Rivers, not refugia, drove diversification in arboreal, sub-Saharan African snakes

Kaitlin Allen[1], Eli Greenbaum[2], Paul Hime[1], Walter Paulin Tapondjou Nkonmeneck [1], Viktoria Sterkhova[1], Chifundera Kusamba[3], Mark-Oliver Rödel[4], Johannes Penner[5], Townsend Peterson[1], and Rafe Brown[1]

[1]University of Kansas
[2]University of Texas at El Paso
[3]Centre de Recherche en Sciences Naturelles
[4]Museum fur Naturkunde - Leibniz-Institut fur Evolutions- und Biodiversitatsforschung
[5]Museum für Naturkunde - Leibniz-Institut für Evolutions- und Biodiversitätsforschung

May 6, 2020

Abstract

The relative roles of rivers and refugia in shaping the high levels of species diversity in tropical rainforests has been widely debated for decades. Only recently has it become possible to take an integrative approach to answer these questions with genomic sequencing and paleo-species distribution modeling. Here, we tested the predictions of the classic river, refuge, and river-refuge hypotheses on diversification in the arboreal West and Central African snake genus Toxicodryas. We used dated phylogeographic inferences, population clustering analyses, machine learning-based demographic model selection, species paleo-distribution range estimates, and climate stability modeling to conduct a comprehensive phylogenomic and historical demographic analysis of this genus. Our results revealed significant population genetic structure within both Toxicodryas species, corresponding geographically to river barriers, and divergence times ranging from the mid to late Miocene. Our demographic and migration analyses supported our interpretation that rivers have represented strong barriers to gene flow among populations since their divergence. Additionally, we found no support for a major contraction of suitable habitat during the last glacial maximum, allowing us to reject both the refuge and river-refuge hypotheses in favor of the river barrier hypothesis. This study highlights the complexity of diversification dynamics in the African tropics and the advantage of integrative approaches to studying speciation in tropical regions.

Key words

Phylogenomics, Historical Demography, Machine Learning, Paleo-distributions, *Toxicodryas*

1. INTRODUCTION

For more than two hundred years, scientists have pondered the most pervasive pattern in biogeography, the latitudinal diversity gradient, a striking pattern of increasing species diversity from the poles toward the equator (Allen & Gillooly, 2006; Darlington, 1957; Darwin, 1859; Hutchinson, 1959; Jablonski, Roy, & Valentine, 2006; Pianka, 1966; Ricklefs & Schluter, 1993; Rohde, 1992; Rosenzweig, 1995; Wallace, 1878). Potential explanations of this pattern have been provided from diverse perspectives, including that tropical regions have a wider array of niches (Buckley et al., 2010; Lamanna et al., 2014; Stevens, 2011), higher primary productivity (Hawkins, Porter, Diniz-Filho, & Alexandre, 2003; Jetz & Fine, 2012; Whittaker, Nogués-Bravo, & Araujo, 2007), higher environmental heterogeneity (Janzen, 1967; Stein, Gerstner, & Kreft, 2014; Stevens, 1989), greater land area (Fine & Ree, 2006; Rosenzweig, 1995; Terborgh, 1973), and higher

climatic stability (Harrison & Noss, 2017; Hawkins, Diniz-Filho, Jaramillo, & Soeller, 2007). In more recent studies of historical tropical biogeography, researchers have focused on speciation processes in major tropical rainforests to explain their high biodiversity (e.g. Cardillo, Orme & Owens, 2005; Jablonski et al., 2006; Ricklefs, 2006; Smith et al., 2017; Weir & Schluter, 2007; Wiens & Donoghue, 2004; Wiens, Sukumaran, Pyron, & Brown, 2009).

Three major allopatric diversification mechanisms have been proposed in the classical literature to explain species diversity in the Amazon: the "river hypothesis" in which species and populations diverged across river barriers (Ayres & Clutton-Brock, 1992; Bates, 1863; Hershkovitz, 1977; Mayr, 1942; Sick, 1967; Wallace, 1853); the "refuge hypothesis" in which forests fragmented during Earth's cold or dry climate cycles (i.e. the Pleistocene glaciation cycles), causing isolation and divergence in small forest patches (Haffer, 1969, 1974, 1982; Prance, 1982; Vanzolini, 1973; Vanzolini & Williams, 1970); and an amalgamate "river-refuge hypothesis" in which speciation was promoted by a combination of river barriers and climate driven vegetation changes (Ayres & Clutton-Brock, 1992; Haffer 1992, 1993). These hypotheses have been widely used in the study of Neotropical biodiversity and the mechanisms of its production (e.g. Gascon et al., 2000; Haffer, 2008; Patton & Silva, 2005; Richardson, Pennington, Pennington, & Hollingsworth, 2001; Weir, 2006). However, because the early scientific focus was primarily on the Amazon (Amorim, 1991; Cracraft, 1985; DeMenocal, 2004; Haffer, 1969, 1997; Plana, 2004; but see Fjeldsa, 1994; Mayr & O'Hara, 1986), and given political instability in tropical Africa (Greenbaum, 2017; Siddig, 2019; Tolley et al. 2016), rigorous testing of the predictions stemming from these hypotheses has been neglected for the West and Central African rainforests until only recently.

Based on pollen core records (Brenac, 1988; Bonnefille & Riollet, 1988; Girese, Maley, & Brenac, 1994; Maley, 1987, 1989, 1991; Maley & Brenac, 1987; Maley & Livingstone, 1983; Sowunmi, 1991) and species distribution data (Colyn, 1987, 1991; Rietkerk, Ketner, & De Wilde, 1995; Richards, 1963; Sosef, 1991), Maley (1996) proposed several rainforest refugia for sub-Saharan Africa that are still widely used today (e.g. Bell et al., 2017; Hughes, Kusamba, Behangana, & Greenbaum, 2017; Huntley, Castellanos, Musher, & Voelker, 2019; Jongsma et al., 2018; Larson, Castro, Behangana, & Greenbaum, 2016; Penner, Wegmann, Hillers, Schmidt & Rodel, 2011, Portik et al. 2017; Fig. 1). Many of these hypothesized refugia are located in highland areas (e.g., the Cameroon Volcanic Line and the Albertine Rift), however, a major fluvial refuge, located in the gallery forests around the Congo River, has been supported by pollen core data (Maley, 1996), and distributional patterns of multiple bird (Huntley, Harvey, Pavia, Boano, & Voelker, 2018; Levinsky et al., 2013), mammal (Colyn, Gautier-Hion, & Verheyen, 1991; Levinsky et al., 2013) and plant taxa (Robbrecht, 1996).

Major river barriers in West and Central Africa include the Volta, the Sanaga, the Ogooue, the Congo, the Niger and the Cross Rivers (Fig. 1). The exact ages of many of these rivers are unknown but are generally estimated to date back to the Late Mesozoic to the Early Cenozoic (80–35 mya; Goudie, 2005; Stankiewicz & de Wit, 2006). However, while the Congo basin is quite old (Flugel, Eckardt, & Cotterill, 2015; Stankiewicz & de Wit, 2006), the present course of the Congo River appears to be much younger, dating to the mid to late Miocene and corresponding to the uplift of the East African Rift (Flugel, et al., 2015; Stankiewicz & de Wit, 2006).

Numerous phylogeographic studies have supported the importance of rivers, refugia, or both as drivers of diversification across disparate plant and animal species. Rivers alone have been shown to be important barriers for some species of primates (Mitchell et al., 2015; Telfer et al., 2003), shrews (Jacquet et al., 2015), and frogs (Charles et al., 2018; Penner et al. 2011; Penner, Augustin & Rodel, 2019; Wieczorek, Drews & Channing, 2000; Zimkus, Hillers & Rodel, 2010), but do not appear to represent an important barrier for many plant species (Dauby et al., 2014; Debout, Doucet, & Hardy, 2011; Hardy et al., 2013; Ley et al., 2014; Lowe, Harris, Dormontt, & Dawson, 2010). Refugia are suggested to have played an important role in the diversification of rodents (Bohoussou et al., 2015; Nicolas et al., 2011; Nicolas, Missoup, Colyn, Cruaud, & Denys, 2012), primates (Clifford et al., 2004; Haus et al., 2013; Tosi, 2008), frogs (Bell et al., 2017; Jongsma et al., 2018), lizards (Allen, Tapondjou, Greenbaum, Welton, & Bauer, 2019; Leache et al.,

2017), birds (Fjeldsa & Bowie, 2008), pangolins (Gaubert et al., 2016), and rainforest plants (Born et al., 2011; Budde, Gonzalez-Martinez, Hardy, & Heuertz, 2013; Dainou et al., 2010; Dauby, Duminil, Heuertz, & Hardy, 2010; Duminil et al., 2015; Faye et al., 2016; Gomez et al., 2009; Hardy et al., 2013; Ley et al., 2014; Ley, Heuertz, & Hardy, 2016; Lowe et al., 2010). In some cases, divergence patterns match both refugial and riverine predictions (Anthony et al., 2007; Barej et al., 2011; Bohoussou et al., 2015; Gonder et al., 2011; Jacquet et al., 2014; Jongsma et al., 2018; Leache et al., 2019; Leache & Fujita, 2010; Marks, 2010; Portik et al., 2017), suggesting that both may have played roles simultaneously—or in combination—in evolutionary diversification. However, because of the spatial overlap of refugia with montane and riverine systems (Hofer, Bersier, & Borcard, 1999, 2000), and the sparse pollen core and fossil records for the tropics (Colinvaux, De Oliveira, Moreno, Miller, & Bush, 1996; Maley & Brenac, 1998), distinguishing between these three hypotheses has been difficult, especially when relying on phylogeographic data alone.

The three major allopatric diversification hypotheses make the following predictions regarding species diversification patterns in tropical African forests (1) river hypothesis: boundaries between population distributions should correspond to riverine barriers and the ages of populations should be relatively old, corresponding to the ages of the rivers; (2) refuge hypothesis: population distributions should be concordant with locations of hypothesized rainforest refugia during cold, dry periods and populations are predicted to be relatively young, possibly corresponding to the Pleistocene glaciation cycles; (3) river-refugia hypothesis: population distributions should be correlated with the locations of rainforest refugia and bounded by rivers barriers, or will have been confined to refugial locations and additionally subdivided by rivers. Finally, the timing of population splits should correspond to ages of rivers but would be expected to show patterns of expansion and contraction dating to the Pleistocene.

In this study, we use the snake genus *Toxicodryas* as a model system to test the predictions of these hypotheses. The genus *Toxicodryas* consists of two large, rear-fanged, venomous West and Central African species, *T. blandingii* and *T. pulverulenta.* The taxonomic placement of this genus is uncertain. They were originally placed in the Asian genus *Boiga* (Schmidt, 1923), and some authors still classify them as such, but recent phylogenetic analyses recover them as the sister genus to the African egg eating snakes, *Dasypeltis* , albeit with weak support (Pyron et al., 2013). Both species in this genus are primarily arboreal, feeding mainly on birds, bats, frogs and chameleons (Akani, Barieenee, & Luiselli, 1998; Chippaux & Jackson, 2019; Nagy et al., 2011; Spawls, Howell, Hinkel, & Menegon, 2018). Because of their general arboreality, these species are predicted to have distributions strongly correlated with forest distribution. In addition, *Toxicodryas* is widely distributed within the Congo River fluvial system and broadly across West and Central Africa, making this genus a suitable system for testing the competing predictions of the river, refugia and river-refugia hypotheses.

Recent advances in paleo-climate modeling and genome-scale DNA sequencing have opened new avenues to testing classic hypotheses of tropical rainforest speciation (Bell et al., 2017; Leache et al., 2019; Portik et al., 2017). In this study, we integrate dated phylogeographic inference, population structure analyses, and machine learning-based demographic modeling to identify the timing of divergence as well as the location and permeability of past and present dispersal barriers. These genetic data are combined with paleo-distribution and climate stability modeling to determine the congruence of historical distributions with the refugial and river-refugial hypotheses. Our results demonstrate that, although population distributions alone could be congruent with any of the three hypotheses, diversification times predate the Pleistocene, a finding that aligns with predictions of the river-barrier hypothesis. Historical demographic analyses support models of no migration among populations since the time of divergence, and migration analyses suggest that the western Congo River represents one of the strongest barriers to recent dispersal. Species paleo-distribution and climate stability modelling show no suggestion of suitable habitat contraction during or since the Pleistocene, allowing us to soundly reject the predictions of refugia hypotheses in favor of the prevailing role of riverine barriers in shaping, structuring, and maintaining diversity in this generally arboreal, forest-associated group of endemic African snakes.

2. MATERIALS AND METHODS

2.1 Sampling

We obtained 20 specimens of *Toxicodryas* (seven *T. blandingii* and 13 *T. pulverulenta*) through fieldwork and from various museums (see Table S1). Sampling was representative of the known range of each species throughout the upper and lower Guinean forest blocks of West and Central Africa including the countries of Guinea, Liberia, Ghana, Cameroon, Gabon, and Democratic Republic of the Congo (DRC). Museum catalog numbers, GenBank accession numbers, and locality data for each specimen are presented in Table S1.

2.2 Genetic data collection, bioinformatic processing, and locus assembly

Tissue samples were preserved in 95% ethanol or RNAlater? (Sigma-Aldrich) and extracted using the Maxwell RSC system (Promega). The nuclear gene c-mos and the mitochondrial gene cytochrome *b*(cyt *b*) were PCR-amplified for each individual using standard primers (c-mos: S67, S68; Lawson, Slowinski, Crother, & Burbrink, 2005; cyt *b* : L4910B, H15720; Burbrink, Lawson, & Slowinski, 2000) and sequenced on an ABI 3730 capillary electrophoresis system. Electropherograms were edited manually in Geneious v5.6.7 (http://www.geneious.com, Kearse et al., 2012) and resulting sequences were aligned in MAFFT v.5 with default parameters (Katoh & Kuma, 2002).

We also sequenced genome-wide anonymous nuclear markers for each individual following a modified version of the ddRADseq protocol of Peterson, Weber, Kay, Fisher, and Hoekstra (2012). For each individual, a total of 300–500 ng of genomic DNA was double digested using the restriction enzymes *Sbf* I (restriction site 5'-CCTGCAGG-3') and*Msp* I (restriction site 5'-CCGG-3'). The resulting double digestion products were then bead-cleaned with AmpureXP beads (Agencourt) and individually barcoded using custom oligonucleotide adapters. Pooled samples were size-selected to a mean insert length of 541 base pairs (bp) (487–595 bp range) with internal standards with a Pippin Prep? (Sage Science, Beverly, MA). Resulting post-ligation products were amplified for eight cycles with a high-fidelity polymerase (Phusion?, New England Biolabs). An Agilent TapeStation was used to determine the final fragment size distribution and concentration of each pool. Library pools were combined in equimolar amounts for sequencing on one Illumina HiSeqX lane (with a 10% *Phi* X spike-in and 150 bp paired-end reads).

Illumina reads from the ddRAD libraries were processed using STACKS v. 2.4 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). Because the ddRAD protocol generates strand-specific libraries, prior to read filtering and assembly, we used a read-stitching approach (Hime, Briggler, Reece, & Weisrock, 2019) to join the first read from an Illumina read pair with the reverse complement of the second, recapitulating the original orientation of fragments in the genome. Stitched reads were quality-filtered and demultiplexed by individual with the process_radtags function in STACKS with the following parameters: demultiplex each library by in-line barcode, check for both restriction enzyme cut sites, remove any read with an uncalled base, rescue barcodes and RAD-Tags, and discard any read with average Phred quality score < 20 over sliding windows of 15% of the total read length. Next, we used STACKS to *de novo* assemble filtered and stitched Illumina read pairs.

We aimed to produce three separate ddRAD data sets, including one for*T. blandingii* , one for *T. pulverulenta* , and a combined data set comprising both species. Because the optimal *de novo*assembly of ddRADseq data can vary widely across taxa (Paris, Stevens, & Catchen, 2017; Shafer et al., 2017), we tested a range of assembly parameters to optimize the recovery of putatively single-copy orthologous loci. Final assembly parameters were selected based on the methods laid out in Paris et al. (2017). According to their recommendations, in USTACKS, we kept m (the minimum number of reads needed to form a stack) at 3 while in CSTACKS, we varied M (the number of mismatches allowed during loci formation) and n (the number of mismatches allowed during catalog formation) until we identified the parameters at which the maximum number of polymorphic loci were available across 80% (r = 0.8) of the population. For our data, this was found to be M = 5 and n = 15. Further parameters were tested in POPULATIONS separately for each species and for the genus as a whole in order to balance missing data and number of polymorphic loci. Within*T. blandingii* and *T. pulverulenta* , the percent missing data was low (5% and 7.3% missing data respectively) and no further processing was needed, and r = 0.8 was used. Because of dissimilarity between

the two species causing high levels of missing data in the combined dataset, further restrictions were implemented. For the genus-wide data set, we set r = 0.5 and p = 4 [p is the minimum number of populations in which a locus must be present (here 4/5)]. This approach increased the number of informative loci, but also the amount of missing data. For each of our three separate data sets, we generated a data set comprising only a single random SNP per locus (for population clustering analyses and demographic modeling), and another data set comprising full-length sequences for all loci (for use in phylogenetic reconstruction).

2.3 Assessing genetic structure

We used multivariate, Bayesian, and admixture-based analyses to assess population structure. In all analyses, clustering algorithms were run on three data sets separately for comparison (*T. blandingii* ,*T. pulverulenta* , and both species combined [genus *Toxicodryas*]). A discriminant analysis of principal components (DAPC) was run using Adegenet v. 2.1.1 (Jombart & Ahmed, 2011). This approach uses discriminant functions to maximize variation among clusters and minimize variation within clusters. The best-clustering scheme was chosen based on Bayesian information criterion (BIC) scores. Numbers of clusters (K) ranging from 1–10 were evaluated and a discriminant function analysis of principal components (DAPC) was performed based on the number of suggested clusters. Ancestry proportions of all individuals were inferred using LEA v. 1.6.0 (Frichot & Francois, 2015) through the Bioconductor v. 3.4 package. The sNMF function was used to assess K values from 1–10, with 20 replicates, estimate individual admixture coefficients, and select the value of K that minimized cross entropy (Francois, 2016; Frichot, Mathieu, Trouillon, Bouchard, & Francois, 2014). Population structure and admixture were also tested using the Bayesian method STRUCTURE v. 2.3.4 (Falush, Stephens, & Pritchard, 2003; Pritchard, Stephens, & Donnelly, 2000). Each data set was evaluated for K=1–10 with 10 runs per K and a MCMC burn-in of 10,000 steps followed by 100,000 steps (Porras-Hurtado et al., 2013). Results were evaluated using the Evanno method (Evanno, 2005) and plotted through the R package pophelper v. 2.3.0 (Francis, 2017).

2.4 Phylogenetic analyses

We conducted a Bayesian time calibrated analysis on our Sanger data set (c-mos and cyt *b*) in Beast v. 2.5.2 (Bouckaert et al., 2019). We used a relaxed log-normal clock and a Yule tree prior assuming a constant lineage birth rate. The species *Farancia erytrogramma* ,*Micrelaps muelleri* , and *Contia longicaudae* were used as outgroups to allow the use of two fossils for calibration, one at the Elapoidea + Colubridae node (minimum age: 30.9 Mya), and one at the *Heterodon* + *Farancia* node (minimum age: 12.08 Mya), with fossil ages and placement based on Head, Mahlow, and Muller (2016). Two runs of 100,000,000 generations were conducted and logged every 10,000 generations. Convergence was assessed using Tracer v. 1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). A burn-in of 10% was used to create a maximum clade credibility tree. Node ages are based on median tree heights.

We analyzed our SNP data set, including all samples of both species of *Toxicodryas* , using both species-tree summary quartet and maximum likelihood phylogenetic methods. The quartet method was implemented through SVDquartets (Chifman & Kubatko, 2014) in PAUP* v. 4.1a166 (Swofford, 2003). We sampled all possible quartets and assessed support using 100 nonparametric bootstraps and species tree topology was summarized with DendroPy v. 4.4.0 (Sukumaran & Holder, 2010). We ran a maximum likelihood analysis of our genus-wide SNP data set in IQtree v. 1.6.12 (Nguyen, Schmidt, von Haeseler, & Minh, 2014) using 10,000 ultrafast bootstraps (Hoang et al., 2018) and the ModelFinder function to choose the best substitution model (Kalyaanamoorthy, Minh, Wong, von Haeseler, & Jermiin, 2017).

2.5 Contemporary migration and genetic diversity

We visualized spatial patterns of gene flow and genetic diversity in each of our three data sets using EEMS (Estimated Effective Migration Surfaces), an approach that uses a population genetic model to compare effective migration rates to expected genetic dissimilarity in order to identify regions where genetic similarity decays more quickly than expected under a model of isolation-by-distance (Petkova, Novembre, & Stephens, 2016). We converted our filtered stacks output to the correct bed filetype using PLINK (Chang et al., 2015), and created a dissimilarity matrix using the BED2DIFFS program in the EEMS package (Petkova et al.,

2016). The outer coordinate file was generated in QGIS v. 3.4 (QGIS Development Team, 2020). We ran the RUNEEMS_SNPS script under several deme sizes (400, 600 and 1000). For each data set, each deme was run for three independent analyses with an MCMC length of 2,000,000 generations, a burn-in of 1,000,000 generations and a thinning interval of 9999 (Petkova et al., 2016). The results were combined, checked for convergence through a visual examination of the trace files, and plotted using the REEMSPLOTS R package (Petkova et al., 2016).

2.6 Demographic modeling and analysis of gene flow

To test for present day migration and historical gene flow between our populations, we used the R package delimitR (Smith & Carstens, 2020; https://github.com/meganlsmith/delimitR). This program uses a binned multidimensional folded site frequency spectrum (bSFS; Smith, Ruffley, Tank, Sullivan, & Carstens, 2017) and a random forest machine learning algorithm to compare speciation models such as no divergence, divergence with and without gene flow, and divergence with secondary contact (Smith & Carstens, 2020). A bSFS was used because it stores the observed frequencies of the minor alleles for multiple populations and bins them to avoid inference problems associated with sampling too few segregating sites (Smith et al., 2017; Terhost & Song, 2015). DelimitR was chosen over more traditional multi-species coalescent methods because of its ability to take historical and current gene flow into account (Leache, Harris, Rannala, & Yang, 2014; Smith & Carstens, 2020). Demographic histories are simulated using the multi-species coalescent model implemented through fastsimcoal2 (Excoffier, Dupanloup, Huerta-Sánchez, Sousa, & Foll, 2013) under a user-specified guide tree and set of priors on divergence times, population sizes, and migration rates. The random forest classifier then creates a user-defined number of decision trees from a subset of the prior. Each decision tree compares the empirical bSFS to the SFS of each simulated speciation model and votes for the most similar model. The demographic model with the largest number of votes is chosen as the best model. Out-of-bag error rates are used to assess the power of the random forest classifier. The posterior probability of the selected model is then calculated by regressing against the out-of-the-bag error rates following Pudlo et al. (2015).

We created folded multi-dimensional site frequency spectrums for the two *T. blandingii* clades and the two Central African *T. pulverulenta* clades using easySFS (https://github.com/isaacovercast/easySFS), a wrapper for [?]a[?]i (Gutenkunst, Hernandez, Williamson, & Bustamante, 2009). The West African *T. pulverulenta* clade was not included because of the low sample size available for this lineage. We simulated 100,000 data sets under four models: no divergence (Model 1), divergence without gene flow (Model 2), divergence with secondary contact (Model 3), and divergence with gene flow (Model 4). Priors for both models were drawn from uniform distributions for population size: 10000 – 1000000 haploid individuals (twice the number of estimated diploid individuals), divergence time: 20000 –2000000 generations, migration rate: 0.000005–0.005 corresponding to 0.05–5 migrants per generation. We then coarsened our empirical site frequency spectra to 10 bins each. Our out-of-bag error rates were calculated, and 500 random forest classifiers were simulated using 100,000 pseudo-observed data sets for each model. A confusion matrix was calculated to determine how often the correct model was selected and posterior probability for the "best" model was estimated for each species.

2.7 Species distribution modeling

Occurrence data for each species was obtained from the specimens used in this study, "expert" identified individual occurrences from GBIF, and research grade locality records from iNaturalist (www.inaturalist.org). This resulted in a total of 43 *T. blandingii* localities and 30 *T. pulverulenta* localities (Fig. S1). Duplicate records were removed, and points were thinned within a distance of 10-kilometers using the spThin package (Aiello-Lammens, Boria, Radosavljevic, Vilela, & Anderson, 2015) in R v. 3.4.4 (R Core Team, 2018). A subset of points from each data set was set aside for model calibration (75%) and internal testing (25%) following Cobos, Peterson, Barve, and Osorio-Olvera (2019).

Environmental data were obtained from the WorldClim database v. 1.4 (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). Fifteen of the 19 bioclim variables were downloaded at a 2.5-minute resolution. We excluded

bio8, bio9, bio18, and bio19 which are known to create artifacts in distribution models (Escobar, Lira-Noriega, Medina-Vogel, & Peterson, 2014). The same 15 variables were used for the Last Glacial Maximum (LGM) under three general circulation models (GCMs): CCSM4, MIROC-ESM, and MPI-ESM-P. In order to reduce spatial autocorrelation, principal component analyses (PCAs) were performed on present bioclim variables and projected to the LGM for the extent of sub-Saharan Africa.

Model calibration areas were defined as a 1000-kilometer buffer around occurrence points for each species. Model calibration, creation, projection, and evaluation were done using the R package kuenm (Cobos et al., 2019). In order to calibrate our models, we created 1479 candidate models for each species by combining three sets of environmental predictors (PCAs 1–6, 1–5, 1–4), 17 possible regularization multipliers (0.1–1.0 at intervals of 0.1, 2–6 at intervals of 1, and 8 and 10), and all combinations of five feature classes (linear = l, quadratic = q, product = p, threshold = t, and hinge = h; Cobos et al., 2019).

Candidate models were run in Maxent (Phillips, Anderson, & Schapire, 2006) and chosen based on significant partial ROC scores (Peterson, Papes, & Soberon, 2008), omission rates of E [?] 5% (Anderson, Lew, & Peterson, 2003), and AICc scores of [?] 2 to minimize model complexity (Warren & Seifert, 2011). These models determined the parameter set used for final model creation.

Final models were created for each species using the full set of occurrence records and the parameters chosen during model calibration. Models were run in Maxent with ten bootstrap replicates and logistic outputs. After models were run in the present, they were projected to the LGM and mid-Holocene for the three GCMs. The mobility-oriented parity (MOP) index was used to test for model extrapolation (Soberon & Peterson, 2005). Models were visualized in QGIS 3.4 and thresholded to 5% to create presence-absence maps. Models from each time period were summed to estimate potential LGM and mid-Holocene distributions as well as continuous stability maps (Devitt, Devitt, Hollingsworth, McGuire, & Moritz, 2013; Yannic et al., 2014).

3. RESULTS

3.1 Genetic data collection, bioinformatic processing, and locus assembly

Our concatenated c-mos and cyt *b* data set (Sanger data set hereafter) consisted of 1237 bp, including indels. Both genes were represented in all samples with the exception of c-mos for the outgroup *Contia longicaudae* . After filtering (see Methods, above), our genus-level ddRAD data set consisted of 2848 loci with 20.7% missing data (here defined as proportion of missing loci across all individuals), and an effective mean per-sample depth of coverage of 78.7x ± 13.6x. Our *T. blandingii* data set consisted of 7231 loci with 5.0% missing data, and an effective mean per-sample depth of coverage of 83.6x ± 12.0x. Our *T. pulverulenta* data set consisted of 4471 loci with 7.3% missing data, and an effective per-sample mean depth of coverage of 77.9x ± 14.6x.

3.2 Phylogenetic structure and divergence dating

Broad-scale phylogenetic relationships estimated in analyses of our Sanger and SNP data sets were identical in topology, with strongly supported internal nodes throughout (Fig. 2; Fig. S2). Our two-locus Sanger tree and our 2848-locus ddRAD SNP trees both supported two divergent lineages of *T. blandingii* , in West and Central Africa, respectively. These same analyses revealed three divergent lineages of *T. pulverulenta* , one from West Africa and two in Central Africa, north and south of the Congo River (Fig. 3). Fossil-calibrated divergence dating suggests that *T. blandingii* and *T. pulverulenta* diverged in the early to mid-Miocene (median age 18.3 Mya). Diversification within each species is estimated to have taken place in the late Miocene to Pliocene, with the two clades in *T. blandingii* diverging around 8.6 Mya, the West African clade of *T. pulverulenta* diverging around 7.0 Mya, and the two Central African clades diverging around 4.1 Mya (Fig. 2).

3.3 Population structure

A comparison of BIC values from the genus-level DAPC analyses suggested a total of five genetic clusters, with two populations in *T. blandingii* and three in *T. pulverulenta* , matching the clades identified in the

phylogenetic analyses (Fig. S3). Our admixture-based method, LEA, identified two distinct genetic clusters at the genus level, corresponding to the two *Toxicodryas* species, and the same two populations for *T. blandingii* and three populations for *T. pulverulenta* as suggested by DAPC (Fig. 4). A low amount of admixture was identified in the Cameroonian sample of *T. blandingii* , and varying levels of admixture were suggested for the Gabonese samples of *T. pulverulenta* (Fig. 4). The population assignment of individuals between the two clustering methods was identical; however, admixture between populations was not detected by DAPC. Similarly, STRUCTURE suggested two populations at the genus level, and two in *T. blandingii* , but combined the Central African clades and suggested two populations, instead of three, for *T. pulverulenta* . Three populations were supported as the second highest ΔK and showed identical admixture proportions to those from LEA. We used five populations for our remaining analyses because multivariate-based analyses such as LEA and DAPC do not make assumptions about Hardy-Weinberg equilibrium and may be preferable over Bayesian methods such as STRUCTURE when sample sizes are small or uneven (Puechmaille, 2016).

3.4 Contemporary migration and genetic diversity

Through our EEMS analyses we identified several present-day barriers to migration, as well as areas of higher- or lower-than-expected genetic diversity. The Dahomey Gap and the western Congo River were supported as major barriers to dispersal when we simultaneously analyzed all data for both species of *Toxicodryas* (Fig. 5a). Areas of lower-than-expected diversity were suggested for the coast of Central Africa and higher levels of West African diversity in in the Upper Guinean rainforest (Fig. 5b). We also ran EEMS individually for *T. blandingii* and *T. pulverulenta* , but these analyses are more difficult to interpret because of the small sample sizes for each individual species (Fig. S4).

3.5 Demographic modeling and analysis of gene flow

Using machine learning-based demographic model selection, we identified divergence without gene flow as the best model for *T. blandingii*with a posterior probability of 0.68, and divergence with gene flow for *T. pulverulenta* with a posterior probability of 0.63 (Fig. 6). For both species, models representing no divergence and divergence with secondary contact received very low support (Tables S2 and S3). The out-of-bag error rate for *T. blandingii* was 17.3% and 22.8% for *T. pulverulenta* . Our values for posterior probability and out-of-bag error rate are similar to those obtained by Smith & Carstens (2020). The confusion matrix and number of votes per model can be found in Tables S2 and S3.

3.6 Distribution modeling

Species distribution modeling suggested widely overlapping ranges for *T. blandingii* and *T. pulverulenta* , with both species documented from both rainforest and woodland habitats (Fig. 7). Paleodistribution models for the LGM suggested a slight northern and southern contraction of suitable habitat for the genus in West and Central Africa. *Toxicodryas pulverulenta* showed evidence of a slight southward range expansion into Angola, while the range of *T. blandingii* remained stable (Fig. 7a). The mid-Holocene distribution was highly similar to the present-day distribution for all data sets (Fig. 7b).

Continuous climate stability maps estimating the areas of persistent suitable habitat from the LGM to the present suggest that the core distribution of each species has remained stable through time (Fig. 7c). Instability in suitable habitat is only found on the edges of the species range, with the greatest potential for distribution change in southern Central Africa. No northward range expansion past the present day was estimated at any time scale in Central Africa, but lesser degrees of northward expansion may have been possible in West Africa.

4. DISCUSSION

The relative roles of rivers and refugia in shaping the high levels of species diversity in tropical rainforests has been widely debated for decades (e.g. Amorim, 1991; Colinvaux, Irion, Räsänen, Bush, & De Mello, 2001; DeMenocal, 2004; Haffer, 1969, 1997; Mayr & O'Hara, 1986; Vitorino, Lima-Ribeiro, Terribile, & Collevatti, 2016). Only recently has it become possible to take an integrative approach to answering these questions with genomic sequencing and paleo-species distribution modeling (Portik et al., 2017; Leaché et al., 2019).

Here we tested alternate predictions of the classic river, refuge, and river-refuge hypotheses for terrestrial faunal diversification using a novel study system: the arboreal African snake genus *Toxicodryas* . We found strong support for the river hypothesis over the refuge and river-refuge hypotheses based on the ages and locations of the populations as well as a lack of support for suitable habitat contraction during the last glacial maximum.

4.1 Species diversification

This study represents the first phylogenetic analysis of the genus *Toxicodryas* . Phylogenetic analyses of our two-locus Sanger data set and 2848-locus RADseq SNP data set reveal two deeply divergent, strongly supported lineages in *T. blandingii* and three in *T. pulverulenta* (Fig. 2; Fig. S2). Although today, the two recognized species are broadly sympatric, clades within each species are generally situated allopatrically across river barriers. The two clades within *T. blandingii* are separated either by the Sanaga River in Cameroon or the Congo River in the DRC. Both rivers have frequently been interpreted as population barriers in other terrestrial vertebrates (Blackburn, 2008; Jongsma et al., 2018; Leaché et al., 2019; Leaché & Fujita, 2010; Portik et al., 2017), but additional sampling and comparative analyses will be needed to determine which river played the most deterministic role in shaping genetic structure in this species. Of the three *T. pulverulenta* clades, one is distributed in West Africa (albeit with limited sampling) and two are distributed in Central Africa, separated by the western Congo River. Our population structure analyses are concordant with phylogenetic analyses supporting five distinct genetic clusters (Fig. 4). Minor levels of admixture appear to have occurred between the *T. pulverulenta* clades separated by the western Congo River, and between the two clades of *T. blandingii* in the sample collected at the Sanaga River (Fig. 4). In both species, the Congo River barrier seems to be stronger in the west where the river is wider, and the current is stronger. In the eastern DRC samples of clades from both species can be found on either side of this river (Fig. 3)

Divergence time estimates from a time-calibrated phylogeny also fail to reject predictions derived from the river-barrier hypothesis. *Toxicodryas blandingii* and *T. pulverulenta* diverged in the early to mid-Miocene, and subsequent intraspecific diversification took place in the late Miocene to the Pliocene (Fig. 2). The Congo River, a barrier in the Central African *T. pulverulenta* (divergence time: ˜4.1 Mya), and a potential barrier in *T. blandingii* (divergence time: ˜8.6 Mya), dates back to the mid-late Miocene (Flügel et al., 2015; Stankiewicz & de Wit, 2006). The Sanaga River, another potential barrier in *T. blandingii* , has a poorly known geological history, but likely dates back to the formation of the Adamawa Plateau in the late Eocene-early Oligocene (Fagny et al., 2016). Similar mid to late Miocene divergence times have been noted for other widespread Central and West African taxa including frogs (Bell et al., 2017; Jongsma et al., 2018; Zimkus et al., 2017), and terrestrial snakes (Portillo et al., 2019), and similar West to Central African distribution splits have been seen in forest cobras (Wüster et al., 2018), frogs (Leache et al., 2019), lizards (Allen et al., 2019), and shrews (Jacquet et al., 2015). The Congo river has been a well-known barrier to many species including primates (Harcourt & Wood, 2012; Mitchell et al., 2015; Telfer et al., 2003), shrews (Jacquet et al., 2015), and frogs (Charles et al., 2018). However, while the timing and locations of population divergences in this study correspond with river barriers, the Miocene was also a time of global climatic change characterized by dramatic cooling and vegetation shifts throughout sub-Saharan Africa (Herbert et al., 2016; Jacobs, 2004; Menegon et al., 2014). Although most research surrounding the role of refugia in driving diversification has focused on the dramatic climate oscillations of the Pleistocene, it is likely that refugia are able to form during any period of climatic change (Haffer, 1997; Hampe & Jump, 2011; Jansson & Dynesius, 2002), but the role of possible older refugia has received little attention in the literature (Hampe & Jump, 2011).

Migration analyses support the western Congo River and the Dahomey Gap as barriers to gene flow in the genus *Toxicodryas* (Fig. 5a). The Dahomey gap is a natural savanna region in West Africa that separates the upper and lower Guinean rainforests, and which has been previously identified as a dispersal barrier for arboreal species (e.g. Rödel, Emmrich, Penner, Schmitz, & Barej, 2014; Schunke & Hutterer, 2005). Both areas also support lower genetic diversity in *Toxicodryas* than expected under a pure isolation-by-distance model (Fig. 5b), emphasizing the biological reality of this barrier for forest-associated, primarily arboreal

vertebrates despite the fact that both *Toxicodryas* species have been found in forest patches within the Dahomey gap. Our demographic analyses further suggest that riverine dispersal barriers between clades are strong, indicating divergence without gene flow between the two *T. blandingii* clades and divergence with minor gene flow across the Congo River in the two Central African *T. pulverulenta* clades (Fig. 6). Contemporary gene flow was ruled out with high confidence in both species (Table S3). In light of the Miocene divergence times and lack of gene flow between these five clades, it is likely that they represent distinct evolutionary lineages and, thus, surveys of morphological data and analyses of phenotypic variation are underway to determine if formal taxonomic revision is justified.

4.2 Paleo-distributions and habitat stability

The nature of the intervening habitat surrounding rainforest refugia during the Pleistocene has been widely debated. Some authors have argued that much of the Central African rainforest was replaced by savannas (DeMenocal, 2004; Maley, 1996; Maley & Brenac, 1998), while others have emphasized the possibility of more subtle shifts in forest composition (i.e., from wet to dry forest; Colinvaux et al., 1996, 2001; White, 1981). *Toxicodryas* species are generally characterized as arboreal across rainforest and woodland habitats and the two species exhibit widely overlapping distributions in West and Central Africa (Chippaux & Jackson, 2019). Our paleo-distribution modeling suggested that no substantial contraction of suitable climate occurred for either species during the LGM (Fig. 7a), and our habitat stability mapping suggested that core ranges of both species have remained stable for the past 22,000 years (Fig. 7c). The greatest potential for habitat expansion in this species appears to be to the south into today's northern Angola and the southern DRC (Fig. 7).

Similar paleo-distribution studies on frogs have suggested substantial habitat contraction in Central Africa during the Pleistocene (Leaché et al., 2019; Portik et al., 2017). In contrast, our inferred widespread habitat stability in *Toxicodryas* may be due to the relatively reduced dependence of arboreal snakes on moist habitats, as reflected by their distribution in both woodland and rainforest. The stability of *Toxicodryas* habitat through the Pleistocene supports the hypothesis that rainforest composition shifted to dryer woodlands surrounding rainforest refugia, instead of a more dramatic shift to strict savannah habitat. Southward shifts in species suitability may correspond with predicted forest distribution shifts of White (1981), suggesting a replacement of lowland rainforest with montane forest habitat.

4.3 Integrative species diversification studies

Prior to the availability of genomic data, dated phylogenies, paleo-distribution modeling, and statistical analyses of historical demography (Carstens & Richards, 2007; Ellegren, 2014; Knowles, 2009; Knowles & Madison, 2002; Luikart, England, Tallmon, Jordan, & Taberlet, 2003), biogeographers commonly observed patterns of species distributions from which they attempted to infer mechanisms of diversification (e.g. Avise, 2000; Templeton, 2001). The majority of rainforest refugial studies were conducted in this descriptive, pattern-based manner, usually employing only single-locus mitochondrial DNA data sets (e.g. Fjeldså & Lovett, 1997; Haffer, 1969; Mayr & O'Hara, 1986). The pitfalls and lack of power in such approaches have been discussed elsewhere (e.g., Knowles & Madison, 2002; Provan & Bennett, 2008; Stewart, Lister, Barnes, & Dalén, 2010), and are not the focus of this study. However, with respect to hypotheses of tropical African species diversification, it is noteworthy that so many of the concepts and hypotheses surrounding refugia have only been evaluated by single-locus data sets during the era of mitochondrial gene phylogeography (but see more recent, multilocus examples in Leaché et al., 2019; Portik et al., 2017).

The complexity of geographic barriers in West and Central Africa, and the association of refugia with areas of high surface relief or riparian zones (Hofer et al., 1999; 2000; Fig. 1), makes it extremely difficult to untangle the relative importance of different diversification mechanisms with distribution data alone (Leaché et al., 2019; Portik et al., 2017). This difficulty is particularly salient in our study system, where distribution data may have suggested the association of populations with hypothesized refugia around the Congo River, Gabon, and in West Africa (refugia 9, 5–8, and 1–3 respectively, Figs. 1, 2). Yet, our dated phylogenies and paleo-distribution models reject the Pleistocene population age and habitat contraction predictions of

the refugial hypotheses in favor of the river barrier hypothesis. These results highlight the importance of using an integrative, multidisciplinary approach to statistically distinguish among competing hypotheses to explain high levels of geographically concentrated species biodiversity. Moving beyond pure pattern-based inference, a deeper and more nuanced understanding of the production, partitioning, and maintenance of diversity in complex landscapes may lead to inference of environmental and evolutionary processes that accumulate terrestrial biodiversity in tropical areas, which coincide in many cases with Global Biodiversity Conservation Hotspots (Hrdina, & Romportl, 2017; Mittermeier, Myers, & Mittermeier, 2000; Mittermeier, Turner, Larsen, Brooks, & Gascon, 2011; Myers, 1988) and other imperiled ecosystems of Earth.

ACKNOWLEDGMENTS

We would like to thank the University of Kansas Biodiversity Institute and the Office of Graduate Studies for funding associated with ddRAD sequencing, and Marlon Cobos for advice regarding niche modeling. We are grateful to all of the people and institutions that lent tissues and extractions for this study, including: Lauren Scheinberg at California Academy of Sciences, Seth Parker at Louisiana State University Museum of Natural Science, Breda Zimkus at Museum of Comparative Zoology, and Addison Wynn at United States National Museum of Natural History. The Brown Lab was supported by the National Science Foundation (DEB 1654388 and 1557053) during the course of genomic data collection at KU. Partial funding for Illumina sequencing in this study came from the Docking Faculty Scholar Fund of the College of Liberal Arts and Sciences and the Biodiversity Institute of the University of Kansas. EG and CK thank their field companions W.M. Muninga, M.M. Aristote, M. Zigabe, A.M. Marcel, M. Luhumyo, J., and F. Akuku, F.I. Alonda, and the late A. M'Mema. The Centre de Recherche en Sciences Naturelles and Institut Congolais pour la Conservation de la Nature provided project support and permits. EG was supported by the Percy Sladen Memorial Fund, an IUCN/SSC Amphibian Specialist Group Seed Grant, K. Reed, M.D., research funds from the Department of Biology at Villanova University, two National Geographic Research and Exploration Grants (nos. 8556-08 and WW-R018-17), the University of Texas at El Paso, and the US National Science Foundation (DEB-1145459). PMH thanks the KU Biodiversity Institute for postdoctoral fellowship support. MOR received funding from the German Research Foundation (DFG VE 183/4-1 & RO 3064/1-2) and the Federal Ministry of Education and Research (BMBF-Project W08 BIOTA-West, 01LC0017). JP is thankful to Michael Barej as well as to D. Langlois, Rene, S. Mazumdar, M. Machon, D. Maougnan, R. Konomou, Marcel, A. Oumar, Mohamed, Ibrahim, Sao Papa, Bayo, Pepe, Daniel, Samuel, Fulton for assistance with field work as well as the Guinean authorities for all necessary permits.

References

Aiello-Lammens, M. E., Boria, R. A., Radosavljevic, A., Vilela, B., & Anderson, R. P. (2015). spThin: An R package for spatial thinning of species occurrence records for use in ecological niche models.*Ecography, 38* , 541–545.

Akani, G. C., Barieenee, I. F., & Luiselli, L. (1998). Observations on habitat, reproduction and feeding of *Boiga blandingii*(Colubridae) in south-eastern Nigeria. *Amphibia-Reptilia, 19,*430–436.

Allen, A. P., & Gillooly, J. F. (2006). Assessing latitudinal gradients in speciation rates and biodiversity at the global scale. *Ecology Letters* , *9* , 947–954.

Allen, K. E., N. Tapondjou, W. P., Greenbaum, E., Welton, L. J., & Bauer, A. M. (2019). High levels of hidden phylogenetic structure within Central and West African *Trachylepis* skinks. *Salamandra* ,*55* , 231–234.

Amorim, D. D. S. (1991). Refuge model simulations: Testing the theory.*Revista Brasileira de Entomologia* , *35* , 803–812.

Anderson, R. P., Lew, D., & Peterson, A. T. (2003). Evaluating predictive models of species' distributions: Criteria for selecting optimal models. *Ecological Modelling* , *162* , 211–232.

Anthony, N. M., Johnson-Bawe, M., Jeffrey, K., Clifford, S. L., Abernethy, K. A., Tutin, C. E., ... Bruford,

M. W. (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.

Avise, J. C. (2000). *Phylogeography: The history and formation of species* . Cambridge, MA: Harvard University Press.

Ayres, J.M.C. & Clutton-Brock, T.H. (1992). River boundaries and species range size in Amazonian primates. *American Naturalist ,140* , 531– 537.

Barej, M. F., Schmitz, A., Menegon, M., Hillers, A., Hinkel, H., Boehme, W., & Rödel, M.-O. (2011). Dusted off–the African *Amietophrynus superciliaris* species complex of giant toads. *Zootaxa ,2772* , 1–32.

Bates, H.W. (1863). *The Naturalist on the River Amazon.* London, UK: John Murray.

Bell, R. C., Parra, J. L., Badjedjea, G., Barej, M. F., Blackburn, D. C., Burger, M., ... Zamudio, K. R. (2017). Idiosyncratic responses to climate-driven forest fragmentation and marine incursions in reed frogs from Central Africa and the Gulf of Guinea Islands.*Molecular Ecology , 26* , 5223–5244.

Blackburn, D. C. (2008). Biogeography and evolution of body size and life history of African frogs: Phylogeny of squeakers (*Arthroleptis*) and long-fingered frogs (*Cardioglossa*) estimated from mitochondrial data. *Molecular Phylogenetics and Evolution , 49* , 806–826.

Born, C., Alvarez, N., McKey, D., Ossari, S., Wickings, E. J., Hossaert-McKey, M., & Chevallier, M.-H. (2011). Insights into the biogeographical history of the Lower Guinea Forest Domain: Evidence for the role of refugia in the intraspecific differentiation of*Aucoumea klaineana* . *Molecular Ecology , 20* , 131–142.

Bohoussou, K. H., Cornette, R., Akpatou, B., Colyn, M., Peterhans, J. K., Kennis, J., .. . Nicolas, V. (2015). The phylogeography of the rodent genus *Malacomys* suggests multiple Afrotropical Pleistocene lowland forest refugia. *Journal of Biogeography , 42* , 2049–2061.

Bonnefille, R. & Riollet, G. (1988). The Kashiru pollen sequence (Burundi): Paleoclimatic implications for the last 40,000 yr BP in tropical Africa. *Quaternary Research , 30* , 19–35.

Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchene, S., Fourment, M., Gavryushkina, A., ... Matschiner, M. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis.*PLoS Computational Biology , 15* , e1006650.

Brenac, P. (1988). Evolution de la vegetation et du climat dans l'Ouest Cameroun entre 25.000 et 11.000 ans BP. Actes Xeme Symposium Ass. Palynologues Langue Franwise. *Travaux de la Section des Sciences & Techniques de l'Institut Francais de Pondichery , 25* , 91–103.

Buckley, L. B., Davies, T. J., Ackerly, D. D., Kraft, N. J., Harrison, S. P., Anacker, B. L., ... McCain, C. M. (2010). Phylogeny, niche conservatism and the latitudinal diversity gradient in mammals. *Proceedings of the Royal Society B: Biological Sciences , 277* , 2131–2138.

Budde, K. B., Gonzalez-Martinez, S. C., Hardy, O. J., & Heuertz, M. (2013). The ancient tropical rainforest tree *Symphonia globulifera* L. f. (Clusiaceae) was not restricted to postulated Pleistocene refugia in Atlantic Equatorial Africa. *Heredity ,111* , 66–76.

Burbrink, F. T., Lawson, R., & Slowinski, J. B. (2000). Mitochondrial DNA phylogeography of the polytypic North American Rat Snake (*Elaphe obsoleta*): A critique of the subspecies concept.*Evolution , 54* , 2107–2118.

Cardillo, M., Orme, C. D. L. & Owens, I. P. F. (2005). Testing for latitudinal bias in diversification rates: An example using New World birds. *Ecology , 86* , 2278–2287.

Carstens, B. C., & Richards, C. L. (2007). Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution: International Journal of Organic Evolution , 61* , 1439–1454.

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics.*Molecular Ecology* , *22* , 3124–3140.

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer data sets. *Gigascience* , *4* , 7.

Charles, K. L., Bell, R. C., Blackburn, D. C., Burger, M., Fujita, M. K., Gvoždík, V., ... Portik, D. M. (2018). Sky, sea, and forest islands: Diversification in the African leaf-folding frog*Afrixalus paradorsalis* (Anura: Hyperoliidae) of the Lower Guineo-Congolian rainforest. *Journal of Biogeography* , *45* , 1781–1794.

Chifman, J., & Kubatko, L. (2014). Quartet inference from SNP data under the coalescent model. *Bioinformatics* , *30* , 3317–3324.

Clifford, S. L., Anthony, N. M., Bawe-Johnson, M., Abernethy, K. A., Tutin, C. E., White, L. J., ... Wickings, E. J. (2004). Mitochondrial DNA phylogeography of western lowland gorillas (*Gorilla gorilla gorilla*). *Molecular Ecology* , *13,*1551–1565.

Chippaux, J. P., & Jackson, K. (2019). *Snakes of Central and Western Africa* . Baltimore, MD: John Hopkins University Press.

Cobos, M. E., Peterson, A. T., Barve, N., & Osorio-Olvera, L. (2019). Kuenm: An R package for detailed development of ecological niche models using Maxent. *PeerJ* , *7* , e6281.

Colinvaux, P. A., De Oliveira, P. E., Moreno, J. E., Miller, M. C., & Bush, M. B. (1996). A long pollen record from lowland Amazonia: Forest and cooling in glacial times. *Science* , *274* , 85–88.

Colinvaux, P. A., Irion, G., Rasanen, M. E., Bush, M. B., & De Mello, J. N. (2001). A paradigm to be discarded: Geological and paleoecological data falsify the Haffer & Prance refuge hypothesis of Amazonian speciation. *Amazoniana* , *16* , 609–646.

Colyn, M. M. (1987). Les Primates des forets ombrophiles de la cuvette du Zaire: Interpretations zoogeographiques des modeles de distribution.*Revue de Zoologie Africaine, Tervuren, 101* , 183–96.

Colyn, M. M. (1991). L'importance zoogeographique du bassin du fleuve Zaire pour la speciation: Le cas des Primates Siens. *Annales Musee Royal de I; Afrique Centrale, Section Zoologie, Tervuren, 264* , 1– 250.

Colyn, M., Gautier-Hion, A. & Verheyen, W. (1991). A re-appraisal of palaeoenvironmental history in Central Africa: Evidence for a major fluvial refuge in the Zaire Basin. *Journal of Biogeography* ,*18* , 403–407.

Cracraft, J. (1985). Historical biogeography and patterns of differentiation within the South American avifauna: Areas of endemism. *Ornithological Monographs* , *36* , 49–84.

Dainou, K., Bizoux, J.-P., Doucet, J.-L., Mahy, G., Hardy, O. J., & Heuertz, M. (2010). Forest refugia revisited: NSSRs and cpDNA sequences support historical isolation in a wide-spread African tree with high colonization capacity, *Milicia excels* (Moracea). *Molecular Ecology* , *19* , 4462–4477.

Darlington, P. J., Jr. (1957). *Zoogeography and the Geographical Distribution of Animals* . New York, NY: John Wiley & Sons, Inc.

Darwin, C.R. (1859). *The Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* . London, UK: John Murray.

Dauby, G., Duminil, J., Heuertz, M., & Hardy, O. J. (2010). Chloroplast DNA polymorphism and phylogeography of a Central African tree species widespread in mature rainforest: *Greenwayodendron suaveolens*(Annonaceae). *Tropical Plant Biology* , *3* , 4–13.

Dauby, G., Duminil, J., Heuertz, M., Koffi, G. K., Stevart, T., & Hardy, O. J. (2014). Congruent phylogeographical patterns of eight tree species in Atlantic Central Africa provide insights into the past dynamics of forest cover. *Molecular Ecology* , *23* , 2299–2312.

Debout, G. D. C., Doucet, J.-L., & Hardy, O. J. (2011). Population history and gene dispersal inferred from spatial genetic structure of a Central African timber tree, *Distemonanthus benthamianus*(Caesalpinioideae). *Heredity* , *106* , 88–99.

DeMenocal, P. B. (2004). African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth and Planetary Science Letters* , *220* , 3–24.

Devitt, T. J., Devitt, S. E., Hollingsworth, B. D., McGuire, J. A., & Moritz, C. (2013). Montane refugia predict population genetic structure in the Large-blotched *Ensatina* salamander. *Molecular Ecology* , *22* , 1650–1665.

Duminil, J., Mona, S., Mardulyn, P., Doumenge, C., Walmacq, F., Doucet, J.-L., & Hardy, O. J. (2015). Late Pleistocene molecular dating of past population fragmentation and demographic changes in African rain forest tree species supports the forest refuge hypothesis. *Journal of Biogeography* , *42* , 1443–1454.

Ellegren, H. (2014). Genome sequencing and population genomics in non-model organisms. *Trends in Ecology & Evolution* , *29* , 51–63.

Escobar, L. E., Lira-Noriega, A., Medina-Vogel, G., & Peterson, A.T. (2014). Potential for spread of the white-nose fungus (*Pseudogymnoascus destructans*) in the Americas: Use of Maxent and Niche A to assure strict model transference. *Geospatial Health* ,*9* , 221–229.

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., Dupanloup, I., Huerta-Sanchez, E., Sousa, V.C., & Foll, M. (2013). Robust demographic inference from genomic and SNP data.*PLOS Genetics* , *9* , e1003905.

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* , *164* , 1567–1587.

Fagny, M. F., Nkouandou, O. F., Temdjim, R., Bardintzeff, J. M., Guillou, H., Stumbea, D., & Boutale, A. (2016). New K–Ar ages of Tchabal Mbabo alkaline volcano massif, Cameroon Volcanic Line and Adamawa plateau (Central Africa). *International Journal of Advanced Geosciences* , *4* , 62–71.

Faye, A., Deblauwe, V., Mariac, C., Richard, D., Sonke, B., Vigouroux, Y., & Couvreur, T. (2016). Phylogeography of the genus*Podococcus* (Palmae/Arecaceae) in Central African rain forests: Climate stability predicts unique genetic diversity. *Molecular Phylogenetics and Evolution* , *105* , 126–138.

Fine, P.V.A. & Ree, R.H. (2006). Evidence for a time-integrated species-area effect on the latitudinal gradient in tree diversity.*The American Naturalist* , *168* , 796–804.

Fjeldsa, J. (1994). Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodiversity & Conservation* , *3* , 207–226.

Fjeldsa, J., & Bowie, R. C. (2008). New perspectives on the origin and diversification of Africa's forest avifauna. *African Journal of Ecology* , *46* , 235–247.

Fjeldsa, J., & Lovett, J. C. (1997). Geographical patterns of old and young species in African forest biota: The significance of specific montane areas as evolutionary centres. *Biodiversity & Conservation, 6* , 325–346.

Flugel, T. J., Eckardt, F. D., & Cotterill, F. P. (2015). The present day drainage patterns of the Congo river system and their Neogene evolution. In De Wit, M. J., Guillocheau, F., & De Wit, M. C. (Eds.) *Geology and Resource Potential of the Congo Basin* . (pp. 315–337). Berlin, Germany: Springer.

Francis, R. M. (2017). Pophelper: An R package and web app to analyze and visualize population structure. *Molecular Ecology Resources* , *17* , 27–32.

Francois, O. (2016). Running structure-like population genetic analyses with R. *R tutorials in Population Genetics, U. Grenoble-Alpes* , 1–9.

Frichot, E., & Francois, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution* , *6* , 925–929.

Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G. & Francois, O. (2014). Fast and efficient estimation of individual ancestry coefficients. *Genetics* , *196* , 973–983.

Gascon, C., Malcolm, J. R., Patton, J. L., da Silva, M. N., Bogart, J. P., Lougheed, S. C., ... Boag, P. T. (2000). Riverine barriers and the geographic distribution of Amazonian species. *Proceedings of the National Academy of Sciences, USA* , *97* , 13672–13677.

Gaubert, P., Njiokou, F., Ngua, G., Afiademanyo, K., Dufour, S., Malekani, J., ... Antunes, A. (2016). Phylogeography of the heavily poached African common pangolin (Pholidota, *Manis tricuspis*) reveals six cryptic lineages as traceable signatures of Pleistocene diversification. *Molecular Ecology* , *25* , 5975–5993.

Girese, P., Maley, J. & Brenac, P. (1994). Late Quaternary palaeoenvironments in the Lake Barombi Mbo (Cameroon) deduced from pollen and carbon isotopes of organic matter. *Palaeogeography, Palaeoclimatology, Palaeoecology, 107* , 65–78.

Gomez, C., Dussert, S., Hamon, P., Hamon, S., de Kochko, A., & Poncet, V. (2009). Current genetic differentiation of *Coffea canephora*Pierre ex. A. Froehn in Guineo-Congolian African zone: Cumulative impact of ancient climatic changes and recent human activities. *BMC Evolutionary Biology* , *9* , 167.

Gonder, M. K., Locatelli, S., Ghobrial, L., Mitchell, M. W., Kujawski, J. T., Lankester, F. J., ... Tishkoff, S. A. (2011). Evidence from Cameroon reveals differences in the genetic structure and histories of chimpanzee populations. *Proceedings of the National Academy of Sciences, USA* , *108* , 4766–4771.

Goudie, A. S. (2005). The drainage of Africa since the Cretaceous.*Geomorphology* , *67* , 437–456.

Greenbaum E. (2017). *Emerald Labyrinth: A Scientist's Adventures in the Jungles of the Congo* . Lebanon, NH: University Press of New England.

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics* , *5* , e1000695.

Haffer, J. R. (1969). Speciation in Amazonian forest birds.*Science* , *165* , 131–137.

Haffer, J. R. (1974). Avian speciation in tropical South America. *Publications of the Nuttall Ornithological Club, 14* , 1–390.

Haffer, J. R. (1982). General aspects of the refuge theory. In Prance, G. T. (Ed.) *Biological Diversification in the Tropics* . (pp. 6–24). New York, NY: Columbia University Press.

Haffer, J. R. (1992). On the "river effect" in some forest birds of southern Amazonia. *Boletim do Museu Paraense Emilio Goeldi. Nova serie. Zoologia* , *8* , 217–245.

Haffer, J. R. (1993). Time's cycle and time's arrow in the history of Amazonia. *Compte Rendu des Seances de la Societe de Biogeographie* , *69* , 15–45.

Haffer, J. R. (1997). Alternative models of vertebrate speciation in Amazonia: An overview. *Biodiversity and Conservation* , *6* , 451–476.

Haffer J. R. (2008). Hypotheses to explain the origin of species in Amazonia. B*razilian Journal of Biology* , *68* , 917– 947.

Hampe, A., & Jump, A. S. (2011). Climate relicts: Past, present, future. *Annual Review of Ecology, Evolution, and Systematics* , *42* , 313–333.

Harcourt, A. H., & Wood, M. A. (2012). Rivers as barriers to primate distributions in Africa. *International Journal of Primatology* , *33* , 168–183.

Hardy, O. J., Born, C., Budde, K., Dainou, K., Dauby, G., Duminil, J., ... Poncet, V. (2013). Comparative phylogeography of African rain forest trees: A review of genetic signatures of vegetation history in the Guineo-Congolian region. *Comptes Rendus Geoscience* ,*345* , 284–296.

Harrison, S., & Noss, R. (2017). Endemism hotspots are linked to stable climatic refugia. *Annals of Botany* , *119* , 207–214.

Haus, T., Akom, E., Agwanda, B., Hofreiter, M., Roos, C., & Zinner, D. (2013). Mitochondrial diversity and distribution of African green monkeys (*Chlorocebus* Gray, 1870). *American Journal of Primatology* , *75* , 350–360.

Hawkins, B. A., Diniz-Filho, J. A. F., Jaramillo, C. A., & Soeller, S. A. (2007). Climate, niche conservatism, and the global bird diversity gradient. *The American Naturalist* , *170* , S16–S27.

Hawkins, B. A., Porter, E. E., Diniz-Filho, F., & Alexandre, J. (2003). Productivity and history as predictors of the latitudinal diversity gradient of terrestrial birds. *Ecology* , *84* , 1608–1623.

Head, J. J., Mahlow, K., & Muller, J. (2016). Fossil calibration dates for molecular phylogenetic analysis of snakes 2: Caenophidia, Colubroidea, Elapoidea, Colubridae. *Palaeontologia Electronica* , *19* , 1–21.

Herbert, T. D., Lawrence, K. T., Tzanova, A., Peterson, L. C., Caballero-Gill, R., & Kelly, C. S. (2016). Late Miocene global cooling and the rise of modern ecosystems. *Nature Geoscience* , *9* , 843–847.

Hershkovitz, P. (1977). *Living New World Monkeys (Platyrrhini)*.Chicago, IL: University of Chicago Press.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high-resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* , *25* , 1965–1978.

Hime, P. M., Briggler, J. T., Reece, J. S., & Weisrock, D. W. (2019). Genomic data reveal conserved female heterogamety in giant salamanders with gigantic nuclear genomes. *G3: Genes | Genomes | Genetics* , *9* , 3467–3476.

Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* , *35* , 518–522.

Hofer, U., Bersier, L.-F., & Borcard, D. (1999). Spatial organization of a herpetofauna on an elevational gradient revealed by null model tests. *Ecology, 80* , 976–988.

Hofer, U., Bersier, L.-F., & Borcard, D. (2000). Ecotones and gradient as determinants of herpetofaunal community structure in the primary forest of Mount Kupe, Cameroon. *Journal of Tropical Ecology* ,*16* , 517–533.

Hrdina, A., & Romportl, D. (2017). Evaluating global biodiversity hotspots–Very rich and even more endangered. *Journal of Landscape Ecology* , *10* , 108–115.

Hughes, D. F., Kusamba, C., Behangana, M., & Greenbaum, E. (2017). Integrative taxonomy of the Central African forest chameleon,*Kinyongia adolfifriderici* (Sauria: Chamaeleonidae), reveals underestimated species diversity in the Albertine Rift. *Zoological Journal of the Linnean Society* , *181* , 400–438.

Huntley, J. W., Harvey, J. A., Pavia, M., Boano, G., & Voelker, G. (2018). The systematics and biogeography of the Bearded Greenbuls (Aves: Criniger) reveals the impact of Plio-Pleistocene forest fragmentation on Afro-tropical avian diversity. *Zoological Journal of the Linnean Society* , *183* , 672–686.

Huntley, J. W., Keith, K. D., Castellanos, A. A., Musher, L. J., & Voelker, G. (2019). Underestimated and cryptic diversification patterns across Afro-tropical lowland forests. *Journal of Biogeography* , *46* , 381–391.

Hutchinson, G. E. (1959). Homage to Santa Rosalia or why are there so many kinds of animals? *The American Naturalist* , *93* , 145–159.

Jablonski, D., Roy, K. & Valentine, J.W. (2006). Out of the tropics: evolutionary dynamics of the latitudinal diversity gradient. *Science* , *314* , 102–106.

Jacobs, B.F. (2004). Paleobotanical studies from Tropical Africa: Relevance to the evolution of forest, woodland and savannah biomes.*Philosophical Transactions of the Royal Society B* ., *359* , 1573–1583.

Jacquet, F., Nicolas, V., Colyn, M., Kadjo, B., Hutterer, R., Decher, J., ... Denys, C. (2014). Forest refugia and riverine barriers promote diversification in the West African pygmy shrew (*Crocidura obscurior* complex, Soricomorpha). *Zoologica Scripta* , *43* , 131–148.

Jacquet, F., Denys, C., Verheyen, E., Bryja, J., Hutterer, R., Kerbis Peterhans, J. C., .. . Nicolas, V. (2015). Phylogeography and evolutionary history of the *Crocidura olivieri* complex (Mammalia, Soricomorpha): From a forest origin to broad ecological expansion across Africa. *BMC Evolutionary Biology* , *15* , 71.

Jansson, R., & Dynesius, M. (2002). The fate of clades in a world of recurrent climatic change: Milankovitch oscillations and evolution. *Annual Review of Ecology and Systematics* , *33* , 741–777.

Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Naturalist* , *101* , 233–249.

Jetz, W., & Fine, P. V. (2012). Global gradients in vertebrate diversity predicted by historical area-productivity dynamics and contemporary environment. *PLoS Biology* , *10* , e1001292.

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics* , *27* , 3070–3071.

Jongsma, G. F. M., Barej, M. F., Barratt, C. D., Burger, M., Conradie, W., Ernst, R., ... Blackburn, D. C. (2018). Diversity and biogeography of frogs in the genus *Amnirana* (Anura: Ranidae) across sub-Saharan Africa. *Molecular Phylogenetics and Evolution* ,*120* , 274–285.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* , *14* , 587.

Katoh, M., & Kuma, M. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* , *30* , 3059–3066.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... Thierer, T. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* , *28* , 1647–1649.

Knowles, L. L. (2009). Statistical phylogeography. *Annual Review of Ecology, Evolution, and Systematics* , *40* , 593–612.

Knowles, L. L., & Maddison, W. P. (2002). Statistical phylogeography. *Molecular Ecology* , *11* , 2623–2635.

Lamanna, C., Blonder, B., Violle, C., Kraft, N. J., Sandel, B., Šímová, I., ... & Buzzard, V. (2014). Functional trait space and the latitudinal diversity gradient. *Proceedings of the National Academy of Sciences, USA* , *111* , 13745–13750.

Larson, T. R., Castro, D., Behangana, M., & Greenbaum, E. (2016). Evolutionary history of the river frog genus *Amietia* (Anura: Pyxicephalidae) reveals extensive diversification in Central African highlands. *Molecular Phylogenetics and Evolution* , *99* , 168–181.

Lawson, R., Slowinski, J.B., Crother, B.I., & Burbrink, F.T. (2005). Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* , *37* , 581–601.

Leaché, A. D., & Fujita, M. K. (2010). Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*).*Proceedings of the Royal Society of London B: Biological Sciences* , 277, 3071–3077.

Leaché, A. D., Grummer, J. A., Miller, M., Krishnan, S., Fujita, M. K., Böhme, W., ... Ofori-Boateng, C. (2017). Bayesian inference of species diffusion in the West African *Agama agama* species group (Reptilia, Agamidae). *Systematics and Biodiversity* , *15* , 192–203.

Leache, A. D., Harris, R. B., Rannala, B., & Yang, Z. (2014). The influence of gene flow on species tree estimation: A simulation study.*Systematic Biology* , *63* , 17–30.

Leaché, A. D., Portik, D. M., Rivera, D., Rödel, M.-O., Penner, J., Gvoždík, V., ... Fujita, M.K. (2019). Exploring rain forest diversification using demographic model testing in the African foam-nest treefrog *Chiromantis rufescens* . *Journal of Biogeography* ,*46* , 2706–2721.

Levinsky, I., Araujo, M. B., Nogues-Bravo, D., Haywood, A. M., Valdes, P. J., & Rahbek, C. (2013). Climate envelope models suggest spatio-temporal co-occurrence of refugia of African birds and mammals. *Global Ecology and Biogeography* , *22* , 351–363.

Ley, A. C., Dauby, G., Kohler, J., Wypior, C., Roser, M., & Hardy, O. J. (2014). Comparative phylogeography of eight herbs and lianas (Marantaceae) in central African rainforests. *Frontiers in Genetics* , *5* , 403.

Ley, A. C., Heuertz, M., & Hardy, O. J. (2016). The evolutionary history of Central African rain forest plants: Phylogeographical insights from sister species in the climber genus *Haumania*(Marantaceae). *Journal of Biogeography* , *44* , 308–321.

Lowe, A. J., Harris, D., Dormontt, E., & Dawson, I. K. (2010). Testing putative African tropical forest refugia using chloroplast and nuclear DNA phylogeography. *Tropical Plant Biology* , *3* , 50–58.

Luikart, G., England, P. R., Tallmon, D., Jordan, S., & Taberlet, P. (2003). The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics* , *4* , 981–994.

Maley, J. (1987). Fragmentation de la foret dense humide africaine et extension des biotopes montagnards au Quatemaire recent: Nouvelles donnees polliniques et chronologiques. Implications paleo-climatiques et biogeographiques. *Palaeoecology of Africa* , *18* , 307–334.

Maley, J. (1989). Late Quaternary climatic changes in the African rain forest: Forest refugia and the major role of sea surface temperature variations. In Leinen, M. & Sarnthein, M. (Eds.) *Paleoclimatology and Paleometeorology: Modern and Past Patterns of Global Atmospheric Transport* (pp. 585–616). Dordrecht, Netherlands: Springer.

Maley, J. (1991). The African rain forest vegetation and palaeoenvironments during late Quaternary. *Climatic Change* ,*19* , 79–98.

Maley, J. (1996). The African rain forest–main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proceedings of the Royal Society of Edinburgh* ,*104* , 31–73.

Maley, J., & Brenac, P. (1987). Analyses polliniques preliminaires du Quaternaire recent de l'Ouest Cameroun: Mise en evidence de refuges forestiers et discussion des problemes paleoclimatiques. *Memoires et Travaux de l'Institut de Montpellier* , *17* , 129–142.

Maley, J., & Brenac, P. (1998). Vegetation dynamics, palaeoenvironments and climatic changes in the forests of western Cameroon during the last 28,000 years BP. *Review of Palaeobotany and Palynology* ,*99* , 157–187.

Maley, J., & Livingstone, D. A. (1983). Extension d'un element montagnard dans le Sud du Ghana (Afrique de l'Ouest) au Pleistocene superieur et a l'Holocene inferieur: Premieres donnees polliniques. *Comptes Rendus de l'Academie des Sciences* , *Paris* ,*296* , 1287–1292.

Marks, B. D. (2010). Are lowland rainforests really evolutionary museums? Phylogeography of the green hylia (*Hylia prasina*) in the Afrotropics. *Molecular Phylogenetics and Evolution* , *55* , 178–184.

Mayr, E. (1942). *Systematics and the Origin of Species from the Viewpoint of a Zoologist.* New York, NY: Columbia University Press.

Mayr, E., & O'Hara, R. J. (1986). The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. *Evolution* , *40* , 55–67.

Menegon, M., Loader, S. P., Marsden, S. J., Branch, W. R., Davenport, T. R. B., & Ursenbacher, S. (2014). The genus *Atheris* (Serpentes: Viperidae) in East Africa: Phylogeny and the role of rifting and climate in shaping the current pattern of species diversity. *Molecular Phylogenetics and Evolution* , *79* , 12–22.

Mitchell, M. W., Locatelli, S., Ghobrial, L., Pokempner, A. A., Clee, P. R. S., Abwe, E. E., ... & Fotso, R. (2015). The population genetics of wild chimpanzees in Cameroon and Nigeria suggests a positive role for selection in the evolution of chimpanzee subspecies. *BMC Evolutionary Biology* , *15* , 3.

Mittermeier, R. A., Myers, N., & Mittermeier, C. G. (2000).*Hotspots: Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions.* Chicago, IL: University of Chicago Press.

Mittermeier, R. A., Turner, W. R., Larsen, F. W., Brooks, T. M., & Gascon, C. (2011). Global biodiversity conservation: The critical role of hotspots. In Zachos, F. E. & Habel, J. C. (Eds.), *Biodiversity Hotspots – Distribution and Protection of Conservation Priority Areas* . (pp. 3–22). Berlin, Germany: Springer.

Myers, N. (1988). Threatened biotas: "Hot spots" in tropical forests. *Environmentalist* , *8* , 187–208.

Nagy, Z. T., Kusamba, Z. C., Tungaluma, G. G., Lokasola, A. L., Kolby, J., & Kielgast, J. (2011). Foraging acrobatics of *Toxicodryas blandingii* in the Democratic Republic of the Congo. *Herpetology Notes* , *4* , 91–92.

Nicolas, V., Missoup, A. D., Denys, C., Kerbis Peterhans, J., Katuala, P., Couloux, A., & Colyn, M. (2011). The roles of rivers and Pleistocene refugia in shaping genetic diversity in *Praomys misonnei* in tropical Africa. *Journal of Biogeography* , *38* , 191–207.

Nicolas, V., Missoup, A. D., Colyn, M., Cruaud, C., & Denys, C. (2012). West-Central African Pleistocene lowland forest evolution revealed by the phylogeography of Misonne's soft-furred mouse. *African Zoology* , *47* , 100–112.

Nguyen, L. T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2014). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* ,*32* , 268–274.

Paris, J. R., Stevens, J. R., & Catchen, J. M. (2017). Lost in parameter space: A road map for stacks. *Methods in Ecology and Evolution* , *8* , 1360–1373.

Patton, J. L., & Silva, M. F. (2005). The history of Amazonian mammals: Mechanisms and timing of diversification. In Bermingham, E., Dick, C.W., & Moritz, C. (Eds.) *Tropical rainforests: Past, present and future.* (pp. 107–126). Chicago, IL: The University of Chicago Press.

Penner, J., Wegmann, M., Hillers, A., Schmidt, M., & Rodel, M.-O. (2011). A hotspot revisited - a biogeographical analysis of West African amphibians. *Diversity and Distribution, 17* , 1077–1088.

Penner, J. Augustin, M., & Rodel, M.-O. (2019) Modelling the spatial baseline for amphibian conservation in West Africa. *Acta Oecologica, 84* , 31–40.

Prance, G.T. (1982). *Biological Diversification in the Tropics* . New York, NY: Columbia University Press.

Richardson, J. E., Pennington, R. T., Pennington, T. D., & Hollingsworth, P. M. (2001). Recent and rapid diversification of a species-rich genus of Neotropical trees. *Science* , *293* , 2242–2245.

Peterson, A.T., Papes, M., & Soberon, J. (2008). Rethinking receiver operating characteristic analysis applications in ecological niche modeling. *Ecological Modelling* , *213* , 63–72.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for *de novo* discovery and genotyping in model and non-model species.*PLoS ONE* , *7* , e37135.

Petkova, D., Novembre, J., & Stephens, M. (2016). Visualizing spatial population structure with estimated effective migration surfaces.*Nature Genetics* , *48* , 94–100.

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling* , *190* , 231–259.

Pianka, E. R. (1966). Latitudinal gradients in species diversity: A review of concepts. *American Naturalist* , *100* , 33–46.

Plana, V. (2004). Mechanisms and tempo of evolution in the African Guineo–Congolian rainforest. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* , *359* , 1585–1594.

Porras-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, Á., & Lareu, M. (2013). An overview of STRUCTURE: applications, parameter settings, and supporting software. *Frontiers in Genetics* , *4* , 98.

Portik, D. M., Leaché, A. D., Rivera, D., Barej, M. F., Burger, M., Hirschfeld, M., ... Fujita, M. K. (2017). Evaluating mechanisms of diversification in a Guineo-Congolian tropical forest frog using demographic model selection. *Molecular Ecology* , *26* , 5245– 5263.

Portillo, F., Stanley, E. L., Branch, W. R., Conradie, W., Rodel, M.-O., Penner, J., ... Eli Greenbaum (2019). Evolutionary history of burrowing asps (Lamprophiidae: Atractaspidinae) with emphasis on fang evolution and prey selection. *PloS ONE* , *14* , e0214889.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* ,*155* , 945–959.

Provan, J., & Bennett, K. D. (2008). Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution* , *23* , 564–571.

Pudlo, P., Marin, J. M., Estoup, A., Cornuet, J. M., Gautier, M., & Robert, C. P. (2015). Reliable ABC model choice via random forests. *Bioinformatics* , *32* , 859–866.

Puechmaille, S. J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* , *16* , 608–627.

Pyron, R. A., Burbrink, F. T., & Wiens, J. J. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology* , *13* , 93.

QGIS Development Team (2020). QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* , *67* , 901–904.

Richards, P. W. (1963). Ecological notes on West African vegetation. II. Lowland forest of the southern Bakundu Forest Reserve. *Journal of Ecology* , *51* , 123–149.

Ricklefs, R. E. (2006). Global variation in the diversification rate of passerine birds. *Ecology* , *87* , 2468–2478.

Ricklefs, R. E., & Schluter, D. (1993). Species diversity: Regional and historical influences. In Ricklefs, R. E. & Schluter, D. (Eds.)*Species Diversity in Ecological Communities: Historical and Geographical Perspectives* . (pp. 350–363). Chicago, IL: University of Chicago Press.

Rietkerk, M., Ketner, P., & De Wilde, J. (1995). Caesalpinioideae and the study of forest refuges in Gabon: Preliminary results.*Bulletin du Museum National d'Histoire Naturelle, Paris, Adansonia* , *17* , 95–105.

Robbrecht, E. (1996). Geography of African Rubiaceae with reference to glacial rain forest refuges. In van der Maesen, L. J. G., van der Burgt, X. M. & van Medenbach de Rooy, J.M. (Eds.), *The Biodiversity of African Plants* . (pp. 564–581). Dordrecht, Netherlands: Kluwer.

Rodel, M.-O., Emmrich, M., Penner, J., Schmitz, A., & Barej, M. F. (2014). The taxonomic status of two West African *Leptopelis*species: *L. macrotis* Schiotz, 1967 and *L. spiritusnoctis*Rodel, 2007 (Amphibia: Anura: Arthroleptidae). *Zoosystematics and Evolution* , *90* , 21–31.

Rohde, K. (1992). Latitudinal gradients in species diversity: The search for the primary cause. *Oikos* , *65* , 514–527.

Rosenzweig, M. L. (1995). *Species Diversity in Space and Time* . Cambridge, UK: Cambridge University Press.

Schmidt, K. P. (1923). Contributions to the herpetology of the Belgian Congo based on the collection of the American Museum Congo Expedition, 1909–1915. Part II. Snakes, with field notes by Herbert Lang and James P. Chapin. *Bulletin of the American Museum of Natural History,*49, 1–146

Schunke, A. C., & Hutterer, R. (2005). The variance of variation: Geographic patterns of coat colouration in *Anomalurops* and*Anomalurus* (Mammalia, Rodentia, Anomaluridae). *Bonner Zoologische Beitrage* , *53* , 169–185.

Shafer, A. B., Peart, C. R., Tusso, S., Maayan, I., Brelsford, A., Wheat, C. W., & Wolf, J. B. (2017). Bioinformatic processing of RAD-seq data dramatically impacts downstream population genetic inference. *Methods in Ecology and Evolution* , *8* , 907–917.

Sick, H. (1967). Rios e enchentes na Amazonia como obstaculo para a avifauna. *Atas do Simposio Sobre a Biota Amazonica* , *5* , 495–520.

Siddig, A. A. (2019). Why is biodiversity data-deficiency an ongoing conservation dilemma in Africa? *Journal for Nature Conservation* , *50* , 125719.

Smith, B. T., Seeholzer, G. F., Harvey, M. G., Cuervo, A. M., & Brumfield, R. T. (2017). A latitudinal phylogeographic diversity gradient in birds. *PLoS Biology* , *15* , e2001073.

Smith, M. L., & Carstens, B. C. (2020). Process-based species delimitation leads to identification of more biologically relevant species. *Evolution* , *74* , 216–229.

Smith, M. L., Ruffley, M., Tank, D. C., Sullivan, J., & Carstens, B. C. (2017). Demographic model selection using random forests and the site frequency spectrum. *Molecular Ecology* , *26* , 4562–4573.

Soberon, J., & Peterson, A. T. (2005). Interpretation of models of fundamental ecological niches and species' distributional areas.*Biodiversity Informatics* , *2* , 1–10.

Sosef, M. S. M. (1991). New species of *Begonia* in Africa and their relevance to the study of glacial rain forest refuges.*Wageningen Agricultural University Papers* , *91* , 120–151.

Sowunmi, M. A. (1991). Late Quaternary environments in equatorial Africa: Palynological evidence. *Palaeoecology of Africa* ,*22* , 213–238.

Spawls, S., Howell, K., Hinkel, H., & Menegon, M. (2018). *Field guide to East African reptiles* . New York, NY: Bloomsbury Publishing.

Stankiewicz, J., & de Wit, M. J. (2006). A proposed drainage evolution model for Central Africa—Did the Congo flow east? *Journal of African Earth Sciences* , *44* , 75–84.

Stein, A., Gerstner, K., & Kreft, H. (2014). Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology Letters* , *17* , 866–880.

Stevens, G. C. (1989). The latitudinal gradient in geographical range: How so many species coexist in the tropics. *American Naturalist* ,*133* , 240–256.

Stevens, R. D. (2011). Relative effects of time for speciation and tropical niche conservatism on the latitudinal diversity gradient of phyllostomid bats. *Proceedings of the Royal Society of London B: Biological Sciences* , *278* , 2528–2536.

Stewart, J. R., Lister, A. M., Barnes, I., & Dalén, L. (2010). Refugia revisited: Individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* , *277* , 661–671.

Sukumaran, J., & Holder, M. T. (2010). DendroPy: A Python library for phylogenetic computing. *Bioinformatics* , *26* , 1569–1571.

Swofford, D. L. (2003). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sunderland, MA: Sinauer Associates.

Telfer, P. T., Souquiere, S., Clifford, S. L., Abernethy, K. A., Bruford, M. W., Disotell, T. R., ... Wickings, E. J. (2003). Molecular evidence for deep phylogenetic divergence in *Mandrillus sphinx* .*Molecular Ecology* , *12* , 2019–2024.

Templeton, A. R. (2001). Using phylogeographic analyses of gene trees to test species status and processes. *Molecular Ecology, 10,*779–791.

Terborgh, J. (1973). On the notion of favorableness in plant ecology. *The American Naturalist* , *107* , 481–501.

Terhorst, J., & Song, Y. S. (2015). Fundamental limits on the accuracy of demographic inference based on the sample frequency spectrum. *Proceedings of the National Academy of Sciences, USA* , *112* , 7677– 7682.

Tolley, K. A., Alexander, G. J., Branch, W. R., Bowles, P., & Maritz, B. (2016). Conservation status and threats for African reptiles. *Biological Conservation* , *204* , 63–71.

Tosi, A. J. (2008). Forest monkeys and Pleistocene refugia: A phylogeographic window onto the disjunct distribution of the*Chlorocebus lhoesti* species group. *Zoological Journal of the Linnean Society* , *154* , 408–418.

Vanzolini, P.E. & Williams, E.E. (1970). South American anoles: geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). *Arquivos de Zoologia, São Paulo* , *19* , 291–298.

Vanzolini, P.E. (1973). Paleoclimates, relief, and species multiplication in equatorial forests. In Meggers, B. J., Ayensu, E. S., & Duckworth, W. D. (Eds.) *Tropical Forest Ecosystems in Africa and South America: A Comparative Review* . (pp. 255–258). Washington D. C.: Smithsonian Press.

Vitorino, L. C., Lima-Ribeiro, M. S., Terribile, L. C., & Collevatti, R. G. (2016). Demographical history and palaeodistribution modelling show range shift towards Amazon Basin for a Neotropical tree species in the LGM. *BMC Evolutionary Biology* , *16* , 213–228.

Wallace, A. R. (1853). *A Narrative of Travels on the Amazon and Rio Negro* . London, UK: Reeve.

Wallace, A. R. (1878). *Tropical Nature, and Other Essays* . London, UK: Macmillan and Company.

Warren, D. L., & Seifert, S. N. (2011). Ecological niche modeling in Maxent: The importance of model complexity and the performance of model selection criteria. *Ecological Applications* , *21* , 335–342.

Weir, J. T. (2006). Divergent timing and patterns of species accumulation in lowland and highland neotropical birds. *Evolution* , *60* , 842–855.

Weir, J. T., & Schluter, D. (2007). The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science* , *315* , 1574–1576.

White, F. (1981). The history of the Afromontane archipelago and the scientific need for its conservation. *African Journal of Ecology* ,*19* , 33–54.

Whittaker, R. J., Nogués-Bravo, D., & Araujo, M. B. (2007). Geographical gradients of species richness: A test of the water-energy conjecture of Hawkins et al. (2003) using European data for five taxa. *Global Ecology and Biogeography* , *16* , 76–89.

Wiens, J. J. & Donoghue, M. J. (2004). Historical biogeography, ecology and species richness. *Trends in Ecology and Evolution* , *19* , 639–644.

Wiens, J. J., Sukumaran, J. S., Pyron, R. A. & Brown, R. M. (2009). Evolutionary and biogeographic origins of high tropical diversity in old world frogs (Ranidae). *Evolution, 64* , 1217–1231.

Wieczorek, A. M., Drewes, R. C., & Channing, A. (2000). Biogeography and evolutionary history of *Hyperolius* species: application of molecular phylogeny. *Journal of Biogeography* , *27* , 1231–1243.

Wuster, W., Chirio, L., Trape, J. F., Ineich, I., Jackson, K., Greenbaum, E., ... Hall, C. (2018). Integration of nuclear and mitochondrial gene sequences and morphology reveals unexpected diversity in the forest cobra (*Naja melanoleuca*) species complex in Central and West Africa (Serpentes: Elapidae). *Zootaxa* , *4455* , 68–98.

Yannic, G., Pellissier, L., Ortego, J., Lecomte, N., Couturier, S., Cuyler, C., ... Cote, S. D. (2014). Genetic diversity in caribou linked to past and future climate change. *Nature Climate Change* ,*4* , 132–137.

Zimkus, B. M., Lawson, L. P., Barej, M. F., Barratt, C. D., Channing, A., Dash, K. M., ... & Gvoždík, V. (2017). Leapfrogging into new territory: How Mascarene ridged frogs diversified across Africa and Madagascar to maintain their ecological niche. *Molecular Phylogenetics and Evolution* , *106* , 254–269.

Zimkus, B. M., Rödel, M. O., & Hillers, A. (2010). Complex patterns of continental speciation: molecular phylogenetics and biogeography of sub-Saharan puddle frogs (*Phrynobatrachus*). *Molecular Phylogenetics and Evolution* , *55* , 883–900.

AUTHOR CONTRIBUTIONS

K.E.A., A.T.P., and R.M.B. designed the study. E.G., C.K., M.-O. R, and J.P. provided tissues. K.E.A., P.M.H. and V.V.S. performed the laboratory work. K.E.A., P.M.H and W.P.T.N analyzed the data. K.E.A wrote the manuscript. E.G., P.M.H., J.P., M.-O. R and R.M.B. edited the manuscript.

DATA AVAILABILITY STATEMENTDNA sequences of c-mos and cyt-*b* will be accessioned on Genbank. Raw reads for ddRAD sequences will be re archived in the Dryad Digital Repository at http://datadryad.org. Bioclimatic data and maxent input files will be re archived in the Dryad Digital Repository at http://datadryad.org.

Hosted file

`image1.emf` available at https://authorea.com/users/317799/articles/447907-rivers-not-refugia-drove-diversification-in-arboreal-sub-saharan-african-snakes

Figure 1. Locations of major rivers and hypothesized refugia (labeled 1–10) in West and Central Africa, adapted from Maley (1996).

Hosted file

`image2.emf` available at https://authorea.com/users/317799/articles/447907-rivers-not-refugia-drove-diversification-in-arboreal-sub-saharan-african-snakes

Figure 2. A time-calibrated Bayesian phylogeny for *Toxicodryas*with c-mos and cyt *b* genes.

Highly supported nodes (PP [?]0.9) are denoted with a black circle. Fossil-calibrated nodes are

denoted with an asterisk. Node bars represent 95% confidence intervals. RADseq phylogenies showed identical topologies

Hosted file

`image3.emf` available at https://authorea.com/users/317799/articles/447907-rivers-not-refugia-drove-diversification-in-arboreal-sub-saharan-african-snakes

Figure 3. *Toxicodryas* clade distributions overlaid onto a map of major rivers and hypothesized rainforest refugia. Clade colors correspond to Figure 2.

Hosted file

`image4.emf` available at https://authorea.com/users/317799/articles/447907-rivers-not-refugia-drove-diversification-in-arboreal-sub-saharan-african-snakes

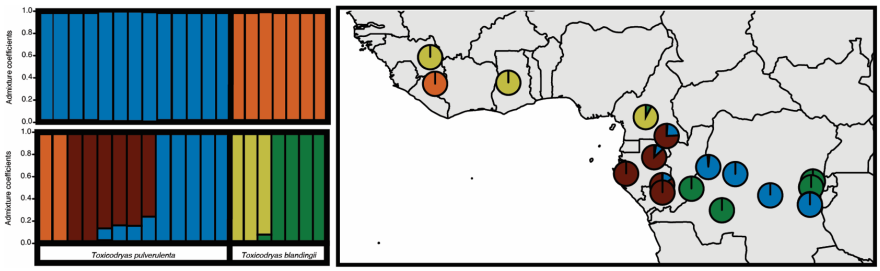

Figure 4. Population structure of the genus *Toxicodryas* . Top: Bar plot of population structure and membership probabilities for K=5 analyzed in LEA. Bottom: Geographic representation of population structure for K=5.

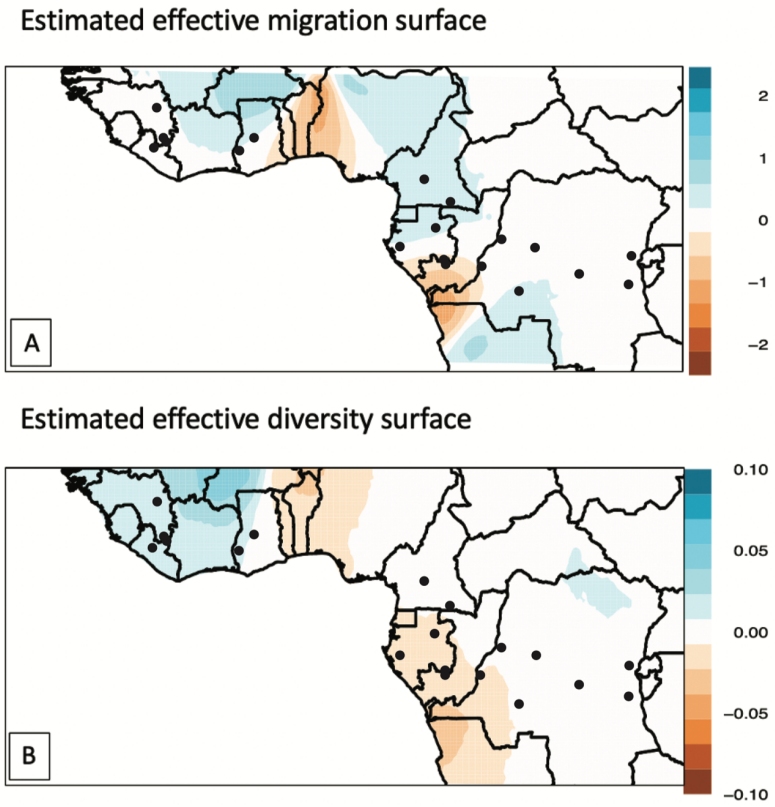

Figure 5. Posterior means for A) the effective estimated migration surface and B) the effective estimated diversity surface for all populations of *Toxicodryas* . In A) blue represents areas of high migration and orange represents areas of low migration, and in B) blue represents areas of high diversity and orange represents areas of low diversity. Genetic diversity and migration rates are plotted on a $\log_{10}$ scale. Sample localities are denoted by black points.

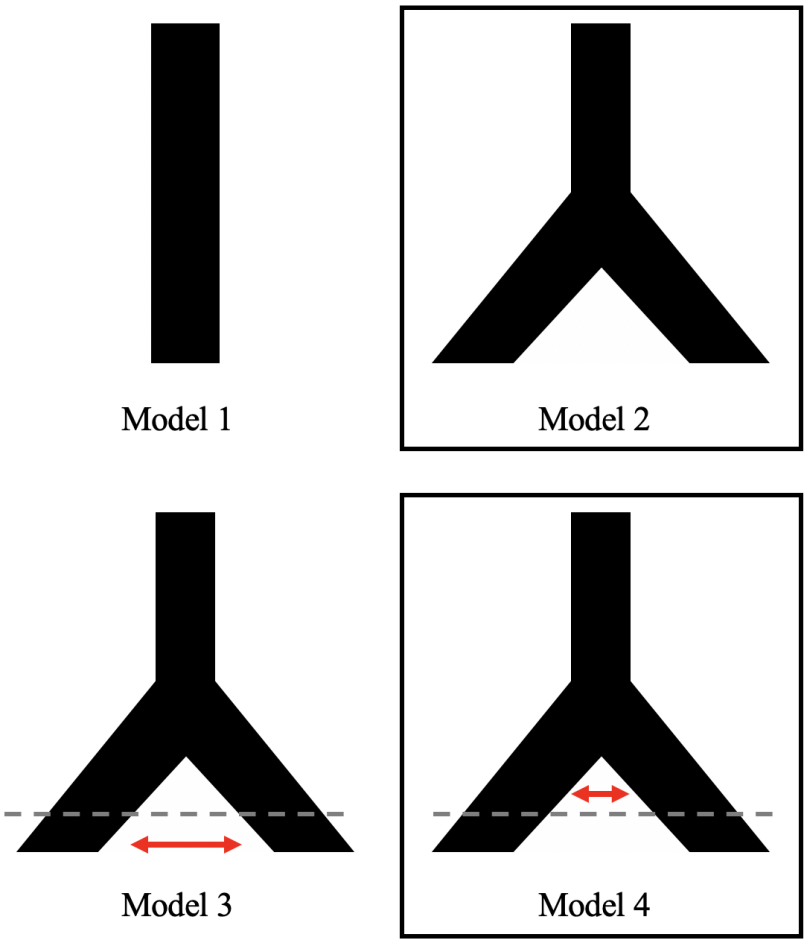

Figure 6. Four demographic models tested using DelimitR. Model 1: no divergence, Model 2: divergence without gene flow, Model 3: divergence with secondary contact, and Model 4: divergence with gene flow. Model 2 was chosen for *Toxicodryas blandingii* , and Model 4 was chosen for the two Central African clades of *T. pulverulenta* .

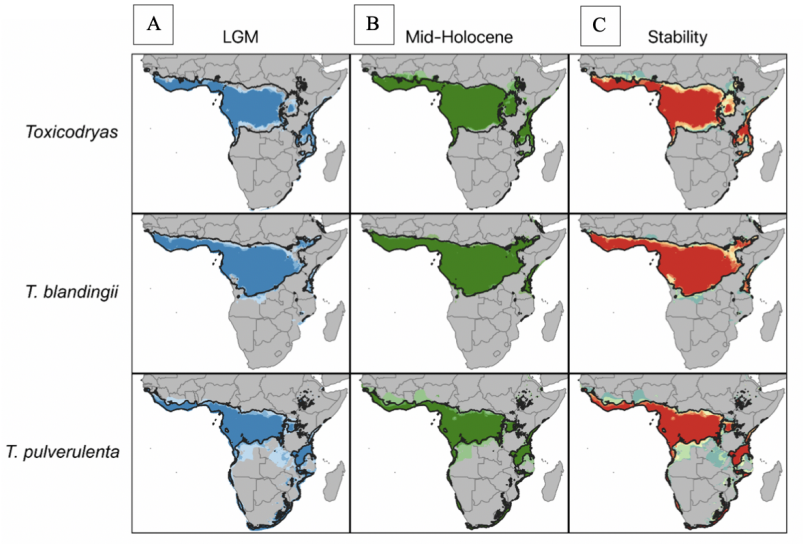

Figure 7. Paleo-distribution models showing A) the suitable habitat for *Toxicodryas* during the last glacial maximum (LGM). The shade of blue represents agreement between global climate models (GCMs) with the darkest blue indicating agreement between all three GCMs and the lightest blue indicating support from only one GCM. B) The suitable habitat for *Toxicodryas* during the mid-Holocene. The shade of green represents agreement between GCMs with the darkest green indicating agreement between all three GCMs and the lightest green indicating support from only one GCM. C) The stability of suitable habitat across the LGM, mid-Holocene, and present, with red indicating high stability and blue indicating low stability.