggests that geographic and ecological distance are concordant. As a result, in our GDM models geographic distance may serve as a proxy for a number of environmental variables directly relevant to patterns of local adaptation and divergence.

Three loci exhibited strong differentiation along a cline from lowland rainforest influenced by a coastal climate regime to montane forest (red areas in Fig. 7C indicate the Cameroon line mountain chain; Fig. 1A insert, S2). Intermediate along this cline were more interior rainforest areas and ecotone habitats (Fig. 7C, S2). Full model GDMs explained 69 - 72% of genetic variation, but did not select geographic distance as an important variable for any of these loci (Table 1). Geographic distance alone explained

very little variation (3 – 12%; Table 1). For two loci (3.98 and 4.102), the gradient of differentiation from coastal lowland rainforest to ecotone to montane habitats coincided with increasing diurnal temperature variation (BIO2), augmented in particular in the montane areas by colder temperatures during the coldest month (BIO5) (Fig. 7C, S2). The effect of BIO5 was apparent despite its small model coefficient, which we confirmed by running an additional GDM excluding BIO5, and which resulted in reduced differentiation of montane areas. Interestingly, for the third locus (4.98), while BIO5 once again explained differentiation of montane habitats, high precipitation of the wettest month (BIO13) and quarter (BIO16) rather than diurnal temperature range characterized adaptive variation in lowland rainforest habitats, and in slightly different areas: along more restricted coastal areas south of the Sanaga (Fig. S2).

For five loci, a pair of rainforest areas north and south of the Sanaga River were differentiated from an intervening "gap" between them and ecotone (Fig. 7D, S2), with ecotone and inland rainforest being characterized by overall drier conditions. The full model explained 40 - 70% of differentiation, and geographic distance was not included for any locus; geographic distance explained only 0 - 3% of variation when run in isolation (Table 1). Variation in the location of the two genetically convergent rainforest areas depended upon the driving variables. For two loci, those areas were associated with high precipitation of the driest month (BIO14; loci 4.105 and 4.129, Fig. S2). For three others (2.141, 4.142, and 4.154), they were differentiated due to substantially lower precipitation of the warmest quarter (BIO18) in the intervening "gap" area (Fig. 7D, S2).

This gap may also be due to lower precipitation of the driest quarter, with which precipitation of the warmest quarter is highly correlated (r = 0.788 at 1,000 random points within the predicted distribution of *T. affinis*). Aside from the seasonal precipitation anomaly along the coast, clines in these loci parallel the rainforest-ecotone gradient. Nevertheless, increasingly conservative α values led to a rapid drop-off of the number of these loci that significantly deviate from neutral expectations (Fig. 5).

Independent effects of environment and distance

Although simple Mantel tests revealed significant IBD and environmental influence on patterns of neutral and outlier differentiation (Table S6), correlations between geographic and environmental distance were typically high (0.81 ± 0.03 SE). Thus, partial tests were necessary in order to discern the effects of geographic distance from those of environment. In these tests, neutral AFLPs showed weakly significant IBA but not IBD, while IBD and not IBA was significant in ND4 (Fig. 8, Table S6). Significant IBD was detected in four out of 15 outlier loci, while significant correlations with environment while controlling for geographic distance were found in all outlier loci. Consistent with the effects of divergent natural selection, these correlations with environment were greater than those for either neutral AFLPs or ND4 except for locus 2.141, which displayed the "gap" pattern with GDM (Fig. 8, S2). Across AFLP markers, both neutral and outliers, environmental effects were always greater than effects of geographic distance (paired t-test on correlation coefficients, d. f. = 15, t = 7.03, P < 0.0001),



Figure 8. Partial Mantel test correlations between genetic distance and geographic distance while controlling for geography, and between genetic distance and environmental distance controlling for geographic distance. For each genetic distance matrix, environmental distance matrices are weighted means of environmental variables selected by GDM, with weights equivalent to the sum of those variables' I-spline coefficients. Data sets to the left of the dashed line are neutral data sets, while those to the right are outlier loci under selection. Significant partial Mantel tests for positive association (i.e. 1-tailed test) are indicated by * $P \le 0.05$, ** $P \le 0.01$, and *** $P \le 0.001$. Across AFLP data sets, The effect of environment while controlling for geographic distance is significantly greater than the effect of geographic distance while controlling for environment (paired t-test on r-values, t = -7.03, $P \le 0.0001$).

suggesting that adaptation to local environments plays an important role in structuring genetic variation.

Historical demography

Ecological niche modeling. Clinal variation observed in neutral AFLPs were consistent with past range dynamics inferred from ecological niche modeling. Ecological niche model predictions of LGM and present-day distributions suggest that *T. affinis* has undergone a substantial range expansion since a shift to warmer and wetter conditions after the LGM (Fig. 9). A number of additional model runs using subsets of the climatic variables in this model produced a qualitatively similar pattern of distribution change (data not shown). Given historical climate variation in Africa driven by glacial and processional cycles (deMenocal 1995), range contractions and expansions are probably recurring phenomena that have repeatedly influenced spatial genetic variation. Predicted range dynamics co-varied with the cline of decreasing precipitation and greater seasonality, with increasing distance from the coast, possibly explaining the large contribution of environmental variables to GDMs of neutral AFLPs.

<u>ND4 diversity</u>. Evidence for range expansion from niche modeling was supported by ND4 mismatch distributions (Fig. S3, Table 2). For areas comprising relatively homogeneous genetic clusters based upon Structure analyses or comprised of unique, divergent ND4 clades (south of Sanaga River, excluding the "+" clade [Fig. S4 A]; the "+" clade found only at Kribi; southwest province; and ecotone), mismatch distributions contained signatures of demographic expansion for all areas, and for spatial expansion in



Figure 9. Predicted distributions of *T. affinis* at A. the last glacial maximum (LGM) and B. the present, generated with a species distribution model (Maxent), indicate postglacial range expansion. Maxent models are based upon a restricted set of climate variables. LGM predictions employ climate data generated by PMIP's CCSM3 general circulation model (see Materials and Methods for details).

in mtDNA gene ND4. Signif	ficant values are in	dicated in boldfa	ice.	
		Geograf	ohic Area ^a	
	South of	-	Southwest	
Diversity metric/test for	Sanaga ^b	Kribi Clade ^b	Province ^b	Ecotone ^b
expansion	(N=48)	(N=16)	(N=33)	(N=48)
# haplotypes	7	4	7	4
<pre># polymorphic sites</pre>	10	ω	17	2
Gene diversity	0.76 ± 0.04	0.69 ± 0.09	0.80 ± 0.04	0.42 ± 0.08
Mean # pairwise differences	3.01 ± 1.60	0.98 ± 0.70	4.23 ± 2.15	0.48 ± 0.42
Nucleotide diversity	$4.0 \times 10^{-3} \pm$	$1.0 \times 10^{-3} \pm$	$6.0 \times 10^{-3} \pm$	$6.93 \times 10^4 \pm$
	3.0×10^{-3}	1.0×10^{-3}	3.0×10^{-3}	6.78×10^{4}
Demographic expansion (SSD ^c)	0.14*	0.01	0.06	7.83×10^{4}
Harpendings raggedness index	0.40**	0.11	0.08	0.140
Spatial expansion (SSD ^c)	0.12*	0.01	0.046	7.31×10^{4}
Fu's <i>Fs</i>	1.80	-0.30	2.43	-0.95
^a Geographic area defined from S	structure analysis of ne	eutral AFLPs.		
^b South of Sanaga = sites 1-3, Kr	ibi Clade = individuals	s from divergent ND	14 clade (see Fig. S4) at site 1;
Southwest Province = sites 6-8,	, Ecotone = $9-12$. See]	Fig. 1 for map of site	es.	

 $^{\rm c}$ Sum of squared deviations from goodness-of-fit tests.

* P<0.05, ** P<0.01

Table 2. Measures of genetic diversity and statistical tests of spatial and demographic expansion

all areas except that south of the Sanaga (Table 2). Fu's tests were not significant, but variation among areas was consistent with signatures of expansion from refugia detected with the other metrics. The largest negative Fs value was for ecotone, while that of the "+" clade in Kribi was slightly negative (Table 2). Positive Fs values were recorded for the group of populations south of the Sanaga River and in Southwest Province, indicating relative population stability in areas near hypothesized refugial areas (Table 2). Additional support for past range expansion comes from lower gene, nucleotide, and haplotype diversity (relative to the number of individuals sampled) in the ecotone than in other areas (Table 2, Fig. S4A). This is likely due to the sequential bottlenecks that occur during range expansion (Hewiit 1996), as ecotone habitats represent the distributional limits of T. affinis determined with Maxent (Fig. 1B), and the terminal end of the expansion front. In the "+" clade from Kribi, negative Fs and reduced genetic diversity relative to the other two groups of forest populations are consistent with haplotypes belonging to a lineage expanding from populations further to the south. Deep genetic divergence of sympatric ND4 clades at Kribi was in conflict with information from AFLPs. Structure analysis of individuals from Kribi at k=2 showed a high degree of admixture within individuals (Fig. S5), strongly suggesting secondary conflict followed by introgression. Finally, GDM of differentiation at ND4 showed a similar clinal pattern to that of neutral AFLPs (Fig. S4B), with no differentiation between hypothesized refugia. An extremely weak river barrier effect was detected, as the model coefficient for the LCP was near zero. Consistent with the lack of such an effect, pairwise F_{ST} between two proximate populations on either side of the Sanaga River (Palm d'Or and Malimba)

was low (0.07), and smaller than all other pair-wise F_{ST} values (0.61 ± 0.04 SE) except for some within-ecotone population pairs. Only 31% of variation was explained by geographic distance in isolation, and it was not included in the full model (Table 1).

Discussion

Genome scans, environmental and niche modeling were found to resolve patterns of nonneutral evolution against a backdrop of species response to historical climate change. Combined, these analyses support a greater role for adaptive evolution along ecological gradients than for allopatric divergence between refugia in generating diversity in equatorial Africa (Smith et al. 1997, 2005a). Genome scans of T. affinis revealed that a relatively large proportion of the genome is under divergent selection, falling close to the mean of 8.5% for 18 reviewed studies (Nosil et al. 2009). Our findings thus add to a growing body of evidence that divergent selection is a strong force influencing genome evolution. While it has been suggested that diversification in the face of Milankovitch cycles occurs primarily in environmentally stable areas (Dynessius and Jansson 2000; Jansson and Dynesius 2002), a large proportion of outliers putatively under selection suggest the opposite. These loci were divergent along the rainforest-savanna gradient, which has undergone periodic contractions since at least the beginning of the Pleistocene. While it is known that introduction into novel habitats can produce adaptive differentiation (Losos et al. 1997), far fewer studies indicate that range expansions facilitate adaptive evolution (Hellberg et al. 2001; Thomas et al. 2001; Zayed and Whitfield 2008). In contrast to past studies based upon species distributions and neutral

genetic markers, we found that neutral genetic divergence between refugia without accompanying ecological divergence is probably ephemeral. However, we identified a previously underappreciated role for refugia in the diversification process. While refugia may not necessarily act as generators of diversity through geographic isolation, they may serve as a source population for range expansions across ecological gradients along which natural selection generates adaptive divergence.

Considering neutral loci without consideration of environmental variation, analyses with Structure revealed three genetic clusters, and two of these may be interpreted as belonging to a distinct, hypothesized rainforest refuge. Individuals unambiguously assigned to one of these clusters were found in close proximity to a hypothesized refuge, and areas of mixed membership corresponded to those populations in the intervening area between the refugia or at their proximate margins. However, at k=2, forest and ecotone were delineated first, and only afterwards are separate refugia clusters resolved. This implies stronger genetic divergence between rainforest and ecotone habitats than between refugia. While consistent with studies on birds demonstrating the evolutionary significance of rainforest-ecotone divergence (Smith et al. 1997, Smith et al. 2005a), genetic structure alone provides little information as to the underlying evolutionary processes producing this pattern, let alone the environmental variables responsible for divergence or the role of mere geographic distance. In conflict with an inference of inter-refuge divergence, ND4 showed little structure between refugial areas (Fig S4). Instead, ecotone haplotypes clustered separate from forest ones, and highly divergent rainforest haplotypes were sympatric as the southernmost sampling

site in Kribi (site 1). At Kribi, the combination of contemporary sympatry, deep mtDNA divergence, and lack of congruent structure in the nuclear genome strongly suggest secondary contact and introgression of lineages previously isolated, perhaps between a larger Cameroonian macrorefugium (sensu Dupont et al. 2000) and one farther south (see Maley 1996). Analysis of neutral AFLP structure confirms this, as the sympatric mtDNA clades show no parallel in neutral AFLPs at k=2, which instead show evidence for rampant admixture (Fig. S3). Persistence of divergence in mtDNA is only maintained because recombination cannot erode the historical, phylogeographical signal—only future dispersal and gene flow spreading mtDNA haplotypes among populations could. The overall conflict between patterns of nuclear and mitochondrial genetic structure, combined with nuclear admixture between refugial areas suggest that, while there is genetic evidence for past geographic isolation between refugia, any resulting inter-refuge neutral divergence is probably ephemeral. Between ecologically differentiated areas, adaptive divergence can inhibit gene flow (Räsänen and Hendry 2008). However, if selection against locally maladapted immigrants is weak, such as might be the case ecologically comparable refugia, introgression during interglacials should eventually erase inter-refuge genetic divergence.

GDM including both geographic distance and environmental variables explained 75% of neutral genetic variation in neutral loci. This pattern of differentiation is at odds with inferences one would make from analyses of spatial genetic structure and phylogeny that do not explicitly include environmental information. GDM failed to detect any differentiation between refugial areas, instead revealing a clinal pattern radiating out from

the coast, and extending toward ecotonal habitats that comprise the range limit of *T*. *affinis* (Fig.7). While geographic distance was the most important variable in a GDM that considered both distance and environmental variables, precipitation variables also contributed substantially. Furthermore, the GDM solely based upon environmental variables (i.e. excluding geographic distance) explained nearly as much neutral variation as the full model (72%).

Why does environmental variation explain so much variation at presumably neutral loci? One explanation is that adaptive divergence between populations generates reproductive isolation allowing individual neutral loci to diverge stochastically via genetic drift, i.e. the "general barriers" mechanism (Räsänen and Hendry 2008; Nosil et al. 2009). If adaptive divergence is greatest between the extremes of rainforest and ecotone, the resulting rainforest-ecotone genetic cline could be generated. Because precipitation was important in the full GDM model and because of the strong association between precipitation and woody vegetation cover (Sankaran et al. 2005; Bucini and Hanan 2007), precipitation probably serves as a proxy for the underlying habitat gradient. Also consistent with the general barriers hypothesis, we found a significant pattern of IBA for neutral loci. Nevertheless, confirmation of general barriers is not possible when IBA is detected at a spatial scale greater than or equal to that of potential gene flow among populations (Nosil et al. 2009). Alternately, adaptive genetic clines create the potential for randomly generated differences in loci affecting mate choice to become associated with and interact with the effects of loci directly responsible for local adaptation. Theoretical work demonstrates that the association of mating and ecological

traits along an ecological gradient can in fact lead to speciation (Doebeli and Dieckmann 2003). Another possibility is that environmental signal in neutral divergence may arise via linkage to loci involved in local adaptation and that carry a selective advantage relative to immigrant alleles (Charlesworth *et al.* 1997). Even so, linkage seems less likely to generate such a strong environmental signal if, as expected, neutral AFLP markers are distributed randomly across the genome, unless the majority of neutral loci exhibit no spatial structure (and thus a few linked loci drive the pattern), or selection is strong and pervasive enough to generate extensive linkage disequilibrium.

We propose a different explanation, namely, that the observed pattern in neutral loci stems from a natural correlation between environmental gradients and levels of habitat suitability, and the resulting spatial dependence of relatedness that arises when populations undergo periodic expansions and contractions induced by climate cycles. Within the range of *T. affinis* in Cameroon (Fig. 1A), the geographic orientation of the rainforest-savanna gradient (Fig. 1B) corresponds closely to that of population expansion since the LGM revealed with ecological niche modeling (Fig. 9) and paleoecological reconstruction of rainforest expansion (Dupont *et al.* 2000); it is also consistent with demographic signal in the mtDNA data (Table 2, Fig. S3). Given that rainforest comprises the core of *T. affinis*' distribution and ecotone habitats are marginally suitable, this is not surprising. In an expanding population, the most likely source of colonists are nearby demes located at the margins of a species' distribution. Consecutive founders should then be more closely related to those demes, producing a genetic cline radiating out from the core habitats that are temporally stable across glacials and interglacials. Both

theoretical and empirical studies support the evolution of such clines arising from colonization during range expansion (Barbujani *et al.* 1995; Austerlitz *et al.* 1997; Le Corre *et al.* 1998). These are most likely for species with limited dispersal, such as *T. afinis*, as long-distance dispersal produces patchiness rather than clines in allele frequencies (Ibrahim *et al.* 1996).

Beyond effects of spatial expansion in the narrow sense, greater IBA than IBD in neutral AFLPs (and the ability of environment to explain neutral genetic structure with GDM) probably results from spatially varying dispersal into the ecotone as a function of variation in precipitation levels that determines the extent of woody vegetation in gallery forests and thus connectivity among them. The directionality of expansion, the sequential bottlenecks that occur at the expansion front (Hewitt 1996), and the environmental heterogeneity of ecotone habitats (Wegmann *et al.* 2006) also explain the low mtDNA haplotype diversity observed in ecotone populations (Fig S4). However, if this is the case, mtDNA should show also show greater IBA than IBD. That is not so may be due to poorer resolution of the trajectory of population expansion with a <700 bp locus than with >100 AFLPs. Alternately, the environmental signal in neutral AFLPs may be the result of our failure to diagnose additional loci under selection that artificially increase the signal of differentiation along the rainforest-ecotone gradient.

GDM of outlier loci juxtaposed to neutral genetic structure suggest that refugial populations play a previously unappreciated role in diversification—not as the geographic template for vicariance but as the source of colonizers of environments that impose novel selection regimes on otherwise rainforest-adapted genomes. Across loci,

parallel, environmentally associated signals of divergent selection were evident along the rainforest-ecotone gradient and to a lesser extent along a gradient between lowland coastal rainforest and montane habitat.

Divergent selection on an additional suite of loci exhibiting the "gap" GDM pattern (Fig. 7D) may further contribute to adaptive divergence along the rainforestecotone gradient. If temporally persistent, the depressed seasonal precipitation between the two hypothesized refugial areas would resolve two refugial areas with similar allele frequencies due to parallel selection pressures. Paleobotanical data indicate the dry interval overlaps considerably with the Littoral Forest type, a degraded forest type characterized by colonist species and a paucity of wet-adapted Caesalpiniaceae species (Letouzey 1968; Maley *et al.* 1990; Maley and Brenac 1998). If not persistent, periods of forest expansion into the dry interval would coalesce two refugial areas into one within which selection pressures would be relatively homogeneous. Coalescence or not, the axis of differentiation occurs along an ecological gradient driven by variation in precipitation, roughly in parallel with that of the rainforest-ecotone gradient. Nevertheless, the fact that these loci are no longer outliers at more conservative α levels call into questions their adaptive significance without additional confirmatory evidence.

Divergent selection in small vertebrates along habitat and elevational gradients is not uncommon (Schneider et al. 1999; Ehinger *et al.* 2002; Ogden and Thorpe 2002; Thorpe *et al.* 2005; Storz *et al.* 2007; Cheviron and Brumfield 2009). And given the known thermal sensitivity of ectothermic reptiles, for *T. affinis* the diurnally and seasonally variable temperatures of ecotone and montane habitats likely represent

selection regimes for physiological traits unlike those in lowland rainforest. Past research suggests selection on physiological traits should reverberate across diverse components of life history and ultimately influence fitness. For example, thermal environments during incubation can influence phenotypic traits in hatchling lizards (Shine et al. 1997; Downes and Shine 1999) and their resulting vulnerability to predators (Downes and Shine 1999). Age and size at reproduction can vary as a result of evolved differences between the extremes of an altitudinal gradient (Rohr 1997). Common garden experiments on the Dominican anole (Anolis oculatus) demonstrate selection-induced variation in morphological traits along a coastal/xeric – montane gradient (Thorpe et al. 2005). In Cameroon, the large geographic distances between the most distant (and most environmentally divergent) rainforest and ecotone habitats may facilitate adaptive genetic divergence because the homogenizing effect of gene flow is relatively small. In contrast, the close geographic proximity of montane to lowland habitats may indicate that adaptive divergence occurs in the face of substantial gene flow, i.e. via divergence-with-gene-flow (Rice and Hostert 1993; Nosil 2008). However, lack of neutral divergence between our montane and lowland forest sites indicate that adaptive divergence has yet to generate structure in neutral loci along the elevation gradient.

Adaptive divergence along the rainforest-savanna gradient can be viewed in the growing framework of theoretical and empirical research on evolution at range margins and in marginal habitats (Bridle and Vines 2006; Kawecki 2008). At the margin (i.e. ecotone), the potential for adaptive evolution depends upon directional selection being strong enough to overcome asymmetric gene flow of locally maladapted alleles from the

range core, i.e. rainforest (Bridle and Vines 2006; Kawecki 2008), or alternately, the ability of dispersal from rainforest (core) into ecotone populations to increase genetic variance permitting more effective response to selection (Barton 2001; Holt et al. 2003). Whether adaptive evolution in *T. affinis* has occurred during range expansion, contraction, or both remains an open question. Theoretical work indicates colonists to new environments can rapidly evolve reproductive isolation from individuals from the ancestral environment (Thiebert-Plante and Hendry 2009) and empirical work demonstrates evolution at an expanding range margin (Hellberg et al. 2001; Thomas et al. 2001; Zayed and Whitfield 2008). Alternately, levels of connectivity between rainforest and ecotone populations likely decrease during cool, dry glacials, with fragmentation and contraction forest leaving some galleries in the ecotone isolated. Analogous to Vanzolini and Williams' (1981) "vanishing refuge" hypothesis, increasingly smaller and more open gallery forests would result in increasingly directional selection for adaptation to open habitats. Theoretical models demonstrate that rapid adaptive evolution occurs in peripheral populations when they become isolated from gene flow (García-Ramos and Kirkpatrick 1997). And the persistence of gallery forests during dry climate phases is supported by phylogenetic evidence in begonias (Plana et al. 2004). Perhaps not coincidentally, an exemplar cited by Vanzolini and Williams (1981) for this "vanishing refuge" model of speciation is *Mabuya arajara*, formerly a congener of T. affinis before African Mabuya were assigned to their own genus.

Regardless of what phase of range change adaptive divergence may occur, our findings suggest that past emphasis on rainforest refugia has been excessive, and that more attention should be paid to disentangling the evolutionary interactions between selection along ecological gradients and the demographic consequences of Milankovitch cycles. The prevailing paradox is that, although demographic influences are often regarded as the generators of spurious signals of selection, the geographic axes along which populations expand and contract are intimately entangled with the environmental axes along which selection can occur. Integrative approaches that disentangle demographic and adaptive signals may not only contribute to a better understanding of diversification, but may also help us anticipate and mitigate the evolutionary consequences of future climate change (Parmesan 2006).

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Table S1. Sampling localitie	es, samp	le size	by se	x, genetics	sampling, an	d habitat type	for T. affinis collected in
Cameroon.							
		Field (collec	tion	Genetic	analysis	
Site	М	щ	5	Total	AFLP	ND4	Habitat
1. Kribi (KRI)	15	24	4	43	20	40	rainforest
2. Mbongwana (MBW)	10	10	с	23	20	11	rainforest
3. Palm d'Or (POR)	20	20	10	50	20	13	rainforest
4. Malimba (MAL)	11	14	7	27	19	10	rainforest
5. Mapenja (MPJ)	12	14	S	31	20	10	montane rainforest
6. Konye (KON)	12	∞	6	22	20	12	rainforest
7. Mundemba (MUN)	6	10	4	23	20	11	rainforest
8. Nguti (NGU)	16	20	4	40	24	10	rainforest
9. Doume (DOU)	8	6	1	18	18	10	lower ecotone
10. Mbakaou (MBK)	12	11	9	29	20	13	upper ecotone
11. Bounou (BOU)	16	13		29	20	14	upper ecotone
12. Bazzama (BAZ)	13	11	2	26	20	11	lower ecotone

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Table S2. Biocl	imatic, vegetation, and elevation variables included as possible	e predictor variables for
Generalized Dis	similarity Modeling of neutral and adaptive genetic variation i	in Trachylepis affinis.
Variable	Environmental attributes	Instrument
Bioclimate:		
BIOI	Annual mean temperature	
BI02	Temperature mean diurnal range	
BI04	Temperature seasonality	
BIO5	Maximum temperature of the warmest month	
BIO6	Minimum temperature of the coldest month	
BIO7	Temperature annual range	
BI08	Mean temperature of the wettest quarter	
BIO9	Mean temperature of the driest quarter	
BIO10	Mean temperature of the warmest quarter	Climate station network
BI011	Mean temperature of the coldest quarter	
BI012	Annual precipitation.	
BI013	Precipitation of the wettest month	
BI014	Precipitation of the driest month	
BI015	Precipitation seasonality	
BIO16	Precipitation of the wettest quarter	
BI017	Precipitation of the driest quarter	
BI018	Precipitation of the warmest quarter	
BIO19	Precipitation of the coldest quarter	
Vegetation (reflect	ince):	
LAIMAX	Annual maximum leat area index (LAI) Normalized difference vecetation index (NDVI) annual maximum	
NDGR	Maximum NDVI during the period of maximum new shoot growth	MODIS satellite
VCF	Vegetation continuous fields percent tree cover	
Vegetation (radar):		
VIMEAN	Mean of radar backScauer Standard deviation of radar hadranthar	Quick Scatteronieter
	JUAILUARI U UCVIAIIOIL UL LAUAL DALASVAIICI	SAICILLIC
Radar Topography		
SRTM	Elevation	Shuttle Radar Topography Mission

I aute 55.	LUCALILIES IU	I Truchiepis allinis used to	UULIU CUUUBICAL	IIICHE IIIONEIS.	
				Specimen Location/	
Latitude	Longitude	Site	Year Collected	Catalog #	Source
10.4803	-10.4433	Somoria	2002	UKBRS ^a /291919	1, 2
11.3544	-12.3742	Banti	2002	UKBRS ^a /291920	1
9.9894	-12.9108	Kindia	2002	UKBRS ⁴ /291921	1
10.8883	-12.3475	Yalague	2002	UKBRS ^a /291922	1
6.4166 ^b	9.8833 ^b	Benakuma	1	MNHN°/2002.742	m
5.4667 ^b	9.8833 ^b	Fontern	I	MNHN°/2002.746	ε
6.36724	-1.03301	Ajenjua Bepo Forest Reserve	2006	MVZ ^d /252603	1
6.36718	-1.0339	Ajenjua Bepo Forest Reserve	2006	MVZ ^d /252605	1
6.32135	-1.03417	Mamang River Forest Reserve	2006	MVZ ^d /252608	1
6.25322	-1.03244	Mamang River Forest Reserve	2006	MVZ ^d /252609	1
6.25874	-1.02571	Mamang River Forest Reserve	2006	MVZ ^d /252610	1
5.87901	0.045290	Shai Hills Production Reserve	2004	MVZ ^d /245340	1
8.33203	0.578970	Kyabobo National Park	2004	MVZ ^d /245341	1
5.29004	-2.63957	Ankasa National Park	2004	MVZ ^d /245350	1
8.34842	0.60111	Kyabobo National Park	2005	MVZ ^d /249744	1
5.33433	-0.69127	Muni Lagoon	1998	UKBRS ^a /290459	1,4
4.83333	7.98333	Eket	1997-2001	ſ	5
5.40289	-7.73228	Grebo	2005	ſ	6
4.75	9.70	Mount Kupe	1994-1995	ſ	7
4.07911	9.17171	Mapenja, Mount Cameroon	2005	UCLA®	I
6.4613	14.23884	Bounou	2004-2005	UCLA [¢]	I
6.30010	12.79470	Mbakaou	2004-2005	UCLA€	I
5.33162	9.41473	Nguti	2004-2005	UCLA ^e	I
3.88463	10.09860	Malimba	2006	UCLA®	I
5.83302	-7.34275	Tai Forest	2001	UCLA ^e	Ι
5.82597	-7.39052	Paule Oula	2001	UCLA ^e	I
5.33098	-4.12905	CSRS	2001	UCLA [¢]	ł
3.57638	10.10832	Palm d'Or	2000	UCLA	I
4.51491	13.93460	Bazzama	2005	UCLA	I
2.72070	9.86746	south of Kribi	2006	UCLA	ł
3.058195	10.42072	Mbongwana	2006	UCLA	I
4.97996	8.916954	Mundemba	2006	UCLA	I

Table S3 I ocalities for Trachylenis affinis used to build ecological niche models

1111	1 1 8 8 9 9 00	2222	fic Papers
UCLA [¢] UCLA [¢] UCLA [¢] UCLA [¢] UCLA [¢]	UCLA [¢] UCLA [¢] 	– – USNM ^t /248837 – USNM ^t /248838	: Center for Tropical A sequence and AFLP nea, West Africa. Scienti
2006 2006 2006 2002 2002	2002 2002 2001 2003 2003 1996	2003 2003 1984 1975 1975	es. ey. ollected by other irmed with DNA ury 2008. tional Park, Guin
Mundemba palm plantation Konye Doume Bringakro Dassioko	Niegre Adipodoume Haute Dodo Forest Cavally Forest Boi-Tano Forest Krokosua Hills Grotte de Dande Cascade de Dimdéfélou	Déré classified forest Diécké classified forest Mount Béro classified forest Saniya, Kilimi National Park Fintonia, Kilimi National Park Abuku Mandinari	rsity Research Center. terature and online mapping resourc Naturelles, Paris. gy, University of California, Berkel gy, University of California, Berkel by AHF, 2001 and 2002 samples oc identity was also molecularly confi thistory, Washington, DC. Initery, Washington, DC. ion Facility, search performed Janua of The herpetofauna Upper Niger Nat
8.87103 9.46096 12.27967 -5.19	-6.57 -5.83 -7.31611 -7.80472 -2.62292 -2.84683 -12.32117 -12.32433	-8.21183 -8.87183 -8.87183 -8.57317 -12.45 -12.45 -16.6558 ^b	Kansas Biodive ermined from li onal d'Histore] ertebrate Zoolo mples collected I teams. Species eum of Natural ersity Informat 'Carr JL (2002) 'History Museu
4.98306 4.96387 5.89497 6.48 5.25	6.08 5.25 4.90222 6.15667 5.525164 6.60919 12.36683 12.36467	7.60367 7.59633 8.13917 9.8 9.63333 13.4042 ^b 13.3728	 ^a University of ^b Locations det ^c Museum Nati ^d Museum of V ^e 2004-2006 sa Research field profiles. ^f National Mus 1 Global Biodiv 2 Greenbaum E

3 Mausfeld-Lafdhiya P et al. (2004) Genetic variation in two African skink species complexes (Reptilia,Scincidae: *Euprepis affinis, Euprepis maculilabris*, based on maximum-likelihood and Bayesian analyses: taxonomic and biogeographic conclusions. *Bonner Zoologische Beitrage*, **52**, 159-177.

Table S3 (continued)

- 4 Raxworthy CJ, Attuquayefio DK (1998) Herpetofaunal communities at Muni Lagoon in Ghana Biodiversity and Conservation, 9, 501-510.
- 5 Akani GC et al. (2002) Community ecology of scincid liards in a swamp forest of south-eastern Nigeria. Russian Journal of Herpetology, 9, 125-134.

Demey R, Peal A. RAP Bulletin of Biological Assessment 44, Center for Applied Biodiversity Science and 6 A Rapid Biological Assessment of North Loma, Gola and Grebo National Forests, Liberia, eds. Hoke P. Conservation International, Washington, DC.

7 Hofer U et al. (2000) Ecotones and gradients as determinants of herpetofaunal community structure in the primary forest of Mount Kupe, Cameroon. Journal of Tropical Ecology, 16, 517-533.

8 A Rapid Biological Assessment of Two Classified Forests in South-western Cote d'Ivoire. eds Alonso LE, Lauginie F, Rondeau, G. RAP Bulletin of Biological Assessment 34, Center for Applied Biodiversity Science and Conservation International, Washington, DC.

Biological Assessment 36, Center for Applied Biodiversity Science and Conservation International, Washington, 9 A Biological Assessment of the Terrestrial Ecosystems of the Draw River, Boi-Tano, Nimiri and Krokosua Hills Forest Reserves, Southwestern Ghana. eds. McCullough, J, Decher J, Kpelle DG. RAP Bulletin of DC:

including the Niokola-Koba National Park, with observation on factors influencing diversity. Tropical 10 Joger U, and Lambert MRK (2002) Inventory of amphibians and reptiles in southeastern Senegal, Zoology, 15, 165-187.

McCullough J, Alonso LE, Diallo MS. RAP Bulletin of Biological Assessment 40, Center for Applied 11 A Rapid Biological Assessment of Three Classified Forests in Southeastern Guinea. eds Wright HE, Biodiversity Science and Conservation International, Washington, DC.

2 Hakansson NT (1981) An annotated checklist of reptiles known to occur in The Gambia. Journal of Herpetology, 15, 155-161.



Figure S1. Distribution of localities for *Trachylepis affinis*, including literature, museum records, and our field sampling, used to build ecological niche models. Localities not included in models because of missing environmental data are not shown.

	Princip	le Component
Trait	1	2
Snout-vent length	0.937	-0.022
Hind limb length	0.927	-0.074
Hind limb span	0.970	-0.078
Fore limb length	0.939	-0.154
Fore limb span	0.940	-0.157
Head depth	0.787	0.607
Gape width	0.904	0.031
Head length	0.947	-0.051

Table S4. Loadings of morphological traits from *Trachylepis affinis* on the first two principle components.

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Figure S2



Figure S2 (continued)



Figure S2 (continued)



Figure S2. GDM spatial predictions of genetic turnover in *Trachylepis affinis* for additional outlier loci purportedly under selection, and variables selected in each GDM model that allows for environmental variables and geographic distance as possible predictors. Colors between panels are not comparable, while within panels, areas with similar shading along the color scale are predicted to be genetically more similar.

Variable	r ^a	P^{a}
BIO1	0.19	0.14
BIO2	0.85	<0.0001
BIO4	-0.15	0.90
BIO5	-0.11	0.69
BIO6	0.51	0.008
BIO7	0.85	<0.0001
BIO8	0.19	0.14
BIO9	0.24	0.096
BIO10	0.16	0.17
BIO11	0.19	0.15
BIO12	0.87	<0.0001
BIO13	0.88	0.0003
BIO14	0.55	0.0002
BIO15	0.58	0.0071
BIO16	0.78	<0.0001
BIO17	0.65	<0.0001
BIO18	0.51	0.0095
BIO19	0.35	0.017
LAIMAX	-0.07	0.64
NDMAX	0.29	0.10
NDGR	-0.14	0.83
VCF	0.53	0.0017
QMEAN	0.42	0.018
QSD	0.78	0.0004
SRTM	0.59	0.0036

Table S5. Mantel correlations betweenenvironmental variables and geographicdistance at sampling localities.

^aSignificant correlations in boldface.
q a	artial	
	ENV r	Р
1 0.03 0.42	0.32	0.041
5 0.35 0.007	0.06	0.34
7 0.46 0.003	0.65	0.0013
1 -0.34 1.00	0.74	<0.0001
7 0.48 0.002	0.67	0.0015
5 0.35 0.0097	0.72	0.0015
7 0.40 0.0073	0.49	0.0017
0.15 0.16	0.81	0.0008
-0.23 0.95	0.32	0.017
7 -0.49 1.00	0.80	<0.0001
1 -0.50 1.00	0.80	<0.0001
3 -0.44 1.00	0.63	0.0003
2 -0.40 0.99	0.66	0.0059
5 -0.53 1.00	0.68	0.001
f -0.11 0.83	0.49	0.0034
-0.34 1.00	0.46	0.0035
9 -0.03 0.59	0.75	0.0005
-spline coefficients for each variat e possible predictors.	le entered into	
6 -0.53 1.00 1 -0.11 0.83 -0.34 1.00 -0.34 1.00 -0.03 0.59 -spline coefficients for each value predictors.	rriab	0.68 0.49 0.46 0.75 uriable entered into

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Figure S3. ND4 mismatch distributions for sets of *T. affinis* populations comprising genetic clusters ascertained from Structure analysis of neutral AFLP loci (filled circles), and those simulated under a demographic expansion (solid line), and under a spatial expansion with constant population size (dashed line). See text for definitions of clusters. All populations showed evidence for demographic expansion based upon differences between observed and simulated estimates of Θ_0 , Θ_1 , and τ (Schneider and Excoffier 1999); similar tests based upon the raggedness index (Harpending 1994) also failed to reject demographic expansion except for S. of Sanaga River. All genetic clusters but S. of Sanaga River showed evidence for spatial expansion (Excoffier 2004).



Figure S4. A. Minimum spanning tree of ND4 haplotypes showing lower haplotypic diversity in the ecotone, and haplotype sharing between the two rainforest genetic clusters identified with Structure. White plus signs and asterisks indicate divergent clusters of haplotypes that are found sympatrically at Kribi (site 1). B. GDM of ND4 F_{ST} distance matrix, with environmental variables and geographic distance as predictors. Percent variation explained are for the full model (D+E), and geographic distance alone (D).



Figure S5. Structure results for 101 neutral AFLP loci for 20 individuals at Kribi, showing evidence for admixture between the two ND4 clades noted by their respective symbols corresponding to those in Fig. S4 A.

References

- Anthony NM, Johnson-Bawe M, Jeffery K, et al. (2007) The role of Pleistocene refugia and rivers in shaping gorilla genetic diversity in central Africa. Proceedings of the National Academy of Sciences, USA, **104** 20432–20436.
- Arévalo E, Davis SK, Sites JW (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome race of the Sceloperus grammicus complex (Phrynosomatidae) in central Mexico. Systematic Biology, 43, 387–418.
- Austerlitz F, Jung-Muller B, Godelle B, Gouyon P-H (1997) Evolution of coalescence times, genetic diversity and structure during colonization. *Theoretical Population Biology*, **51**, 148–164.
- Avise JC (2000) *Phylogeography: the History and Formation of Species*. Harvard University Press, Boston, Massachusetts.
- Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. Ecography, doi: 10.1111/j.1600-0587.2009.05807.x.

- Barbujani B, Sokal RF, Oden NL (1995) Indo-European origins: a computer-simulation test of five hypotheses. *American Journal of Physical Anthropology*, **96**, 109–132.
- Barton NH (2001) Adaptation at the edge of a species' range. In: Integrating Ecology and Evolution in a Spatial Context (eds Silvertown J, Antonovics J), pp. 365–392.
 Cambridge University Press, Cambridge, United Kingdom.
- Beaumont MA (2005) Adaptation and speciation: what can F_{ST} tell us? *Trends in Ecology* and Evolution, **20**, 435–440.
- Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, **13**, 969–980.
- Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society of London, Series B, Biological Sciences, 263, 1619–1626.
- Bohonak AJ (2002) IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity*, **93**, 153–154.
- Bonin A, Pompanon F, Taberlet P (2005) Use of amplified fragment length polymorphism (AFLP) markers in surveys of vertebrate diversity. In: *Molecular*

Evolution: Producing the Biochemical Data, Part B Methods in Enzymology, Vol. 395 (eds. Zimmer EA, Roalson E), pp. 145–161. Academic Press, New York, New York.

- Bonin A, Taberlet P, Miaud C, Pompanon F (2006) Explorative genome scan to detect candidate loci for adaptation along a gradient of altitude in the formon frog (*Rana temporaria*). *Molecular Biology and Evolution*, **23**, 773–783.
- Bowie RCK, Fjeldså J, Hackett SJ, Bates JM, Crowe TM (2006) Coalescent models reveal the relative roles of ancestral polymorphism, vicariance, and dispersal in shaping phylogeographical structure of an African montane forest robin. *Molecular Phylogenetics and Evolution*, **38**, 171–188.
- Bridle JR, Vines TH (2006) Limits to evolution at range margins: when and why does adaptation fail? *Trends in Ecology and Evolution*, **22**, 140–147.
- Bucini G, Hanan NP (2007) A continental-scale analysis of tree cover in African savannas. *Global Ecology and Biogeography*, **16**, 593–605.
- Caballero A, Quesada H, Rolán-Alvarez E (2008) Impact of amplified fragment length polymorphism size homoplasy on the estimation of population genetic diversity and the detection of selective loci. *Genetics*, **179**, 539–554.

- Campbell D, Bernatchez L (2004) Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Molecular Biology and Evolution*, **21**, 945–956.
- Carnaval AC, Hickerson MJ, Haddad CFB, Rodrigues MT, Moritz C (2009) Stability predicts genetic diversity in the Brazilian Atlantic Forest hotspot. *Science*, **323**, 785–789.
- Castellano S, Balletto E (2002) Is the partial Mantel test inadequate? *Evolution*, **56**, 1871–1873.
- Charlesworth B, Nordborg M, Charlesworth D (1997) The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genetics. Research*, **70**, 155–174.
- Cheviron ZA, Brumfield RT (2009) Migration-selection balance and local adaptiation of mitochondrial haplotypes in rufous-collared sparrows (*Zonotrichia capensis*) along an elevational gradient. *Evolution*, **63**, 1593–1605.
- Clegg SM, Degnan SM, Moritz C, et al. (2002) Microevolution in island forms: the roles of drift and directional selection in morphological divergence of a passerine bird.
 Evolution, 56, 2090–2099.

Coyne JA, Orr HA (2004) Speciation. Sinauer Associates, Sunderland, Massachusetts.

Crosswhite DL, Fox SF, Thill RE (1999) Comparison of methods for monitoring reptiles and amphibians in upland forests of the Ouachita Mountains. *Proceedings of the Oklahoma Academy of Sciences*, **79**, 45–50.

DeMenocal PB (1995) Plio-Pleistocene African climate. Science, 270, 53-59.

- Doebeli M, Dieckmann U (2003) Speciation along environmental gradients. *Nature*, **421**, 259–264.
- Downes SJ, Shine R (1999) Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? *Oecologia*, **120**, 9–18.
- Dupont LM, Jahns S, Marret F, Ning S (2000) Vegetation changes in equatorial West Africa: time-slices for the last 150ka. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **155**, 95–122.
- Dynesius M, Jansson R (2000) Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proceedings* of the National Academy of Sciences, USA, **97**, 9115–9120.

- Ehinger M, Fontanillas P, Petit E, Perrin N (2002) Mitochondrial DNA variation along an altitudinal gradient in the greater white-toothed shrew, *Crocidura russula*. *Molecular Ecology*, **11**, 939–945.
- Elith J, Graham CH, Anderson RP *et al.* (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129–151.
- Endler JA (1982) Pleistocene forest refuges: fact or fancy? In: *Biological Diversification in the Tropics* (ed Prance GT), pp. 641–657.Columbia University Press, New York, New York.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14, 2611– 2620.
- Excoffier L (2004) Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Molecular Ecology*, **13**, 853–864.
- Excoffier L, Laval G, Schneider S (2006) ARLEQUIN version 3.1: an integrated software package for population genetics data analysis. University of Berne, Computational and Molecular Population Genetics Lab, Berne, Switzerland.

- Ferrier S, Manion G, Elith J, Richardson K (2007) Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. *Diversity and Distributions*, **13**, 252–264.
- Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.
- Funk DJ, Nosil P, Etges WJ (2006) Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences, USA*, **103**, 3209–3213.
- García-Ramos G, Kirkpatrick M (1997) Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, **51**, 21–28.
- Graham CH, Ron SR, Santon JC, Schneider CJ, Moritz C (2004) Integrating phylogenetics and ecological niche models to explore speciation mechanisms in Dendrobatid frogs. *Evolution*, **58**, 1781–1793.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95– 98.

- Hansen MC, DeFries RS, Townshend JRG, et al. (2002) Towards an operational MODIS continuous field of percent tree cover algorithm: Examples using AVHRR and MODIS data. Remote Sensing of the Environment, 83, 303–319.
- Hellberg ME, Balch DP, Roy K (2001) Climate-driven range expansion and morphological evolution in a marine gastropod. *Science*, **292**, 1707–1710.
- Hernandez PA, Graham CH, Master LL, Albert DL (2006) The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, **29**, 773–785.
- Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature, 405, 907-913.

- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land area. *International Journal of Climatology*, 25, 1965–1978.
- Hoffmann AA, Blouin MS (2004) Historical data refute recent range contraction as a cause of low genetic diversity in frog populations. *Molecular Ecology*, **13**, 271–276,

- Holt RD, Gomulkiewicz R, Barfield M (2003) The phenomenology of niche evolution via quantitive traits in a 'black-hole' sink. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **270**, 215–24
- Hranitz JM, Baird TA (2000) Effective population size and genetic structure of a population of collard lizards (*Crotaphytus collaris*), in central Oklahoma. *Copeia*, 2000, 786–791.
- Huey RB, Kingsolver JG (1989) Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology and. Evolution*, **4**, 131–135.
- Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282–291.
- Jansson R, Dynesius M (2002) The fate of clades in a world of recurrent climate change:
 Milankovitch oscillations and evolution. *Annual Review of Ecology and Systematics*,
 33, 741–777.
- Jahns J (1996) Vegetation history and climate changes in West Equatorial Africa during the Late Pleistocene and Holocene, based upon a marine pollen diagram from the Congo fan. *Vegetation History and Archaeobotany*, **5**, 207–213.

- Kawecki TJ (2008) Adaptation to marginal habitats. Annual Review of Ecology, Evolution, and Systematics, **39**, 321–342.
- Kiehl JT, Gent PR (2004) The community climate system model, version 2. *Journal of Climate*, **17**, 3666–3682.
- Le Corre V, Roussel G, Zanetto A, Kremer A (1998) Geographic structure of gene diversity in *Quercus petraea* (Matt.) Liebl. III. Patterns of variation identified by geostatistical analyses. *Heredity*, **80**, 464–473.
- Letouzey R (1968) Etude phytogéographic du Cameroun. Encyclopédie Biologique 69. Paul Lechevalier, Paris.
- Linder HP (2001) Plant diversity and endemism in sub-Saharan tropical Africa. Journal of Biogeography, 28, 169–182,
- Long DG, Drinkwater MR, Holt B, Saatchi S, Bertoia C (2001) Global ice and land climate studies using scatterometer image data. *EOS Transaction of the American Geophysical Union*, **82**, 503.
- Losos JB (1990) Ecomorphology, performance capability, and scaling of West Indian Anolis lizards: an evolutionary analysis. Ecological Monographs, **60**, 369–388.

- Losos JB, Warheit KI, Schoener TW (1997) Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature*, **387**, 70–73.
- Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, **3**, 91–99.
- Maley J (1996) The African rainforest-main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proceedings of the Royal Society of Edinburgh*, **104B**, 31–73.
- Maley J, Brenac P (1998) Vegetation dynamics, palaeoenvironments and climate changes in the forests of western Cameroon during the last 28,000 years B.P. *Reviewof Palaeobotany and Palynology*, **99**, 157–187.
- Maley J, Livingstone DA, Giresse P,, et al. (1990) Lithostratigraphy, volcanism,
 paleomagnetism and palynology of Quaternary lacustrine deposits from Barombi
 Mbo (West Cameroon): preliminary results. Journal of Volcanology and Geothermal
 Research, 42, 319–335.
- Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, **18**, 189–197.

- Manly BFJ (1997) Randomization and Monte Carlo Methods in Biology, 2nd edition. Chapman and Hall, New York, New York.
- Massot M, Hue RB, Tsuji J, van Berkum FH (2003) Genetic, prenatal, and postnatal correlates of dispersal in hatchling fence lizards (*Sceloporus occidentalis*). *Behavioral Ecology*, **14**, 650–655.
- Mausfeld P, Vences M, Schmitz A, Veith M (2000) First data on the molecular phylogeography of scincid lizards of the genus *Mabuya*. *Molecular Phylogenetics* and Evolution, 17, 11–14.
- Mayr E, O'Hara RJ (1986) The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. *Evolution*, **40**, 55–67.
- McKinnon JS, Mori S, Blackman BK, et al., (2004) Evidence for ecology's role in speciation. Nature, 429, 294–298.
- Moritz C, Patton JL, Schneider CJ, Smith TB (2000). Diversification of rainforest faunas: an integrated molecular approach. *Annual. Review of. Ecology and. Systematics*, **31**, 533–563.

- Mullen LM, Hoekstra HE (2008) Natural selection along an environmental gradient: a classic cline in mouse pigmentation. *Evolution*, **62**, 1555–1570.
- Myneni RB, Hoffman S, Knayazikhin Y et al. (2002) Global products of vegetation leaf area and fraction absorbed PAR from year one of MODIS data. Remote Sensing of the Environment, 83, 214–231.
- Nielsen R (2005) Molecular signatures of natural selection. *Annual Review of Genetics*, **39**, 197–218.
- Nix H (1986). A biogeographic analysis of Australian elapid snakes. In: *Atlas of Elapid Snakes of Australia* (ed Longmore R), pp 4–15. Australian Government Publishing Service, Canberra, Australia.
- Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, **18**, 375–402.
- Nosil P, Egan SP, Funk DJ (2008) Heterogeneous genomic differentiation between walking-stick ecotypes: "isolation by adaptation" and multiple roles for divergent selection. *Evolution*, **62**, 316–336.

Ogden R, Thorpe RS (2002) Molecular evidence for ecological speciation in tropical habitats. *Proceedings of the National Academy of Sciences*, USA, **99**, 13612–13615.

Ohta T (1973) Slightly deleterious mutant substitutions in evolution. Nature, 246, 96–98.

- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics, **37**, 637–669.
- Pease KM, Freedman AH, Pollinger JP et al. (2009) Landscape genetics of California mule deer (Odocoileus hemionus): the roles of ecological and historical factors in generating differentiation. Molecular Ecology, 18, 1848–1862.
- Phillips S, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.
- Pinot S, Ramstein G, Harrison SP, et al. (1999) Tropical paleoclimates at the Last Glacial
 Maximum: comparison of Paleoclimate Modeling Intercomparison Project (PMIP)
 simulations and paleodata. *Climate Dynamics*, 15, 857–874.
- Plana V, Gascoigne A, Forrest LL, Harris D, Pennington RT (2004) Pleistocene and pre-Pleistocene Begonia speciation in Africa. Molecular Phylogenetics and Evolution, 31, 449–461.

- Posada D (2006) ModelTest server: a web-based tool for the statistical selection of models of nucleotide substitution online. *Nucleic Acids Research*, **34**, W700–W703.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Quérouil S, Verheyen E, Dillen M, Colyn M (2003) Patterns of diversification in two
 African forest shrews: Sylvisorex johnstoni and Sylvisorex ollula (Soricidae,
 Insectivora) in relation to paleo-environmental changes. Molecular Phylogenetics and
 Evolution, 28, 24–37.
- Ramsay JO (1988) Monotone regression splines in action. *Statistical Science*, **3**, 425–461.
- Räsänen K, Hendry AP (2008) Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters*, **11**, 624–636.
- Raufaste N, Rousset F (2001) Are partial mantel tests adequate? *Evolution*, **55**, 1703–1705.

- Ray N (2005) PATHMATRIX: a GIS tool to compute effective distances among samples. Molecular Ecology Notes, 5, 177–180
- Ray N, Currat M, Excoffier L (2003) Intra-deme molecular diversity in spatially expanding populations. *Molecular Biology and Evolution*, **20**, 76–86.
- Rice WR, Hostert EE (1993) Perspective: laboratory experiments on speciation: what have we learned in 40 years? *Evolution*, **47**, 1637–1653.
- Rieseberg LH, Widmer A, Arntz AM, Burke JM (2002) Directional selection is the primary cause of phenotypic diversification. *Proceedings of the National Academy of Sciences, USA*, **99**, 12242–12245.
- Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.
- Rohr DH (1997) Demographic and life-history variation in two proximate populations of a viviparous skink separated by a steep altitudinal gradient. *Journal of Animal Ecology*, **66**, 567–578.
- Rousset F (2002) Partial Mantel tests: reply to Castellano and Balletto. *Evolution*, **56**, 1874–1875.

Roy MS (1997) Recent diversification in African greenbuls (Pycnonotidae: Andropadus) supports a montane speciation model. Proceedings of the Royal Society of London, Series B, Biological Sciences, 264, 1337–44.

- Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DNASP: DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.
- Sankaran M, Hanan NP, Scholes RJ,, et al. (2005) Determinants of woody cover in African savannas. Nature, 438, 836–849.
- Schneider CJ, Smith TB, Larison B, Moritz C (1999) A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia.
 Proceedings of the National Academy of Sciences, USA, 96, 13869–13873.
- Schneider S, Excoffier L (1999) Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites:
 Application to human mitochondrial DNA. *Genetics*, **152**, 1079–1089.
- Shine R, Elpick MJ, Harlow PS (1997) The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology*, **78**, 2559–2568.

- Smith TB, Calsbeek R, Wayne RK et al. (2005a) Testing alternative mechanisms of evolutionary divergence in an African rain forest passerine bird. Journal of Evolutionary Biology, 18, 257–268.
- Smith TB, Wayne RK, Girman DJ, Bruford MW (2000) The importance of ecotones in rainforest speciation. In: *Rainforests: Past and Future* (eds Moritz C, Bermingham E, Dick C), pp. 149–165. University of Chicago Press, Chicago, Illinois.
- Smith TB., Saatchi S, Graham C, Slabbekoorn H, Spicer G (2005b) Putting process on the map: why ecotones are important for preserving biodiversity. In: *Phylogeny and Conservation* (eds Purvis A, Gittleman J, Brooks T), pp. 166–197. Cambridge University Press, Cambridge, United Kingdom.
- Smith TB, Wayne RK, Girman DJ, Bruford MW (1997). A role for ecotones in generating rainforest biodiversity. *Science*, **276**, 1855–1857.
- Stevenson RD, Peterson CR, Tsuji J (1985) The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiological Zoology*, 58, 46–57.
- Storey JD, Tibshirani R. (2003) Statistical significance for genome-wide experiments. Proceedings of the National Academy of Sciences, USA, 100, 9440–9445.

- Storz JF, Sabatino SJ, Hoffmann FG *et al.* (2007) The molecular basis of high-altitude adaptation in deer mice. *PLoS Genetics*, 3, **e45**, 0448–0459.
- Sumner J, Rousset F, Estoup A, Moritz C (2001) 'Neighbourhood' size, dispersal and density estimates in the prickly forest skink (*Gnypetoscincus queenslandiae*) using individual genetic and demographic methods. *Molecular Ecology*, **10**, 1917–1927.
- Tchouto MGP, de Wilde JJFE, deBoer WF, van der Maesen LJG, Cleef AM (2008) Bioindicator species and Central African rain forest refuges in the Campo-Ma'an area, Cameroon. *Systematic Biodiversity*, doi:10.1017/ S1477200008002892.
- Thibert-Plante X, Hendry AP (2009) Five questions on ecological speciation addressed with individual-based simulations. *Journal of Evolutionary Biology*, **22**, 109–123.
- Thomas CD, Bodsworth EJ, Wilson RJ, et al. (2001) Ecological and evolutionary processes at expanding range margins. *Nature*, **411**, 577–581.
- Thorpe RS, Reardon JT, Malhotra A (2005) Common garden and natural selection experiments support ecotypic differentiation in the Dominican anole (*Anolis oculatus*). *American Naturalist*, **165**, 495–504.

- Vanzolini PE, Williams EE (1981) The vanishing refuge: a mechanism for ecogeographic speciation. Papéis Avulsos Zoology, São Paulo, 34, 251–255.
- Vekemans X, Beauwens T, Lemaire M, Roldan-Ruiz I (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology*, 11, 139–151.
- Vitt, LJ, Caldwell JP, Zani PA, Titus TA (1997) The role of habitat shift in the evolution of lizard morphology: evidence from tropical *Tropidurus*. *Proceedings of the National Academy of Sciences*, USA, **94**, 3828–3832.
- Vitt LJ, Zani PA, Caldwell JP (1996) Behavioral ecology of *Tropidurus hispidus* on isolated rock outcrops in Amazonia. *Journal of Tropical Ecology*, **12**, 81–101.
- Vos P, Hogers R, Bleeker M, et al. (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23, 4407–4414.
- Waltari E, Hijmans RJ, Peterson AT, et al. (2007) Locating Pleistocene refugia:
 comparing phylogeographic and ecological niche model predictions. PLoS ONE, 2, e563, 1–11.

- Wegmann D, Currat M, Excoffier L (2006) Molecular diversity after a range expansion in heterogeneous environments. *Genetics*, **174**, 2009–2020.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wright S (1943) Isolation by distance. Genetics, 28, 114–138.

- Wronski T, Hausdorf B (2008) Distribution patterns of land snails in Ugandan rain forests support the existence of Pleistocene forest refugia. *Journal of Biogeography*, 35, 1759–1769.
- Zayed A, Whitfield CW (2008) A genome-wide signature of positive selection in ancient and recent invasion expansions of the honey bee *Apis mellifera*. *Proceedings of the National Academy of Sciences, USA*, **105**, 3421–3426.
- Zhivotovsky LA (1999) Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology*, **8**, 907–913.

Modeling the effects of anthropogenic habitat change on savanna snake invasions into African rainforest

Introduction

Habitat destruction and fragmentation is a leading cause of biodiversity loss (Fahrig 2003; Hoffmeister *et al.* 2005), particularly in tropical rainforests, where species richness and rates of anthropogenic land conversion reach some of the highest levels of any ecosystem (Achard *et al.*2002; Wright 2005). Although there has been considerable emphasis on the loss of rainforest species, less attention has been paid to the impacts of biological invasions (Wright 2005). Invasions are frequently mediated by habitat disturbance, facilitating dispersal into previously unsuitable habitats (Vitousek *et al.* 1997). Although invasive species typically refers to human-introduced non-native species, the term also describes the expansion of native species into adjacent biomes where they do not normally occur.

In addition to human-caused habitat loss, climate change may also alter habitats and animal and plant populations in profound ways (Parmesan 2006). Ranges of species are expected to shift as a function of their physiological tolerances (Parmesan 2006). Shifts in temperature are accompanied by changes in precipitation, which affects the distribution of vegetation (IPCC 2007). Populations unable to move or adapt quickly may face extinction (Parmesan 2006). Combined, impacts of habitat conversion and climate change are likely to have dramatic and complex effects. The combined effects of climate and habitat fragmentation on species distributions, however, are poorly understood, especially for tropical ecosystems.

Ectothermic species, such as snakes, are highly dependent on ambient thermal environments, which through their effects on body temperature influence physiology, locomotor performance, behavior, habitat use, and ultimately, fitness (Stevenson *et al.* 1985; Row and Blouin-Demers 2006). Because of this sensitivity, snakes are ideal for investigating the impacts of habitat alteration and climate warming. At a local scale rainforest fragmentation creates invasion opportunities for savanna species by producing clearings with more savanna-like thermal attributes. These clearings also create edge effects that invaders can exploit: hotter, drier conditions persisting within fragments up to hundreds of meters from the rainforest-clearing boundary (Laurance *et al.* 2002) and increased microclimate heterogeneity (Camargo and Kapos 1995), which depend, in part, on edge structure (Camargo and Kapos 1995; Didham and Lawton 1999). Global warming alters temperature gradients and seasonality on a continental scale, which may lead to regional distribution shifts by temperature-sensitive species.

In equatorial Africa human population expansion, logging, agriculture, and firewood harvesting create open canopy vegetation that structurally resembles savanna. Consequently, species that are typically restricted to savanna habitats now occur in disturbed areas within the rainforest zone (e.g., Thiollay 1986; Akani *et al.* 1999). In addition to these effects, Africa is predicted to be the continent most vulnerable to the effects of climate change (IPCC 2007). Determining invasion potential resulting from climate change or habitat destruction is important because invaders may displace or

reduce densities of existing species (Case and Bolger 1991), cause extinction (Savidge 1987), and have deleterious ecosystem-level effects (Hoffmeister *et al.* 2005).

To generate present and future distribution models of three Central African snake species, which typically inhabit savanna but are also found in human-disturbed forest habitats, we integrated contemporary point-locality data and bioclimatic and satellite remote-sensing data in a species-distribution modeling framework. Species distribution models use information on environmental variables at species localities to construct an environmental envelope that approximates a species' ecological niche, and then the models project the niche onto geographic space to predict distribution of a species.

Because our study species have not historically occurred in rainforest, we first tested the hypothesis that rainforest is climatically unsuitable for savanna snakes. We did this by building distribution models with only climate variables and snake localities from the savanna. Next, we addressed the proposed mechanism underlying invasion, specifically, how human alteration of rainforest may create savanna-like habitats with suitable microclimates. To test this hypothesis, we built models that included only finescale remotely sensed data on vegetation from MODIS and savanna localities. Finally, to estimate the relative contributions of climate and vegetation in the predictions and to provide an assessment of invasion potential, we constructed models that included all environmental variables and all snake localities. Our specific objectives were to predict the contemporary distributions of the three snake species in Cameroon; assess the relative influence of vegetation cover and current climate on the snakes' ability to invade the rainforest zone; and explore how climate change might influence invasions.

Methods

Study Area and Species

We conducted our study in Cameroon, Central Africa, a country with dense rainforest in the south and savanna in the north (Fig. 1). Between these two biomes is an ecotone of rainforest fragments, gallery forest, and savanna (Letouzey 1968). There is substantial variation in level of anthropogenic disturbance, temperature, and precipitation regimes along the rainforest-savanna gradient, making it an ideal setting to test the joint effects of forest fragmentation and climate change on species distributions.

We studied three snake species that are broadly distributed in the savanna. In the rainforest zone these species are found only in human-disturbed habitats, which suggests a recent invasion. We chose these species because available point-locality data sets were sufficiently large for modeling, their taxonomic status is reasonably certain and they are absent in undisturbed rainforest (Akani *et al.*1999; Chippaux 1999; Sprawls *et al.* 2002) but have been recently observed in rainforest.

The night adder (*Causus maculatus*, 88 localities; Fig. 2) is a nocturnal viper found in savanna and human-disturbed regions of forest (e.g., gardens, plantations, and urban areas) and semidesert (Akani *et al.* 1999; Chippaux 1999; Sprawls *et al.* 2002). It preferentially feeds on amphibians and, as a result, uses wet microhabitats (Luiselli *et al.* 2004). Reproduction in this species is seasonal, with oviposition occurring most frequently at the beginning of the wet season, which coincides with the reproduction of its amphibian prey (Luiselli *et al.* 2004).



Figure 1. Ecoregions of Cameroon showing the distribution of rainforest and savanna habitats (from CARPE 1997).



Figure 2. Point localities, probabilities of occurrence, and predicted distributions under present conditions for three snakes that are potentially invasive in Cameroon's rainforests. Color scale indicates probability of occurrence and thus invasion potential, with larger values indicating greater probability of occurrence. Areas with probability of occurrence larger than the balance threshold define a species' likely distribution. Tan

Figure 2 (continued)

areas are those below the balance threshold. Distributions are derived from models with (a) climate and elevation variables and savanna points only, (b) fine-scale MODIS vegetation variables and savanna points only, and (c) climate, elevation, MODIS, and QSCAT variables and all point localities. Black and white crosses indicate localities in the savanna and rainforest, respectively. Black contour lines outline ecoregions within the rainforest (see Fig. 1). White squares in (c) indicate areas enlarged to the right. Within the enlargements, the area of high predicted probability of occurrence in the upper right corner is an area of extensive clearcutting, and the diagonal line running from the lower left corner represents clearing along the road between Yaoundé and Bertoua. The olympic lined snake (*Dromophis lineatus*, 33 localities; Fig. 2) is a diurnally active, oviparous colubrid inhabiting moist savannas, damp grasslands, floodplains, lake shores, marsh edges, and plantations (Menzies 1966; Chippaux 1999; Sprawls *et al.* 2002). It feeds on amphibians and small mammals (Villiers 1975).

The African house snake (*Lamprophis fuliginosus*, 86 localities; Fig. 2) is a nocturnal, oviparous colubrid inhabiting savanna and mosaics of forest and humancreated savanna ("derived savanna"). It is highly tolerant of urban land use and cultivation. It preferentially feeds on small mammals (Akani *et al.* 1999; Chippaux 1999; Sprawls *et al.* 2002; Luiselli *et al.* 2005).

Because lower temperatures under the canopy might pose less of a physiological constraint to nocturnal species—whose peak activity times coincide with the coolest part of the diurnal cycle—we hypothesized that *C. maculatus* and *L. fuliginosus* would display greater invasion potential than *D. lineatus*. Rainforest habitats contain greater per-area abundance of amphibian prey than savanna, but intensive human land use leads to elevated rodent densities. Thus, we did not attempt to make *a priori* predictions with respect to invasion potential on the basis of dietary preferences. Here, we defined invasion as the penetration of areas within the rainforest zone.

All locality data were compiled during a recent countrywide survey of reptiles by a Cameroon-based project (CAMHERP) and will be in a forthcoming atlas of the reptiles of Cameroon. Our data points (spanning 1998–2003) included GPS-referenced sites of reptiles captured by CAMHERP herpetologists and those collected by local field assistants in villages, surrounding forest, and on farms. Although there may be some bias

in collection toward inhabited areas, this would actually underestimate invasion areas. Because localities in the rainforest would only be associated with extreme levels of vegetation clearing proximal to human settlement and not other disturbed forest habitats, models would underpredict invasions of disturbed habitats farther from inhabited areas. Area coverage included most 0.5° grid cells within country boundaries.

Environmental Data

We obtained data on 19 bioclimatic variables from WorldClim (version 1.4; Hijmans *et al.* 2005) gridded to 1-km resolution and selected a subset to capture major variation in annual means and seasonality of temperature and precipitation that likely serve as limiting factors for reptiles. Specifically, we selected annual mean temperature, temperature mean diurnal range, temperature seasonality, minimum temperature of the coldest month, annual precipitation, precipitation of the wettest quarter, and precipitation of the driest quarter. Future projections of these climate layers were also obtained from WorldClim and stem from a simulation of the CCM3 general circulation model under doubling of CO_2 (Govindasamy *et al.* 2003). Depending on the emission scenario, a doubling of CO_2 may be reached anytime from the middle to the end of this century (IPCC 2007).

Remote-sensing data provided measurements of variables such as topography, tree cover, vegetation density (e.g., leaf area), and seasonality. To quantify spatial and temporal vegetation patterns, we used the monthly 1-km leaf area index (LAI) data derived from MODIS reflectance over a 5-year period (2000–2004; Myneni *et al.* 2002).

To further reduce effects of persistent cloud cover and natural interannual variability in the data, we created monthly climatologies by averaging the five years of data. We then used these composites to generate two vegetation variables: annual maximum LAI and LAI annual seasonality (difference between annual maximum and minimum months). As an additional fine-scale metric of land cover, we used the MODIS-derived vegetation continuous field (VCF) product as a measure of the percentage of tree canopy cover at 1km resolution (Hansen et al. 2002). The VCF came from 2001 MODIS data and separated open, fragmented, and deforested areas from those of intact old-growth forests. We also generated monthly composites of global Ouick Scatterometer (OSCAT; Long et al. 2001) microwave measurements for 2001 and processed them to produce two variables: annual mean (QMEAN) and standard deviation of radar backscatter (QSD). The QSCAT data layers were reaggregated from their 2.25-km native resolution to 1 km to match the resolution of the other predictor variables. The QSCAT radar measurements are sensitive to surface roughness, surface moisture, leaf water content, and other seasonal attributes, such as deciduousness of vegetation. They also have the added advantage of being unbiased by cloud cover. Finally, we included elevation data at 1-km resolution from the Shuttle Radar Topography Mission (SRTM).

Covariance among environmental variables may limit interpretation with respect to which variables best explain predicted distributions. To assess covariance among the 13 environmental variables, we computed a cross-correlation matrix with 1000 random points sampled throughout the study area. Only seven of 78 bivariate correlations had values of r > 0.75, which suggests the selected variables carry a considerable amount of

unique information. The model algorithm we used (Maxent) is largely insensitive to covariance among variables (S. Phillips, personal communication).

Species-Distribution Modeling

We modeled species distributions with Maxent (version 3.0), a recently developed general-purpose algorithm for presence-only data (Phillips et al. 2006). In a recent, comprehensive, inter-model comparative study, Maxent ranked among the highest performers (Elith et al. 2006). Other researchers prefer biophysical models for predicting reptile distributions (Kearney and Porter 2004). Although such approaches are useful for deriving hypotheses pertaining to mechanisms through which thermal constraints limit distributions (Kearney and Porter 2004; Crozier and Dwyer 2006), they require extensive species-specific information on physiological responses to ambient climate. Such data are lacking for most tropical species, including ours. Furthermore, biophysical models focus on the fundamental niche and do not account for non-climatic influences, such as interspecific interactions, unless physiological parameters are supplemented with information on relevant biotic features such as community composition. In contrast, correlative models derived from environmental envelopes (such as Maxent) can capture biotic and abiotic effects and at large spatial scales are suitable for making predictions concerning the likely effects of climate change (Pearson and Dawson 2003). Although distributions of ectotherms may be limited by the thermal environment, there is also abundant evidence for biotic interactions of large effect in tropical snakes (Madsen and Shine 2000; Luiselli 2006; Madsen et al. 2006). Because we focused on distributions at a
countrywide scale and wished to incorporate all potentially significant factors influencing invasions, an environmental-envelope modeling approach was implemented.

For each species, we built three different species distribution models for presentday conditions. First, to test the hypothesis that undisturbed rainforest is climatically unsuitable for our focal species, we built models with only bioclimatic variables and elevation. We included elevation because it captures steep elevation associated climatic gradients. To characterize the climatic envelope of the noninvasive range of our species, we restricted this analysis to localities from (1) savanna, (2) the northernmost margin of the forest-savanna mosaic containing natural savanna vegetation, and (3) Cameroon Highland forests, which contain a mixture of shrub, woodland, and savanna vegetation and thus have natural habitat for our focal species (Fig. 1). Our climate data were relatively coarse and did not allow us to quantify the microclimates our study species presumably use in the rainforest. But, given historical correlations between climate and vegetation cover at regional scales (in the absence of substantial habitat alteration by humans), we used climate data to validate the known, historical exclusion of our study species from the rainforest. This test was complementary to the second test that only had vegetation variables and savanna localities (see below) because it allowed us to rule out invasions facilitated by suitable macroclimates in the rainforest zone.

Second, to test the hypothesis that large-scale human alteration and fragmentation of rainforest has made it more suitable for invasions, we built a set of models with only the MODIS remote-sensing variables. These variables are suitable to identify rainforest alterations at spatial scales on the order of 1 km (close to their original resolution), but

cannot discern deforestation pattern at smaller scales. If the mechanism that underlies invasion is the creation of more savanna-like habitat and microclimates (Vitt *et al.* 1998; Foley *et al.* 2005), then localities within the savanna should predict invasion within the rainforest biome. In other words, we used the remotely sensed variables as surrogates for microclimatic variation in the affected rainforest zone. As with the first set of models, we excluded rainforest localities, but included them in a second step to evaluate how well the models predicted distributions in rainforest.

Finally, in a third set of models, we used all available information on climate, elevation, and vegetation to quantify the relative contributions of climate and vegetation and to determine the extent of current snake distributions. Because we wanted to characterize the environmental envelope of both the native and invasive distributions, we included rainforest localities. However, because the climate variables are interpolated surfaces, they have extremely coarse effective resolution (due to the low density of the station network) and thus do not capture microclimatic variation. Thus, inclusion of rainforest sample points introduced a bias in the species-climate relationship: models inferred that rainforest climates are suitable and overpredicted invasion potential in the rainforest zone. However, failure of our vegetation variables to detect areas of small-scale deforestation that are potentially suitable habitat for savanna snakes would lead to underprediction of invasion potential. With these considerations in mind, our models defined reasonable approximations of invasion potential. To reduce some of the potential bias, we included QSCAT variables, which have a 2.25-km nominal resolution. These data were slightly coarser than the MODIS variables, but have proven effective in

distinguishing rainforest from savanna biomes (Buermann *et al.* 2008). Furthermore, because QSCAT measurements are sensitive to canopy moisture and roughness, they captured aspects of climate at finer resolution than the bioclimatic variables. They also reduced potential overprediction resulting from edge effects: presences in pixels of high rainforest cover estimated by MODIS that result from close proximity to other pixels with low vegetation cover.

To explore how future climate change may influence invasion potential, we projected the present-day climate-species relationship onto future estimates of temperature and precipitation data that stem from the CCM3 CO₂ doubling scenario (Govindasamy et al. 2003). In this experiment, we only included climate variables because we did not know how vegetation would change in the future. We excluded elevation because of its confounding association with contemporary climate. To identify which climate variables were most important in the projection of the species' responses to a doubling of CO₂, we ran sensitivity analyses in which the climate variables were held constant except for the variable of interest and visually compared climate-change maps with model predictions. To evaluate potential problems with predictions due to climate shifts outside current climate space, we examined Maxent output maps that identify areas of "clamping," where Maxent reduces projected future climate variables to their present maximum within the study area, if they are projected to exceed it. Although future climate projections (and particularly those for precipitation) contain uncertainty (Neelin et al. 2006), the CCM3-based projected increases in temperature and precipitation over

our study region are largely consistent with projections derived from ensemble means of 22 different climate models (IPCC 2007).

We tested spatial accuracy of the predictions with threshold-dependent (omission and predicted area) and threshold-independent (area under the receiver operator curve [AUC]) measures following Phillips et al. (2006). We created 10 random data partitions, with 60% of the point localities assigned for training and 40% for testing. We performed threshold-independent tests through the Accumetric Test Performance Analysis toolbox (Vida 1993). To facilitate threshold-dependent tests, we evaluated model performance at the balance threshold, which balances intrinsic (training) omission, proportional predicted area, and cumulative threshold, and, hence, minimizes over- and underprediction (Phillips 2005). For the models in which only vegetation variables and savanna localities were used, we also estimated how well the models predicted rainforest localities by calculating omission error at the balance threshold and across all thresholds (in terms of test AUC). Here we used the average predicted probability of presence within a 5-km radius to account for imprecision in georeferencing of locality and remote-sensing data. We defined the rainforest zone according to ecoregion boundaries (Fig. 1) and considered areas within that zone with predicted probabilities of occurrence above the balance threshold to constitute the invasion area; increasing probabilities of species occurrence indicated greater invasion potential.

Results

Distribution models for all species performed well. The probability of predicting a species absent when it was actually present (test omission error) was generally low at the balance threshold (Table 1). At that threshold all but one model performed significantly better than random at the 0.05 level, and all models were significant at the 0.10 level (Table 1). Across all thresholds (threshold independent), all model performances were highly significant (P < 0.005; on the basis of test AUC against random). Test AUC values were generally close to training AUC except for the climate-only simulations in which there was a tendency for the models to overfit. In general, these test results from 10 partitions indicated the models were powerful in discriminating suitable from unsuitable habitats.

For all three species, with the exception of minor incursions, models derived exclusively from climate and elevation variables did not predict invasions into the rainforest zone (Fig. 2a). This suggests that, when undisturbed, rainforest is climatically unsuitable. In the predictions for *C. maculatus* and *D. lineatus*, precipitation of the driest quarter (PDQ) was the most important variable. This variable exhibited greater values in the rainforest zone, particularly near the coast, and had a negative relationship with habitat suitability. Elevation was most important for *L. fuliginosus* (Table 2) and had a positive relationship with habitat suitability. This was due to higher temperature and lower rainfall on the Adamaoua plateau, which makes up a large portion of the savanna in Cameroon. For *C. maculatus* and *L. fuliginosus*, lower temperature diurnal range in the rainforest also limited invasion potential.

Species	Predicted area	Test omission rate	Training AUC	Test AUC
Causus maculatus				
climate only	0.567 ± 0.010	0.065 ± 0.017	0.788 ± 0.005	0.693 ± 0.010
vegetation only	0.577 ± 0.011	0.036 ± 0.011	0.803 ± 0.006	0.789 ± 0.009
climate and vegetation	0.668±0.031	0.057±0.013	0.848 ± 0.004	0.748 ± 0.006
Dromophis lineatus				
climate only	0.476 ± 0.011	0.142 ± 0.028	0.878 ± 0.005	0.776 ± 0.012
vegetation only	0.473 ± 0.012	0.020 ± 0.020	0.849 ± 0.007	0.847 ± 0.014
climate and vegetation	0.569±0.027	0.078 ± 0.020	0.896±0.007	0.826 ± 0.011
Lamprophis fuliginosus				
climate only	0.556 ± 0.015	0.044 ± 0.011	0.826 ± 0.003	0.781 ± 0.008
vegetation only	0.571 ± 0.015	0.039 ± 0.014	0.830 ± 0.003	0.802 ± 0.006
climate and vegetation	0.423 ± 0.007	0.086 ± 0.014	0.904±0.003	0.833±0.007

^{*a*} Estimates are means \pm SE from 10 random partitions of the data.

Models that included only MODIS vegetation variables and savanna localities showed extensive areas of invasion within the rainforest zone for C. maculatus and L. fuliginosus and to a much lesser extent for D. lineatus (Fig. 2). For all species tree cover was the most important variable (Table 2). Habitat suitability was negatively correlated with tree cover, and predictions of invasion in rainforest along major roadways and near urban centers were consistent with our hypothesis that human removal of rainforest vegetation facilitates invasion by creation of savanna-like microhabitats in the rainforest zone. For predicted probabilities of occurrence at known rainforest localities, omission error rates derived from the balance threshold were variable: 15%, 80%, and 50% for C. maculatus, D. lineatus, and L. fuliginosus, respectively. The relatively high omission errors for the latter two species were to some extent due to our choice of a conservative threshold, but also resulted from remote-sensing data not capturing smaller-scale deforestation that facilitates invasion. Even so, threshold-dependent (at balance threshold) and threshold-independent tests with rainforest point localities and predicted area restricted to the rainforest zone indicated that the predictions were significantly better than random for C. maculatus (both tests, P < 0.001; AUC = 0.754) and L. fuliginosus (both tests, P<0.001; AUC = 0.637). For D. lineatus, both tests were marginally non-significant (P = 0.133; AUC = 0.643), likely due to the small sample size (N=5) for test localities.

Models incorporating climate and remote-sensing (including QSCAT) variables in conjunction with all point localities predicted an invasion front extending deep into the Northwest Congolian Lowland Forest for all species (Fig. 2c). Present-day patterns of

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	Model Scenario	Climate only C. maculatus	D. lineatus	L. fuliginosus	Vegetation only	C. maculatus	D. lineatus	L. fuliginosus	Climate and	vegetation	C. maculatus	D. lineatus	L. fuliginosus	^{<i>a</i>} Boldface indicate: ^{<i>b</i>} SRTM = elevatior seasonality, BIO6 wettest quarter B	VCF = % tree cov

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species distribution within the rainforest zone were determined by a combination of vegetation variables. This is clearly visible in the pixellated distribution of highersuitability values within the rainforest (Fig. 2c), which contrasts with the smooth, clinal variation of the coarser bioclimatic variables (Fig. 2a). In addition, even though the coarser-resolution QSCAT and climate variables partially obscured the effect of finescale vegetation patterns, increased habitat suitability due to anthropogenic reduction of forest cover was still apparent along the major transportation corridor between the cities of Yaoundé and Bertoua and in an area of past rainforest clearcutting (Fig. 2c, see enlargement). Predicted distributions of C. maculatus and L. fuliginosus also extended into the Cross River Forest, and L. fuliginosus showed the greatest propensity for invading the Congolian Coastal Forest (Fig. 2c). For all species, tree cover contributed most to the overall model (Table 2). For C. maculatus and L. fuliginosus, QSD explained proportions of the model comparable to tree cover (Table 2). Differential levels of invasion potential among the three species supported the hypothesis that nocturnal species exhibit greater invasion potential: extent of invaded areas in the rainforest zone (on the basis of balance threshold) were $133,267 \text{ km}^2$, $103,467 \text{ km}^2$, and $148,319 \text{ km}^2$ for C. maculatus (nocturnal), D. lineatus (diurnal), and L. fuliginosus (nocturnal), respectively. The importance of rainforest alteration was not confounded by climate because within the rainforest zone, correlations between climate and vegetation (including QSCAT) variables were extremely weak (mean absolute value of bivariate correlations at 1000 random points within rainforest, $r = 0.148 \pm 0.018$ SE.

The reduction of invasion potential within the Congolian Coastal Forest relative to models built only with vegetation variables was due to climatic constraints. Percent contributions of variables to models, combined with sensitivity analyses that excluded bioclimatic and QSCAT variables in a stepwise fashion, demonstrated that for *C*. *maculatus* this pattern was driven by greater PDQ, for *D. lineatus* by larger values for all precipitation variables combined, and for *L. fuliginosus* predominantly by lower temperature diurnal range and by higher PDQ. These comprehensive models suggest that human alteration of vegetation facilitates savanna snake invasions, but that climatic constraints may limit these invasions in portions of the rainforest biome.

Future climate change was predicted to cause a reduction in habitat suitability in the rainforest zone for all species largely because of the projected trend toward wetter conditions (Fig. 3). These changes were most dramatic for *C. maculatus* and *L. fuliginosus*, rendering substantial areas of the rainforest-savanna ecotone and lower savanna climatically unsuitable. Increase in PDQ was an important driver of reductions of habitat suitability for these two species. Decreasing temperature diurnal range and a more spatially variable pattern of change in precipitation of the wettest quarter also contributed to suitability reductions for *C. maculatus* and *L. fuliginosus*, respectively. The smaller reductions in suitability predicted for *D. lineatus* were not clearly explained by any single variable, which suggests small changes in several variables were the cause. We did not observe any clamping effects, which indicates projection of future climate outside of the current climate space did not occur.



Figure 3. Changes in predicted probability of occurrence of (a-c) three snake species as a result of climate change predicted by the CCM3 general circulation model under a doubling of CO₂. Future distributions were generated through projecting the present-day climate-species relationship derived from savanna points and climate layers only onto the future climate. Map values equal the difference between the future and present predicted distributions (see Fig. 2a), with negative and positive values indicating areas of decreasing and increasing probability of occurrence, respectively.

Discussion

Deforestation and the effects of climate change are dramatically transforming ecosystems worldwide. The rapidity and pervasiveness of these impacts requires a better understanding of how they operate in concert, yet few researchers have taken such an approach. To our knowledge ours is the first study that attempts to simultaneously examine the effects of habitat alteration and future climate change for equatorial African reptiles. Our results show that human disturbance is creating favorable condition for the invasion of three snakes into the rainforest zone. In contrast, predictions of future climate change indicate rainfall will increase in the rainforest zone, which will likely inhibit invasions. Thus, the effects of deforestation and climate change are complex and potentially opposing forces on invasion dynamics.

Our models provide evidence that human alterations of vegetation are leading to invasions within the rainforest. First, models with savanna occurrences and climate variables supported our hypothesis that the rainforest zone is climatically unsuitable. This suggests that for invasion to occur in the rainforest, a release from climatic constraints is necessary. Second, models with savanna occurrences and vegetation variables supported our hypothesis that human alteration of rainforest vegetation facilitates invasions. These models predicted invasions within the rainforest and identified areas of anthropogenic habitat modification (along roadways and in logged and urbanized areas) as suitable habitat. Third, models including all environmental variables predicted extensive invasion areas within the rainforest zone. Vegetation variables were the drivers of invasions, and models predicted fine-scale, pixellated invasion patterns in rainforest despite the

smoothing effects of climate and QSCAT variables. This was due to the dominant influence of satellite-based fine-scale data on tree cover, which indicated snake species were more likely to occur in areas with limited canopy cover resulting from land conversion.

Habitat suitability does not guarantee occupancy because invasions also depend on dispersal success (Peterson 2003). Nevertheless, given the extensive amount of disturbance that has already occurred and the large and increasing road network (Nolte *et al.* 2001), dispersal is unlikely to be a limiting factor. In light of this and the potential sources of under- and overprediction mentioned above, we believe our models represent reasonable approximations of where species will invade. Human-mediated increases in habitat suitability may be due to increased insolation in more open habitats created by rainforest removal. This would lead to thermal conditions at potential nest sites being more similar to those in savanna. For nocturnal reptiles warmer nest sites can be a limiting resource in areas where dense canopy cover reduces nest-site temperatures (Pringle *et al.* 2003). Thus, although we found greater invasion potential for nocturnal than diurnal species (consistent with our hypothesis that nocturnal species have lower thermal sensitivity), this pattern may actually be due to land-use-facilitated increases in the availability of thermally suitable daytime refugia.

Because we constructed models that both isolated and combined climate and vegetation variables, we were able to determine how unsuitable climate might partially constrain invasions driven by anthropogenic alteration of rainforest. Within the Congolian Coastal Forest, prevailing wet conditions limited invasions relative to those

predicted by models that included only vegetation variables. The greater predicted invasion areas in the Northwest Congolian Lowland Forest relative to the Congolian Coastal Forest suggest that, as one moves away from the coast, climates become more suitable and human effects on vegetation overcome the constraints imposed by the rainforest macroclimate.

Invasions may also be constrained by seasonal differences between the rainforest and savanna. In Cameroon rainforest is characterized by a short rainy season followed by a long one; the single and shorter savanna wet season lags months behind the onset of the wet season in rainforest. Oviparous tropical reptiles time reproduction to coincide with optimal nest moisture conditions and to avoid lethal waterlogging of eggs (Brown and Shine 2006). Timing may also be linked to prey availability because precipitationregulated abundance of prey influences demographic, reproductive, and ecological characteristics of tropical snakes (Madsen and Shine 2000; Brown *et al.* 2002; Madsen *et al.* 2006). In particular, oviposition in *C. maculatus* is timed to coincide with reproduction of amphibian prey at the beginning of the wet season (Luiselli *et al.* 2004). If oviposition in the savanna corresponds to a period of relatively wetter conditions in the rainforest, invaders may deposit eggs when moisture levels are too high and produce offspring after the peak in prey availability has passed. Such maladaptive timing might substantially reduce reproductive success (Parmesan 2006).

For each of the three species, projected changes in future climates led to decreased habitat suitability in the rainforest zone due to a trend toward increased precipitation and tended to counteracted invasions caused by deforestation. Although

savanna ectotherms would be expected to be able to take advantage of future temperature increases in the rainforest, our results suggest the benefits from warming may be outweighed by the negative consequences of increased precipitation. Analogous to present-day exclusions from coastal areas noted earlier, the wetter conditions in the future may accentuate the temporal mismatch between important life-history traits and features of the biotic environment and may limit the availability of dry sites for oviposition. Conclusions with respect to future climate projections should, nevertheless, be regarded as tentative because there remains substantial uncertainty concerning patterns of future precipitation change (IPCC 2007). Furthermore, because future projections did not include vegetation variables and because human removal of rainforest is ongoing, whether an actual reduction in invasion potential due to climate change would occur is unclear.

Conclusions

As rainforest habitats of Central Africa continue to be degraded, snake species currently found in disturbed and savanna habitats are predicted to invade the rainforest zone. Because invading snake species may have severe impacts on native vertebrate populations (Savidge 1987; Case and Bolger 1991), conservation strategies for equatorial Africa should take into account the possibility of these invasions. Our models predict that future climate change will counteract the effects of rainforest removal and reduce invasion potential, due to projected increases in precipitation in the rainforest. Thus, climate change may actually mediate the effects of human removal of rainforest, which

illustrates how anthropogenic habitat alteration and climate change are likely to have complex impacts on biodiversity. Nevertheless, because there is substantial uncertainty associated with projecting precipitation trends in tropical Africa, better predictions of future precipitation and analyses that incorporate such uncertainty are crucial. Additional studies are also needed to identify the outcome of interactions between bioclimatic variables that drive broad-scale species distributions, microhabitat variation due to human alterations of rainforest, and population-level processes that determine persistence of species within the invaded range.

References

- Achard F, Eva HD, Stibig H-J et al. (2002) Determination of deforestation rates of the world's humid tropical forests. *Science*, **297**, 999–1002.
- Akani GC, Barieenee IF, Capizzi D, Luiselli L (1999) Snake communities of moist rainforest and derived savanna sites of Nigeria: biodiversity patterns and conservation priorities. *Biodiversity and Conservation*, 8, 629–642.
- Brown GP, Shine R (2006) Why do most tropical animals reproduce seasonally? Testing hypotheses on an Australian snake. *Ecology*, **87**, 133–143.
- Brown GP, Shine R, Madsen T (2002) Responses of three sympatric snake species to tropical seasonality in northern Australia. *Journal of Tropical Ecology*, **18**, 549–568.

- Buermann W, Saatchi S, Graham CH et al. (2008) Predicting species distributions across the Amazonian and Andean regions using remote sensing data. Journal of Biogeography, 35, 1160–1176.
- Camargo JLC, Kapos V (1995) Complex edge effects on soil moisture and microclimate in central Amazonian forest. *Journal of Tropical Ecology*, **11**, 205–221.
- Case TJ, Bolger DT (1991) The role of introduced species in shaping the distribution and abundance of island reptiles. *Evolutionary Ecology*, **5**, 272–290.
- Central African Regional Program for the Environment (CARPE) (1997) Regional Ecological Regions. University of Maryland, College Park, Maryland. Available from http://carpe.umd.edu/ (accessed February 2006).

Chippaux JP (1999) Les Serpents d'Afrique Occidentale et Centrale. IRD Editions, Paris.

- Crozier L, Dwyer G (2006) Combining population-dynamic and ecophysiological models to predict climate-induced range shifts. *American Naturalist*, **167**, 853–866.
- Didham RK, Lawton JH (1999) Edge structure determines the magnitude of changes in microclimate and vegetation structure in tropical forest fragments. *Biotropica*, **31**, 17–30.

- Elith J, Graham CH, Anderson RP *et al.* (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129–151.
- Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics, 34, 487–515.
- Foley JA, DeFries R, Asner GP et al. (2005) Global consequences of land use. Science, 309, 570–574.
- Govindasamy B, Duffy PB, Coquard J (2003) High-resolution simulations of global climate, part 2: effects of increased greenhouse gases. *Climate Dynamics*, 21, 391–404.
- Hansen MC, DeFries RS, Townshend JRG, Sohlberg RA, Dimiceli C, Carroll M (2002)
 Towards an operational MODIS continuous field of percent tree cover algorithm:
 examples using AVHRR and MODIS data. *Remote Sensing of Environment*, 83, 303–319.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978.

 Hoffmeister TS, Vet LEM, Biere A, Holsinger K, Filser J (2005) Ecological and evolutionary consequences of biological invasion and habitat fragmentation. *Ecosystems*, 8, 657–667.

Intergovernmental Panel on Climate Change (IPCC) (2007) Climate Change 2007: Climate Change Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change: Summary for Policymakers. IPCC, Geneva.

- Kearney M, Porter WP (2004) Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology*, **85**, 3119–3131.
- Laurance WF, Lovejoy TE, Vasconcelos HL et al. (2002) Ecosystem decay of Amazonian forest fragments: a 22-year investigation. Conservation Biology, 16, 605– 618.
- Letouzey R (1968) Etude Phytogéographique du Cameroun Encyclopédie Biologique LXIX. Lechevalier, Paris.
- Long DG, Drinkwater MR, Holt B, Saatchi S, Bertoia C (2001) Global ice and land climate studies using scatterometer image data. *EOS Transactions American Geophysical Union*, **82**, 503.

- Luiselli L (2006) Resource partitioning and interspecific competition in snakes: the search for general geographic and guild patterns. *Oikos*, **114**, 193–211.
- Luiselli L, Akani GC, Rugiero L, Politano E (2005) Relationships between body size, population abundance and niche characteristics in the communities of snakes from three habitats in southern Nigeria. *Journal of Zoology (London)*, **265**, 207–213.
- Luiselli L, Akani GC, Angelici FM, Politano E, Ude L, Wariboko SM (2004) Aspects of the ecology of a population of *Causus maculatus* from southern Nigeria. *Amphibia-Reptilia*, **25**, 99–104.
- Madsen T, Shine R (2000) Rain, fish and snakes: climatically driven population dynamics of Arafura filesnakes in tropical Australia. *Oecologia*, **124**, 208–215.
- Madsen T, Ujvari B, Shine R, Olsson M (2006) Rain, rats, and pythons: climate-driven population dynamics of predators and prey in tropical Australia. *Austral Ecology*, **31**, 30–37.

Menzies JI (1966) The snakes of Sierra Leone. Copeia, 2, 169–179.

- Myneni RB, Hoffman S, Knyazikhin Y et al. 2002. Global products of vegetation leaf area and fraction absorbed PAR from year one of MODIS data. *Remote Sensing of Environment*, **83**, 214–231.
- Neelin JD, Münnich M, Su H, Meyerson JE, Holloway CE (2006) Tropical drying trends in global warming models and observations. *Proceedings of the National Academy of Sciences, USA*, **103**, 6110–6115.
- Nolte C, Kotto-Same J, Moukam A et al. (2001). Land-use Characterization and Estimation of Carbon Stocks in the Alternatives to Slash-and-Burn Benchmark Area in Cameroon. Resource and Crop Management Resource Monograph 28. International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Parmesan C (2006) Ecological and evolutionary responses to climate change. Annual Review of Ecology, Evolution, and Systematics, 37, 637–669.
- Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the distribution of species: are bioclimatic envelope models useful? *Global Ecology and Biogeography*, 12, 361–371.
- Peterson AT (2003) Predicting the geography of species' invasions via ecological niche modeling. *Quarterly Review of Biology*, **78**, 419–433.

- Phillips S, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.
- Phillips S (2005) A Brief Tutorial on Maxent. AT&T Research, Florham Park, New Jersey. Available from http://www.cs.princeton.edu/~schapire/maxent/tutorial/tutorial.doc (accessed June 2006).
- Pringle RM, Webb JK, Shine R (2003) Canopy structure, microclimate, and habitat selection by a nocturnal snake, *Hoplocephalus bungaroides*. *Ecology*, **84**, 2668–2679.
- Row JR, Blouin-Demers G (2006) Thermal quality influences effectiveness of thermal regulation, habitat use, and behaviour in milk snakes. *Oecologia*, **148**, 1–11.
- Savidge JA (1987) Extinction of an island forest avifauna by an introduced snake. Ecology, **68**, 660–668.
- Sprawls S, Howell K, Drewes R, Ashe J (2002) A Field Guide to the Reptiles of East Africa. Academic Press, San Diego, California.
- Stevenson RD, Peterson CR, Tsuji JS (1985) The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiological Zoology*, **58**, 46–57.

- Thiollay JM (1986) Alteration of raptor communities along a succession from primary rainforest to secondary rainforest habitats on two continents. *Ibis*, **128**, 172.
- Vida S (1993) A computer program for non-parametric receiver operating characteristic analysis. *Computer Methods and Programs in Biomedicine*, **40**, 95–101.
- Villiers A (1975) Les Serpents de l'ouest Africain. Initiations et Etudes Africaines. Number II. 3rd edition. University of Dakar, Dakar, Senegal.
- Vitousek PM, Harold AM, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494–499.
- Vitt LJ, Avila-Pires TCS, Caldwell JP, Oliveira VRL (1998) The impact of individual tree harvesting on thermal environments of lizards in Amazonian rain forest. *Conservation Biology*, **12**, 654–664.
- Wright SJ (2005) Tropical forests in a changing environment. *Trends in Ecology and Evolution*, **20**, 553–560.

Human Impacts Flatten Rainforest-Ecotone Gradient and Reduce Adaptive Diversity

Introduction

Recent empirical (Smith *et al.* 1997b; Schneider *et al.* 1999; Ogden and Thorpe 2002; Slabbekoorn and Smith 2002; Seehausen *et al.* 2008) and theoretical (Endler 1977; Gavrilets 2000; Doebeli and Dieckmann 2003) research suggests divergent selection along ecological gradients is an important evolutionary force indiversification and speciation. Fundamental to these assertions is that the strength of natural selection leading to reproductive divergence and speciation is related to the steepness of the environmental gradient (Endler 1977; Doebeli and Dieckmann 2003). Generally, steeper ecological gradients should translate into stronger differential selection that will overcome the homogenizing effects of gene flow and more likely lead to diversification and speciation (Endler 1977; Räsänen and Hendry 2008) (see *SI Text*). In contrast, the flattening of ecological gradients, such as that caused by human landscape modification, may weaken the strength of divergent selection. This may tip the balance in favor of the homogenizing effects of gene flow, undermining the potential for differentiation (Räsänen and Hendry 2008).

In equatorial Africa, the gradient between rainforest and the forest-savanna transition zone or ecotone is believed to be important for the generation of rainforest biodiversity (Smith *et al.* 1997b; Slabbekoorn and Smith 2002; Smith *et al.* 2005b). As a result, human alterations of this gradient may have significant evolutionary

consequences. African rainforests are under substantial human pressure (Achard et al. 2002), and deforestation resulting from logging and road construction is predicted to increase (Laporte et al. 2007; Buys et al. 2006). In the vast ecotone between rainforest and savanna, comprised of mosaics of gallery forests and wooded savannas, the abundance of woody vegetation is principally determined by anthropogenic disturbance (Sankaran et al. 2005). However, because the magnitude of temporal change in woody vegetation within the ecotone is much smaller relative to deforestation in rainforest, we focus on how rainforest loss influences the slope of the rainforest-ecotone gradient. We use newly available data on tree cover, derived from measurements from the Moderate Resolution Imaging Spectrometer (MODIS) sensors onboard NASA's Terra and Aqua satellites, to quantify the steepness of the gradient across West and Central Africa. We also use a time series of tree cover data based on measurements from the Advanced Very High Resolution Radiometer (AVHRR) sensor onboard NOAA's satellite fleet to examine recent trends that may be indicative of ongoing deforestation. Greater deforestation in West Africa relative to Central Africa provides a framework for assessing the evolutionary implications of changes to the steepness of the rainforestecotone gradient for species distributed in both regions. To examine the effects of gradient flattening, we contrast patterns of morphological divergence in the rainforest passerine the little greenbul (A. virens) which was previously shown to show morphological divergence along the rainforest-ecotone gradient in Central Africa (Smith et al. 1997b).

Materials and Methods

Field sampling

Field sampling was conducted at 17 sites in West Africa (Ivory Coast) and Central Africa (Equatorial Guinea and Cameroon) between 1993 and 2001 (Table S1, Fig. 1B). Vegetation at forest sites includes primary and secondary lowland rainforest (Letouzey 1968; Louette 1981; Smith *et al.* 1997b; Smith *et al.* 2005a). Mosaics of savanna, forest patches, and gallery forest are classified as ecotone (Letouzey 1968; Louette 1981; Longman and Jenik 1992; Smith *et al.* 1997b; Smith *et al.* 2005a). Montane sites are described elsewhere (Smith and McNiven 1993; Smith *et al.* 2000). Mist netting protocols, morphological measurements, and DNA sample storage follow Smith *et al.* (2005a). All morphological measurements were taken by TBS. We restricted our analyses to adult males (N=126) because sample sizes were larger. Juveniles were distinguished from females using a PCR protocol that identifies a gene on the W chromosome (Ellegren 1996).

AVHRR-based NDVI trend analysis for detecting recent deforestation

To illustrate large-scale patterns of deforestation, we used an 18-year AVHRR record from the Pathfinder program (1982 – 2000) recorded by NOAA/NASA satellites (Agbu and James 1994; Smith et al. 1997a) at 8 km resolution. Areas where tree cover decreased greater than 10% during this time period, computed from the AVHRR time series by DeFries *et al.* (1995, 1999, 2000, 2002), were overlaid onto the MODIS tree cover map.



Figure 1. Distribution of biomes and sampling sites (circles) for *A. virens*. A. Land cover map based upon MODIS IGBP classification at 1km spatial resolution for 2000 (Friedl *et al.* 2002). This classification includes information on vegetation cover and seasonality, and defines areas that, despite the extent of current deforestation, encompass suitable ecological conditions for rainforest. Areas classified as forest are broadly consistent with White's reconstruction of historical rainforest distribution (White 1983). B. Present rainforest based on MODIS percent tree cover from 2001 (Hansen *et al.* 2002) and study

Figure 1 (continued)

sites in West and Central Africa (see Table S1 for sampling information). Sampling in West Africa coincides with a region where rainforest should occur, but where there have been large losses of tree cover, leading to convergence in habitat structure between the rainforest and ecotone zones. Red pixels indicate areas of recent deforestation (1982 – 2000), based upon tree cover data from measurements by the AVHRR satellite sensor (DeFries *et al.* 2002). These trends indicate that deforestation and gradient flattening in the recent past were greater in West than in Central Africa. C. Projection of the association between PC1 of morphology and tree cover, estimated with least-squares linear regression, shows that deforestation in West Africa has led to the flattening of the morphological cline along the rainforest-ecotone gradient. In contrast, Central Africa, which has experience much less deforestation, shows no such pattern.

Tree cover gradient analysis for West and Central Africa

To determine whether the slope of the gradient in tree cover was different between Ivory Coast and Cameroon/Equatorial Guinea (where we conducted field studies on A. virens), we reaggregated the MODIS tree cover map to 5-km resolution. For each region, we then calculated mean tree cover for each latitudinal row with > 50 grid cells. Because the location of the rainforest-ecotone boundary differs in latitude between West and Central Africa, to make plots comparable we shifted the plot for West Africa by -2.5°. We determined the relative location of this boundary from the IGBP landcover map (see Fig. 1A). We tested the hypothesis that the slope of the rainforest-savanna tree cover gradient is shallower in West than in Central Africa with ANCOVA, with latitude and region as factors, and a latitude * region interaction term. In doing so, we truncated the regional data sets so as to start at the latitude at which the maximum mean tree cover was located. This effectively excludes areas outside the relevant latitudinal range in Central Africa, and those areas containing natural inland savannas in Gabon. The longitudinal range for latitudinal averaging of tree cover was $\approx 7.53^{\circ}W - 2.94^{\circ}W$ for West Africa (Ivory Coast), and $\approx 8.60^{\circ}\text{E} - 16.24^{\circ}\text{E}$ for Central Africa (Cameroon, Equatorial Guinea, and Gabon).

Analysis of contemporary morphological variation

For live-captured birds, morphological variables included principal component 1 (size axis), principle component 2 (shape axis), and log-transformed tarsus length, wing length, tail length, and upper mandible length. Before performing principle components analyses (PCA) across geographic regions, we verified that their respective covariance matrices

were either equal or proportional (Flury 1988), using the program CPC, available at <u>http://www.uoregon.edu/~pphil/programs/cpc/cpc.htm</u>. We also evaluated size-corrected traits, calculated as residuals from linear regressions of log-transformed traits on log-transformed tarsus length. As feeding performance is more a function of absolute bill morphology and food resources, we did not perform body size corrections on upper mandible length.

To establish the relationship between morphology and tree cover, we performed univariate least-square weighted regressions of site means for each PC or log-transformed trait on percent tree cover, with sites weighted by sample size. We include montane and island sites in this analysis in order to take maximal advantage of sampling, and because we expected the relationship between morphology and tree cover to be general across habitats. Percent tree cover was collected from a 2001 tree cover map available at 500m resolution, constructed from measurements made by the MODIS satellite (Hansen et al. 2002). To account for georeferencing error, we used the average percent tree cover from a 2.5 x 2.5 km pixel centered on each study site. These tests are already conservative, because of georeferencing error, and because climate variables that may influence morphology are not correlated with the large amount of variation in tree cover within the rainforest zone due to human activity. As a result, a climate-morphology relationship would reduce our ability to detect a similar relationship with tree cover. Thus, we did not apply a correction factor to control for the familywise error rate arising from multiple individual tests. However, we point out that applying such a correction would only influence the significance of the regression of size-corrected wing length on tree cover.

The normality of site means used in the regressions of each morphological trait were confirmed with Shapiro-Wilk W tests (all P>0.05). For each morphological trait that was significantly associated with tree cover, we generated a predictive map by inputting the tree cover map into the corresponding regression equation, using the Spatial Analyst Raster Calculator in ArcGIS 9 (ESRI, Inc. 2006). These projections were then aggregated to 5-km resolution, and back-transformed for traits that were log-transformed for the regression analyses.

To determine whether loss of morphological differentiation between rainforest and ecotone habitats was greater in West than in Central Africa, we analyzed variation in PC axes and log-transformed morphological traits between rainforest and ecotone. We restricted our analyses to lowland rainforest and ecotone sites. Because tarsus length was not normally distributed within these habitats even after log-transformation, and to accommodate for small sample sizes in some comparisons, we evaluated morphological divergence with Wilcoxon rank sum tests (2-tailed, α =0.05). We excluded PC2 and upper mandible length from these and subsequent analyses, because these traits are not significantly associated with percent tree cover with which we define the rainforestecotone gradient (Table S2). We applied Dunn-Sidak correction (Sokal and Rohlf 1995) for multiple forest-ecotone comparisons, within regions, for the six traits showing a significant association with tree cover ($\alpha = 0.05$; $\alpha' = 1 - (1 - \alpha)1/6 = 0.0085$). However, corrections were found not to influence results, forest-ecotone comparisons in Central Africa remained highly significant, and those for West Africa not significant (P>0.05) even when not applying Dunn-Sidak correction.

Because sampling in West Africa was more limited relative to Central Africa, we tested whether the lack of morphological divergence between habitats in West Africa was due to chance, because of sampling of unrepresentative individuals. For each morphological trait we found significant morphological divergence in Central but not West Africa, we bootstrapped without replacement 1,000 samples from the Central Africa data equal in size to the West Africa samples. We then calculated the probability of observing this pattern by chance as the proportion of replicates where the absolute morphological divergence between rainforest and ecotone was less than or equal to that observed in West Africa. We also tested whether the lack of morphological divergence in West Africa was due to chance sampling of atypical populations (see SI Text). We confirmed that the lack of morphological divergence between rainforest and ecotone habitats in West Africa was not due to higher levels of between-habitat gene flow than in Central Africa, by calculating mean between-habitat F_{ST} from matrices provided in Smith *et al.* (2005a).

Analysis of historical morphological variation

To confirm that regional differences in rainforest-ecotone morphological divergence were due to greater deforestation in West Africa, and not to a persistent historical pattern, we collected morphological data from historical specimens of *A. virens* deposited at the Natural History Museum at Tring, United Kingdom (Table S3). As with live-captured birds, TBS took all measurements. In order to obtain adequate sample sizes while excluding individuals whose collection date may have post-dated extensive deforestation,

we included only individuals collected before 1935. We also excluded individuals for which locality information was not available or did not permit an assignment to either rainforest or ecotone habitat with reasonable confidence. Locality and habitat information were obtained by a combination of historical records (e.g., accounts of particular collectors' expeditions such as those published in *Ibis*), georeferencing using online gazetteers and Google Earth (Google, Inc. 2007), and contemporary tree cover from MODIS. Localities falling within areas of high contemporary tree cover were presumed to have historically been covered by rainforest. We excluded specimens with insufficient information to make a habitat assignment with reasonable confidence. Although sex information was obtained for most individuals, museum tags for eleven older historical specimens did not contain sex information, and lack of any distinctive plumage or other variation prevented us from classifying the sex of these individuals. We chose to include these individuals because doing so did not statistically influence our results except in the case of tarsus length, and this trait is not significantly sexually dimorphic in live-caught birds from Central African rainforest where we have large sample sizes and sufficient statistical power (Wilcoxon rank sum test on log-transformed tarsus length, male N=46, female N=40, $\chi^2=0.0054$, P=0.94). Dunn-Sidak corrections for multiple comparisons within time periods (α '=0.013) did not influence any comparison.

Very few individuals from the museum collection that met our criteria were from ecotone habitats. Most of the historical sampling took place near to the coast where there is predominantly forest. We recomputed the PCA for contemporary samples excluding individuals from ecotone, montane, and island habitats, and similarly performed a

separate PCA for the historical samples. Unlike the analyses of contemporary rainforestecotone differentiation, we did not include size-corrected wing and tail, because for museum specimens regressions of these traits on tarsus length were not statistically significant. Significance of differences between regional rainforest populations were computed with Wilcoxon rank sum tests as noted above. All statistical analyses were performed using JMP 7.0 (SAS Institute, Inc. 2007), with the exception of bootstrapping, which was implemented with a Microsoft Excel spreadsheet macro.

Results

Deforestation Impacts on Rainforest-Ecotone Gradient

The spatial distribution of MODIS tree cover data for 2001(Hansen *et al.* 2002) for regions where rainforest occurred historically indicates that West Africa has lost much more of its forest than Central Africa (Fig. 1) (White 1983; Friedl *et al.* 2002). The extent of human impacts on forest cover have been so extensive that many regions of West Africa today have tree cover comparable to, and in some cases, lower than ecotone habitats (Fig. 1B). In the regions where we collected morphological data, this spatial pattern in forest cover results in a significantly shallower rainforest-ecotone gradient in Ivory Coast relative to Cameroon and Equatorial Guinea (ANCOVA, Region * Latitude effect on percent tree cover *F*=345.2, *P*<0.0001) (Fig. 2). The magnitude of differences in levels of rainforest tree cover and gradient slope between the two regions are the net result of human rainforest removal on a multi-decadal timescale. Coarse-scale AVHRR-



Figure 2. Depiction of rainforest-ecotone gradients using MODIS percentage tree cover plotted against latitude for West and Central Africa, showing greater flattening of gradient in West than Central Africa. For each region, mean tree cover was calculated for each 5km latitudinal band and plotted along the south-north direction. For better comparison, the rainforest-ecotone gradient for West Africa is shifted southward by 2.5° to account for the shift in latitudinal position of the rainforest-ecotone boundary between West and Central Africa. The dashed line indicates the approximate position of the rainforest-ecotone boundary, and the solid black lines indicate least-square regression lines fit to the regional trends in tree cover-by-latitude trends. based trends in tree cover from 1982 to 2000 provide a temporal perspective, and confirm larger losses of tree cover in West Africa (Fig. 1B) (DeFries *et al.* 2002).

Contemporary Morphological Diversity.

Previous research on African passerine birds suggests that natural selection across the rainforest-ecotone gradient has led to divergence in morphological and behavioral traits important in fitness and potentially speciation (Smith *et al.* 1997b; Smith *et al.* 2005b; Slabbekoorn and Smith 2002). To examine how the flattening of the forest-ecotone gradient may have impacted phenotypic diversity, we contrast morphological divergence between West and Central Africa in the common, sedentary bird species *A. virens* found in both rainforest and gallery forests of the ecotone (Smith *et al.* 1997b) (Table S1). This species exhibits divergence in both morphology (Smith *et al.* 1997b) and song (Slabbekoorn and Smith 2002) across the gradient, and has been shown to be sensitive to habitat disturbance, exhibiting morphological, song and plumage differences as a function of tree cover (Smith *et al.* 2008).

To verify that tree cover is an important determinant of morphology, we perform weighted least-square regressions of the first two principle components derived from morphological traits typically important to fitness (Grant 1986), as well as regressions of individual traits, on percent tree cover data from 2001. Morphology is significantly associated with percent tree cover, including PC1 (overall size), tarsus, and both absolute and size-corrected wing and tail length (Table S2). These significant associations are all
in the direction expected from observed patterns of rainforest-ecotone divergence in Central Africa (Fig. 3), with birds becoming more ecotone-like as forest cover decreases.

Projections based on regressions of morphology traits on tree cover show that deforestation has led to a severe flattening of the morphological cline along the rainforest-ecotone gradient in West Africa, with the exception of Sierra Leone, where considerable forest remains (Fig. 1C, S1). In contrast, in Central Africa clines remain relatively intact, the rainforest-ecotone gradient is steeper and morphology is significantly divergent along the gradient in PC1, tarsus length, and both absolute and size-corrected wing and tail length (Fig. 3). We can detect no divergence in West Africa. Because sampling in West Africa is more limited than in Central Africa, we test whether the lack of morphological divergence between habitats in West Africa might be due to the chance effects of sampling. A bootstrap resampling approach shows that the lack of morphological differentiation in West Africa relative to Central Africa is extremely unlikely to have occurred due to chance. (PC1, P<0.001; absolute and size-corrected wing length, P<0.001; tail length, P<0.0001; size-corrected tail length, P=0.013; tarsus length, P<0.001) (see SI text). Inter-regional differences in gene flow between rainforest and ecotone populations cannot explain the observed lack of morphological divergence in West Africa, as pairwise F_{ST} between habitats is actually higher in West Africa than in Central Africa (West Africa, $F_{ST} = 0.058 \pm 0.005$ SE, N=2; Central Africa, $F_{ST} =$ 0.013 ± 0.002 SE, N=20) Thus, our results suggest the loss of morphological divergence between habitats depends upon the extent of tree cover along the gradient, and that the



Figure 3. Differences in morphological characters between rainforest (blue) and ecotone (red) habitats for adult male A. virens in West and Central Africa. Morphological differences between forest and ecotone populations are smaller in West Africa than in Central Africa consistent with a flatter envrionmental gradient. Error bars indicate ± 1 SE. about the mean; sample sizes are adjacent to means. PCs are based upon tarsus, wing, tail, and upper mandible length. Size-corrected traits are computed from regressions of log-transformed measurements on log-transformed tarsus length. Measurements of individual traits are in millimeters. Significant Wilcoxon rank sum tests comparing rainforest and ecotone habitats within domains are indicated by ******* $P \leq 0.001$. Sample sizes and results of regressions of morphological traits on tree cover are provided in Table S2.

loss of morphological diversity in traits important in fitness (Grant 1986) has been greater in West Africa where deforestation has been greater.

Pre-deforestation Morphological Diversity

We provide further support for a loss of variation with an analysis of museum specimens of A. virens from the late 1800's and early 1900's, before the mechanization and subsequent acceleration of large-scale deforestation following World War II (Williams 2003). Ecotone specimens from this period were too scarce to analyze, however, consistent with the high levels of deforestation in West Africa, rainforest populations from West and Central Africa are morphologically more divergent today than they were historically (Fig. 4). The only character that did not show this pattern was tarsus length, but here the historical direction of divergence between regions is opposite to the contemporary one (Fig. 4). In other words, the relative increase in tarsus length in West Africa expected as habitat becomes more open after deforestation, has reversed the historic pattern. A shift in West Africa towards more ecotone-like morphology is likely responsible for the increase in divergence between regions, and occurs in all traits in which we detected significant rainforest-ecotone divergence in Central Africa. Furthermore, the shift is consistent with the direction of morphological change in A. virens when humans clear rainforest (Smith et al. 2008). Thus, temporal changes in morphology over the last 100 years in West Africa appear to be tracking the conversion of rainforest to more open, ecotone-like habitats.



Figure 4. Comparison of historical and contemporary morphological differences between West (blue) and Central (green) Africa rainforest populations of *A. virens*. Regional populations are more morphologically divergent now than historically, due to West African populations becoming more ecotone-like as a result of deforestation. Historical ecotone specimens were too scarce for comparisons. Error bars indicate ± 1 SE about the mean; sample sizes are adjacent to means. PC1 is based upon tarsus, wing, tail, and upper mandible length, and to facilitate visualization are normalized, within time period, by the largest individual value so that PCs scale from -1 to +1. Measurements of individual traits are in millimeters. Significant Wilcoxon rank sums tests comparing regions within each time interval are indicated by ** $P \leq 0.01$, and *** $P \leq 0.001$.

Discussion

The flattening of the rainforest-ecotone gradient caused by human destruction of rainforest appears to be erasing a signature of divergence-with-gene-flow (Rice and Hostert 1993), a pattern previously described for *A. virens*, and thought to be important in speciation (Smith *et al.* 1997b; Slabbekoorn and Smith 2002; Smith *et al.* 2005b; Nosil 2008). Should land use practices in Central Africa continue on their present trajectory (Laporte *et al.* 2007), flattening of the rainforest-ecotone gradient is likely to lead to losses of phenotypic diversity akin to that observed in West Africa, a prediction supported by the inverse relationship between morphological traits and tree cover. Because growing evidence suggests directional selection is the primary driver of phenotypic diversification (Rieseberg *et al.* 2002), and ecological divergence may be tied to reproductive isolation for incipient or recently speciated species (Kitano *et al.* 2008). Furthermore, it may reduce adaptive variation, and potentially lower rates of diversification for the many taxa whose distributions span the rainforest-ecotone gradient.

While there has been much focus on population declines and extinctions of rainforest species as a result of deforestation (Pimm and Raven 2000), our results suggest that the threats to ecological gradients and the important evolutionary processes that take place along them in promoting diversity need greater attention by conservation decision makers (Cowling and Pressey 2001; Smith and Grether 2008). This is a need made more urgent given that Africa is predicted to be the continent most severely affected by climate

warming (IPCC 2007), where any loss of diversity may limit the ability of populations to adapt to changing environmental conditions (Parmesan 2006).

We provide the first evidence linking the loss of an evolutionarily significant environmental gradient with a decline in adaptive diversity. However, the ecological signatures associated with deforestation suggest a larger global-scale phenomenon. In West Africa, deforestation and gradient flattening have been associated with the loss of rainforest species and incursions by savanna-adapted species into previously forested regions (Kofron and Chapman 1995; Thiollay 1998). Such incursions arising from deforestation have also recently been documented in Cameroon (Lawton et al. 1998; Freedman et al. 2009) where rainforest-ecotone divergence in A. virens currently persists, suggesting an early signal of gradient deterioration. Similar changes associated with rainforest deforestation have also been documented in Southeast Asia (Sodhi et al. 2004) and South America (Julien and Thiollay 1996; de M. Bastos et al. 2005). Within South America, the flattening of environmental gradients in the Andes appears to be particularly acute (Thiollay 1996; Brooks et al. 2002). To preserve both the pattern of biodiversity and the processes that produce and maintain it, greater attention should focus on the preservation of gradients (Smith and Grether 2008). With as much as two thirds of the world's terrestrial land area impacted by human activities (Mace et al. 2005) gradient flattening is likely common, but its corresponding impacts on adaptive diversity and implications for conservation in a changing world are not fully recognized.

Supporting Information

SI Text

Gradient slopes

In some instances, speciation may be inhibited when gradients are too steep. One simulation study (Doebeli and Dieckmann 2003) demonstrates that along very steep gradients, dissimilar phenotypes are spatially close, and thus compete strongly. This precludes evolutionary branching because by eliminating the gradient-induced frequency dependence that, in the underlying Lotka-Volterra model, drives the diversification process. Consistent with this, recent empirical research on cichlid fish found less adaptive divergence along the steepest gradients (Seehausen *et al.* 2008). However, the rainforest-ecotone gradient is a large-scale phenomenon that is distributed across over 1000 km, and for the vast majority of species it will not be perceived as a steep environmental gradient.

Population re-sampling analyses

In addition to the bootstrapping analyses (see Materials and Methods section), we also calculated the probability that morphological divergence in West Africa was less than in Central Africa due to sampling atypical populations. Specifically, we calculated rainforest-ecotone divergence between all possible sets of two rainforest and two ecotone populations from Central Africa, with weighted means for each habitat calculated using the sample sizes of the corresponding populations in West Africa. For each trait, this produced 300 replicates. For four of six traits, the probability of sampling populations lacking forest-ecotone divergence is extremely low (PC1, P<0.001; absolute wing and tail length, P<0.001; size-corrected wing length, P=0.013). For tarsus length and size corrected wing length 20% and 21% of replicates had a level of forest-ecotone divergence less than or equal to that observed in West Africa, respectively. However, for each trait, the lack of forest-ecotone divergence was driven almost exclusively by one Central African population. For tarsus length, 96% of these replicates showing low divergence involved comparisons including Betare Oya. For size-corrected tail length, 88% of these replicates involved comparisons including Meiganga. Thus, for four of six traits, the lack of morphological divergence along the gradient cannot be explained by chance sampling of populations. Because low divergence in tarsus length and sizecorrected tail length were each driven by different populations, it is unlikely that those traits would simultaneously exhibit low divergence by chance alone. Multiplying their respective probabilities of low divergence, there is only a four percent chance that those traits would simultaneously show low forest-ecotone divergence. We can therefore conclude that the effects of non-random sampling can be ruled out as the cause for the lack of morphological divergence in West Africa for five of six traits.

·····				. —	% Tree	
Site	Latitude	Longitude	Country [†]	Habitat	Cover	N
1. Paule Oula	5.826°N	7.391°W	IC	Forest	49.6	4
2. Lamto	6.216°N	5.027°W	IC	Ecotone	19.7	9
3. Marahoue	7.028°N	5.948°W	IC	Ecotone	24.9	2
4. CSRS	5.331°N	4.129°W	IC	Forest	13.7	5
5. Bioko*	3.743°N	8.721°E	EG	Forest	64.4	7
6. Elende	2.216°N	9.793°E	EG	Forest	69.1	10
7. Kribi	2.731°N	9.872°E	CAM	Forest	77.7	4
8. Sakbayeme	4.038°N	10.574°E	CAM	Forest	56.5	16
9. Nkwouak	3.870°N	13.316°E	CAM	Forest	74.0	8
10. Zobefame	2.659°N	13.397°E	CAM	Forest	79.1	3
11. Lac Lobeke	2.311°N	15.762°E	CAM	Forest	79.5	5
12. Tchabal Mbabo*	7.252°N	12.058°E	САМ	Montane	33.6	4
13. Tchabal Gandaba*	7.743°N	12.716°E	CAM	Montane	30.9	14
14. Tibati	6.504°N	12.588°E	CAM	Ecotone	28.3	8
15. Wakwa	7.271°N	13.526°E	CAM	Ecotone	18.2	5
16. Ngaoundaba	7.133°N	13.698°E	CAM	Ecotone	28.0	5
17. Meiganga	6.517°N	14.300°E	CAM	Ecotone	17.6	6
18. Betare Oya	5.563°N	14.091°E	CAM	Ecotone	28.3	13

Table S1. Coordinates, habitat classification, percent tree cover, and sample sizes for *A. virens* captured and measured by TBS in West and Central Africa.

* Sites are used in weighted regressions of morphological traits on percent tree cover, but not for comparisons of morphological divergence between rainforest and ecotone habitats between West and Central Africa.

† IC = Ivory Coast, EG = Equatorial Guinea, CAM = Cameroon.

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_Trait	r^2	_+/- *	P^{\dagger}	N (Observations) [‡]	
PC1	0.51	_	0.0009	18 (125)	
PC2	0.01		0.68	18 (125)	
Tarsus length (mm) [§]	0.39	_	0.006	18 (128)	
Wing longth (mm)	0.48		0.0015	10 (120)	
wing length (min)	(0.32)∥	(0.32) [∥] –		10 (120)	
Tail length (mm)	0.57		0.0004	18 (125)	
	(0.45)∥	-	(0.002)	10 (125)	
Upper mandible length (mm) [§]	0.04		0.41	18 (128)	

Table S2. Weighted least-squares regressions of morphological traits on percent
 tree cover.

* Indicates direction of correlation, where significant, between morphology and tree cover.

[†]Boldface indicates significance.

^c Number of populations, with parentheses indicating the total number of individuals.

[§] Regressions of log-transformed trait values on log-transformed percent tree cover.

Regressions use residuals from linear regressions of log-transformed trait values on log-transformed tarsus length, which are then regressed on percent tree cover.
 Parentheses indicate results for traits corrected for size using tarsus length.

	Year		
Catalogue #	Collected	Locality	Country
1902.7.15.5	1901	Efulen	CAM
1904.7.18.118	1904	Efulen	CAM
1905.1.24.131	1904	Dja River	CAM
1905.1.24.126	1904	Dja River	CAM
1906.12.14.93	1905	Efulen	CAM
1906.12.14.90	1905	Efulen	CAM
1906.12.14.94	1905	Efulen	CAM
1911.5.31.270	1905	Bitye	CAM
1911.5.31.271	1907	Bitye	CAM
1969.44.56	1907	Bitye	CAM
1920.6.26.406	1908	Assobam, Bumba River	CAM
1920.6.26.407	1908	Assobam, Bumba River	CAM
1924.11.2.137	1922	Bitye	CAM
1911.12.23.1632	1900	Prahsu	GC
1936.2.21.308	1900	Prahsu	GC
1911.11.18.124	1910	Sekondi	GC
1934.3.16.502	1934	Goaso, Ashanti	GC
1934.3.16.504	1934	Goaso, Ashanti	GC
1934.3.16.506	1934	Mampong, Ashanti	GC
58.1.4.109	1858	Ashanti	IC
76.5.23.606	1876	Fantee	IC
76.5.23.643	1876	Fantee	IC
76.5.23.646	1876	Fantee	IC
76.5.23.338	1876	Fantee	IC
76.5.23.645	1876	Fantee	IC
76.5.23.604	1876	Fantee	IC
95.5.1.718	1895	Fantee	IC
95.5.1.715	1895	Fantee	IC
95.5.1.714	1895	Denkera	IC
1911.11.18.126	1911	Nanna Kru	LI
99.8.10.27	1893	Grand Cape	LI
1930.12.3.290	1930	Kankordu	SL
1930.12.3.289	1930	Sandaru	SL

Table S3. Historical specimens of A. virens measured at the NaturalHistory Museum at Tring.

* CAM = Cameroon, GC = Gold Coast (Ghana), IC = Ivory Coast, LI = Liberia, SL = Sierra Leone.



Figure S1. Additional projections of associations between morphology and tree cover, estimated with least-squares linear regression.

References

- Achard F, Eva HD, Ssstibig H-J et al. (2002) Determination of deforestation rates of the world's humid tropical forests. *Science*, **297**, 999–1002.
- Agbu PA, James ME (1994) The NOAA/NASA Pathfinder AVHRR Land Data Set User's Manual. Goddard Distributed Active Archive Center, Greenbelt, Maryland.
- Brooks TM, Mittermeier RA, Mittermeier CG *et al.* (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology*, **16**, 909–923.
- Buys P, Deichmann U, Wheeler D (2006) Road network upgrading and overland trade expansion in sub-Saharan Africa: Policy Research Working Paper WPS 4097. World Bank, Washington, DC.
- Cowling RM, Pressey RL (2001) Rapid plant diversification : Planning for an evolutionary future. *Proceedings of the National Academy of Sciences*, USA **98**, 5452–5457.
- DeFries RS, Field CB, Fung I et al. (1995) Mapping the land surface for global atmosphere– biosphere models: toward continuous distributions of vegetation's functional properties. *Journal of Geophysical Research*, **100**, 20867–20882.

- DeFries RS, Townshend JRG, Hansen M (1999) Continuous fields of vegetation characteristics at the global scale at 1-km resolution *Journal of Geophysical Research*, **104**, 16911–16923.
- DeFries RS, Hansen MC, Townshend JRG, Janetos AC, Loveland TR (2000) A new global 1 km data set of percent tree cover derived from remote sensing. *Global Change Biology*, **6**, 247–254.
- de M. Bastos, EG, de Araújo AFB, da Silva HR (2005) Records of the rattlesnakes *Crotalus durissus terrificus* (Laurenti) (Serpentes, Viperidae) in the State of Rio de
 Janeiro, Brazil: a possible case of invasion facilitated by deforestation. *Revista Brasileira de Zoologia*, 22, 812–815.
- DeFries RS, Houghton RA, Hansen MC, Field CB, Skole D, Townshend J (2002) Carbon emissions from tropical deforestation and regrowth based on satellite observations for the 1980s and 1990s. Proceedings of the National Academy of Sciences, USA, 99, 14256–14261.
- Doebeli M, Dieckmann U (2003) Speciation along environmental gradients. *Nature*, **421**, 259–264.

- Ellegren H (1996) First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proceedings of the Royal Society of London*, **263**, 1635–1641.
- Endler JA (1977) Geographic Variation, Speciation, and Clines, Monographs in Population Biology 10. Princeton University Press, Princeton, New Jersey.

ESRI, Inc.(2006) ArcGIS 9. ESRI, Inc., Redlands, California.

- Flury B (1988) Common Principal Components and Related Multivariate Models. Wiley, New York.
- Freedman AH, Buermann W, Lebreton M, Chirio L, Smith TB (2009) Modeling the effects of anthropogenic habitat change on savanna snake invasions into African rainforest. *Conservation Biology*, **23**, 81–92.
- Friedl MA, McIver DK, Hodges JCF et al. (2002) Global land cover mapping fromMODIS: algorithms and early results. *Remote Sensing of Environment*, 83, 287–302.
- Funk DJ, Nosil P, Etges WJ (2006) Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences, USA*, **103**, 3209–3213.

- Gavrilets S (2000) Waiting time to parapatric speciation. *Proceedings of the Royal* Society London, Series B, 267, 2483–2492.
- Google, Inc. (2007) Google Earth, Version 5.0. Google Inc., Mountain View, California, available at http://earth.google.com/.
- Grant PR (1986) *Ecology and Evolution of Darwin's Finches*. Princeton University Press, Princeton, New Jersey.

Hansen MC, DeFries RS, Townshend JRG et al. (2002) Towards an operational MODIS continuous field of percent tree cover algorithm: examples using AVHRR and MODIS data. Remote Sensing of Environment, 83, 303–319.

3

Intergovernmental Panel on Climate Change (IPCC) (2007) Climate Change 2007: Climate Change Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fourth assessment report of the Intergovernmental Panel on Climate Change: Summary for Policymakers. IPCC, Geneva.

Julien M, Thiollay J-M (1996) Effects of rain forest disturbance and fragmentation: comparative changes of the raptor community along natural and human-made gradients in French Guiana. *Journal of Biogeography*, **23**, 7–25.

- Keith S, Urban EK, Fry CH (1992) The Birds of Africa. Academic Press, New York, New York.
- Kitano J, Bolnick DI, Beauchamp DA *et al.* (2008) Reverse evolution of armor plates in the threespine stickleback *Current Biology*, **18**, 769–774.
- Kofron CP, Chapman A (1995) Deforestation and bird species composition in Liberia, West Africa. *Tropical Zoology*, **8**, 239–256.
- Laporte NT, Stabach JA, Grosch R, Lin TS, Goetz SJ (2007) Expansion of industrial logging in Central Africa. *Science*, **316**, 1451.
- Lawton JH, Bignell DE, Bolton B *et al.* (1998) Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. *Nature*, **391**, 72–76.
- Letouzey R (1968) Etude phytogeographique du Cameroun, Volume. 69, Encyclopedie Biologie. Lechevalier, Paris.
- Longman KA, Jenik J (1992) Forest-savanna boundaries: general considerations. In: *Nature and Dynamics of Forest-Savanna Boundaries* (eds Furley, PA, Proctor J, Ratter JA, pp. 3–20. Chapman and Hall, New York.

- Louette M (1981) The birds of Cameroon. An annotated checklist. Verhandelingen van de Koninklijke Academie voor Wetenschappen, Letteren en Schone Kunsten van België, Klasse der Wetenschappen, **43**,1–295.
- Mace GM, Masundire H, Baillie J et al. (2005) Biodiversity. In: Millenium Ecosystem
 Assessment: Ecosystems and Human Well-being: Current State and Trends, Vol. 1
 (eds Hassan R, Scholes R, Ash N), pp. 77–122. Island press, Washington, DC.
- Nosil P (2008) Speciation with gene flow could be common. *Molecular Ecology*, **17**, 2103–2106.
- Ogden R, Thorpe RS (2002) Proceedings of the National Academy of Sciences, USA, 99, 13612–13615.
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics, **37**, 637–669.

Pimm SL, Raven P (2000) Extinction by numbers. Nature, 403, 843-845.

Räsänen K, Hendry AP (2008) Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters*, **11**, 624–636.

- Rice WR, Hostert EE (1993) Laboratory experiments on speciation: What have we learned in 40 years? *Evolution (Lawrence, Kansas)*, **47**, 1637–1653.
- Rieseberg LH, Widmer A, Arntz AM, Burke JM (2002) Directional selection is the primary cause of phenotypic diversification. *Proceedings of the National Academy of Sciences, USA*, **99**, 12242–12245.
- Sankaran M, Hanan NP, Scholes RJ *et al.* (2005) Determinants of woody cover in African savannas. *Nature*, **438**, 846–849.

SAS Institute, Inc. (2007) JMP, Version 7. SAS Institute Inc., Cary, North Carolina.

- Schneider CJ, Smith TB, Larison B, Moritz C (1999) A test of alternative models of diversification in tropical rainforests: Ecological gradients vs. rainforest refugia.
 Proceedings of the National Academy of Sciences, USA, 94, 13869–13873.
- Seehausen O, Terai Y, Magalhaes IS et al. (2008) Speciation through sensory drive in cichlid fish. Nature, 455, 620–626.
- Slabbekoorn H, Smith TB (2002) Habitat-dependent song divergence in the little greenbul: an analysis of environmental selection pressures on acoustic signals. *Evolution (Lawrence, Kansas)*, 56, 1849–1858.

- Smith PM, Kalluri SNV, Prince SD, DeFries RS (1997a) The NOAA/NASA pathfinder AVHRR 8-km land data set. *Photogrammetric Engineering and Remote Sensing*, **63**, 12-31.
- Smith TB, Grether G (2008) in *Conservation Biology: Evolution in Action*, eds. Carroll,S. P. and Fox, C. W. (Oxford Univ. Press, Oxford), pp 85–98.
- Smith TB, McNiven D (1993) Preliminary survey of the avifauna of Mt. Tchabal Mbabo, west-central Cameroon. *Bird Conservation International*, **3**, 13–19.
- Smith TB, Holder K, Girman D, O'Keefe K, Larison B, Chan Y (2000b) Comparative avian phylogeography of Cameroon and Equatorial Guinea: Implications for conservation. *Molecular Ecology*, **9**, 1505–1516.
- Smith TB, Wayne RK, Girman DJ, Bruford MW (1997b) A role for ecotones in generating rainforest biodiversity. *Science*, **276**, 1855–1857.
- Smith TB, Calsbeek R, Wayne RK, Holder KH, Pires D, Bardeleben C. (2005a) Testing alternative mechanisms of evolutionary divergence in an African rainforest passerine bird. *Journal of Evolutionary Biology*, **18**, 257–268.

- Smith TB, Wayne RK, Girman DJ, Bruford MW (2005b) Evaluating the divergencewith-gene-flow model in natural populations: the importance of ecotones in rainforest speciation. In: *Rainforests: Past, Present, and Future* (eds Bermingham E, Dick CW, Moritz C), pp 148–165. University of Chicago Press, Chicago.
- Smith TB, Milá B, Grether GF et al. (2008) Evolutionary consequences of human disturbance in a rainforest bird species from Central Africa. Molecular Ecology, 17, 58–71.
- Sodhi NS, Pin Koh L, Brook BW, Ng PKL (2004) Southeast Asian biodiversity: an impending disaster. *Trends in Ecology and Evolution*, **19**, 654–660.
- Sokal RR, Rohlf FJ (1995) Biometry: the Principles and Practice of Statistics in Biological Research. 3rd Edition. W. H. Freeman, New York, New York.
- Thiollay J-M (1996) Distributional patterns of raptors along altitudinal gradients in the northern Andes and effects of forest fragmentation. *Journal of Tropical Ecology*, **12**, 535–560.
- ThiollayJ-M (1998) Long-term dynamics of a tropical savanna bird community. Biodiversity Conservation, 7, 1291–1312.

White F (1983) Vegetation of Africa - a Descriptive Memoir to Accompany the Unesco/AETFAT/UNSO vegetation map of Africa. Natural Resources Report XX.(UNESCO, Paris.

Williams M (2003) Deforesting the Earth: From Prehistory to Global Crisis. University of Chicago Press, Chicago, Illinois.