

A mid-Pleistocene rainforest corridor enabled synchronous invasions of the Atlantic Forest by Amazonian anole lizards

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Abstract

Shifts in the geographic distribution of habitats over time can promote dispersal and vicariance, thereby influencing large-scale biogeographic patterns and ecological processes. An example is that of transient corridors of suitable habitat across disjunct but ecologically similar regions, which have been associated with climate change over time. Such connections likely played a role in the assembly of tropical communities, especially within the highly diverse Amazonian and Atlantic rainforests of South America. Although these forests are presently separated by open and dry ecosystems, paleoclimatic and phylogenetic evidence suggest that they have been transiently connected in the past. However, little is known about the timing, magnitude and the distribution of former forest connections. We employ sequence data at multiple loci from three codistributed arboreal lizards (*Anolis punctatus*, *Anolis ortonii* and *Polychrus marmoratus*) to infer the phylogenetic relationships among Amazonian and Atlantic Forest populations and to test alternative historical demographic scenarios of colonization and vicariance using coalescent simulations and approximate Bayesian computation (ABC). Data from the better-sampled *Anolis* species support colonization of the Atlantic Forest from eastern Amazonia. Hierarchical ABC indicates that the three species colonized the Atlantic Forest synchronously during the mid-Pleistocene. We find support of population bottlenecks associated with founder events in the two *Anolis*, but not in *P. marmoratus*, consistently with their distinct ecological tolerances. Our findings support that climatic fluctuations provided key opportunities for dispersal and forest colonization in eastern South America through the cessation of environmental barriers. Evidence of species-specific histories strengthens assertions that biological attributes play a role in responses to shared environmental change.

Keywords: Amazon, *Anolis*, approximate Bayesian computation, Atlantic Forest, phylogeography

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Introduction

Shifts in the geographic distribution of habitats over time can promote dispersal and vicariance, thereby

influencing large-scale biogeographic patterns and ecological processes. For instance, topographic discontinuities (e.g. mountains, rivers) and environmental barriers (e.g. dry corridors separating wet ecosystems) can prevent the homogenization of species pools while favouring genetic divergence between closely related disjunct taxa (Graham *et al.* 2004). Whenever those barriers are

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disrupted, organisms may have an opportunity to colonize adjacent suitable areas and hence encounter and interact with new species and environments (Petren *et al.* 2005). The ecological opportunities promoted by these habitat shifts may lead to evolutionary radiations (Hughes & Eastwood 2006).

The establishment of suitable habitat corridors across previously disjunct, ecologically similar regions has been widely linked to climate change over time. For example, the allopatric distribution of closely related mountaintop endemics has been tied to downhill dispersal during former cold periods, followed by uphill movement in subsequent warmer times, allowing species to reach nearby peaks (Kozak & Wiens 2006). Similarly, climate-mediated sea level fluctuation has been invoked in cross-island divergences (Brown *et al.* 2013). These cases support the claim that climatic variation plays a major role in the establishment of spatial patterns of biodiversity not only by constraining species ranges (Carnaval *et al.* 2014), but also by enabling species exchange between biologically distinct geographic regions.

Climate-mediated dispersal is thought to have played a key role in the assembly of tropical biotas, including those of Amazonia and the Atlantic Forest in South America, two of the most diverse ecosystems on Earth. These rainforest blocks are currently separated by the Caatinga (xeric scrublands) and the Cerrado (savannas) domains, which prevent dispersal of rainforest-associated organisms. However, there is substantial evidence that Amazonian and Atlantic Forest biotas have been linked through time. Ubiquitous biotic exchange in the past is supported by present-day floristic similarity (Bigarella *et al.* 1975; Santos *et al.* 2007) and disjunct distribution patterns of many animal and plant species (e.g. Fiaschi & Pirani 2009; Gehara *et al.* 2014; Ribeiro-Júnior 2015; Rocha *et al.* 2015). Sister relationships between species and clades restricted to either Amazonia or the Atlantic Forest indicate that *in situ* diversification following dispersal has contributed to the high levels of endemism that characterize both systems (e.g. Costa 2003; Fouquet *et al.* 2012a, b; Batalha-Filho *et al.* 2013). However, limited data are available on the timing, frequency and magnitude of these former connections. Did they happen through narrow corridors of suitable habitat, or was former climate change enough to enable major fusion of the forest domains that are now physically isolated?

Paleoenvironmental data shed light on the climatic drivers of former biotic exchange between major South American rainforests. Oxygen isotope records (Cheng *et al.* 2013), speleothem and travertine deposition patterns (Auler *et al.* 2004) and paleoclimatic simulations (Sobral-Souza *et al.* 2015) suggest that climatic variation through time has favoured pulses of rainforest

expansion. These data support a scenario of intermittent periods of increased precipitation that date back at least 900 ky (Auler *et al.* 2004) and match the oscillations of the precession component of Earth's orbital cycles (every ~20 ky; Cheng *et al.* 2013). It has been hypothesized that phases of increased humidity during the Quaternary may have enabled the establishment of forest corridors between eastern Amazonia and the northern Atlantic Forest (through present-day northeastern Brazil), as well as between southwestern Amazonia and the southern Atlantic Forest (through southwestern Brazil). These two routes are consistent with the distribution patterns of bird species that occur disjunctively in both domains (Cheng *et al.* 2013), as well as with spatial patterns of phylogenetic structure observed among other vertebrate taxa (e.g. Costa 2003; Fouquet *et al.* 2012a, b; Gehara *et al.* 2014). However, most divergence time estimates indicate pre-Quaternary splits between Amazonian and Atlantic Forest lineages (i.e. older than ~2.6 Ma), which precedes the time frame of the paleoenvironmental data available by millions of years (e.g. Costa 2003; Fouquet *et al.* 2012a, b; Batalha-Filho *et al.* 2013; Gehara *et al.* 2014; Rodrigues *et al.* 2014). Here, we present an investigation of intraspecific genetic structure in species that occur in both forests – whose populations presumably diverged more recently – to more effectively address the impact of Late Quaternary climate change on the connectivity of South American rainforests.

To investigate the magnitude of former connections between Amazonia and the Atlantic Forest, we capitalize on the fact that demographic syndromes such as population expansion and bottlenecks leave contrasting genetic signatures in the local biota (Beaumont *et al.* 2002; Fagundes *et al.* 2007; Carnaval *et al.* 2009), and use current patterns of genetic diversity to test alternative biogeographic and demographic hypotheses. Specifically, if short-term or spatially restricted forest corridors enabled movement between Amazonia and the Atlantic Forest, we expect to detect a signal of colonization by a small subset of individuals from the source population into the colonized region. In this case, a population bottleneck would have occurred along with dispersal into the new area, followed by population expansion. Alternatively, long-lasting or extensive connections might have favoured high levels of gene flow between Amazonia and the Atlantic Forest. Subsequent isolation of forest blocks could have led to vicariance and divergence, with no population bottlenecks. Finally, if forest connections happened cyclically – as suggested by the documented climatic fluctuations (Cheng *et al.* 2013) – one also may expect that populations in different forest blocks have experienced some level of gene flow after divergence.

To assess these alternative historical hypotheses, we utilize extensive geographic sampling of three distantly

related lizard species (divergence > 49 Ma; Prates *et al.* 2015): the true anoles *Anolis punctatus* and *Anolis ortonii* (Dactyloidae), and the bush anole *Polychrus marmoratus* (Polychrotidae). These species are similar in their arboreal habits and association with forests, but differ in their tolerance to transitional habitats: *P. marmoratus* explores forest edges, while the two *Anolis* are associated with closed-canopy forests (Vitt *et al.* 2003; Kawashita-Ribeiro & Ávila 2008; Ribeiro-Júnior 2015). The ancestral ranges of these three species have been traced back to Amazonia, but the Amazonian regions that served as sources of expansion remain unclear (Prates *et al.* 2016). To allow proper biogeographic reconstruction, we perform targeted sampling in areas that have likely been connected by forest corridors, as suggested by the available paleoclimatic (Cheng *et al.* 2013; Sobral-Souza *et al.* 2015) and phylogenetic (Costa 2003; Batalha-Filho *et al.* 2013; Gehara *et al.* 2014) data. To test between alternative historical scenarios, we use coalescent simulations, approximate Bayesian computation (ABC; Beaumont 2010; Csilléry *et al.* 2010) and empirical sequence data at six unlinked loci from 159 sampled specimens. Employing a hierarchical ABC approach (Hickerson *et al.* 2007), we then test whether colonization of the Atlantic Forest happened synchronously across the three species.

Material and methods

Molecular sampling

We generated DNA sequences for 102 specimens of *Anolis punctatus* (Dactyloidae), 34 *Anolis ortonii* and 23 *Polychrus marmoratus* (Polychrotidae) collected in Brazil (list of voucher numbers and locality information in Table S1, Supporting information). Our sampling targeted localities in the Atlantic Forest as well as in eastern and southwestern Amazonia, which were presumably connected to the Atlantic Forest through forest corridors (Cheng *et al.* 2013). As outgroups, we sampled two individuals of each of the following species: *A. dissimilis*, *A. fuscoauratus*, *A. phyllorhinus*, *A. scyphus*, *A. tandai*, *A. trachyderma*, *A. transversalis* and *P. liogaster*. The mitochondrial gene *NADH dehydrogenase subunit 2* (ND2) and the flanking *tryptophan transfer RNA* (tRNA-Trp) gene were sequenced as per Jezkova *et al.* (2009). Additionally, five nuclear genes were sequenced: the *recombination-activating gene 1* (RAG1), as per Gartner *et al.* (2013), the KIAA2018 ortholog, following Portik *et al.* (2012), and the *dynein axonemal heavy chain 3* (DNAH3), *nerve growth factor beta polypeptide* (NGFB) and *synuclein alpha-interacting protein* (SNCAIP) following Townsend *et al.* (2008, 2011). Sequences were edited with *Geneious Pro 6* (Biomatters,

Auckland), aligned with the *Geneious* algorithm and deposited in GenBank (Accession numbers KM204350-4, KM598666-749 and KX760196-1163).

Heterozygous positions in nuclear genes were called with *Geneious* plugin *Find Heterozygotes*, using a 0.90 overlap threshold. For coalescent-based analyses, the haplotypic phase of heterozygotes was determined using PHASE 2.1.1 (Stephens & Donnelly 2003), with a 0.90 probability threshold and a parent-independent mutation model. Input files for PHASE were prepared in SEQPHASE (Flot 2010). For phylogenetic analyses, models of nucleotide evolution for each locus were determined with JMODELTEST 2.1 (Darriba *et al.* 2012) implementing the Bayesian information criterion for model selection (Sullivan & Joyce 2005).

Phylogenetic inference

To characterize the historical relationships among samples, which may help to identify former routes of dispersal, we used the multilocus data set to generate phylogenetic trees under a coalescent framework, using the **Beast* tool in BEAST 1.8.3 (Drummond *et al.* 2012). To assign individuals to putative independently evolving lineages (sometimes referred to as 'species'), as required by **Beast*, we used a Bayesian implementation of the *Generalized Mixed Yule-Coalescent model* (GMYC; Pons *et al.* 2006). GMYC models the splits on a phylogeny as either divergences between 'species' or as coalescent events within 'species'. Based on the assumption that the rate of within-species coalescence is much larger than the rate of divergences between species, the method aims to find a threshold that distinguishes these two branching types on a target phylogeny. Its Bayesian implementation (*bGMYC*, Reid & Carstens 2012) accounts for phylogenetic uncertainty and error by integrating over the posterior distribution of trees from a Markov chain Monte Carlo (MCMC) procedure. To delimit putative independently evolving lineages within our target lizard taxa, we implemented *bGMYC* on the highly structured mitochondrial gene trees, also inferred with BEAST. Specifically, *bGMYC* was applied to 500 posterior trees from the mitochondrial BEAST runs. Using the *bgmyc.multiphyl()* function in R 3.0.2, we implemented 50 000 MCMC steps (with 40 000 steps as burn-in), sampling every 100 steps.

In all BEAST analyses (i.e. mitochondrial gene trees and multilocus coalescent-based trees), we ran three independent chains of 100 million steps, sampling every 10 000 steps. Analyses were performed for each target species separately and employed a strict molecular clock. Convergence and stationarity of model parameters were assessed in TRACER 1.6 (available from <http://beast.bio.ed.ac.uk/Tracer>) to ensure effective sample

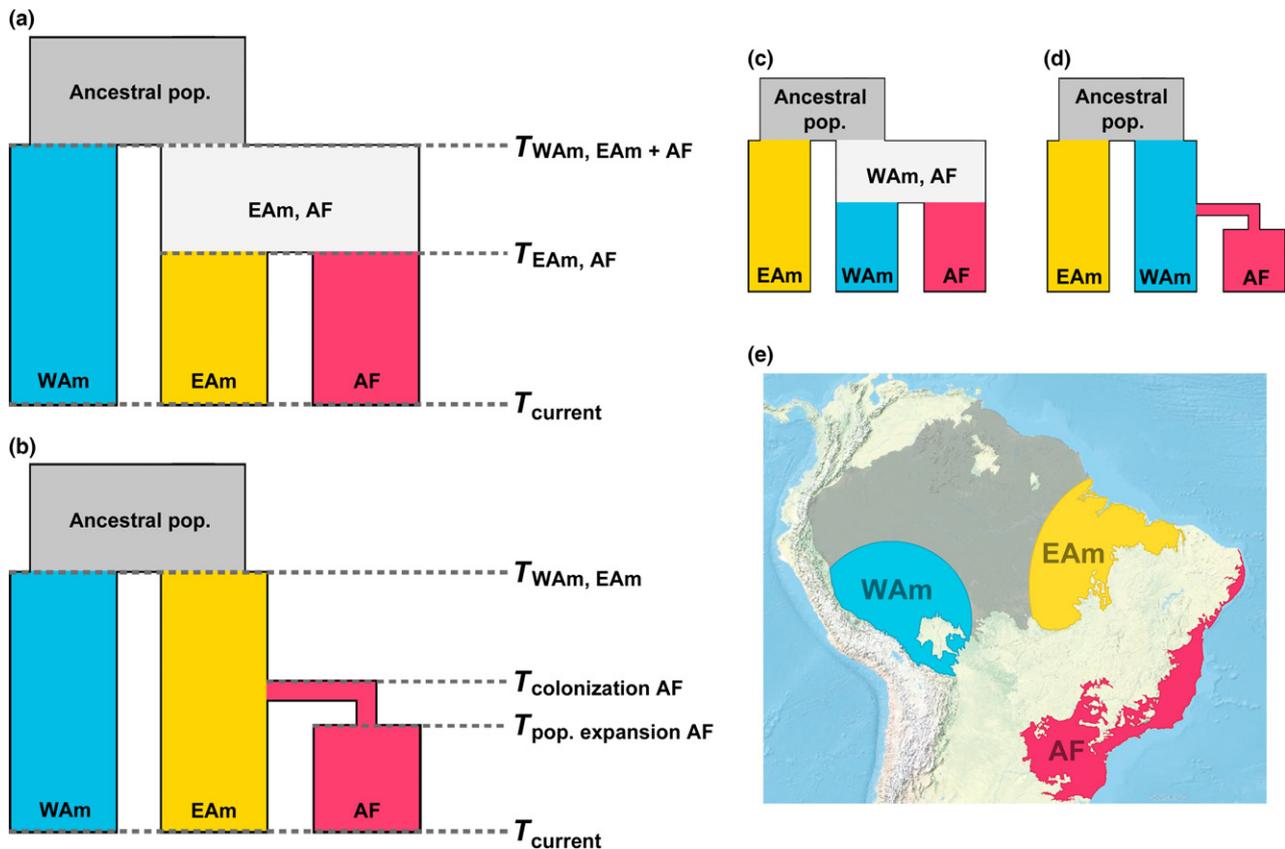


Fig. 1 Demographic scenarios tested with coalescent simulations and approximate Bayesian computation (ABC). (a) Northeastern vicariance: an ancestral population occurring in both eastern Amazonia (EAm) and in the Atlantic Forest (AF) is split due to expansion of open and dry domains in presently dry northeastern South America. (b) Northeastern dispersal: a founder population colonizes the AF from EAm, with population expansion following a bottleneck. Scenarios (c) (southwestern vicariance) and (d) (southwestern dispersal) are similar to (a) and (b), respectively, with the difference that the southwestern (instead of eastern) Amazonia acts as the source of dispersal into the Atlantic Forest. T = time. (e) Map showing the three regions considered in this study. Please refer to on-line version of this manuscript for a color version of this figure.

sizes > 200. Runs were combined in LOG COMBINER 1.8.3, with 10% of each run discarded as burn-in. A maximum clade credibility tree was summarized with TREE ANNOTATOR 1.8 (Drummond *et al.* 2012). Resulting topologies were visualized in FIGTREE 1.4 (available from <http://tree.bio.ed.ac.uk/software/figtree>).

Testing alternative demographic models of dispersal across forest domains

We used coalescent simulations and ABC to test which Amazonian region (eastern vs. southwestern) most likely acted as the colonization source for the Atlantic Forest populations and to test whether colonization was followed by a population bottleneck, as expected as a result of spatially or temporally restricted connections across domains. Our approach hence compared the observed (i.e. sampled) genetic data to data simulated through a coalescent framework under competing demographic scenarios. Both empirical and simulated

sequences were recapitulated into informative summary statistics that capture patterns of genetic variation (Beaumont 2010; Csilléry *et al.* 2010). For each of our three focal species, previous research found that Atlantic Forest samples compose a clade nested among Amazonian ones, suggesting a single invasion of the Atlantic Forest from Amazonia (Prates *et al.* 2015, 2016). We therefore tested alternative historical scenarios that differ by (i) which Amazonian lineages (eastern or southwestern) are more closely related to Atlantic Forest samples, thus potentially indicating connection routes, and (ii) whether there was a founder event during colonization of the Atlantic Forest (indicating a bottleneck followed by pronounced population expansion), or whether population sizes remained relatively constant in each forest block (indicating vicariant subdivision of a large ancestral population). Four scenarios were simulated and compared (Fig. 1). In scenario A (northeast vicariance), an ancestral population occurring in both eastern Amazonia and the northern Atlantic Forest was

split following expansion of open and dry domains in northeastern South America. In scenario B (northeast dispersal), a founding population colonized the northern Atlantic Forest from eastern Amazonia through a forest corridor in the presently dry belt of northeastern South America, with subsequent population expansion within the Atlantic Forest. Scenarios C (southwest vicariance) and D (southwest dispersal) are similar to A and B, respectively, with the difference that southwestern (instead of eastern) Amazonia acted as the source of individuals into the Atlantic Forest (Fig. 1). In the case of *P. marmoratus*, we limited our analyses to scenarios A and B due to the lack of individual samples from southwestern Amazonia.

Individuals from the two better-sampled (*Anolis*) species were assigned to either an eastern or southwestern Amazonian group according to their location in relation to longitude 60°W (east or west). This longitude has been proposed as the approximate region where two major Amazonian macroclimatic systems meet (Cheng *et al.* 2013) and broadly corresponds to a known biogeographic break for several Amazonian taxa (e.g. Hal & Harvey 2002; Geurgas & Rodrigues 2010; Fernandes *et al.* 2013; d'Horta *et al.* 2013). It also marks a region of contact for two genetic groups within the Amazonian *A. punctatus*, as inferred through a genetic clustering analysis (Prates *et al.* 2016).

Previous clustering analyses of *A. punctatus* DNA sequences recovered a major break between eastern and western Amazonian samples, as well as two distinct genetic clusters in western Amazonia: one restricted around the Brazil–Peru border, and the other spanning a much larger range in central and southwestern Amazonia (Prates *et al.* 2016). This pattern was not observed in Amazonian *A. ortonii*, for which substructure was not detected (Prates *et al.* 2016). To ensure that the spatial groups used in our demographic analyses did not violate panmixia, and given the limited availability of samples for the *A. punctatus* cluster occurring at the Brazil–Peru border, our demographic analyses included only samples of the more broadly distributed western *A. punctatus* cluster (i.e. that occurs in central and southwestern Amazonia).

For each lizard species independently, we performed 4 million coalescent simulations (1 million simulations under each of the four demographic scenarios) followed by ABC inference with DIYABC 2.1 (Cornuet *et al.* 2014). For each locus, the following four summary statistics were computed: mean of pairwise genetic difference within groups, Tajima's *D* (Tajima 1989), number of segregating sites between groups, and pairwise F_{st} (Weir & Cockerham 1984), totaling 72 summary statistics (given the six sampled loci and three spatial groups). These summary statistics were chosen based

on preliminary runs which confirmed that (i) they can discriminate among the tested scenarios and (ii) the empirical data were contained within the space of simulated data as outlined by these summary statistics (Cornuet *et al.* 2014). To verify that the observed genetic data were contained within the space of simulated data, we performed a principal component analysis on the simulated summary statistics. Then, we assessed the statistical support of each scenario by calculating Euclidean distances between the observed and each simulated data set, using a logistic regression on the summary statistics (Beaumont 2010). We estimated the posterior probability of each scenario based on the 2000 simulated data sets (0.05% of the total simulations) that were closest to the observed data. Posterior distributions of population parameters were estimated under the best-fit scenario, using the 1000 simulated data sets that were closest to the observed data.

To evaluate the accuracy of the model selection procedure (i.e. to verify whether the alternative scenarios tested can be discriminated based on summary statistics), we simulated 1000 pseudo-observed data sets under each scenario, such that the true scenario was known for each pseudo-observed data set. We used the summary statistics to calculate Euclidean distances between each pseudo-observed data set and the simulated data, based on 1 million simulated data sets. Lastly, we calculated the proportion of pseudo-observed data sets correctly identified by the model selection procedure (Cornuet *et al.* 2014).

Priors for model parameters were set as follows: divergence times between regions ~ uniform [0.1, 5] million years, assuming a generation time of 1 year in anole lizards to convert prior values from number of generations to years (Jezkova *et al.* 2009; Tollis *et al.* 2012; Muñoz *et al.* 2013); effective population size in each region ~ uniform [0.01, 5] million; effective population size during a founder event ~ uniform [0.001, 0.1] million; and effective population size prior to a vicariant event ~ uniform [0.01, 5] million. An HKY85 substitution model (Hasegawa *et al.* 1985) was implemented for each gene. Mutation rate priors were set as ~ uniform [10^{-11} , 10^{-9}] per site per generation for the five nuclear loci, and as ~ uniform [10^{-9} , 10^{-7}] per site per generation for the mitochondrial locus. Prior ranges for mutation rates were based on the number of substitutions among sequences of *Anolis* and *Polychrus* species and on estimated divergence times between these species based on fossil calibrations (Prates *et al.* 2015). In each simulation, the time of Atlantic Forest colonization was constrained to be more recent than the time of coalescence between the eastern and southwestern Amazonian groups. Likewise, the time of a population bottleneck in the Atlantic Forest was constrained to be

more recent than the time of coalescence between the Atlantic Forest group and its Amazonian source.

Testing synchronous dispersal between species across forest domains

After determining which Amazonian region was the most likely source of Atlantic Forest colonization, we used a hierarchical approximate Bayesian computation approach to test whether colonization of the Atlantic Forest happened synchronously in these three species. With that, we compared the sampled genetic data to data simulated under alternative divergence models differing in their combinations of synchronous or idiosyncratic divergence times between pairs of populations of distinct species. We used *dpp-msbayes* as implemented in the PYMSBAYES package (Oaks 2014), an extension of *msbayes* (Huang *et al.* 2011) which implements a Dirichlet process over the hyperprior specifying the number of divergence events (Oaks *et al.* 2013). A total of 2 million simulations were performed, and the posterior probability of alternative divergence models was estimated based on the 1000 (0.05%) simulated data sets that were closest to the observed data. We did not resort taxa and did not apply a regression correction method because these procedures may bias *msbayes* model results (Oaks *et al.* 2013; Oaks 2014).

Posterior estimates of demographic parameters from DIYABC analyses were used as a guide to set prior distributions in *dpp-msbayes*, yet modified to encompass broader intervals to better account for uncertainty and error in DIYABC estimates, as follows: tau \sim gamma [1, 0.0], based on estimates of mutation rates and divergence times between populations; theta \sim gamma [1, 0.005], based on estimates of mutation rates and population sizes; and the magnitude of population bottlenecks \sim beta [1, 1], which allows a wide range of bottlenecks strengths, including no bottleneck. We allowed theta and the magnitude of bottlenecks to differ between each pair of populations. The substitution rate of the mitochondrial locus was set as 10 times faster than that of nuclear genes (from DIYABC estimates), and the transition-to-transversion rate ratio of the HKY substitution model (Hasegawa *et al.* 1985), implemented in *dpp-msbayes*, was estimated for each locus of each species separately using JMODELTEST 2.1 (Darrriba *et al.* 2012). The concentration parameter of the Dirichlet process hyperprior was set as \sim gamma [1000, 0.0014], such that equal prior probability was assigned to divergence models where all or no taxa diverged synchronously, and such that higher prior probability was assigned to models with intermediate numbers of divergence events (as there are three possible models in which two of three taxa codiverge). These settings rendered our analysis

conservative with regard to the occurrence of synchronous divergences between the three target species.

To test the hypothesis of divergence followed by gene flow between Amazonia and the Atlantic Forest, a result expected if forest connections were recurrent through time, we compared the statistical fit of divergence models that incorporated migration to those that did not. In this case, the migration rate prior was set as \sim gamma [1, 0.01], while the remaining model parameter settings were kept the same.

To test whether there is a bias towards synchronous divergences in our *dpp-msbayes* analyses, we simulated 1000 pseudo-observed data sets under a scenario of asynchronous divergences (i.e. enforcing three independent divergence times), which were then analysed under the scenario used for inference (described above), based on 500 000 simulated data sets. With that, we assessed the frequency of (i) the number of inferred divergence events (which was known to be three) and (ii) the posterior probability of one synchronous divergence (which was known to be incorrect). Moreover, to assess the robustness of our estimates of the dispersal index of divergence times (the variance/mean of the divergence times across all population pairs) to the inclusion of a migration parameter, we simulated 1000 pseudo-observed data sets under the scenario incorporating migration, which were then analysed under the scenario of no migration based on 500 000 simulated data sets.

Results

Phylogenetic structure

The *bGMYC* analyses identified 10 putative independently evolving lineages within *Anolis punctatus*, 17 in *Anolis ortonii* and seven in *Polychrus marmoratus*. Most lineages were restricted to the Atlantic Forest, eastern Amazonia or southwestern Amazonia. A single lineage encompasses all Atlantic Forest samples of *A. punctatus* (J in Fig. 2a); this lineage also includes samples from two sites in the eastern Amazonian border in the Brazilian states of Pará and Mato Grosso (PA and MT in Fig. 2, respectively). Atlantic Forest samples of *A. ortonii* composed four lineages, while three lineages represent *P. marmoratus* in this region.

For the three species, coalescent-based phylogenetic analyses recovered Atlantic Forest lineages as nested within Amazonian lineages, supporting the hypothesis that ancestral ranges trace back to Amazonia (Fig. 2). Moreover, in the case of *A. punctatus* and *A. ortonii* (the two species with available samples for all three regions), phylogenetic analyses found Atlantic Forest lineages to be more closely related to those from eastern Amazonia than to those from southwestern Amazonia.

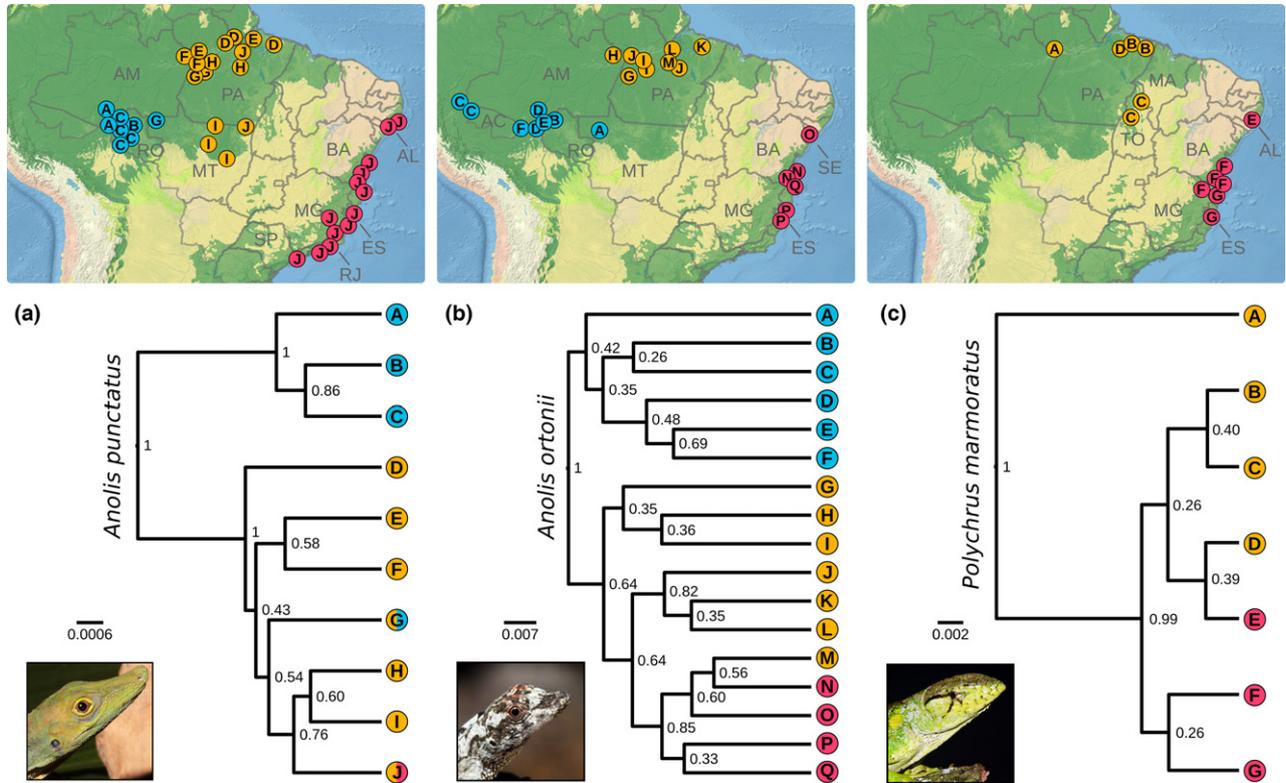


Fig. 2 Sampled sites and coalescent-based phylogeny of *Anolis punctatus* (a), *Anolis ortonii* (b) and *Polychrus marmoratus* (c) based on a multilocus data set, depicting the relationships between putative independently evolving lineages as inferred based on *b*GMYP. Colours correspond to the spatial groups used in analyses of alternative demographic scenarios in *DIYABC*, as follows: blue, southwestern Amazonia; orange, eastern Amazonia; red; Atlantic Forest. Brazilian state acronyms on maps are as follows: AC, Acre; AL, Alagoas; AM, Amazonas; BA, Bahia; ES, Espírito Santo; MA, Maranhão; MG, Minas Gerais; MT, Mato Grosso; PA, Pará; RJ, Rio de Janeiro; RO, Rondônia; SE, Sergipe; SP, São Paulo; TO, Tocantins.

Southwestern Amazonian lineages of *A. punctatus* compose a maximally supported clade (PP = 1), while eastern Amazonian and Atlantic Forest lineages compose another major clade (PP = 1). In the case of *A. ortonii*, the four Atlantic Forest lineages compose a moderately supported clade along with one lineage from eastern Amazonia in the state of Pará (PP = 0.85). Similar to *A. ortonii*, Atlantic Forest *P. marmoratus* are paraphyletic (Fig. 2). However, for both *A. ortonii* and *P. marmoratus*, nodal support values for the relationships between lineages were generally low (Fig. 2).

While phylogenetic relationships between Atlantic Forest lineages were poorly supported in the coalescent-based analyses, mitochondrial gene trees further support the view that Atlantic Forest samples are more closely related to the eastern than to the southwestern Amazonian samples (Figs S1–S3, Supporting information). Furthermore, these trees nest the southern Atlantic Forest samples within those from the northern Atlantic Forest. In *A. punctatus*, for instance, a clade of individuals from northeastern Brazil, sampled in the state of Alagoas (AL in Fig. 2), is sister to all remaining

Atlantic Forest samples (PP = 0.95). Within the latter group, a clade from the state of Bahia (BA in Fig. 2) is recovered as sister to a clade composed of samples distributed in more southern sites (PP = 1). Similar patterns were recovered within *A. ortonii* and *P. marmoratus* (Figs S1–S3, Supporting information).

Best-fit historical scenarios

Based on coalescent simulations and ABC, we find that the observed genetic data are consistent with the hypothesis that the better-sampled *A. punctatus* and *A. ortonii* colonized the Atlantic Forest from eastern Amazonia. For these lizards, alternative scenarios positing colonization of the Atlantic Forest from southwestern Amazonia received low to no support (PP < 0.03).

The best-fit demographic scenario differed between the three species. For *A. punctatus* (PP = 0.99) and *A. ortonii* (PP = 0.68), results favour the scenario of a population bottleneck associated with the colonization of the Atlantic Forest, followed by pronounced population expansion in the Atlantic Forest (Fig. 1b). On the other

hand, the best-fit scenario for *P. marmoratus* (PP = 0.81) was that of an ancestral population occurring in both eastern Amazonia and in the Atlantic Forest which was then split between domains, with no detectable population bottleneck involved (Fig. 1a).

Posterior parameter estimates based on best-fit scenarios for *A. punctatus*, *A. ortonii* and *P. marmoratus* support mid-Pleistocene divergences between Atlantic Forest and eastern Amazonian lineages, dating back to 545 kya (median value; 95% credibility interval (CI) = 0.19–1.62 Ma) in *A. punctatus*, 501 kya (CI = 0.16–1.24 Ma) in *A. ortonii* and 740 kya (CI = 0.14–2.37 Ma) in *P. marmoratus* (posterior estimates of all model parameters in Table 1; plots of parameter estimates presented in Fig. S4, Supporting information). Effective population size estimates suggest that the initial pool of Atlantic Forest colonizers was ca. 95-fold smaller than that of the ancestral eastern Amazonian population in *A. punctatus* and ca. 85-fold smaller in *A. ortonii* (Table 1), supporting a pronounced population bottleneck following dispersal into the Atlantic Forest in the two *Anolis*. The DIYABC results also suggest that *A. punctatus* and *A. ortonii* underwent an 89-fold and 35-fold population expansion in the Atlantic Forest, respectively, once it was colonized. By contrast, no population bottleneck or subsequent population expansion was detected in the Atlantic Forest samples of *P. marmoratus* (Table 1). Median substitution rates were estimated as 1.27×10^{-8} , 1.72×10^{-8} and 8.2×10^{-9} substitutions per site/year for the mitochondrial locus of *A. punctatus*, *A. ortonii* and *P. marmoratus*, respectively, and ranged between 1.31×10^{-10} and 7.92×10^{-10} substitutions per site/year for each nuclear marker of the three species (see Table S2, Supporting information, for substitution rates estimated for all loci).

Model validation based on principal component analyses of the summary statistics confirmed that the observed data were contained within the space of simulated data, in all three species (Fig. S4, Supporting information). Posterior error rates based on pseudo-observed data sets were estimated as 0.19 for both *A. punctatus* and *A. ortonii* and 0.09 for *P. marmoratus*, suggesting that simulated scenarios were overall mutually identifiable.

Best-fit codivergence models

We found support for synchronous divergences between eastern Amazonian and Atlantic Forest lineages across *A. punctatus*, *A. ortonii* and *P. marmoratus* (PP = 0.48). We recovered significantly lower support for a scenario of completely temporally idiosyncratic divergences (PP < 0.09), as well as for each of the three scenarios in which two of the three species have codiverged (PP < 0.17). This result is further supported by the

Table 1 Posterior estimates of the time of divergence (*T*, in millions of years) between lizards in the Atlantic Forest (AF) and eastern Amazonia (EAm), the time of population bottlenecks in the Atlantic Forest, and effective population sizes (*N_e*, in millions of individuals), based on best-fit scenarios in DIYABC analyses. Credibility intervals (CI) of 95% are indicated

	Median	Mean	Mode	Lower 95% CI	Upper 95% CI
<i>Anolis punctatus</i>					
<i>T</i> EAm-AF divergence	0.545	0.628	0.450	0.194	1.620
<i>T</i> AF population expansion	0.290	0.337	0.240	0.092	0.833
<i>N_e</i> WAm	1.860	1.950	1.610	0.961	3.510
<i>N_e</i> EAm	3.360	3.280	3.770	1.540	4.850
<i>N_e</i> AF bottleneck	0.035	0.040	0.008	0.003	0.094
<i>N_e</i> AF after expansion	3.120	3.160	3.000	1.680	4.650
<i>Anolis ortonii</i>					
<i>T</i> EAm-AF divergence	0.501	0.565	0.455	0.156	1.240
<i>T</i> AF population expansion	0.276	0.342	0.110	0.032	1.020
<i>N_e</i> WAm	2.700	2.740	2.410	1.430	4.390
<i>N_e</i> EAm	4.530	4.400	4.600	3.040	4.980
<i>N_e</i> AF bottleneck	0.054	0.053	0.064	0.005	0.098
<i>N_e</i> AF after expansion	1.870	2.090	1.190	0.232	4.650
<i>Polychrus marmoratus</i>					
<i>T</i> EAm-AF divergence	0.740	0.876	0.482	0.136	2.370
<i>N_e</i> EAm-AF ancestor	2.190	2.320	1.400	0.761	4.280
<i>N_e</i> EAm after vicariance	1.290	1.560	0.681	0.191	4.220
<i>N_e</i> AF after vicariance	1.260	1.370	1.170	0.336	3.050

dispersion index of divergence times (the variance/mean of the divergence times across all population pairs), which equalled zero (CI = 0–0.83). In agreement with the DIYABC analyses, posterior estimates from *dpp-msbayes* suggest that synchronous divergences between Amazonian and Atlantic Forest populations of the three species happened at around 950 kya (CI = 0.20–2.08 Ma), assuming an average substitution rate across loci of 2.42×10^{-9} per generation (from DIYABC estimates).

An analysis incorporating postdivergence migration between eastern Amazonian and Atlantic Forest populations received slightly lower support than one that did not incorporate gene flow, yet posterior probabilities were very similar between models (PP = 0.48 vs. 0.52). This result suggests that the genetic data (or the summary statistics implemented in *dpp-mrbayes*) do not provide enough signal to capture migration across forest blocks following initial population divergence.

Validation analyses based on pseudo-observed data sets confirm that our *dpp-msbayes* analyses were not biased towards a scenario of synchronous divergences (Fig. S5, Supporting information). Validation analyses also confirm that our estimates of the dispersal index of divergence times are robust to the incorporation of a migration parameter (Fig. S5, Supporting information).

Discussion

This study provides insights about likely routes of former forest connections and the directionality of dispersal between Amazonia and the Atlantic Forest. An Amazonian origin for *A. ortonii*, *A. punctatus* and *P. marmoratus* agrees with patterns of phylogeographic structure previously recovered for birds (Batalha-Filho *et al.* 2013), rodents and marsupials (Costa 2003), and other lizards (Pellegrino *et al.* 2011). Our findings support the hypothesis that past forest expansion in north-eastern South America promoted forest connections and enabled biotic exchange between these two major forest blocks (Cheng *et al.* 2013). They also reinforce the view that climatic variation over time has led to taxonomically similar and historically linked communities between eastern Amazonia and the northern component of the Atlantic Forest.

Our investigation sheds light on the timing of historical biotic exchange between Amazonia and the Atlantic Forest and supports the hypothesis that documented precipitation shifts during the Quaternary have been key drivers of forest cover change in South America (Cheng *et al.* 2013). The conclusion that the three focal lizard species expanded synchronously into the Atlantic Forest in the mid-Pleistocene is consistent with paleoenvironmental data available; patterns of travertine deposition suggest pulses of rainforest expansion over the xeric Caatinga as much as 900 kya (Auler *et al.* 2004), which is close to the range of colonization times that we estimated for *A. punctatus*, *A. ortonii* and *P. marmoratus*. Because the amplitude of recurring precipitation increase in northern South America varied over time, matching the precession component of Earth's orbital cycles (Cheng *et al.* 2013), it is possible that connections and biotic exchange between Amazonia and the Atlantic Forest were restricted to (or more pronounced at) time periods in which levels of precipitation were particularly high. Our data suggest that one such pivotal event may have occurred around 950 thousand years ago.

A scenario of recurrent forest connections could have potentially led to periodic re-establishment of gene flow after initial population separation. Paleontological and geochemical studies have inferred pulses of rainforest expansion around present-day northeastern Brazil

happening as recently as the Holocene (de Oliveira *et al.* 1999; de Vivo & Carmignotto 2004; Cheng *et al.* 2013). However, our tests of gene flow following initial divergence were inconclusive, providing equal support for historical models that incorporated postdivergence migration and models that did not. Intriguingly, phylogenetic patterns in a range of taxa indicate no repeated dispersal events between Amazonia and the Atlantic Forest following initial colonization (e.g. Geurgas & Rodrigues 2010; Pellegrino *et al.* 2011; Fouquet *et al.* 2012a, b; Rodrigues *et al.* 2014), in spite of the opportunity provided by subsequent periods of increased humidity and presumed forest expansions.

Due to the cyclical nature of Late Quaternary climate change (Cheng *et al.* 2013), one could expect to detect signals of population size shifts in the Atlantic Forest after colonization. However, our analyses estimated that the pulse of population expansion in Atlantic Forest *A. punctatus* and *A. ortonii* (Table 1) precedes multiple subsequent cycles of climate change documented for this region (Cheng *et al.* 2013). The fact that we detected no signatures of more recent population shifts suggests that patterns of genetic diversity of the two *Anolis* were strongly shaped by the initial colonization event. However, more recent demographic changes have been inferred for these three species in the Atlantic Forest based on population-level analyses using reduced genomic data (Prates *et al.* 2016). That study recovered population expansion in *A. punctatus* around 60 kya, which is later than the time of expansion estimated from our DIYABC analyses of this species (~290 kya). In the case of *A. ortonii* and *P. marmoratus*, Prates *et al.* (2016) found signatures of population contraction in the Atlantic Forest around 72 and 81 kya, respectively, which contrasts with population expansion in *A. ortonii* (~276 kya) and no size change in *P. marmoratus* based on our DIYABC analyses. Given the different estimated times and population trends (in the case of *A. ortonii* and *P. marmoratus*) between Prates *et al.* (2016) and the present investigation, it seems likely that these two studies captured distinct and consecutive demographic events. If so, it is possible that the genetic data sets used (i.e. six loci vs. thousands of single nucleotide polymorphisms) provide signals of distinct demographic events which have occurred at different time scales. Nevertheless, it is currently unclear whether and how results based on traditional and next-generation genetic data sets are comparable.

We find that anole lizards do not fit to a scenario of colonization through former forest corridors around present-day southwestern Brazil, which have been implicated in instances of closely related taxa occurring in both southern Atlantic Forest and southwestern Amazonian or Andean forests (Batalha-Filho *et al.* 2013;

Cheng *et al.* 2013). Based on divergence time estimates among bird taxa, it has been suggested that southern connections between these two rainforest blocks generally predate 5.6 Ma, whereas northern connections were more recent, between 5.5 and 4.17 Ma (Batalha-Filho *et al.* 2013). Old divergences (dating to the Miocene and Oligocene) have been also reported for a number of lizard clades that show disjunct distribution across forests, such as between species of *Enyalius* (Rodrigues *et al.* 2014) and between the sister genera *Leposoma* and *Loxopholis* (Pellegrino *et al.* 2011) and *Chatogecko* and *Coleodactylus* (Geurgas & Rodrigues 2010). The same was found between frog species of *Adelophryne* (Fouquet *et al.* 2012a) and *Adenomera* (Fouquet *et al.* 2014) and between the genera *Dendrophryniscus* and *Ama-zophrynella* (Fouquet *et al.* 2012b). However, spatial patterns of phylogenetic structure suggest that most of these old divergences represent northern colonizations, similar to our recently diverged populations. This pattern disagrees with the idea that different dispersal routes were restricted to distinct time scales (Batalha-Filho *et al.* 2013).

Lastly, our results indicate that the expansion of suitable habitats and associated opportunities for dispersal resulted in distinct demographic patterns among species. While we recovered remarkable temporal congruence in the colonization of the Atlantic Forest between the focal lizards, the best-fit historical demographic scenarios differed among them. ABC analyses on the observed genetic data are consistent with a history of pronounced population expansion in *A. punctatus* and *A. ortonii* after colonization of the Atlantic Forest. By contrast, results for *P. marmoratus* are consistent with the subdivision of a large, panmictic ancestral population. The effective population size of *P. marmoratus* in the Atlantic Forest at the time of colonization was estimated as two orders of magnitude larger than that of the two other species in the same region (Table 1). These findings agree with the view that former reconfiguration of South American rainforests had species-specific impacts on the local fauna (Prates *et al.* 2016). We hypothesize that the contrasting patterns observed across these three species may be associated with distinct tolerances to forest fragmentation and to a matrix of open and drier environments (Caatinga and Cerrado). This idea is consistent with available information on the ecology and distribution of these species, as *P. marmoratus* has been recorded in transition zones between Amazonian forests and Cerrado savannas, while *A. ortonii* and *A. punctatus* seem to be strictly associated with closed-canopy wet forests (Vitt *et al.* 2003; Kawashita-Ribeiro & Ávila 2008; Ribeiro-Júnior 2015). In the case of *P. marmoratus*, some capacity to tolerate transition areas between South American

rainforests and their adjacent settings may have enabled higher levels of gene flow under environmental conditions that were rather restrictive for the two *Anolis* species. This hypothesis has profound implications for biogeographic studies: despite the magnitude of corridors between regions, the apparent biogeographic history of a species may be strongly determined by its dispersal capacity within and across patches of suitable habitat, which could confound the distinction between dispersal and vicariance.

Based on ample geographic sampling of genetic diversity in three codistributed lizard species, we track historical relationships between populations and the timing of former biotic exchange between highly diverse tropical rainforest domains. The spatial patterns of genetic structure indicate that cessation of environmental breaks as a result of inferred climatic fluctuations across time provided key opportunities for dispersal and species exchange between regions. Intriguingly, we found this influx of species to be asymmetric, in agreement with the hypothesis that some regions have acted mostly as providers and others as receivers of biodiversity (Santos *et al.* 2009). Our results also point to species-specific responses to the former expansion of suitable habitats, strengthening assertions that biological attributes may play an important role by shaping responses to shared environmental change (Paz *et al.* 2015; Zamudio *et al.* 2016). Understanding whether and how climate change over time has led to opportunities for dispersal and biotic exchange, and how different taxa have explored such opportunities, will contribute to investigations of community assembly in other biologically diverse systems.

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Data accessibility

All sequence data were deposited in the GenBank (Accession numbers KM204350-4, KM598666-749, and KX760196-1163).

Supporting information

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

Fig. S1 Mitochondrial gene tree of *Anolis punctatus*.

Fig. S2 Mitochondrial gene tree of *Anolis ortonii*.

Fig. S3 Mitochondrial gene tree of *Polychrus marmoratus*.

Fig. S4 Plots of the PC analyses and of the posterior distributions of model parameters from DIYABC analyses.

Fig. S5 Plots of the validation analyses based on pseudo-observed datasets in *dpp-msbayes* analyses.

Table S1 Sampled specimens, including locality information.

Table S2 Posterior estimates of substitution rates for the sampled loci (in substitutions per DNA site/year), from DIYABC analyses.